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Par Elena, HEGO

Tolérance au Cu chez *Agrostis capillaris* L. : du phénotype vers les mécanismes moléculaires

Sous la direction du Dr. Michel MENCH

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Résumé

Des populations tolérante (métallicole: M) et sensible (non-métallicole: NM) d'*Agrostis capillaris* L. ont été exposées à des doses croissantes de Cu (1-50 µM) pour étudier la tolérance au Cu par une approche pluridisciplinaire. Selon les paramètres phénotypiques (biomasse, longueur des feuilles et symptômes visuels), les plantes M ont une meilleure croissance aux expositions supérieures à 10 µM Cu. Les concentrations en Cu des tissus reflètent une rétention racinaire (phénotype d'exclusion) et une réduction de la translocation vers les feuilles quand le stress augmente. En excès de Cu, le protéome soluble racinaire présente des altérations du métabolisme énergétique chez M et NM, plus marquées chez NM (glycolyse, cycle de Krebs /phosphorylation oxydative). Le protéome foliaire indique des impacts sur les phases claires et obscures de la photosynthèse chez M et NM, et un besoin plus important en acides aminés soufrés (augmentation des cystéine et méthionine synthases). Chez NM, l'augmentation d'enzymes de la glycolyse, de la voie des pentoses phosphates et du cycle de Calvin indiquent un besoin énergétique accru, tandis que la stimulation des chaperonnes et des processus de synthèse protéique suggère des impacts sur le métabolisme des protéines et celle des enzymes redox un stress oxydatif plus fort. Plusieurs protéines, surexprimées ou accumulées, interviendraient dans la tolérance au Cu chez M, en protégeant le métabolisme des protéines (HSP70, racines et feuilles) et en augmentant les mécanismes anti-oxydants (ascorbate péroxydases), de détoxification (GST et aldéhyde déshydrogénase) et de protéolyse (peptidase et protéasomes, racines).

Mots clés : pseudo-metallophyte, excluder, Cu-tolérance, protéome soluble.

Pluridisciplinary study of Cu tolerance in *Agrostis capillaris* L.: from phenotype to molecular mechanisms.

Abstract

Cu-tolerant (metallicolous: M) and sensitive (non-metallicolous: NM) populations of *Agrostis capillaris* L. were exposed to increasing Cu concentrations (1-50 µM) to investigate Cu tolerance by a pluridisciplinary approach. Phenotypic parameters (biomass production, shoot length, and visual symptoms) indicated a higher growth and a better fitness of M plants over 10 µM Cu. Plant Cu concentrations indicated root Cu retention ('excluder' phenotype) and a reduced root-to-shoot translocation with increasing Cu stress. Based on root soluble proteome energy metabolism was altered by Cu excess in both populations with stronger impacts in NM (glycolysis, Krebs cycle/oxidative phosphorylation). Changes in shoot proteome showed impacts on both light dependent and independent photosynthesis phases in both populations, and an enhanced need in S-containing amino-acids (up-regulation of cysteine/methionine synthases). In NM leaves, increase of enzymes involved in glycolysis, pentose phosphate pathway and Calvin cycle indicated a stimulation of energy metabolism, while enhanced protein synthesis processes and protein chaperones suggested impacts on protein metabolism and increase of redox enzymes indicated a higher oxidative stress. Several over-expressed or accumulated proteins may be pivotal for Cu tolerance in M plants, for protecting protein metabolism (Heat shock protein 70kDa, roots and leaves), increasing anti-oxidative (ascorbate peroxidases, roots) – detoxification (Glutathione S-transferase and aldehyde dehydrogenase, roots) and proteolysis (peptidase and proteasome subunits) processes.

Keywords: pseudo-metallophyte, excluder, Cu-tolerance, soluble proteome.

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Synthèse des travaux

Deux populations d'*Agrostis capillaris*, l'une tolérante (M) et l'autre sensible (NM) à l'excès de Cu, issues respectivement d'un site contaminé en Cu et d'un site non-contaminé, ont été sélectionnées pour leur plasticité phénotypique afin d'étudier la réponse des plantes à l'excès de Cu et d'identifier les mécanismes impliqués dans la tolérance au Cu en utilisant une approche pluridisciplinaire.

Le premier chapitre est une étude bibliographique des effets phytotoxiques de l'excès de Cu sur les plantes, réalisée en intégrant les connaissances à plusieurs échelles, des études de plein champ aux déterminants moléculaires identifiés par la protéomique. En excès, le Cu est phytotoxique, mais certaines espèces végétales, dont *A. capillaris*, appelées pseudo-métallophytes, ont une plasticité phénotypique pour la tolérance aux métaux (métalloïdes), dont Cu, avec des populations tolérantes (Métallicole : M) et sensibles (Non-Métallicole : NM). Ces espèces sont des modèles utiles pour l'étude des mécanismes physiologiques et moléculaires impliqués dans la tolérance au Cu, en comparant ces populations M et NM en condition de stress.

La section 8, 'Plasticité phénotypique de la tolérance aux métaux chez *Agrostis capillaris*', destinée à la publication, met l'accent sur la tolérance aux métaux chez des populations d'*A. capillaris* et a permis de formuler plusieurs hypothèses sur des mécanismes potentiellement impliqués dans la tolérance au Cu des populations M.

Afin d'identifier les processus moléculaires impliqués dans la réponse à l'excès de Cu chez *A. capillaris* et dans la tolérance au Cu de la population M, l'expression différentielle du protéome soluble en réponse aux expositions croissantes en Cu a été comparée entre les populations M et NM. Une expérience exploratoire (Chapitre 2), conduite en 2008 et publiée sous forme d'article par le journal 'Proteomics' (DOI: 10.1002/pmic.201300168), a analysé le protéome soluble racinaire des populations M et NM d'*A. capillaris* exposées à 5 doses de Cu (1, 5, 10, 15 et 30 µM Cu, hydro-culture sur perlite pendant 2 mois).

19 protéines avec une expression différentielle ont été identifiées en utilisant la spectrométrie de masse (LC-MS/MS) et des bases de données d'ESTs. Aux fortes expositions en Cu (15-30 µM), les surexpressions de la triosephosphate isomerase et la fructose bisphosphate aldolase suggèrent des altérations de la glycolyse dans les racines NM et une production accrue de glycérone-P et de méthylglyoxal. Chez cette population, la diminution de l'expression des tubulines indiquerait des impacts sur le cytosquelette, et l'augmentation des 5-methyltetrahydropteroyltriglutamatehomocysteine méthyltransferase (metE) et S-adenosyl-

méthionine (SAM) synthase (SAMS) refléterait une stimulation de la synthèse d'éthylène. Parallèlement, des quantités accrues de L-méthionine et S-adénosylméthionine faciliteraient la production de nicotianamine (NA), impliqués dans la chélation du Cu et de L-cystéine, nécessaire pour la synthèse de glutathion (GSH).

Cette première étude, exploratoire, suggère que la tolérance au Cu de la population M d'*A. capillaris* ne résulterait pas d'un mécanisme unique mais plutôt de la coopération de plusieurs processus, incluant une meilleure détoxification des ions superoxydes (augmentation de l'expression d'une [Cu/Zn] superoxyde dismutase).

Les chapitres III, IV et V correspondent aux différentes parties d'une même expérience (Fig. 1), dont le but est de réaliser une étude pluridisciplinaire de la tolérance au Cu chez *A. capillaris*, en comparant des populations M et NM d'une trentaine d'individus soumises à des doses croissantes de Cu (1, 5, 10, 15, 20, 25, 30, 40 et 50 µM, hydro-culture sur perlite pendant 3 mois). L'exposition a été chronique, de la germination à la récolte, et les doses sélectionnées pour simuler l'homéostasie et l'excès. La perlite a permis d'apporter de la silice aux végétaux et de simuler une porosité plus proche d'un sol, favorisant le respect de l'ultrastructure des racines.

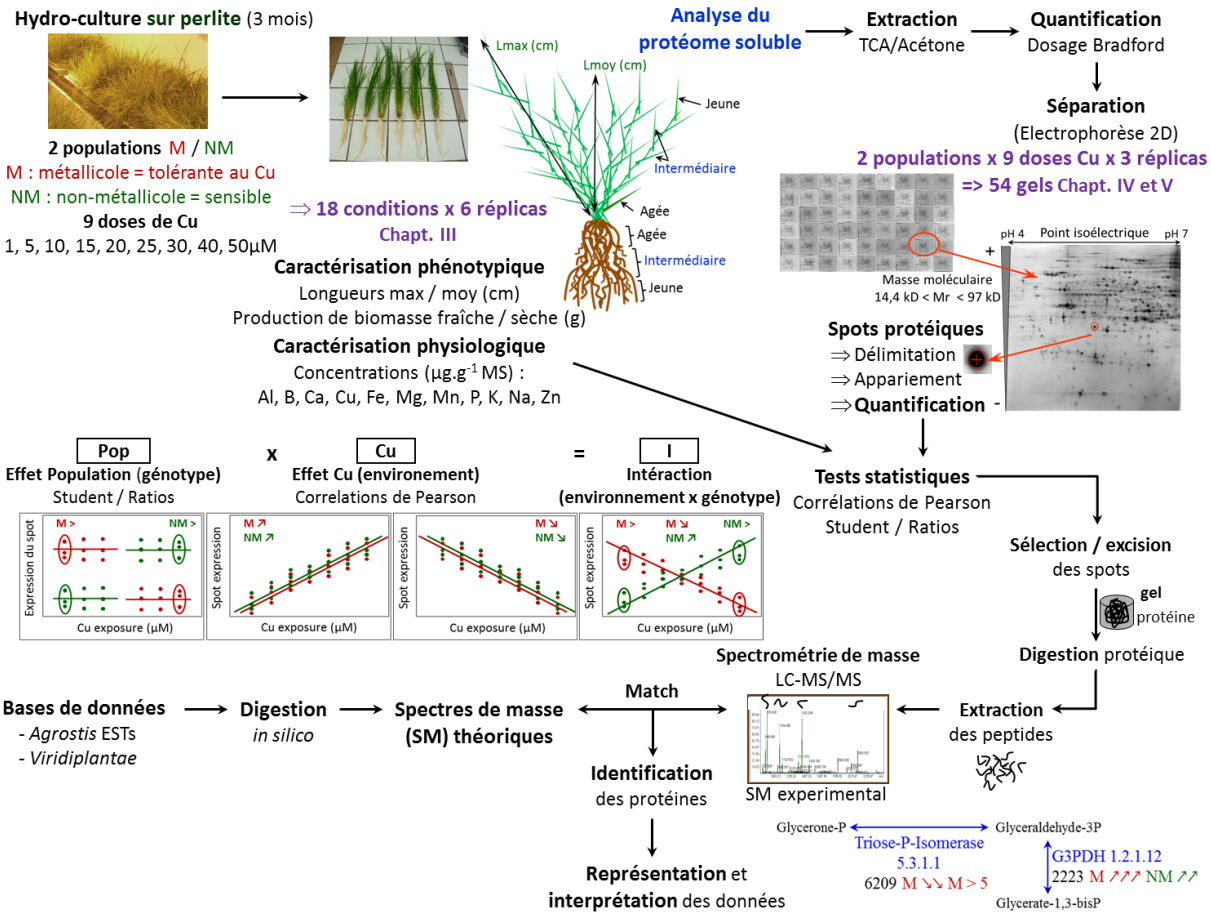

Figure 1 : Résumé du protocole expérimental des expériences présentées dans les chapitres III, IV et V, avec la présentation des outils statistiques.

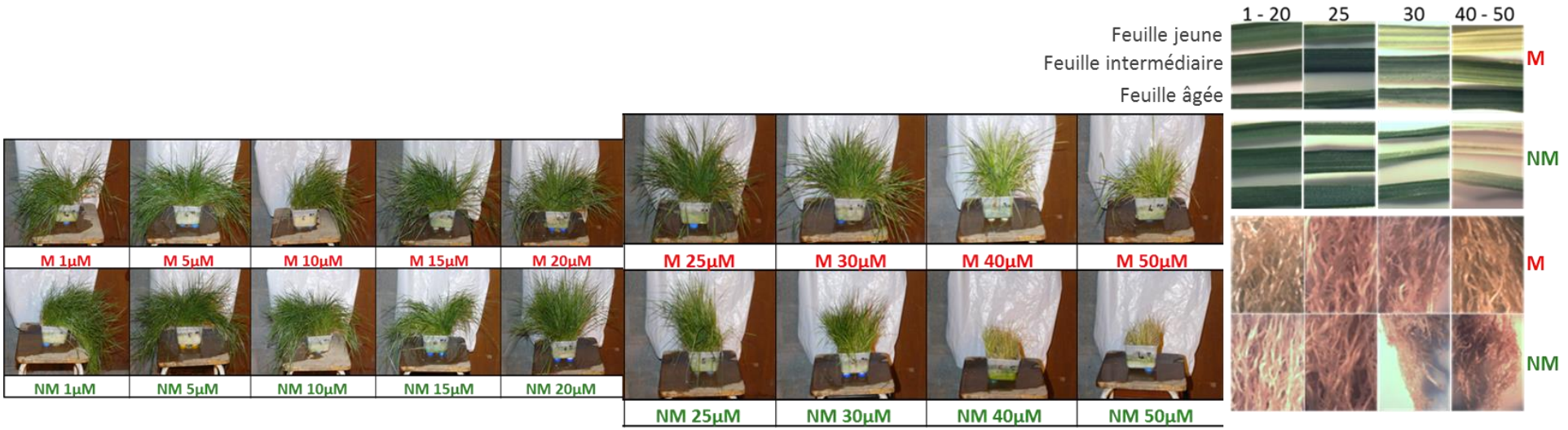

Figure 2 : Impacts du Cu (1-50 µM Cu) sur la croissance des populations M (rouge) et NM (vert) d'*Agrostis capillaris* et symptômes foliaires et racinaires.

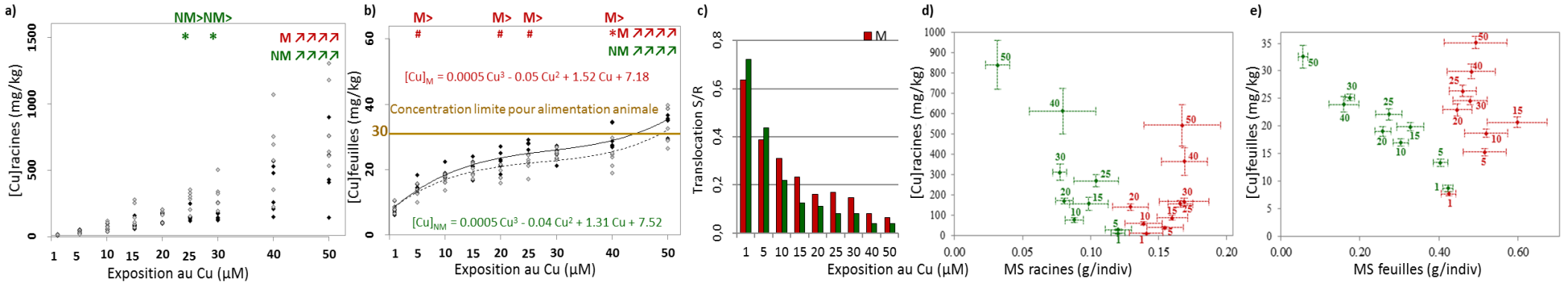

Figure 3: Concentrations en Cu dans (a) les racines et (b) les feuilles, et (c) Facteur de transfert (Cu feuilles/Cu racines) des populations M (rouge/noir) et NM (vert/gris) d'*A. capillaris* exposées à des doses croissantes de Cu (1-50 µM). Relation entre les concentrations en Cu et la production de biomasse (MS) dans (d) les racines et (e) les feuilles des populations M (rouge) et NM (vert).

La croissance des plantes a été caractérisée par les longueurs maximales (Lmax) et moyennes (Lmoy) des parties aériennes, ainsi que par la production moyenne de biomasse fraîche (FW) et sèche (DW) par individu. L'excès de Cu réduit drastiquement la croissance et la biomasse des individus NM alors que celles des individus M restent constantes ou diminuent légèrement. Pour des doses de Cu supérieures à 10 µM, la croissance des populations M est significativement supérieure, quel que soit le paramètre mesuré. Aux fortes expositions (25-50 µM Cu), des symptômes phytotoxiques, i.e. racines coralloïdes avec coloration jaune brun foncée, chloroses des feuilles jeunes, sont visibles chez les deux populations mais plus marqués chez NM (Fig. 2).

Les concentrations en Al, B, Ca, Cu, Fe, Mg, Mn, P, K, Na, et Zn ont été mesurées dans les racines et les feuilles. L'augmentation des concentrations racinaires et foliaires en P et K suggèrent un besoin accru avec l'augmentation du stress en Cu, mais la diminution des concentrations en K après 25 µM Cu chez NM indique soit une réduction du prélèvement, soit une fuite liée à l'altération de l'intégrité membranaire. La diminution des concentrations en Fe laisse supposer une déficience dans les parties aériennes des 2 populations qui expliquerait, au moins en partie, les chloroses observées aux fortes expositions en Cu.

Chez la population M, un double mécanisme a pour conséquence de réduire les concentrations foliaires en Na, avec un stockage plus important dans les racines (25-40 µM Cu) et une translocation plus faible pour l'ensemble des expositions en Cu testées. Pour Ca, un prélèvement réduit dans les racines expliquerait la diminution de ses concentrations foliaires.

L'augmentation des concentrations en Cu dans la solution nutritive (exposition) entraîne un accroissement des concentrations tissulaires en Cu, plus marqué dans les racines que dans les feuilles (Fig. 3a, b). Il indique une rétention de Cu dans les racines (phénotype d'exclusion) mais aussi une diminution de la translocation quand le stress en Cu augmente (ratios feuilles/racines, Fig. 3c). L'existence d'un(e) plus faible prélèvement/accumulation du Cu dans les racines des plantes M n'est suggérée qu'aux expositions moyennes en Cu (25-30 µM Cu) par des concentrations plus faibles chez M (Fig. 3a) ; l'existence d'une translocation réduite est réfutée par les concentrations foliaires en Cu supérieures chez M à 5, 20, 25 et 40 µM Cu (Fig. 3b). L'augmentation du Cu dans les tissus (feuilles et racines) est positivement corrélée avec la diminution de biomasse pour la population NM mais aucune corrélation n'existe pour M (Fig. 3d, e). Ces résultats suggèrent une meilleure homéostasie cellulaire du Cu chez les individus M, hypothèse étudiée par l'analyse du protéome soluble des racines et des feuilles intermédiaires (3 réplicas pour chaque condition expérimentale : population x Cu, Fig. 1).

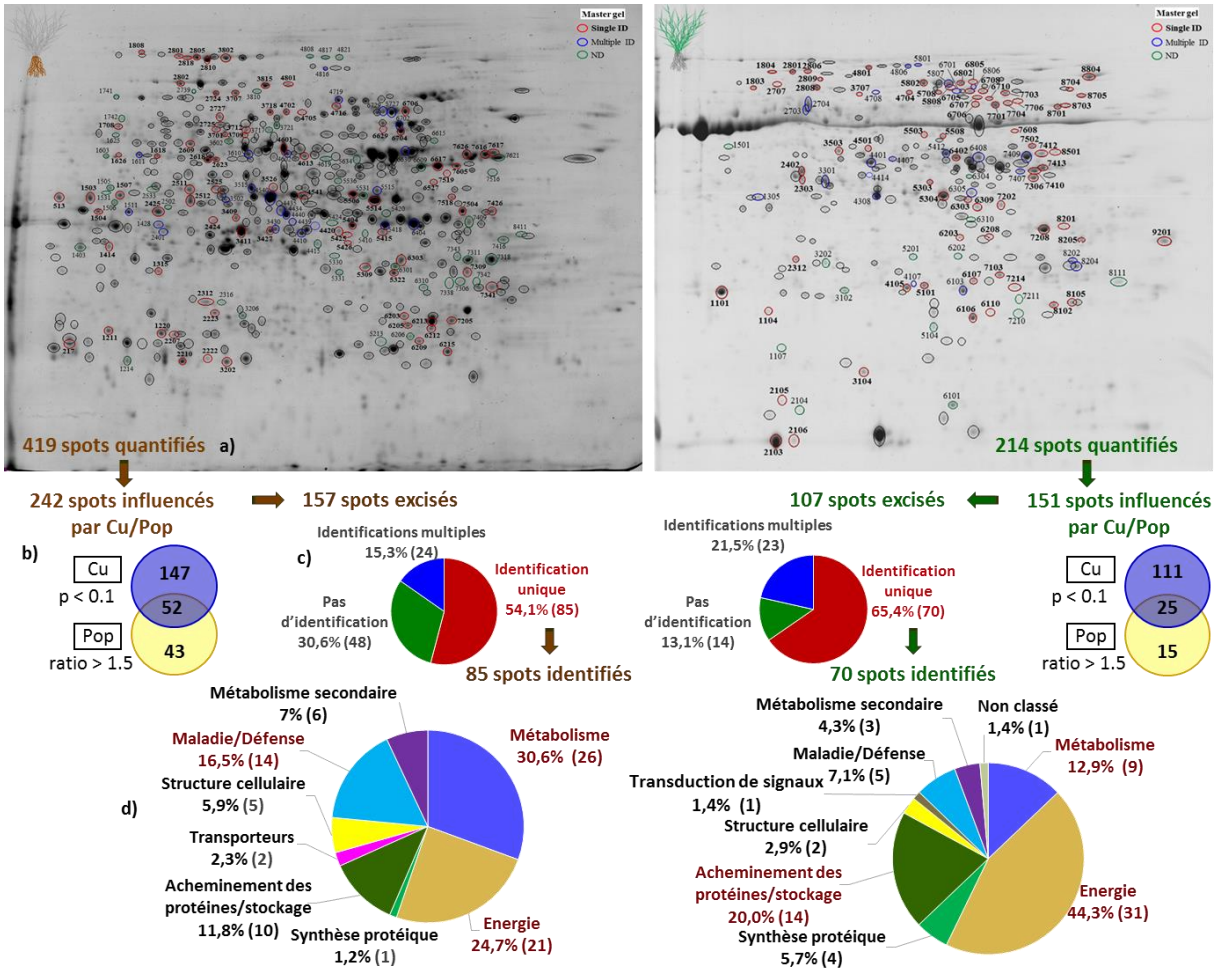

Figure 4 : Résumé de (a) la distribution des spots (gels de référence avec spots excisés) ; (b) des analyses statistiques ; (c) de l'identification des spots excisés (en bleu : 2 ou 3 identifications probable ; en vert : pas d'identification probable ; en rouge : 1 identification unique) ; (d) de la classification des spots avec une identification unique selon les catégories fonctionnelles définies par Bevan *et al.* (1998), pour le protéome soluble des racines (à gauche) et des feuilles (à droite) d'*A. capillaris*.

Après extraction (acide trichloracétique/acétone), les protéines solubles ont été séparées par électrophorèse 2D (gradient linéaire de pH 4-7, bleu de Coomassie). L'analyse des images des gels (PDQuest, 54 gels 2D) a permis de délimiter et de quantifier 419 spots pour les racines et 214 pour les feuilles (gels de référence, Fig. 5a). L'effet du Cu a été testé avec des corrélations de Pearson (pval < 0.1) et l'effet Pop avec des ratios (ratio > 1.5, Fig. 1). Parmi les 242 (racines) et 151 (feuilles) spots influencés par le Cu et /ou la population (diagramme de Venn modifié, Fig. 5c), 157 et 151 spots ont été respectivement sélectionnés dans les racines et les feuilles (pval < 0.05 et ratio > 1.5), excisés, puis analysés en spectrométrie de masse pour déterminer leur identité probable. Environ 46% et 35% de ces spots n'ont pu être identifiés mais 85 et 70 spots ont été associés à une identification unique, puis classés selon les catégories fonctionnelles définies par Bevan *et al.* (1998 ; Fig. 5d). Ces identifications, associées au sens de variations sont présentées pour les racines (Fig. 6, 85 spots) et les feuilles (Fig. 7, 70 spots).

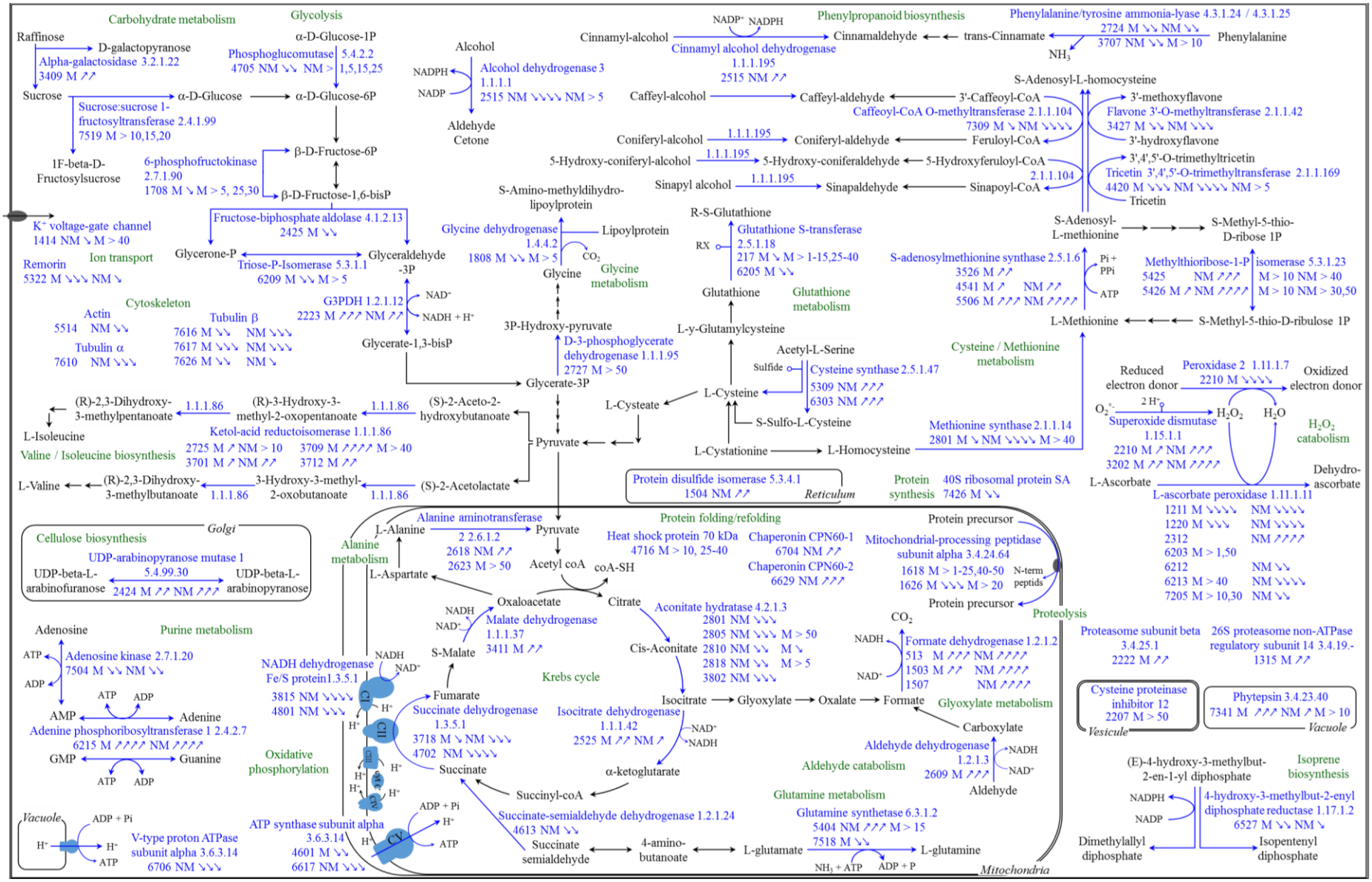

Figure 5 : Fonctions et variations des protéines identifiées (en bleu) dans les processus métaboliques (racines).

Les enzymes sont représentées par leur nom et EC. Les données proviennent du chapitre IV. M / NM: population métallicole / non-métallicole d'*A. capillaris*.

↗ / ↘: corrélation positive / négative (Pearson); p-val: 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; M/NM > 1-50: population avec sur-expression à 1-50 µM Cu (ratio > x1.5).

Dans les racines M et NM, l'excès de Cu altère le métabolisme énergétique, avec un besoin accru en pouvoir réducteur (augmentation de la glycéraldéhyde-3P-déshydrogénase, G3PDH), mais une réduction de la production d'ATP (diminution de l'ATP synthase), associée à une augmentation de la respiration cellulaire (formate déshydrogénase).

Dans les racines de la population NM, une limitation des processus énergétiques et des dommages plus importants sur le métabolisme des protéines sont respectivement suggérés par la diminution de protéines impliquées dans le cycle de Krebs et le transport d'électron (aconitases, succinate déshydrogénase, NADH déshydrogénase Fe/S protéine et V-type proton ATPase) et l'augmentation de plusieurs protéines chaperonnes (CPN60-1, CPN60-2 et protéine disulfide isomérase ou PDI). L'excès de Cu a des impacts négatif sur le cytosquelette des deux populations (diminution de tubulines β), plus marqués chez NM (diminution de tubuline α et actine). L'augmentation, dans les racines NM, de deux cystéine synthases indique un besoin accru en acides aminés soufrés et la diminution d'une méthionine synthase, une limitation de la production de méthionine. La production plus forte de S-adénosylméthionine (SAM), suggérée par l'augmentation des SAM synthétases pourrait jouer un rôle dans la tolérance au Cu, en stimulant la synthèse de nicotianamine, de glutathion ou d'éthylène.

Dans les racines M, la coopération de plusieurs enzymes du métabolisme des carbohydrates pour approvisionner la glycolyse est suggérée par l'augmentation d'une α-galactosidase et la sur-expression d'une sucrose:sucrose 1-fructosyltransférase et une 6-phosphofructokinase pyrophosphate-dépendante aux concentrations intermédiaires en Cu. L'augmentation linéaire de la G3PDH, en opposition au plateau observé pour la population NM, insinue un approvisionnement en NADH plus important aux fortes expositions en Cu (40-50 µM). Plusieurs protéines potentiellement impliquées dans la tolérance des plantes M ont pu être identifiées. L'augmentation des malate (MDH) et isocitrate (IDH) déshydrogénases contribuerait à la chélation du Cu libre dans les cellules, via la synthèse accrue d'acides malique et citrique, tandis que l'augmentation de deux protéasomes et d'une phytepsin, associée à la sur-expression d'une peptidase, permettrait une protéolyse plus efficace, limitant l'accumulation de protéines non-fonctionnelles ou dégradées. Les expressions plus importantes d'une 'heat-shock' protéine (HSP 70KDa), de plusieurs ascorbate péroxydases, et d'une glutathion-S-transférase, aux moyennes et fortes expositions en Cu, associées à l'augmentation d'une aldéhyde déshydrogénase, sous-entendent une protection plus efficace du métabolisme des protéines, et des mécanismes antioxydant et de détoxification renforcés chez cette population.

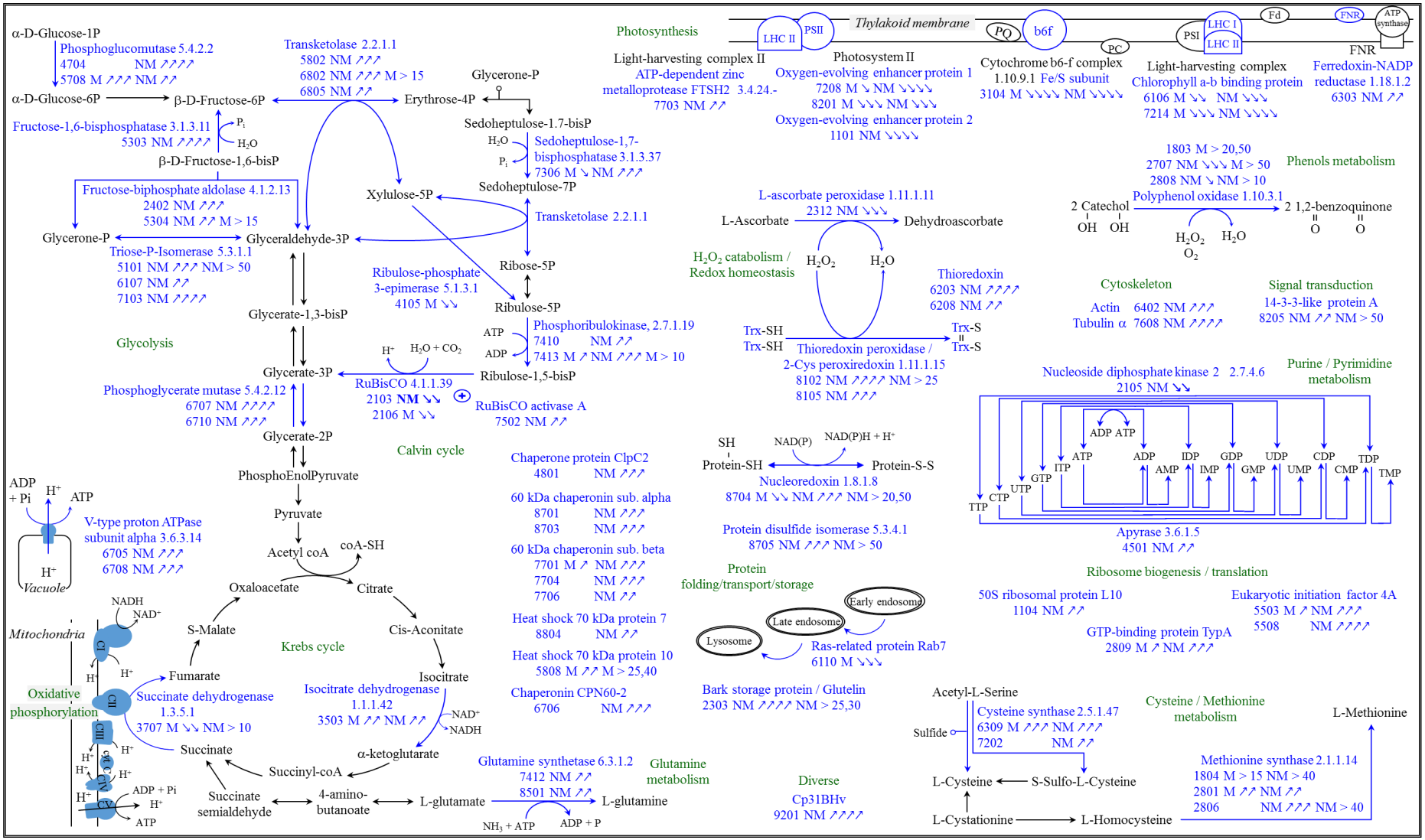

Figure 6 : Fonctions et variations des protéines identifiées (en bleu) dans les processus métaboliques (feuilles).

Les enzymes sont représentées par leur nom et EC. Les données proviennent du chapitre V. M / NM: population métallicole / non-métallicole d'*A. capillaris*. ↗ / ↘: corrélation positive / négative (Pearson); p-val: 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; M/NM > 1-50: population avec sur-expression à 1-50 µM Cu (ratio > x1.5).

Les deux populations présentent des altérations de la photosynthèse au niveau moléculaire, avec la diminution de plusieurs protéines impliquées dans les réactions de transferts d'électron (OEE, Cytochrome b6-f complexe, Chlorophylle a-b binding protéine) et d'assimilation du carbone (RuBisCO) ; mais des dommages oxydants plus importants chez NM sont proposés par l'augmentation d'une métalloprotéase et d'une ferrédoxine réductase. La relation entre les chloroses enregistrées à l'échelle de la plante et la déficience en Fe dans les feuilles est accréditée au niveau moléculaire par la diminution de la sous unité Cytochrome b6-f complexe Fe/S, une protéine impliquée dans la photosynthèse et contenant du Fe. Chez les deux populations, l'augmentation de cystéine et méthionine synthases indique un besoin accru en acides aminés soufrés, impliqués dans la synthèse de glutathion (GSH), nicotianamine (NA), polyamines ou phytochélatines (PC), qui participent à la chélation du Cu libre.

Dans les feuilles NM, une stimulation du métabolisme énergétique est suggérée par l'augmentation d'ATPases, et d'enzymes impliquées dans la glycolyse (phosphoglucomutase, fructose-bisphosphate aldolase, triosephosphate isomérase, et phosphoglycérate mutase) ou le cycle de Calvin (sédoheptulose-1,7-bisphosphatase, RuBisCO activase et phosphoglycérate mutase). La stimulation des processus de synthèse protéique (eukaryotic initiation factor 4A, 50S ribosomal protéine L10 et GTP-binding protéine TypA) et l'induction de plusieurs protéines chaperonnes (ClpC2, 60kDa chaperonin, chaperonin CPN60-2, nucléorédoxine et PDI) indiquent des impacts sur le métabolisme des protéines, alors que l'induction de thiorédoxine et thiorédoxine péroxydases reflète un stress oxydant plus important.

Comme dans les racines, une Heat shock protéine 70kDa est sur-exprimée dans les feuilles M et peut contribuer à protéger le métabolisme des protéines.

Une approche transcriptomique (qPCR) a été menée dans un dernier temps (Chapitre VI), afin de complémenter l'étude de la tolérance au Cu et d'évaluer l'accumulation différentielle d'ARN correspondant à des protéines d'intérêt, impliquées dans la réponse au Cu et identifiées lors de l'expérience préliminaire. L'application d'une telle technique sur cet intervalle d'exposition au Cu apparait relativement limitée ; l'intervalle entre les doses et les changements (tant moléculaires que phénotypiques) induits par le Cu sont trop importants pour comparer les conditions. Cependant, cette expérience a permis de réaliser des banques d'ARN et de construire, tester et valider un couple de primer efficaces pour 19 des 20 gènes sélectionnés : 8 gènes de référence (*EF1*, *RuBisCO*, *Ubi*, *ABC*, *APRT*, *Cyc*, *L2* and *YLS 8*) et 12 gènes d'intérêt (*Act 101*, *Act 3*, *GAPDH*, *Glx I*, *MetE*, *SAMS*, *Cu/Zn-SOD*, *TIM*, *Tub alpha*, *HMA5* et *NAS*).

Ce travail a permis de relier des symptômes phénotypiques à des impacts au niveau moléculaire. La réduction de croissance peut être expliquée, au moins en partie, par des dommages sur la photosynthèse et sur le métabolisme énergétique dans les racines. L'excès de Cu entraîne des changements complexes sur une large variété de processus cellulaires, incluant le métabolisme énergétique, les processus antioxydants et de détoxification, le métabolisme des protéines et du soufre (S). Des impacts moléculaires de l'excès de Cu, sur les métabolismes énergétique et des protéines dans les racines et les feuilles, expliquent les symptômes plus importants chez la population NM.

L'identification de plusieurs protéines, potentiellement impliquées dans la tolérance au Cu de la population M, confirme la coopération de multiples processus (comme suggéré par les résultats de l'étude préliminaire), incluant une meilleure protection du métabolisme des protéines dans les feuilles et les racines (HSP70), un renforcement des processus de protéolyse, des mécanismes antioxydant et de détoxification.

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Caution for readers

The first chapter of this thesis '*Cu in plants, from field pollution to cellular impacts'* consists in a bibliographic survey on the phytotoxic effect of Cu excess, from plants to cells. This part aimed to integrate the knowledge obtained from field experiment to proteomic approaches. Section 8 of this chapter, '*Phenotypic plasticity for metal-tolerance in Agrostis capillaris*' focuses on the previous reports of metal tolerance among and between populations of *A. capillaris*. Once corrected and finalized, this section will be submitted as Review. This first part permitted to formulate several preliminary hypotheses about the mechanisms underlying the higher Cu-tolerance in the metallicolous population.

In the second chapter is presented a preliminary experiment, initiated in 2008, which was designed to compare the differential accumulation of root soluble proteins in response to increasing Cu exposure (1-30 µM Cu) in two Cu-tolerant (Metallicolous, M) and non-tolerant (Non-Metallicolous, NM) populations of *Agrostis capillaris* exposed. This work was submitted to the journal 'Proteomics' as a peer-reviewed paper at the end of April 2013 and accepted recently. The corresponding bibliography list was presented at the end of this chapter to respect the article form. However, for all other chapters the bibliography list was placed at the end of the manuscript, in a form of a general alphabetically-ordered list of publications.

Chapters III, IV and V correspond to complementary parts of a multidisciplinary approach, written as independent experiment for further publication. This work aimed to characterize the plant response to increasing Cu exposure (1-50 µM Cu) in both M and NM populations, and to elucidate the mechanisms underlying the higher tolerance of the M population under Cu excess. *A. capillaris* plants were cultivated under increasing Cu exposure for three months, mimicking a long-term exposition to Cu stress, from germination to harvest. Chapter III presents the variations of plant growth, biomass production and concentrations of several elements in tissues, while chapters IV and V respectively describes the differential accumulation of soluble root and leaf proteins under increasing Cu stress. Chapter VI presents an attempt to complement and enlarge the multidisciplinary approach, by testing the feasibility of a transcriptomic procedure, on these two populations and this range of Cu exposure. This last work aimed at evaluating the differential RNA accumulation of a selected set of proteins under Cu excess. First part of the 'General discussion' consists in the comparison of root and leaf proteomic profiles, then results of the chapters III, IV and V were discussed together in the second part of this chapter to draw a global picture of Cu-induced impacts on plants and to gain clues about the mechanisms underlying the higher tolerance in metallicolous populations.

CHAPTER I: Cu in plants, from field pollution to cellular impacts

1. Cu contamination in soils: sources, dispersion and remediation

Cu is widely used for three main economic sectors of human activities, i.e. industries, farming activities and domestic purposes.

Industrial purposes consist in a large range of production or transformation processes, such as wood treatment, metallurgical and mining activities, electricity, Cu-based pesticides, paper or automobile production, oil refinery etc. (Bes, 2008). In Aquitaine, 15% of the 191 industrial sites inventoried are concerned by a Cu contamination (Basol, 2008) and many of them host activities linked to fungicides production and wood / paper production.

Median Cu concentration in upper layers of French soils depends on their texture and varies from 3 mg $Cu.kg^{-1}$ for sandy substrates to 17 mg $Cu.kg^{-1}$ for clay soils. When concentrations exceed 35 mg $Cu.kg^{-1}$, an investigation for Cu contamination is highly recommended (Baize, 1997).

Cu sources from farming activities come from the use of Cu as food additive in animal farming, the application of Cu-based fungicides and pesticides, and the spreading of solid and liquid manures. One of the best known Cu-based pesticide is the Bordeaux mixture [$Ca(OH)_2$ + CuSO4] which has been used for a long time in orchards, and which application induces Cu remaining in cultivated soil (Hirst *et al.*, 1961; Byrde *et al.*, 1965). Bordeaux mixture has also been extensively used in the past decades to protect vines against pathogen attacks, including mildews. As a result total Cu concentrations in soils can be up to 100 mg $Cu.kg^{-1}$ soil in old or abandoned vineyard soils and around 60-70 mg $Cu.kg^{-1}$ soil in more recent vineyards. Although a high proportion of Cu (between 40 and 50%) is bound to organic matter and to amorphous inorganic colloids, reducing the adverse effect of Cu toxicity, risks of Cu exposition underlying new land uses of old or abandoned vineyard remain present (Fernandez-Calvino *et al.*, 2008).

However, many other Cu-based compounds have been tested and used as fungicides, such as copper oxychloride and cupric oxides or hydroxides (Holmes and Storey, 1962; Till and Fish, 1964). Application of metal enriched sewage sludge also contributes to enhance metal concentrations and mobility in soils, which pose risks of groundwater contamination and biological receptors exposition (Yeganeh *et al.*, 2010).

Third use of Cu concerns domestic application, through fertilization of private soils with domestic composts or non-controlled application of pesticides (Adriano 1986, Baize, 1997, Arias *et al.*, 2002; Brun *et al.*, 2003; Acemioglu and Alma, 2004; Copper development association, 2008).

In a contaminated site, there are different ways of dispersal from soil source to other ecosystem compartments. Cu may reach superficial or below-ground waters through leaching or percolation; atmosphere and closed soils through flight of thin soil particles due to aerial and water erosion. Cu excess in soils leads to exposition of biological receptors, directly, by breath of soil particles, drinking of contaminated water, ingestion of soil particles or Cu uptake in soil solution; and indirectly, through food chain contamination, initiated by Cu uptake in plants and soil feeding organisms (Fig. 1).

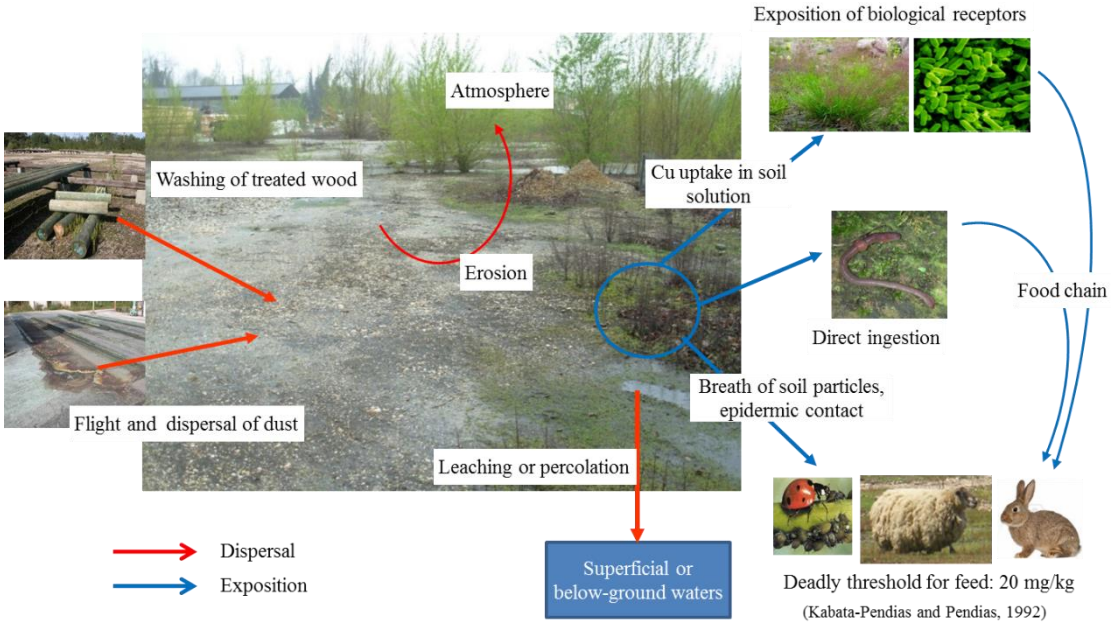

Figure 1: Dispersal exposition ways on a Cu contaminated soil.

In France, management of contaminated soils and environment protection are an obligation for industrial enterprises since the law of n° 76-663 of 19/07/76 relative to ICPE (Classified Installations for Environmental Protection), reinforced by the circular of 08/02/2007, edited by the Ministry of Ecology, Sustainable Development and Energy (http://www.developpement-durable.gouv.fr/Circulaire-du-8-fevrier-2007,19383.html).

Physical and chemical options exist to reduce excessive expositions and related risks; however, soil excavation and physical/chemical washing with or without granulometry sorting affect soil properties and fertility, destruct biodiversity together with being expensive (Pilon-Smits, 2005; Padmavathiamma and Li, 2007). Biological alternative to soil engineering, phytoremediation approaches avoid soil excavation and lead to restoration of soil and ecosystem functioning, such as production of usable biomass. These technics, based on the properties and functioning of plants and associated microorganisms, aim to reduce to an acceptable level the migration of contaminants and risks linked to contaminated soils. They provide efficient and poorly invasive solutions, with low cost and promote restoration of biodiversity and ecosystem services like carbon sequestration and biomass production.

Two main processes can be distinguished, extraction/degradation and immobilization, also called phytostabilization (Pilon-Smits, 2005; Padmavathiamma and Li, 2007). During phytoextraction, metal(loid) is taken up from soil, then translocated and accumulated in shoots, which may be harvested and valorized, leading to decrease of labile pool in soil. Phytoextraction technics have been improved by the addition of organic or inorganic soil amendments, which enable plants to take up higher amounts of metal(loid)s (Meers *et al.*, 2008). Cu immobilization (phytostabilization) in Cu-contaminated soils using tolerant plants limits the mobility of metals (decrease of labile pool) in soil solution by binding on organic matter and accumulation in the rhizosphere.

Plant cover increases soil stability, texture and water retention and limit metal dispersion through limitation of wind or water erosion and leaching/lixiviation (Fig. 1). Phytostabilization has also been improved by the association with soil amendments to either increase plant growth or decrease Cu availability to plant roots. For example, addition of organic compost increases Cu fixation on organic colloids, enabling a limitation of Cu bioavailability in soil solution, but also improve soil structure and properties, and favor plant growth as nutriment source (Nwachukwu and Pulford, 2009; Karami *et al.*, 2011). Phytoremediation technics are also adapted for contaminated waters, through construction of wetlands with macrophytes and the associated micro-organisms (Marchand *et al.*, 2010; Rai, 2008).

Selection of tolerant species, adapted to stressful environments, represents a key step for application of phytoremediation. Cu pollution had many impacts on living organisms, and to grow on contaminated soils, plants need to develop mechanisms of tolerance together with particular phenotypic traits. The General context of this work fits into the improvement of phytoremediation, notably through the selection of tolerant species, for which it is necessary to improve the knowledge on Cu tolerance mechanisms in plants. Hypothesis of this work is that a multi-scale approach on a species exhibiting high phenotypic plasticity for Cu-tolerance is a key to elucidate mechanisms underlying the development of Cu-tolerant populations on contaminated soils.

2. Impacts on plant community

On Cu-contaminated soils, the composition of plant communities are strongly modified, with a low diversity and the dominance of a few number of species, often belonging to *Asteraceae* and *Poaceae* families (Lepp *et al.*, 1997; Baize, 1997; Vogeler *et al.*, 2008; Bes, 2008). Wu and Kruckeberg (1985), reported different composition between a Cu-mine waste

soil and the surrounding meadow, with quantitative and qualitative differences in the dominant species. Few species developed on both soils but the distribution remained soil-dependent.

3. Plant phenotype regarding Cu-tolerance and accumulation

Two main strategies have been identified to tolerate high Cu exposure, avoidance and accumulation. Plants with avoidance strategy exhibit an "excluder" phenotype, the Cu is accumulated in roots and root-to-shoot translocation is reduced. On the opposite, plants with "accumulator / hyperaccumulator" phenotype exhibit increase of foliar concentrations.

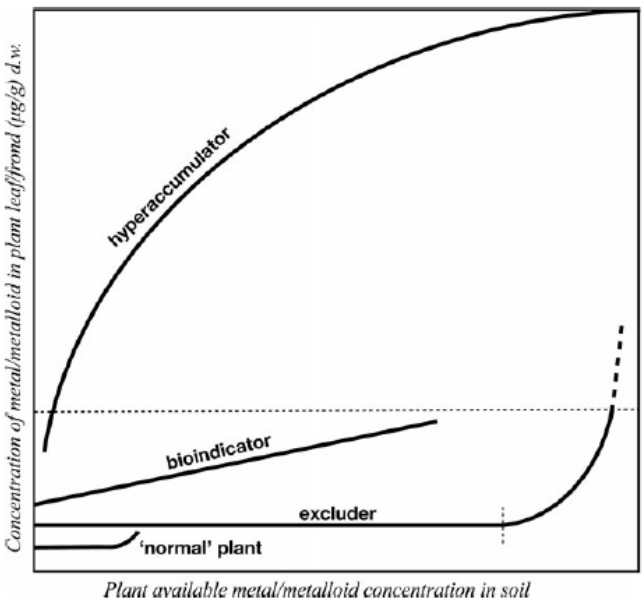

Fig. 1 Conceptual response diagram for uptake of metals and metalloids in plant leaves/fronds, after Baker (1981) and with minor alterations from McGrath et al. (1999). 'Normal' plants can only tolerate low concentrations of bioavailable metals/ metalloids in soil, before they die due to acute phytotoxicity. Excluders however can grow over a wide range of phytotoxic available metals before physiological mechanisms cannot control and allow unregulated uptake, resulting in death of the plant. Bioindicators take up metals over a wider range than 'normal' plants and the concentrations in plant leaves reflect that of the soil, until phytotoxicity prevents further growth and causes death of the plant. Hyperaccumulators are able to withstand 'normal' plants, bioindicators and excluders, and because of competitive disadvantages and greater sensitivity to fungal and pathogen infections, most do not occur over non-metal-enriched soils, depicted by the latent start of the line in the diagram. The dotted baseline indicates the hyperaccumulator threshold for the different metals and metalloids: 100 µg/g for Cd, Se and Tl, 300 µg/g for Cu, Co and Cr, 1000 µg/g for Ni, As, and Pb, 3000 µg/ g for Zn, and 10,000 µg/g for Mn. Note that the response line for hyperaccumulators represents the possible behaviour of such plants, individual plant concentrations are scattered around the line, and most hyperaccumulators occupy only a small portion of the line (above the hyperaccumulation threshold)

Figure 2: Description of plant phenotypes, from (Van der Ent *et al.*, 2013)

3.1. Bio-indicators species

As results of many toxicity tests on different plants species (Wang and Keturi, 1990), a list of 10 test plants has been recommended by US Environmental Protection Agency as bioindicators to test the toxicity of pesticides and various substances. These bioindicator species include *Solanum lycopersicum*, *Cucumis sativus*, *Lactuca sativa*, *Glycine max*, *Brassica oleracea*, *Avena sativa*, *Lolium perenne*, *Allium cepa*, *Daucus carota* and *Zea mays* (US EPA, 1996). This list has been enlarged by other organizations with most used species for standard toxicity tests (OECD, 2003), and these species are currently used as plant-based bioassays to evaluate the toxicity/genotoxicity of contaminated-soils, sediments or industrial wastewaters (Charles *et al.*, 2011; Siddiqui *et al.*, 2011), as this kind of routine tests have very low cost and are reproducible.

3.2. Hyperaccumulator species

Hyperaccumulation of Ni, Zn, Cd, Mn, As and Se has been identified in plant species but for Pb, Cu Co, Cr and thallium (Tl), the existence of hyperaccumulators needs more proofs to be confirmed (Van der Ent *et al.*, 2013). Typical elemental concentrations of metals and metalloids in plant shoots have been established around 1.5 µg/g for Ni, 50 µg/g for Zn, 0.05 µg/g for Cd, 1 µg/g for Pb, 10 µg/g for Cu, 0.2 µg/g for Co, 1.5 µg/g for Cr, 200 µg/g for Mn, 0.02 µg/g for Tl, 0.1 µg/g for As and 0.02 µg/g for Se. Several reviews on hyperaccumulation mechanisms and hyperaccumulator species have recently been written and mainly focus on Cd and Zn (Verbruggen *et al.*, 2009; Verbruggen *et al.*, 2013; Maestri *et al.*, 2010; Van der Ent *et al.*, 2013).

The ability to hyperaccumulate metals in above-ground tissues without phytotoxic effects has evolved in at least 500 plant species, mainly from the *Brassicaceae* family (Krämer, 2010). In fact, Zn and Cd hyperaccumulation seems to be limited mainly to the *Brassicaceae* family, with only few other species able to accumulate Zn and Cd. For example, *Thlaspi caerulescens* is one of the best Zn and Cd hyperaccumulators, known to evolve ecotypes with marked difference in their degree of tolerance (Tuomainen *et al.*, 2006).

Cu-hyperaccumulation is poorly found in plants as most species accumulate Cu in roots and have a very low translocation factor, *i.e.* < 0.05 for Sunflower, alfalfa, fodder radish and Italian ryegrass (Vamerali *et al.*, 2011). However, some copper hyperaccumulator species (cited in Van der Ent *et al.*, 2013) have been reported in Congo (32 species), China (*Elsholtzia splendens* or *Commelina communis*), Sri Lanka (5 species with Cu > 1,000 µg/g) and Salajar Island (7 species with 300 > Cu > 600 µg/g). Some species found on highly Cu contaminated soils and able to accumulate Cu in their shoots, up to concentrations higher than 1,000 mg/kg, are called cuprophytes. In the Cu-rich soils of the Katangan and Zambian copperbelt, at least 40 out the 500 plant species recorded are considered as endemic of Cu-rich and called "absolute cuprophytes" (Faucon *et al.*, 2009). For example, shoot Cu concentrations higher than 1300 mg/kg were measured in a small annual *Scrophulariaceae*, *Crepidorhopalon perennis*, which is endemic to the Katangan copperbelt (Democratic Republic of Congo, Africa, Faucon *et al.*, 2009). *Haumaniastrum katangense* from the *Lamiaceae* family, also called the Katangan "copper flower", colonizes Cu-enriched soils and has been used as bioindicator for such soils (Chipeng *et al.*, 2010).

Recently, the ability of *Brassica juncea* L. to accumulate high levels of Cu and Zn has been used to synthetize Cu/Zn nanoparticles, indicating a new opportunity to valorize shoot biomass produced during phytoextraction procedures (Qu *et al.*, 2012).

3.3. Tolerant species

Several organisms are tolerant to metals, i.e. iron, nickel, lead, zinc, cobalt, silver, cadmium or copper, or metalloids, i.e. boron, silicon, arsenic, antimony. This part presents some species known to be Cu-tolerant and/or evolve tolerant populations, but does not consist in an exhaustive list.

Some pluricellular algae have been studied for Cu-tolerance, the case of unicellular algae will not be presented. Cu-tolerant populations have been reported for the marine alga *Ectocarpus siliculosus* (Dillw.) Lyngbye (Hall, 1980) and Cu-tolerant ecotypes of this species were more recently studied with a proteomic approach (see section 8, Ritter *et al.*, 2010). Ability of the marine alga *Scytosiphon gracilis* to colonize Cu-contaminated areas together with field and laboratory experiments indicate that Cu-tolerance is linked to a rapid and reversible antioxidant response, and that this tolerance may be constitutive for the genus *Scytosiphon* (Contreras *et al.*, 2010).

Cultivars of *Matricaria chamomilla* (2 tetraploids 'Lutea' and 'Unknown' and one diploid 'Novbona') were compared for Cu uptake and impacts on physiology, when exposed to 60 µM Cu for 7 days. Root water content and dry weight is more reduced in diploid cultivar but lignin accumulation and cinnamylalcohol dehydrogenase activity are the highest. Phenylalanine ammonia-lyase activity is stimulated in tetraploid but reduced in diploid roots, which contain higher amount of Cu and soluble phenols in tissues but lower potassium content (Kováčik *et al.*, 2011)

Many species from genus *Silene* evolve metal tolerant populations in Europe, including Cu, such as *S. vulgaris* (Kováčik *et al.*, 2010), *S. maritima* (Baker, 1978; Cobon and Murray, 1983), *S. cucubatus* (SO_2, Cu, Zn, Dueck *et al.*, 1987) and some have even been characterized as full metallophyte, such as *Silene cobalticola*, which is endemic of Cu/Co contaminated soil in Zaïre (Baker *et al.*, 1983)

Becium homblei, native from Zambia and belonging to the *Labiateae* family, has early been studied for its ability to grow on highly Cu-contaminated soils (more than 15 000 ppm) and to accumulate more than 50 or 100 mg Cu/kg DW in both roots and shoots, tightly bound within tissues, probably to protein complexes as total nitrogen does increase proportionally to Cu content, and not as free ionic form in cytoplasm (Reilly, 1969). For this species, higher Cu concentrations occur in leaves compared to roots. Further investigations have indicated that 17% of the total Cu is bound to leaf cell wall as stable organic complexes, whereas in cell juice and water extracts of leaf tissues, Cu is complexed with polypeptides and amino-acids (Reilly

et al., 1970). History of studies on this species was presented in a review paper in 1999 (Brummer and Woodward).

Plantago lanceolata L. populations originated from various contaminated soils (Zn, Pb, Cu or As) were compared to one from a control and uncontaminated soil and exhibited higher tolerance to the metal present in collection soil than control population (Pollard, 1980). This work confirmed the already reported Zn-tolerance in population grown on Zn mine soils but also reported high As- and moderate Cu-tolerance. In the case of As, even the population from uncontaminated soil exhibited the potential to evolve highly tolerant individuals, suggesting high frequency of appearance in few generations (Pollard, 1980). Copper tolerance is still under debate for this species, as some works provided evidence of Cu-tolerance (Pollard, 1980) whereas others concluded to an incapacity to evolve Cu-tolerant individuals (Gartside and McNeilly, 1974). A link between establishment of this species on Cu-contaminated soils and the presence of abnormal Zn concentrations in those soils has also been pointed out but this relation and its consequences remain unclear (Pollard, 1980).

Only a low number of Legume species are reported on Cu-contaminated soils. Some of them, *Lupinus bicolor* and *Lotus purshianus*, exhibit tolerant populations collected on Cu-mine waste soils (25-935 µg $Cu.g^{-1}$ soil), which are more tolerant to Cu than populations of the same species from surrounding meadow (0.1-1.5 µg $Cu.g^{-1}$ soil; Wu and Kruckeberg, 1985).

Populations of *Deschampsia cespitosa*, collected on a Cu/Ni smelter complex, exhibited clear differentiation for their tolerance to these particular metals when compared to the population grown on uncontaminated soil, based on relative root growth and on frequency distribution of tolerance index calculated from root growth. However this population has also a higher tolerance to Al, Pb and Zn, as compared to the control population, despite a high overlap of tolerance distribution among populations for these three metals. Tolerance to Zn and Pb is only partial, as exposed plants never reach the size of plants grown in control conditions, as observed for Cu or Ni tolerance (Cox and Hutchinson, 1980). This study contradicted ideas that tolerance to a metal does not confer tolerance to another and that multiple tolerance occurs only on multi-contaminated soils.

Agrostis capillaris, the subject of this work, has been long time studied for its ability to evolve metal-tolerant populations; history of studies concerning this species is presented in a special section (see section 6).

3.4. Higher need in metal for metal-tolerant populations

Some examples of a higher need in metal for proper germination and growth have been reported in metallophyte species, or in metallicolous populations of pseudo-metallophyte species. For example, a lower germination of tolerant populations of *Deschampsia cespitosa* on uncontaminated compared to contaminated soil support the hypothesis of a higher need for metal in tolerant plants to maintain correct cell functioning (Cox and Hutchinson, 1980).

Untreated seeds of the cuprophyte *Haumaniastrum katangense* exhibit germination lower than 15%, whereas pre-treatment with either copper or fungicide improves germination rate and combination of Cu or pesticide with washing and heat exposure, increases germination rate above 80%. Growth is maximal at 12 µM Cu, while at control Cu concentration (0.5 µM) it is only one third of maximal growth, indicating a higher Cu need to achieve optimal growth (Chipeng *et al.*, 2010)

4. Different use of proteomic approaches

The proteomic tool is mainly used for three purposes, elucidate differential protein expression in response to treatments (‘expression proteomics’); analysis of protein complex structures (‘structural proteomics’) and characterization of protein-protein interactions (functional proteomics; Monsinjon and Knigge, 2007).

These last two purposes are not discussed as this work aims to study differential protein expression in response to Cu. Proteomic has been used to study various biotic interactions such as interactions with microbes and pathogens or symbiosis but also plant development in response to abiotic stresses (Cánovas *et al.*, 2004; Rossignol *et al.*, 2006; Jorrin *et al.*, 2007).

In ecotoxicology, ‘expression proteomic’ may be used in two ways, the ‘identity-based approach’ aims to elucidate mechanisms underlying toxicological effects of stresses while ‘Pattern-only approach’ aims to identify some sets of protein spots which can be used as biomarker patterns of environmental stress/pollution exposure, without any attempt of protein identification (Monsinjon and Knigge, 2007).

The following sections focus only on ‘identity-based approaches’ as the purpose of this work is to understand the molecular mechanisms underlying plant response to Cu excess.

4.1. Plant response to metal(loid)s

Selection of tolerant plant species (or cultivars) may improve crop cultures or efficiency of phytoremediation trials, so proteomic approaches are used to examine plant responses to abiotic stresses. These technics could give new pieces of evidence to understand the molecular mechanisms underlying tolerance to abiotic stresses in photosynthetic organisms such as plants or algae. Numerous studies exist about plant response to metal(loid) excess, including Cd (Jorrin *et al.*, 2007; Ahsan *et al.*, 2008; Zacchini *et al.*, 2009; Zhang *et al.*, 2009; Zhao *et al.*, 2011; Gomes *et al.*, 2012; Marmiroli *et al.*, 2013; Weng *et al.*, 2013), or Al (Yang *et al.*, 2007; Chen and Lin, 2010).

One study exists about *A. capillaris* response to arsenic and arsenate, in leaves of plants grown for one month in metal-free conditions, then exposed for 8 days (Duquesnoy *et al.*, 2009). As altered photosynthesis processes, as shown by the identification of degraded fragments of RuBisCO and the up-regulation of several oxygen-evolving enhancer proteins.

Some reviews focus on plant response to metal(loid)s excess. For example, Hossain *et al.*, (2013) did study metal stress-related proteins involved in sequestration, detoxification and antioxidant defense systems and primary metabolism.

Only works targeting Cu excess are described in this section. Few studies have been conducted on plant responses to Cu exposure at a proteomic level, i.e. in leaf segments of *Oryza sativa* floated in solutions containing 250 µM Cu for 72h (Hajduch *et al.*, 2001); in seedlings of *Phaseolus vulgaris* exposed to 15 or 50 µM Cu for 7 days (Cuypers *et al.*, 2005); in roots and leaves of *Elsholtzia. splendens* plants exposed to 100 µM Cu for 3 or 6 days (Li *et al.*, 2009); and in *Cannabis sativa* seedlings exposed to 150mg/L $CuSO_4$ for six weeks, after germination in metal-free solution (Bona *et al.*, 2007).

Leaf segments of *O. sativa* were floated in 250 µM Cu solution, but also in solutions containing 250 µM Cd, Hg, Li, Zn or Sr (Hajduch *et al.*, 2001). Accumulation of RuBisCO large and small subunits are severely reduced by Cu, Cd and Hg excess, less sharply by Co and Li but not altered by Zn or Sr. Additionally, increased accumulation of degraded products of RuBisCO indicates that metals directly impact carbon assimilation in altering enzyme integrity.

Whereas *P. vulgaris* seedlings shoots don't exhibit any significant variation in protein patterns under moderate Cu excess (15 µM), two protein spots, pathogenesis-related (PR) protein PvPR1 and a 17.4 kDa protein, homologue of *A. thaliana* thylakoid luminal, appear under high Cu exposure (50 µM). In roots, all identified proteins belong to the PR-10 family: two spots, identified as an intracellular pathogenesis-related protein (PR) and a previously

unidentified member of PR-10 family, matched to PvPR1 and/or PvPR2, appear under moderate Cu excess (15 µM) and increase under high Cu (50 µM), another PvPR2 spot appears only at 50 µM, whereas a newly identified PR-10 protein is Cu down-regulated (Cuypers *et al.*, 2005).

Long-term response to Cu excess was investigated in roots of *C. sativa* seedlings exposed to 150mg/L $CuSO_4$ for six weeks, after germination in metal-free solution (Bona *et al.*, 2007). Cu stress induces down-regulation of seven proteins, i.e. enolase, cyclophilin, ABC transporter substrate-binding protein, glycine rich RNA binding protein, putative peroxidase and elicitor inducible protein; up-regulation of five proteins, i.e. aldo/keto reductase, putative auxin induced protein, 40S ribosomal protein S20, formate dehydrogenase and actin, and disappearance of two protein spots, i.e. thioredoxin-dependent peroxidase and 60S ribosomal protein L12.

As Cu-tolerant species and good candidate for application of phytoremediation of Cu-contaminated soils, variation in root and leaf proteomes of four-weeks-old *E. splendens* plants were investigated after exposition to 100 µM Cu for 3 or 6 days (Li *et al.*, 2009). 45 protein spots, involved in many cellular processes such as energy metabolism, signal transduction, regulation of transcription and translation, redox homeostasis and cell defense, are either up- or down-regulated in roots. Only 6 spots vary in shoots, and most were degraded fragments of RuBisCO, indicating impacts on photosynthetic activity. The decreased accumulation of a multi-copper oxidase in leaves has been suggested to confer resistance to oxidative stress by increasing the ascorbic acid content.

Some works on Cu-tolerance exist also on algae, such as *Scytosiphon gracilis* exposed to 100 $\mu g.L^{-1}$ for 4 days (Contreras *et al.*, 2010) and on the yeast *Rhodotorula mucilaginosa* (Irazusta *et al.*, 2012) and may be potentially useful for studying Cu-tolerance in plants.

In *S. gracilis* exposed to 100 $\mu g.L^{-1}$ for 4 days, several protein spots increase under Cu stress and are potentially involved in the control of Cu-induced oxidative, i.e. a peroxiredoxin, able to cope oxidative stress by reducing H_2O_2; a phosphomannomutase, which, by increasing production of mannose 1-phosphate, a precursor of cell wall polysaccharides, was suggested to enhance the buffering capacity of the algal cell wall; a glyceraldehyde-3-phosphate dehydrogenase, which was suggested to attenuate the negative effects of Cu-induced oxidative stress by maintaining energy and reducing power; ABC transporters, suggested to regulate transport of GSH-metal complex into vacuole or proteasome subunit, suggested to remove damaged proteins (Contreras *et al.*, 2010). In the RCL-11Cu-resistant strain of the yeast *R. mucilaginosa*, exposure to 0.5 mM Cu for 48h up-regulates the expression of 16 protein spots, of which ten have been identified as heat shock proteins (3 Hsp88, 6 Hsp70 and 1 Hsp60), four

as methionine synthase and two as superoxide dismutase and beta-glucosidase. These results suggested that Cu-resistance in this yeast is linked to over-expression of stress-related proteins such as HSPs, acting as protein chaperones, or SOD, involved in peroxide detoxification, and to increase in methionine content. Changes in glycolipids content and proportion, related to changes in beta-glucosidase accumulation, may also play a role in physical and structural stabilization of the membrane (Irazusta *et al.*, 2012).

Role of Cu/Zn-SOD in Cu tolerance has been studied using transgenic *Arabidopsis* seeds constitutively over-expressing Cu/Zn-SOD of *Potentilla atrosanguinea* (PaSOD), exposed to Cu during germination (Gill *et al.*, 2012). Transgenic seeds exhibit higher germination percentage and lower time to germinate, indicating that over-expression of PaSOD in *Arabidopsis* enhances tolerance to Cu. 39 protein spots are differentially expressed between transgenic and wild type (WT) under Cu stress (1 mM Cu). 14 spots, up-regulated by Cu, are recorded only in transgenics, and related to ammonia assimilation, ester hydrolysis, respiratory component synthesis, development and detoxification. Up-regulation of a protein homologue with 26S proteasome AAA-ATPase subunit RPT5a in transgenics compared to WT under Cu, is also suggested as defense mechanism against Cu. However, as different set of proteins are involved in seed germination then during plant growth, mechanisms of Cu-tolerance cannot be compared in this work, which focused on adult plants and not seedlings

4.2. Comparison between sensitive and tolerant cultivars/populations/genotypes

As differences in efficiency of homeostasis and detoxification processes may explain the higher tolerance of metallicolous individuals, some proteomic studies have focused on comparison between populations, genotypes or cultivars, exhibiting large difference in metal tolerance, i.e. metal-tolerant vs metal-sensitive, to gain information on molecular mechanisms underlying this enhanced tolerance.

4.2.1. Cu-tolerance

Only few comparisons between Cu-tolerant and sensitive populations/cultivars/genotypes were conducted, one study focused on the alga *Ectocarpus siliculosus* exposed to 50 µg Cu/L during 10 days (Ritter *et al.*, 2010), and another on roots of *Oryza sativa* varieties exposed to 8µM Cu for 3 days (Song *et al.*, 2013),

Cu response under chronic stress (50-150 µg $Cu.L^{-1}$ for 10 days) was examined in Cu-tolerant and sensitive strains of *E. siliculosus*, a brown alga able to develop in Cu-enriched environments (Ritter at al., 2010). Cu excess induced strain-specific up-regulation of different proteins related to energy, glutathione metabolism and protein metabolism (HSPs). Over-

expression in the tolerant strain of two spots related to photosynthesis, i.e. PSII Mn-stabilizing protein and fucoxanthine chlorophyll a–c binding protein, suggested their involvement in Cu tolerance. Higher expression of proteins involved in glycolysis and pentose phosphate pathway, i.e. transketolase, fructose bisphosphate aldolase phosphoribulokinase, and glyceraldehyde-3-phosphate dehydrogenase, indicated higher energy production in the tolerant strain.

In the comparison of Cu stress responses of two *O. sativa* varieties differing in their levels of Cu tolerance (Song *et al.*, 2013), pre-germinated seedlings of Cu-tolerant (B1139) and Cu-sensitive (B1195) varieties were cultivated in normal nutrient solution for 7 days then exposed to 8µM Cu for 3 days and compared to non-exposed plants. 34 protein spots were differently expressed under Cu-stress in at least one variety, i.e. antioxidative defense, redox regulation, stress response, sulfur and glutathione (GSH) metabolism, carbohydrate metabolism and signal transduction. Nine protein spots, i.e. putative cysteine synthase, probable serine acetyltransferase 3, L-ascorbate peroxidase 1, putative glutathione S-transferase 2, and thioredoxin-like 3-3, increased more in Cu-tolerant B1139 compared to sensitive B1195 and one putative glutathione S-transferase was detected only in Cu-tolerant under Cu stress. Results indicated that most differentially expressed proteins were involved in redox regulation, and sulfur and GSH metabolism, suggesting that higher tolerance in tolerant variety was due to better maintaining of Cu-homeostasis.

Cu-tolerance has been investigated in a plant growth promoting copper-resistant bacterium, *Pseudomonas* spp., by generating a library of transposon mutants, and selecting a copper-sensitive mutant, CSM2, disrupted in clpA gene (ATP-dependent Clp protease), which was further compared to the wild type (WT) using metabolomic and proteomic approaches. Growth of mutants did not differ from WT at 0 or 2 µM Cu, but was significantly lower at 4 µM and suppressed at 4.5 µM while the WT survived by reducing cell size and slowing cell division. The disruption of ClpA in CSM2 caused differential expression of 21 spots, of which 5 were excised for more than three-fold changes between WT and CSM2 grown without copper. Two spots, DnaJ-class molecular chaperone and HpcH/HpaI aldolase, were 8 times more abundant in CSM2, while three, glycosyl transferase and ubiquinone biosynthesis protein, respectively involved in tRNA processing, carbohydrate metabolism and energy production were 3.5 to 4.3 times more abundant in WT. All these five spots were strongly up-regulated in WT grown in 4 mM copper. Results suggested a direct role of Clp protease, including ClpA, in copper resistance in degrading the damaged proteins or prevent their irreversible aggregation under copper stress but also in up-regulating amino acids (L-proline and L-isoleucine), sugars (glycerol-3-phosphate and alpha-D-glucopyranoside), , or enzymes involved in tRNA processing, tRNA (guanine-N(7)-)-methyltransferase (Li *et al.*, 2012).

4.2.2. Tolerance to other abiotic stresses

Numerous other studies focused on understanding metal(loid) tolerance in comparing plant species, populations, or cultivars. Al tolerance was investigated in roots of rice cultivars (Arenhart *et al.*, 2013), in leaves of *Glycine max* cultivars exposed to 10 µM Al for 6, 51 or 72 hours (Duressa *et al.*, 2011), and of *Hordeum vulgare* cultivars and genotypes exposed to 0, 50 or 200 µM Al for 3 days (Dai *et al.*, 2013). All studies indicated that stress altered different sets of proteins between tolerant and sensitive plants.

Several have been conducted on *Agrostis* spp. Proteomic response to heat stress (30 or 40°C for 2 or 10 days) has been characterized in roots (Xu and Huang, 2008) and leaves (Xu and Huang, 2010a) of 60-days-old clonal plants from two *Agrostis* species differing in their thermo-tolerance, the heat-tolerant *A. scabra* and the heat-sensitive *A. stolonifera*. In roots of both species, heat stress induced a reduction of amino acid synthesis, including methionine, serine, and glycine, but a role of serine and sulfur metabolism in root thermo-tolerance was suggested by the up-regulation of phosphoserine aminotransferases and ATP sulfurylase only in the tolerant species. Additionally, the implication of a sucrose synthase in the thermo-tolerance, in regulating sucrose metabolism to support glycolysis supply, was suggested by its up-regulation in *A. scabra*, and down-regulation in *A. stolonifera*. Heat stress disturbed carbon degradation and electron transport chain in mitochondria, as shown by the down-regulation of 16 energy-related proteins. Heat also impaired protein folding in *A. stolonifera* roots while the up-regulation of heat shock protein Sti (stress-inducible protein) only in *A. scabra* may protect protein metabolism. Two glutathione-S-transferase were up-regulated in both species but more accumulated in *A. scabra*, while another GST and one SOD increased only in heat-tolerant *A. scabra*, suggesting that a better control of active oxygen species also contributed to thermo-tolerance (Xu and Huang, 2008). In leaves, up-regulated proteins spots belong to four main functional categories, i.e. metabolism, energy, protein destination/storage and intracellular traffic, while down-regulated protein spots belonged to metabolism, energy (20%), transcription, protein destination/storage, cell structure and disease/stress defense. Heat stress down-regulated several enzymes involved in photorespiration in at least one species (Hydroxypyruvate reductase, alanine aminotransferase, serine hydroxymethyltransferase, glycine decarboxylase), indicating inhibition of photorespiration with increasing temperature (Xu and Huang, 2010a).

Differential accumulation of salt-responsive proteins was investigated, using a 2D-DIGE approach, in roots and shoots of a salt-sensitive ‘Penncross’ and a tolerant ‘Penn-A4’ cultivars of *Agrostis stolonifera* (Xu *et al.*, 2010). Higher tolerance of ‘Penn-A4’ cultivar was associated

in roots with better maintenance of energy metabolism (higher accumulation of isocitrate dehydrogenase, phosphogluconate dehydrogenase or enolase and up-regulation of aldolase, ferredoxin-NADP reductase and GAPDH) and alteration of ion transport (probable sequestration of Na in the vacuole) through higher vacuolar H^+-ATPase accumulation. In leaves, cultivars were able to maintain the production of ATP and NADH but not the carbon assimilation, as enzymes related to light reactions (cytochrome f, OEE, PSI subunit N, light-harvesting complex I and cytochrome b6–f complex iron/sulfur subunit) were up-regulated while those involved in dark reactions (RuBisCO large subunits, RuBisCO activase, phosphoglycerate kinase and chloroplastic aldolase) were down-regulated. Higher tolerance of 'Penn-A4' cultivar was associated in leaves with better maintenance of glycolysis activity (higher accumulation of aldolase and GAPDH spots), better maintaining of thylakoid integrity (UDP-sulfoquinovose synthase), stimulation of polyamine biosynthesis (methionine synthase), cell wall loosening proteins (beta-D-glucan exohydrolase), and antioxidant defense mechanisms (increasing accumulation of GST, CAT and APX). These 'Penncross' and 'Penn-A4' *A. stolonifera* cultivars were also tested for water stress response (Xu and Huang, 2010b).

5. Biological roles, impacts and mechanisms of tolerance

As bivalent cation with oxido-reductive properties and essential oligo-element for plants, Cu plays many roles in cell functioning such as enzyme cofactor and has to be thinly controlled to avoid both deficiency and excess. However, only the excess aspect will be further discussed, as the purpose is to identify the mechanisms enabling tolerance to excess Cu.

5.1. Impacts on plant growth

Lots of reviews exist about impacts of Cu excess impacts in plants (Bertrand and Poirier, 2005; Yruela, 2005; Pilon *et al.*, 2006). Cu excess impacts root growth and architecture, leading to the so-called coralloid architecture and disturbs nutrient uptake. Cu competes with Fe during root transport, so increasing Cu uptake resulting in a decreased Fe uptake (Song *et al.*, 2014). Reduction of chlorophyll content, sensitivity to photo-inhibition but also Cu accumulation in tissues, induced by excess Cu, is alleviated in *Phaseolus vulgaris* by adding Fe in the growth medium, which indicates that Cu outcompetes Fe uptake, inducing Fe-deficiency (Patsikka *et al.*, 2002). In soybean plants, a comparison between two types of Cu exposition, i.e. leaf treatment or Cu supplementation in hydroponic medium, has revealed that Fe/Cu antagonism only occurs after root treatment, suggesting that Cu mostly competes with Fe-uptake in roots (Bernal *et al.* 2007).

Root elongation is inhibited when meristem cells become excessively damaged. Production of primary roots is reduced while the one of lateral roots is increased. Lignification of cell wall reduces cell growth, function and division (Llugany *et al.* 2003). Water and nutrient are reduced due to early suberisation of roots. Growth of aerial parts slows down, biomass is reduced and foliar epinasty occurs, together with the appearance of phytotoxic symptoms such as chlorosis, necrosis, discolorations and bronzing (Yruela, 2005). Reduction of leaf thickness results from cell and tissue modifications, including reduction of inter-cell spaces or reduction of cell growth and structure of thylakoids is modified (Sanchez *et al.*, 2014).

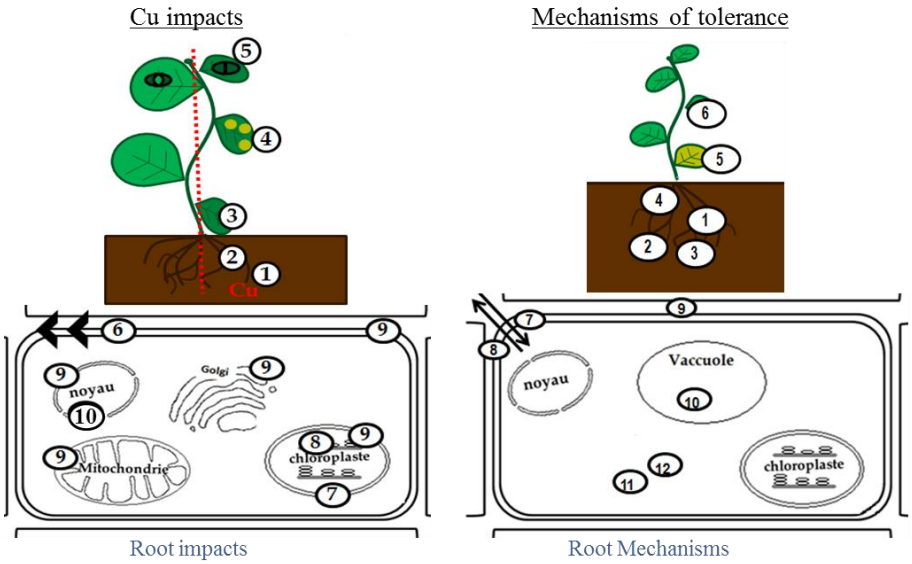

Figure 3: Cu impacts on plants and mechanisms enabling Cu tolerance.

Different strategies/mechanisms may lead to enhance Cu tolerance in plants. The first option to enhance Cu tolerance does consist in preventing Cu entry in cells, through rhizospheric mechanisms or modification of Cu influx/efflux. Strategies of root-to-shoot translocation differ between excluders and hyperaccumulators, and this section will focus on excluder plants such as *A. capillaris*.

For these plants, Cu translocation is very low as Cu is stored in roots to limit Cu content in leaves. Roots represent then the first organ exposed to Cu excess, but also the first barrier to protect leaves from Cu toxicity. Through a more efficient Cu storage in roots or a reduced root-to-shoot Cu translocation, leaves may be protected from increase of external Cu. Once in cells, a large range of molecular processes exist to maintain Cu homeostasis and assure proper transport and delivery of Cu. The reinforcement of these homeostasis processes may enhance Cu tolerance of root and shoot cells. Lastly, a more efficient management of oxidative stress, with better detoxification and repair processes may also protect cells from Cu toxicity.

5.2. Rhizospheric mechanisms underlying limitation of Cu entry in cells

Rhizospheric mechanisms may both limit or enhance Cu uptake in roots, or help in Cu-storage within root tissues. Moreover, these processes may be linked to plant action on soil through chemical reactions or may involve symbiosis with microorganisms. As this work aimed to understand the Cu response in plants and because of culture mode excluding soil use (hydro-culture), rhizospheric mechanisms underlying Cu-tolerance were poorly presented.

5.2.1. Root exudates

Root exudates can contain a large variety of compounds, including organic acids, acid phosphatases, phenolic substances, and phytosiderophores and play various functions to support nutrient uptake by plants, such as modification of soil solution pH and/or reduction/increase of metal availability/uptake (Meers *et al.*, 2008). Organic acids, including acetic, oxalic, tartaric, malic, citric, propionic, and lactic acids function as chelating agents, able of solubilizing mineral soil components such as metal(loid)s. For example, phytosiderophores are involved in Fe uptake strategy II of grass plants and oxalate and citrate decrease Cu^{2+} sorption in soil (Meers *et al.*, 2008). In case of accumulator/hyperaccumulator species, organic exudates may aim at increasing metal solubility, enabling higher uptake from soil solution. Rhizodeposition and exsudation of organic compounds can also reduce Cu solubility in soil solution (Mench *et al.*, 2010; Dousset *et al.*, 2001). For *Lupinus albus*, grown in hydroponic system and exposed to 0.5, 20 or 62 µM Cu for 40 days, increase in soluble and high molecular mass phenols into the solution was reported at 20 µM, with any difference in dry matter compared to control (0.5 µM) solution, suggesting that complexation of Cu^{2+} in rhizosphere and soil solution participate to Cu-tolerance through direct binding (Jung *et al.*, 2003).

5.2.2. Associations with microorganisms

In soil, plants provide association with diverse microorganisms, such as endophyte bacteria or mycorrhiza, which may contribute, directly or indirectly, to enhance Cu tolerance. Associated microorganisms may favor plant growth through improvement of nutrient uptake, but also accumulate Cu in their tissues. Studies dealing with the effect of mycorrhizal fungi on metal uptake by host plants have provided conflicting results among species, experimental conditions, types of substrates and contamination levels and types (Malcová *et al.*, 2003). However, association with tolerant population of mycorrhizal fungi has been suggested to enhance metals-tolerance in plants (Griffioen, 1994; Hall, 2002), by decreasing the translocation of metals in plant cells or by supplying nutrients and water to counterpart the adverse soil conditions. The beneficial effect of inoculation with arbuscular mycorrhizal fungi was recently suggested to be related to an improvement of phosphorus nutrition rather than to a reduction of toxic element transfer to plant tissues (Neagoe *et al.*, 2013). The involvement of plant-associated bacteria in trace element mobilization and phytoextraction was recently discussed in a review by Sessitsch *et al.* (2013).

Effect of mycorrhiza association on Cu-tolerance is still under debate and may depend on host and symbiotic species, type of contaminant and level of exposure. For example, infection of *Betula papyfera* seedlings by 4 ectomycorrhizal species originated from contaminated soils (*Laccaria proxima*, *Lactarius hibbardae, Lactarius rufus* and *Schleroderma flavidum*) have different effects on growth under Cu and Ni exposure, depending on both metal and exposure level but also on the symbiotic species inoculated. In the absence of metal addition, mycobiont species didn't influence seedlings growth but influenced the degree of infection, which was positively correlated with root biomass and negatively with shoot biomass. Copper was more phytotoxic than Ni, as growth was reduced at low and high Cu exposure (32 and 63 µM Cu) compared to control, with more drastic reduction at high exposure. Inoculation did not affect growth at low Cu but at high Cu, a negative effect of symbiosis was reported on growth of shoots, especially in seedlings inoculated with *L. rufus*, whereas roots weight did not differ. Positive correlation was found between both root and shoot growth and degree of infection only in *S. flavidum*-inoculated seedlings. However, differences at high Cu couldn't be related to Cu-uptake or translocation. At high Cu or Ni exposure, inoculated species affected P content in roots and *S. flavidum* inoculated seedlings exhibited the highest P concentrations at high Cu, but no relationship between P status and metal-tolerance appeared. At Cu and Ni exposure, Fe decreased in leaves and increased in roots. Fe status was strongly influenced by fungal inoculation only in metal-free and high Cu solutions (63 µM), with higher Fe content in *L.*

proxima and *L. rufus* seedlings. Fe in roots did not differ at low Cu, but *L. hibbardae* seedlings had lower Fe at high Cu (Jones and Hutchinson, 1986).

5.2.3. Plasma membrane and cell wall

To enter into plant cells, Cu needs to be transported across cell walls and plasma membranes. Two families of Cu-transporters have been identified in plants, first includes the "Heavy Metal P-type ATPase", also called HMAs and second "Copper transporters" or COPTs. COPTs belong to the "CTR family", which members were found in mammals and yeast (Hall and Williams, 2003; Sancenon *et al.*, 2004; Grotz and Guerinot, 2006). Cell wall constitutes one of the first defense barrier to tolerate excess Cu, as Cu may be bound directly by pectins and glycoproteins, enabling accumulation and limiting Cu entry in cells (Qian *et al.* 2005). Accumulation of Cu in the cell walls was suggested to contribute to Cu detoxification in root tips of cucumber plants (Song *et al.*, 2014)

The plasma membrane also plays an important role in protecting cells against excess Cu entry by reducing Cu influx and/or enabling an enhanced active efflux (Hall, 2002). This strategy exists in *Holcus lanatus* where As-tolerance is linked to a decrease in As uptake by disappearance of a high-affinity transporter (Meharg and Macnair, 1991), but no example of this strategy has been clearly identified for Cu stress. In *Silene armeria* exposed to 0.1-20 µM Cu, a better protection of meristem and limited influx are pointed out for being responsible of better tolerance in metallicolous population (Llugany *et al.*, 2003). In *Agrostis capillaris*, integrity and functions of the plasma membrane are impacted by Cu toxicity, as shown by an increase of ions leakage, such as K^+ efflux (Wainwright and Woolhouse, 1977). A better protection of membrane integrity may provide an enhanced tolerance to Cu stress by maintaining correct membrane functioning, permeability and properties. For example, a lower lipid peroxidation has been measured in a tolerant ecotype of *Holcus lanatus* compared to a non-tolerant one, when exposed to increasing As exposure (Hartley-Whitaker *et al.*, 2001).

A high affinity Cu transporter, COPT1, which belongs to a five-member family (COPT1-5), has been isolated from *Arabidopsis thaliana* cDNA by complementation of a defective yeast mutant. Based on homology, CTR2 gene has been identified in yeast. Depletion in this gene leads to enhanced resistance to Cu excess while overexpression increased sensitivity to excess Cu but resistance to Cu deficiency (Kampfenkel *et al.*, 1995). The role of COPT1 in Cu transport has been confirmed in *Arabidopsis* using CaMV35S::COPT1 antisense transgenic plants (Sancenon *et al.*, 2004). A role in Cu homeostasis during Cu deficiency is suggested for COPT1 and COPT2, as they are able to restore growth of yeast mutants impaired in Cu uptake, but exhibited decreased mRNA levels in the presence of Cu. A role in pollen development is

also suggested based on investigations on antisense COPT1 lines (Grotz and Guerinot, 2006). Several other Cu transporters of the COPT family, COPT1-7 are characterized from rice, as containing high cysteine and methionine (Yuan *et al.*, 2011; Kochian *et al.*, 2012). A Fe transporter, YS1, which is involved in uptake of iron complexed with mugineic acid (MA) and functions as Fe(III)–MA/H^+ symporter, may also transport Cu and be involved in Cu uptake, when complexed to MA (Haydon *et al.*, 2007).

The alternative of reducing Cu influx in cells to limit Cu content is to increase efflux. HMA5, a member of the HMA family, is predicted to participate to Cu efflux from the cytoplasm, but the final destination, which might be out of the cell or into an organelle for sequestration, remains unclear. HMA5 interacts with at least two Cu chaperones, ATX1 (Anti-oxidant) and CCH (Copper Chaperone), from which it can recruit Cu.

5.3. Cu homeostasis, cellular impacts and molecular mechanisms of tolerance

As essential oligo-element, Cu is necessary for many metabolic processes, as cofactor of several enzymes. Because of its important functions, plants have evolved a complex set of mechanisms to maintain correct Cu homeostasis. Several reviews have already been written about Cu homeostasis and tolerance in plants (Clemens, 2001; Yruela, 2005; Clemens, 2006; Grotz and Guerinot, 2006; Burkhead *et al.*, 2009; Ravet and Pilon, 2013; Yruela, 2013).

5.3.1. Intracellular trafficking

Once inside cells, Cu is bound to chaperones or chelates to avoid free Cu in cells and to be correctly transported to their biological target. Several proteins acting as metallochaperones are involved in intra-cellular trafficking of Cu. Function of CCH (Copper Chaperone) in plants has been inferred from the function of its homolog Atx1 from yeasts, which delivers Cu from the cytoplasm to the RAN1 transporter, and has been linked to the specific Cu delivery to P-type ATPases, at the post-Golgi membrane (Puig *et al.*, 2007). A role in recycling Cu from senescing leaves has also been suggested (Grotz and Guerinot, 2006). AtCCs, a homologue of the yeast Ccs1 Cu chaperone, which delivers Cu to a Cu/Zn superoxide dismutase, has been characterized in *Arabidopsis* and localized in chloroplasts, where it has been suggested to maintain proper Cu levels for plastocyanin and Cu/Zn SODs (Abdel-Ghany *et al.*, 2005). A third Cu chaperone, COX17 (Cytochrome Oxidase) may participate to deliver Cu to the cytochrome oxidase complex within mitochondria (Yruela, 2005; Grotz and Guerinot, 2006).

At least three transporters are related to Cu transport in plant chloroplasts, PAA1, PAA2 and HMA1. Several dysfunctions related to Cu deficiency were identified from leaves of *paa1* mutants, i.e. decrease in chloroplastic Cu content, lack in functional holoplastocyanin but

accumulation of apoplastocyanin and decreased in Cu/Zn SOD activity, indicating that **PAA1** (*Arabidopsis* P-type ATPase also named HMA6) transports Cu through chloroplast envelopes into stroma. While PAA1 could be localized on chloroplast perimeter by fluorescence, location of **PAA2** (also named HMA8), another metal-transporting ATPase similar to PAA1, could only be restrained to chloroplasts, but it remains unclear. Based on holoplastocyanin levels and CSD2 activity, PAA2 has been suggested to transport Cu across thylakoid membranes, from chloroplast stroma to thylakoid lumen, cooperating with PAA1 to supply Cu to chloroplasts. Another Heavy Metal P-type ATPase, **HMA1** has been localized in chloroplast envelopes and related to Cu transport into chloroplasts, as *hma1* mutants exhibited lower Cu levels in chloroplasts, lower SOD activity and photosensitivity. Function of the P-type ATPase **RAN1** (Responsive-to-antagonist), also called HMA7, has been inferred by analogy to Ccc2p transporter from *Saccharomyces cerevisiae*, and involved in the supply of ethylene receptors (ETR1) at the Golgi membrane (Hall and Williams, 2003; Grotz and Guerinot, 2006).

5.3.2. Energy metabolism

Reduction of growth has been linked to both disturbance of roots functioning but also to direct impacts on photosynthetic apparatus, which limit carbon fixation. Growth of aerial parts slows down, biomass is reduced and foliar epinasty occurs, together with the appearance of phytotoxic symptoms such as chlorosis, necrosis, discolorations and bronzing (Yruela, 2005). Cu excess reduced Fe accumulation in chloroplasts, leading to chlorotic symptoms by interfering directly with chlorophyll synthesis (Reilly and Reilly, 1973).

In leaf tissues, Cu concentrations exceeding 20 to 30 µg $Cu.mg^{-1}$ DW are toxic to most plant species (Patsikka *et al.*, 2002). In chloroplasts, Cu is an essential cofactor for plastocyanin, a Cu-containing protein involved in electron transport during photosynthesis processes. Located in the thylakoid lumen, plastocyanin acts as a mobile electron carrier between the cytochrome b6f complex and the reaction center of photosystem I. In *Arabidopsis*, the role of the two plastocyanin isoforms (PETE1 and PETE2) has been studied using mutant lines. Although a functional redundancy, both isoforms are differentially regulated in response to low or high Cu supply. PETE1 is essential for electron transport under Cu deficiency, as its expression is not altered by Cu depletion while PETE2 is down-regulated, leading to reduced electron transport. PETE2, in addition to its participation in electron transport, is involved in the buffering of excess Cu in chloroplasts (Abdel-Ghany *et al.*, 2009). Impaired photosynthetic activity and increased respiration result from the disturbance of electron transport, thylakoid and chloroplast structures together with the decrease/denaturation of pigments (Mocquot *et al.*, 1996; Yruela, 2005).

At a proteomic level, only scarce information is available about plant response to Cu excess; Cu induces differential accumulation of proteins related to glycolysis and respiration and mitochondria is a major target of Cu toxicity. ATP synthase subunit beta is down-regulated in *E. splendens* roots after exposition to 100 µM Cu for 3 or 6 days (Li *et al.*, 2009). Indicating Cu impacts on photosynthetic activity, several degraded fragments of RuBisCO have been identified in leaves of *E. splendens* plants after exposition to 100 µM Cu for 3 or 6 days (Li *et al.*, 2009) and in leaf segments of *O. sativa* floated in solutions containing 250 µM Cu for 72h (Hajduch *et al.*, 2001).

5.3.3. Protein metabolism

Under Cu excess, total content of soluble proteins decreases down to 50% in sunflower (Jouili and El Ferjani, 2003) or *Solanum melongena* (Körpe and Aras, 2011) roots or shoots. Cu toxicity on protein metabolism is due to the direct interaction (binding) between Cu and thiols functions (-SH), which leads to activity inhibition, structural disruptions, or substitution with other essential elements (Hall, 2002). Cu affects transcription and translation, protein folding, and protein degradation. Metal impacts on protein synthesis are still unclear and differs on their nature, physiological roles and species. In roots of *C. sativa*, Cu exposure induced the up-regulation of a 40S ribosomal protein S20 but the down-regulation of a 60S ribosomal protein L12 (Bona *et al.*, 2007).

Induction of protein chaperones, protein disulfide isomerase (PDI) or heat shock proteins (HSP) by Cu exposure may protect cell against Cu toxicity. Better maintenance and repairing of proteins, together with a better proteolysis of damaged/misfolded proteins may contribute to enhance tolerance in plants. No evidence exists about up-regulation of PDIs by Cu excess but a down-regulation has been recorded in response to As in roots of *O. sativa* (Ahsan *et al.*, 2008). Role of heat shock proteins (HSPs), which are low molecular mass proteins, remains controversial concerning the Cu-tolerance and more largely, metal tolerance. These HSPs may be classed by their molecular mass, such as low (10kDa), or high (90kDa), and their accumulation is induced by different abiotic stresses (Wollgiehn and Neumann, 1999; Hall, 2002). In *Armeria maritime*, a small HSP, HSP17, is expressed in roots of individuals grown on contaminated soil (Hall, 2002). Up-regulation of various HSPs has been recorded at a proteomic level in plants exposed to metal(loid) excess, including Cu. A HSP90 is up-regulated in rice roots by 8 µM Cu (Song *et al.*, 2013), while a HSP70 is down-regulated in *E. splendens* roots exposed to 100 µM Cu (Li *et al.*, 2009). In Cu-sensitive Es32 and Cu-tolerant Es524 strains of *E. siliculosus*, one HSP10 and one HSP70 are respectively up-regulated by 50 and 150 µg Cu/L (Ritter *et al.*, 2010). In the Cu-tolerant yeast *R. mucilaginosa*, several spots

identified as Hsp88 (3 spots), Hsp70 (6 spots) and Hsp60 are up-regulated by Cu excess (Irazusta *et al.*, 2012).

5.3.4. Chelation and storage

Cu chelation is realized by several distinct compounds. Once bound to molecular chelators, metals may be transported into vacuoles to be stored in inactive form (Hall, 2002). Amino- and organic acids are potential ligands due to the reactivity of Cu with amine functions (-NH), thiols (-SH) and carboxyles (-COOH, Clemens, 2001; Hall, 2002). Increases in free amino acids content, particularly of S-containing amino acids, occur in leaves of *M. chamomilla* cultivars exposed to Cu and probably contribute to their Cu-tolerance by chelating excess Cu (Kováčik *et al.*, 2011). Proteomic studies indicate that metal(loid) excess greatly affect sulfur metabolism, with differential expression of cysteine (CS) and methionine (MS) synthases. Two CS increase in roots of Cu-tolerant and sensitive rice varieties exposed to Cu (Song *et al.*, 2013) and others are up-regulated by Al (Yang *et al.*, 2007; Yang *et al.*, 2012) or As (Ahsan *et al.*, 2008). The glutathione (GSH) is a thiol tripeptide composed of glutamine, cysteine and glycine (γGlu-Cys-Gly) and formed by the consecutive action of γ-glutamylcysteine synthetase (γECS) and glutathione synthetase (GSS). Due to its thiol residues, GSH may chelate Cu and it is also involved in antioxidative activities (see following section 5.3.4).

Other cysteine-rich peptides like phytochelatins (PCs) or metallothioneins (MTs) are high-affinity ligands able to chelate metals including Cu (Mc Bride *et al.*, 1998; Clemens, 2001; Hall, 2002). PCs are synthetized from GSH by phytochelatin synthase (γ-glutamylcysteine synthetase) and are composed by motifs with a general structure $(\gamma\text{-Glu Cyst})_n$-Gly which may be repeated from 2 to 11 times. MTs are polypeptides classified in two main groups, Class 1, which shares alignments with mammalian MTs and Class 2, which does not exhibit such alignment, but MT3 and MT4 types have also been identified in plants (Hall, 2002). Accumulation of such metal-binding peptides may play a role in metal-tolerance including Cu-tolerance, by increasing chelation of free Cu and by reducing ROS production. However, MT and PC role in still unclear and remains controversial among species and metal(loid)s.

In *H. lanatus*, PCs content increased with Cu exposure (Hartley-Whitaker *et al.*, 2001), whereas in other studies no relation was found between metal exposure and PCs production. In *Silene vulgaris* or *S. paradoxa*, tolerance of metallicolous individuals has been attributed to amplification of MT genes (Van Hoof *et al.*, 2001; Mengoni *et al.*, 2003). When *Saccharomyces cerevisae* was transformed to express MTs gene from *Arabidopsis thaliana*, sensitivity to Cu was suppressed (Zhou and Goldsbrough 1994).

Different patterns of protein accumulation are reported for S-adenosylmethionine (SAM) synthase (SAMS) under various abiotic stresses, including Cu. Under low Cu exposure (8 µM Cu for 3 days), SAMS accumulation is up-regulated in roots of a Cu-tolerant (x 2.1) and a sensitive (x 1.6) varieties of *O. sativa* (Song *et al.*, 2013), while it was down-regulated in roots of *E. splendens* under high Cu exposure (1.5 and 2.4-fold decrease after 3 and 6 days at 100 µM Cu; Li *et al.*, 2009). SAM also acts as direct precursor for nicotianamine (NA), through nicotianamine synthase (Shojima *et al.*, 1990; Higuchi *et al.*, 1994) and indirect precursor for glutathione (GSH) through its conversion to cysteine via the trans-sulfuration pathway (Lu, 2000; Brosnan and Brosnan, 2006). NA is a key player in Cu homeostasis, for long distance Cu transport in xylem and phloem, Cu distribution, and accumulation (Pich *et al.*, 1996; Haydon *et al.*, 2007; Manara, 2012) but its role in Cu-tolerance remains controversial, as it is induced in *B. carinata* xylem sap in case of Cu deficiency but not in Cu excess (Irtelli *et al.*, 2009) whereas a Cu-induced rise in NA may reflect interspecies variations concerning Cu impacts (Pich *et al.*, 1996). SAM is also a direct precursor of ethylene (Brosnan and Brosnan, 2006), which is involved in growth, development, and stress signaling notably during senescence.

Organic acids are involved in root-to-shoot metal translocation, for example, citrate is the major Fe chelator in xylem sap (Manara, 2012). Citric, malic and oxalic acids, or histidine are involved in chelation and vacuolar storage (Rauser, 1999). Increasing production of such compounds could confer higher tolerance to Cu exposure. Phenols are also low molecular weight antioxidants which metabolites may scavenge ROS directly or through enzymatic reactions, but they may also directly chelate metals to reduce free content in cells. The role of high and low molecular mass phenolic compounds in Cu-tolerance by chelation has been demonstrated in *Lupinus albus* roots exposed to Cu (0.5, 20 or 62 µM Cu for 40 days; Jung *et al.*, 2003).

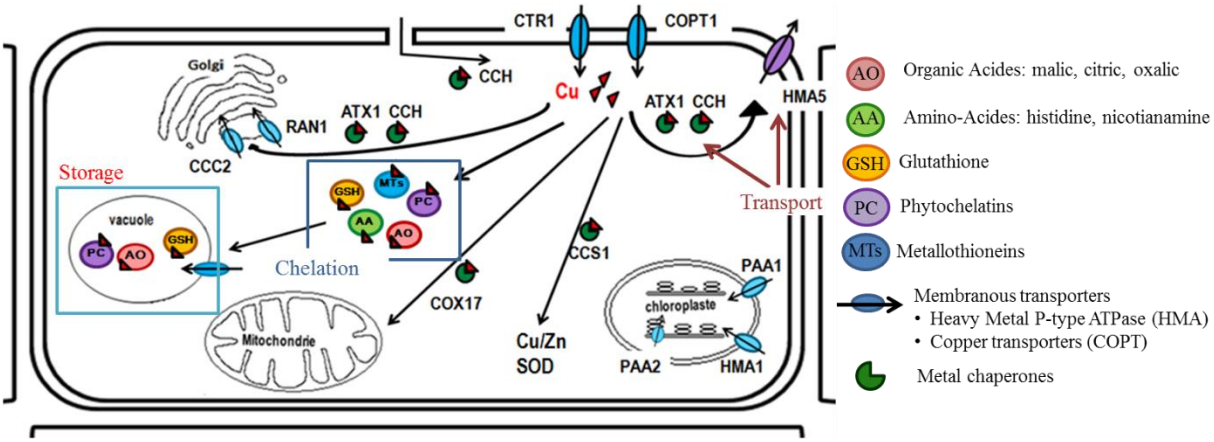

Figure 4: Cellular mechanisms underlying Cu homeostasis and tolerance (adapted from Clemens 2001; Hall and Williams 2003; Bertrand *et al.*, 2005; Yruela, 2005; and Pilon *et al.*, 2006)

5.3.5. Control of oxidative stress

Avoidance of oxidative stress with more efficient homeostasis is suggested as responsible for Cu-tolerance, rather than a better detoxification of induced oxidative stress (Pilon *et al.*, 2006). To avoid oxidative stress and maintain correct cell homeostasis, amounts of free Cu in cells have to be controlled, by limiting entrance and translocation together with favoring its storage, chelation and detoxification by intervention of chelates, chaperones or antioxidant enzymes (Yruela, 2005; Grotz and Guerinot, 2006). Limitation of root-to-shoot translocation permits to reduce oxidative stress in shoots reducing disturbance of photosynthetic apparatus. One avoiding strategy is the increase of biomass production to limit intra-cellular concentrations. In *Lotus purshianus*, tolerance index based on root length are three times higher in metal-tolerant population (Wu and Lin, 1990).

Due to its properties as bivalent cation, free Cu within cells catalyzes formation of reactive oxygen species (ROS) and other radicals through Haber-Weiss and Fenton reactions, i.e. $O_2^- + Cu^{2+} \leftrightarrow Cu^+ + O_2$, or $H_2O_2 + Cu^+ \leftrightarrow Cu^{2+} + OH\cdot + OH^{-}\cdot$ (Noctor and Foyer, 1998; Hall, 2002). Oxidative stress, which is defined as the imbalance in favor of production and accumulation of free oxygen radicals and other oxidants (Kehrer, 2000), needs to be controlled to avoid oxidative damages. Toxicity of $O_2^{\cdot -}$ and H_2O_2 accumulation is to create oxidative stress by initiating reaction cascades producing destructive compounds, such as lipid peroxides (Noctor and Foyer, 1998), which affect functioning of cell membranes (Hall, 2002), but also causes protein oxidation and induces irreversible DNA damages leading to cell death (Hall and Williams, 2003; Yruela, 2005; Pilon *et al.*, 2006; Bes, 2008). However, in plants, ROS are involved in signal transduction pathway. ROS contents are perceived by proteins, enzymes or receptors and trigger cascades of signal transduction, involving for example Ca^{2+}-binding proteins, calmodulin, G-protein activation or serine/threonine protein kinase (Mittler *et al.*, 2004). Lifetime of ROS in cells depends on antioxidant systems protecting cell functioning. This system includes enzymatic and non-enzymatic compounds with low molecular mass able to interrupt the chain of redox reactions (Noctor and Foyer, 1998).

Two major antioxidant enzyme families catalyze direct ROS degradation, i.e. superoxide dismutases (SODs) and catalases (CAT) (Noctor and Foyer, 1998, Mittler *et al.*, 2004).
SODs are metalloenzymes, classed by their metal cofactors in three groups, Cu/Zn-, Fe- and Mn-SODs, that catalyze the dismutation of superoxide radicals into hydrogen peroxide:

$2\ O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2\cdot$.

The resulting H_2O_2 is decomposed by CAT in the following reaction: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2\cdot$.

H_2O_2 is also detoxified through the ascorbate/glutathione pathway (AsA/GSH), where H_2O_2 is used as electron receptor for the oxidation of AsA or GSH to monodehydroascorbate (MDHA) or GSSG by ascorbate (APx) or glutathione (GPx) peroxidases. AsA acts also as a direct ROS scavenger. MDHA may either be reduced to AsA by MDHA reductase (MDHAR) using NAPDH as electron donor or disproportionated non-enzymatically to AsA and dehydroascorbate (DHA). DHA can also been reduced to AsA by DHA reductase (DHAR), which acts with oxidized glutathione (GSH) as electron donor (Fig. 5).

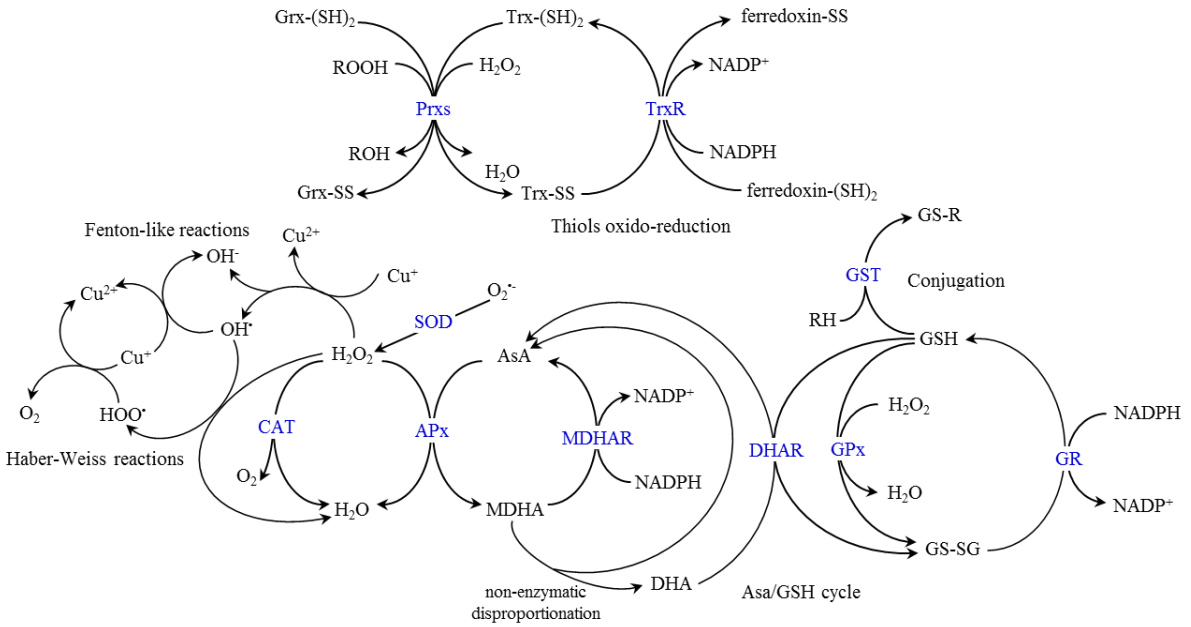

Figure 5: Production and detoxification of reactive oxygen species (ROS) in plants under Cu stress. AsA: ascorbate; APx: ascorbate peroxidase EC:1.11.1.11; CAT: catalase EC:1.11.1.6; DHA: dehydroascorbate; DHAR : DHA reductase EC:1.8.5.1; GSH: reduced glutathione; GS-SG: oxidized glutathione (or glutathione disulfide); GR: glutathione reductase EC:1.8.1.7; GPx: glutathione peroxidase EC:1.11.1.9; GST: glutathione-S-transferase EC:2.5.1.18; Grx: glutaredoxins; MDHA: monodehydroascorbate; MDHAR: MDHA reductase EC:1.6.5.4; Prxs: peroxiredoxins EC:1.11.1.15 ; ROOH: alkyl hydroperoxides; ROH: alcohols; SOD: superoxide dismutase EC:1.15.1.1; Trx: thioredoxin; TrxR: thioredoxin reductase (Ferredoxin-TrxR, EC 1.8.7.2 and NADPH-TrxR, EC:1.8.1.9). Adapted from Noctor and Foyer, 1998; Clemens, 2001; Hall et Williams 2003; Bertrand *et al.*, 2005; Yruela, 2005; Pilon *et al.*, 2006.

GSH is considered as a major antioxidant in plants. It may be oxidized (GS-SG) by DHAR, but also by GSH peroxidase (GPx), which participates to the degradation of H_2O_2. GS-SG is then reduced by glutathione reductase (GR) to maintain balance between both forms. GSH may also be used for conjugation with various substrates by the glutathione-S-transferase (GST). Homoglutathione (hGSH) is another tripeptide (γGlu-Cys-βAla), formed by the consecutive action of γ-glutamylcysteine synthetase (γECS) and homoglutathione synthetase (hGSS), which exhibits similar properties that GSH and may replace it in some plant species.

Other thiol peroxidases, peroxiredoxins (Prxs) catalyze the reduction of H_2O_2 or alkyl hydroperoxides (ROOH) to water or the corresponding alcohols (ROH), respectively, using preferentially thioredoxin (Trx) as an electron donor, but also other thiol active proteins such as glutaredoxin (Grx) or cyclophilin: ROOH + Trx-$(SH)_2$ → ROH + Trx-S_2 + H_2O. Once oxidized, Trx are regenerated by Trx reductases (TrxR), ferredoxin-TrxR, and NADPH-TrxR (Fig. 5). All these ROS-scavenging processes take place in different plant cell compartments as presented in the figure 2 from Mittler *et al.*, 2004 (Fig. 6).

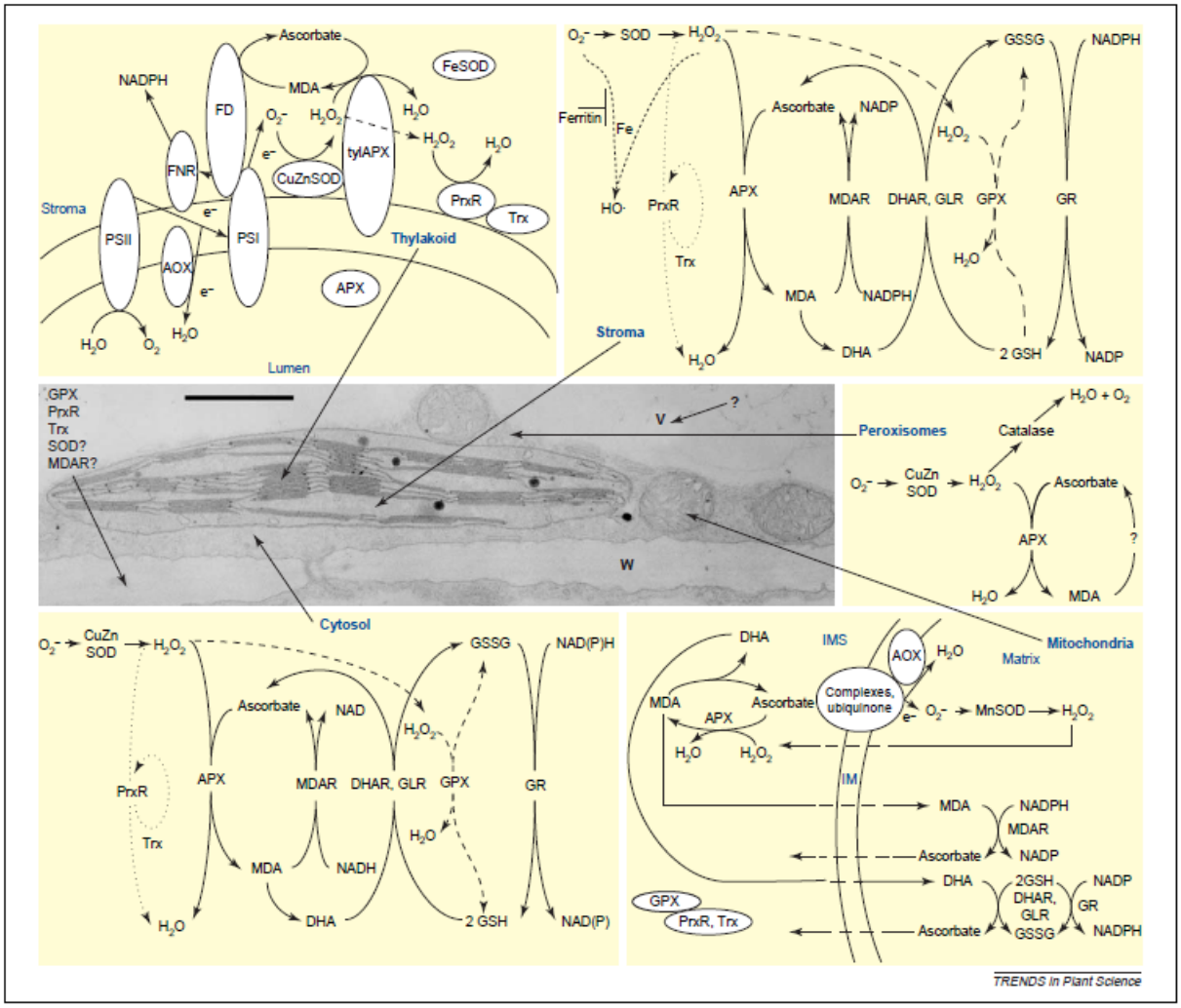

Figure 2. Localization of reactive oxygen species (ROS) scavenging pathways in plant cells. A transmission electron micrograph of a portion of a plant cell is used to demonstrate the relative volumes of the different cellular compartments and their physical separation (middle left). The enzymatic pathways responsible for ROS detoxification are shown. The water–water cycle detoxifies O_2^- and H_2O_2, and alternative oxidase (AOX; *Immutans*) reduces the production rate of O_2^- in thylakoids [top left; in some plants iron superoxide dismutase (FeSOD) might replace CuZnSOD in the chloroplast]. ROS that escape this cycle and/or are produced in the stroma undergo detoxification by SOD and the stromal ascorbate–glutathione cycle. Peroxiredoxin (PrxR) and glutathione peroxidase (GPX) are also involved in H_2O_2 removal in the stroma (top right). ROS produced in peroxisomes during photorespiration, fatty acid oxidation or other reactions are decomposed by SOD, catalase (CAT) and ascorbate peroxidase (APX) (middle right). SOD and other components of the ascorbate–glutathione cycle are also present in mitochondria. In addition, AOX prevents oxidative damage in mitochondria (bottom right). In principle, the cytosol contains the same set of enzymes found in the stroma (bottom left). However, these are encoded by a different set of genes and the major iron-chelating activity in the cytosol responsible for preventing the formation of HO· radicals is unknown. The enzymatic components responsible for ROS detoxification in the apoplast and cell wall (W) are only partially known, and the ROS-scavenging pathways at the vacuole (V) are unknown. Membrane-bound enzymes are depicted in white, GPX pathways are indicated by dashed lines and PrxR pathways are indicated by dotted lines in the stroma and cytosol. Although the pathways in the different compartments are mostly separated from each other, H_2O_2 can easily diffuse through membranes and antioxidants such as glutathione and ascorbic acid (reduced or oxidized) can be transported between the different compartments. Abbreviations: DHA, dehydroascrobate; DHAR, DHA reductase; FD, ferredoxin; FNR, ferredoxin NADPH reductase; GLR, glutaredoxin; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; IM, inner membrane; IMS, IM space; MDA, monodehydroascorbate; MDAR, MDA reductase; PSI, photosystem I; PSII, photosystem II; Trx, thioredoxin; tyl, thylakoid.

Figure 6: Localization of ROS scavenging pathways (Figure 2, from Mittler *et al.*, 2004)

Some studies indicate stimulated activities of these enzymes in response to Cu excess. In *P. vulgaris* roots exposed to 15µM Cu, activities of all enzymes belonging to AsA-GSH cycle increase, i.e. APx, MDHAR, DHAR and GR, as well as the concentrations in GSH and AsA (Gupta *et al.*, 1999). CAT activities are stimulated in *Helianthus annuus* exposed to 50 µM Cu (Jouili and El Ferjani, 2003) and extreme stimulation of CAT and GPX occurred in roots of *Matricaria chamomilla* cultivars exposed to Cu (Kováčik *et al.*, 2008). At the proteomic level, only few of these enzymes were identified as differentially regulated by Cu excess. Up-regulation of APX and GST spots has been reported in roots of rice cultivars exposed to 8 µM Cu for 3 days, with more marked increases in the tolerant one, suggesting a role in Cu-tolerance (Song *et al.*, 2013). Induction of APx spots were also recorded in response to other metal(loid)s (Ahsan *et al.*, 2009), including Cd (Weng *et al.*, 2013) or Al (Yang *et al.*, 2012). GST spots were also up-regulated in response to Cd (Alvarez *et al.*, 2009; Zhao *et al.*, 2011; Weng *et al.*, 2013), Al (Yang *et al.*, 2007; Navascués *et al.*, 2012; Yang *et al.*, 2012) or As (Ahsan *et al.*, 2008).

6. Phenotypic plasticity for metal-tolerance in *Agrostis capillaris*

Agrostis capillaris is one of the most commonly herbaceous species found on metals-contaminated soils; its ability to evolve metal-tolerant populations has been studied for at least 50 years and marked it out as a good candidate for phytostabilization of Cu-contaminated soils, as presented in the following review.

Potential use of tolerant-populations of *Agrostis capillaris* in phytoremediation

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Abstract

Agrostis capillaris is a pseudo-metallophyte able to grow on dry, poor and acidic soils, extensively studied for its ability to evolve populations tolerant to high stressor levels, such as metal excess and salt stress. Populations from highly contaminated areas show a higher tolerance to metal excess than populations originated from uncontaminated areas. This species can store metals in its roots, limiting metal accumulation in shoots. An excluder phenotype with a root-to-shoot ratio lower than 0.3 exists for Cu and Pb.

Such metal tolerant *A. capillaris* populations can contribute to seed bank and vegetation cover and enhance soil biological activity, which promote the ecological restoration of viable vegetation, ecosystem interaction and services on contaminated soils. Such characteristics and the various metal-tolerant populations evolved, make this species relevant for a potential use in phytostabilization of contaminated soils and for studying mechanisms underlying metal(loid) tolerance by comparing tolerant and sensitive populations exposed to metal(loid) excess.

6.1. Introduction

One option to unravel mechanisms underlying plant metal tolerance is to examine species and populations adapted to stressful environment, since these plants may evolve and retain the mechanisms enabling their survival. Some plant species having an intra-specific variability for adapting to (a)biotic stressful environments are identified, whereas others do not show any tolerance (Gartside and McNeilly, 1974; Marchand, 2012). Among them, *Agrostis capillaris* populations have evolved tolerance to various metal contaminations. This review aimed at summarizing the knowledge on populations of *A. capillaris* differing by their tolerance levels to various stresses and identifying the mechanisms underlying the higher tolerance of populations established on contaminated soils.

In addition, the potential use of metal-tolerant *A. capillaris* populations for phytoremediation of metal-contaminated soils is examined. Various methods can be used to remediate metal(loid) contaminated soils. Ecological alternative to soil excavation, phytoremediation regroups several options with two main processes, removal (extraction) and containing (immobilization or stabilization) (Pilon-Smits, 2005; Padmavathiamma and Li, 2007). All options consist in using plants with tolerant phenotypes to create a plant cover, which will improve soil micro-conditions and restore microbial activity and ecosystem services such as C sequestration and reduced contaminant dispersion by erosion and water (Vangronsveld *et al.*, 1995; Boon *et al.*, 1998; Padmavathiamma and Li, 2007).

Relevant plant species for phytoremediation of metal(loid)-contaminated soils must present several characteristics, common to all options, i.e. be native to avoid biological invasions, have relative fast growth, high soil coverage, tolerance to abiotic/biotic stresses, low-input production (energy, costs) and low nutrient/water requirements. However different phenotypes are needed: for phytoextraction, species are selected for their ability to translocate and accumulate high metal(loid) amounts in aerial parts, which can after be harvested, exported and valorized. For phytostabilization, species are chosen for their ability to store metal(loid)s in the root system, limiting translocation to aerial part as non-lethal concentrations in tissues (excluder phenotype) to limit transfer into food chain (Ruttens *et al.*, 2006, Padmavathiamma and Li, 2007). Because only very few species are Cu-hyperaccumulators, remediation options for Cu contaminated soils are mostly immobilization (phytostabilization), by restoring vegetal cover of tolerant species, with or without addition of organic and inorganic amendments.

Use of metal(loid)-tolerant plants for phytoremediation of contaminated areas is not recent (Vangronsveld *et al.*, 1995, Römkens *et al.*, 1999), and examples of phytostabilization of contaminated soils using *A. capillaris* tolerant populations/cultivar already exist.

The use of *A. capillaris* and *Festuca rubra* metal-tolerant cultivars, screened from commercial cultivars for their Zn tolerance, together with the incorporation of a coal fly ash (beringite) and compost combination into the contaminated soil has resulted in a rapid and effective re-vegetation of the bare area of a Zn smelter (6150 mg Zn, 17 mg Cd, 660 mg Cu and 1375 mg Pb.kg $^{-1}$ DW soil; Vangronsveld *et al.*, 1995). Although tolerant plants are able to grow on this contaminated soil, without amendment, addition of beringite enables them to survive under higher Zn exposure and drastically reduces toxicity symptoms and shoot Zn concentrations.

Potential of adding soluble inorganic phosphate, alone or together with growth of *A. capillaris* tolerant plants (‘Parys’ cultivar) to form insoluble metal phosphates and then

immobilize metal excess in contaminated soils has been studied (Cotter-Howells, 1996). Results evidence the potential of *Agrostis* to fix Pb and Zn in forming insoluble metal phosphates, which may, with time, affect the bulk solid speciation of metals in mine-waste soils, leading to effective immobilization in soil.

Plant species diversity is low in highly metal(loid) contaminated soils. Persistence and high contribution to seed bank composition has been found for *A. capillaris* on contaminated soil indicated that establishment of tolerant populations may highly contribute to restore seed bank (Meerts and Grommesch, 2001).

Tolerant population of *A. capillaris* have spontaneously colonized lysimeters filled with Zn, Cu, Cd, and Pb contaminated soils from a Zn smelter and on which aided-phytostabilization was tested, with a single addition of various amendments (Ruttens *et al.*, 2006). As *A. capillaris* was found in all treated soils and was the only species on untreated soil, it was used to record effectiveness of chemical amendment tested, by measuring shoot metal concentrations. Whereas on untreated soil *Agrostis* plants exhibited Zn and Cd concentrations exceeding background metal concentrations in grasses and Cu-concentration at the upper-limit of common range, all amendments were successful to reduce significantly shoot metal concentrations.

A phytoremediation trial of As-contaminated soil has been conducted with the As-hyperaccumulator *Pteris vittata* and tolerant population of *A. capillaris* grown on separate lysimeters (Cattani *et al.*, 2009).

Effects of lime addition have been tested on contaminated soils and three species were compared to examine the effects of metal tolerance strategies, i.e. hyperaccumulator *Pelargonium sp.* or excluder *Silene vulgaris* and *A. capillaris*, on metal uptake, mobility, and bioavailability of metals after amending (Benz, 2013). Results indicated a positive effect of plants growth on soil, especially for the two metal excluders, which both increase soil pH and decrease available fractions of Al^{3+} and Cu^{2+}, whereas *Pelargonium* plants does not change pH but decreases available fractions of Cu, Zn, and Al by accumulation in tissues. For all species, liming decreases metal concentrations in shoots.

Restoring a vegetal cover, notably using metallicolous *A. capillaris* cv. 'Parys', can counterpart Cu impacts by increasing pH, dissolved organic carbon (DOC) and Ca in soil solution enough to reduce both the total dissolved Cu concentration and the free metal activity in Cu-contaminated soil (Römkens *et al.*, 1999). It also promotes conversion of ammonia to nitrate in soil (Römkens *et al.*, 1999), soil microbial activity, bacterial growth and presence of nematodes in Cu-contaminated soil (Boon *et al.*, 1998; Vogeler *et al.*, 2008). With time,

establishment of *A. capillaris* can decrease Cu concentration in upper layers of soil (Dahmani-Muller *et al.*, 2000) and enhance establishment of less-tolerant species (Bes, 2008).

Based on these works, *A. capillaris* tolerant populations may be useful for phytostabilization processes of contaminated soils and particularly adapted for aided-phytostabilization of metal(loid) contaminated soils.

6.2. Ecological requirements

Agrostis capillaris, formerly called *A. tenuis* Sibth. (McCain and Davies, 1983; Humphreys and Nicholls, 1984), belongs to the genus *Agrostis* (*Poaceae*) which comprises more than 200 species. These C3 plant species is a tetraploid (2n = 4x = 28) with a genome composition listed as $A_1A_1A_2A_2$ or $A_2A_2A_2A_2$. It is a perennial grass with thin leaves, spreading by rhizomes and stolons, which is used for erosion control and on fairways and tees in golf courses (Rajasekar *et al.*, 2007; Rotter *et al.*, 2007; Dinler and Budak, 2008).

This species has long seed persistency in soil (Bossuyt *et al.*, 2007) and is able to grow under adverse abiotic conditions reflecting its high tolerance to partial shade, acidic, poor or dry soils (Smith, 1972; Osborne and Whittington, 1981; Dixon, 1986, Dunsford *et al.*, 1998; Bech *et al.*, 2012). Population differentiation in *A. capillaris* is a major effect of the environment. Short distances (50 m or less) may isolate populations from others, so this species is able to evolve to very local environmental conditions (Bradshaw, 1959). Sudden change from predominantly tolerant to predominantly non-tolerant individuals occurs over a distance of one meter when Cu increases in soil. Despite high gene flows from non-tolerant to tolerant populations, the latest maintain their identity because of the strong selection pressure favoring tolerant individuals (McNeilly, 1968; McNeilly and Antonovics, 1968). This illustrates the ability of *A. capillaris* to evolve populations with various phenotypes in a small area if the originated habitats are different (Bradshaw, 1959; McNeilly, 1968; McNeilly and Antonovics, 1968; Smith, 1972). *Agrostis capillaris* diversity is identified at the genetic level with AFLP markers, which suggests a potential value for cultivar improvement (Zhao *et al.*, 2006).

6.3. Localization of metal-tolerant ecotypes of *A. capillaris* on contaminated sites

Agrostis capillaris colonizes contaminated and disturbed soil surfaces and is a dominant species on multi-contaminated sites, such as Pb/Zn mine soil (Baker *et al.*, 1986), Zn refinery (Griffioen *et al.*, 1994), former metallurgical factory (Cu, Zn and Pb; Dahmani-Muller *et al.*, 2000), soil contaminated by metal-rich dust (Zn, Pb, and Cd; Meerts and Grommesch, 2001), waste disposal site near a lead smelter (Zn, Cu, Cd and Pb; Sudova *et al.*, 2008), and

contaminated soils from a former antimony mine (Sb, As, Pb and Cu; Bech *et al.*, 2012). Tolerant populations of this species are reported on soils mainly contaminated by Cu, such as a Cu/Pb mine soil (Thompson and Proctor, 1983), Cu/Zn mine/refinery (Benz, 2013), metal processing factories with Cu smelting and refining, Cu-Cd alloy production and brass (Cu-Zn) foundry (Cu, Cd, Zn, Dickinson *et al.*, 1996), a copper rod rolling factory (Lepp *et al.*, 1997), Cu mine soils (McNeilly, 1968; McNeilly and Antonovics, 1968; Griffioen *et al.*, 1994), and a wood preservation site (Bes *et al.*, 2010). Its ability to survive on both contaminated and uncontaminated soils marks it out as a pseudo-metallophyte (Dahmani-Muller *et al.*, 2000; Sudova *et al.*, 2008).

6.4. High metal-tolerance and variability among and within populations

High intra-specific variability in metal-tolerance levels occurs among populations from Cu-contaminated and normal pasture sites, with metallicolous populations (M, Cu-tolerant) having higher growth and showing less symptoms than non-metallicolous ones (NM, non-tolerant) on contaminated conditions (Nicholls and McNeilly, 1985; Symeonidis *et al.*, 1985a and 1985b; Lepp *et al.*, 1997; Vogeler *et al.*, 2008; Sudova *et al.*, 2008). Variability in Cu-tolerance exists among and within populations from various Cu-contaminated sites (Gregory and Bradshaw, 1965; Nicholls and Mc Neilly, 1979 and 1985). Grown on Cu-contaminated soils, *A. capillaris* plants accumulate more Cu in roots, with limited translocation to shoot, and typical shoot-to-root ratio of an excluder phenotype (Dahmani-Muller *et al.*, 2000; Ernst, 2006). Excluder phenotypes are also identified for Pb (Dahmani-Muller *et al.*, 2000; Malcová *et al.*, 2003), Cd (Dahmani-Muller *et al.*, 2000) and As exposures (Austruy *et al.*, 2013) but not for Zn, with a ratio around 0.5 (Dahmani-Muller *et al.*, 2000).

Variability among and within populations occurs in populations originated from non-contaminated sites and commercial seeds. When sufficiently large, they are able to evolve few Cu-tolerance individuals just after one cycle of selection, even if it is in lower frequency than in Cu-tolerant populations (Gartside and McNeilly, 1974; Walley *et al.*, 1974, Sudova *et al.*, 2008). For instance, some commercial non-tolerant seeds of *A. capillaris* were sowed in the soil of a Cu rod rolling factory and after only two years tolerant populations were present even in most contaminated areas (Lepp *et al.*, 1997).

Flowering time differ between Cu-tolerant and sensitive populations: flowering of tolerant population begins and ends early, suggesting a possible isolation among populations at beginning and ending of flowering period (McNeilly and Antonovics, 1968). Mining activities are impacting seed bank by destroying the vegetation, which leads to soil erosion that also depletes seed bank. A new seed bank can develop after colonization by tolerant species, with a

low species diversity, absence of common species found in this type of grassland and dominant contribution of *A. capillaris* population (Meerts and Grommesch, 2001). Results are similar at a wood preservation site, with dominance of *A. capillaris* in vegetation and seed bank and low diversity in species (Bes *et al.*, 2013). The distinct pattern between plant species found in seed bank and vegetation indicates a selection through environmental pressure (Lepp *et al.*, 1997). However, Cu exposure impacts germination of tolerant populations of *A. tenuis* in some studies with a reduction of both germination and growth when the proportion of contaminated soil increased, even if it was to a lesser extent for the 'Parys' ecotype compared to non-tolerant population (Gartside and McNeilly, 1974; Walley *et al.*, 1974),

Selection of tolerant individuals would occur at three even four levels: first during flowering stage, with selection of plants able to produce flower and with earlier flowering plants crossed together (McNeilly and Antonovics, 1968), secondly during persistence in soil (Lepp *et al.*, 1997), thirdly during germination, with only seeds possessing some tolerance being able to germinate, and lately during the plant life, with the survival of plants which possess enough tolerance to maintain growth until maturity (Gartside and McNeilly, 1974; Walley *et al.*, 1974).

Variability between- and within-populations are found on Pb/Zn contaminated soils with large differences in shoot Pb and Zn concentrations for a given Pb or Zn concentration in soil (Barry and Clark, 1978). *Agrostis capillaris* populations sampled from a Pb mine and a rough grazing have been tested on Pb/ Zn-contaminated mine soil (Goginan mine, UK; Bradshaw, 1960) Based on roots length, the mine population shows greater resistance to Pb and Zn exposure. Root length highly varies between individual plants within the tolerant population. Tolerant individuals exist in *A. capillaris* populations growing on Zn-contaminated soil beneath electricity pylons, whereas plants collected at a minimal distance of 50 m from the pylons didn't show any tolerance. However, the tolerance levels vary among and within populations collected beneath different pylons (Al-Hiyaly *et al.*, 1988; Al-Hiyaly *et al.*, 1993). This selection of tolerant plants, together with the high variability within populations have been confirmed for *A. capillaris* but also recorded in other plant species, e.g. *A. stolonifera*, *Anthoxanthum odoratum*, *Deschampsia cespitosa*, and *Festuca ovina*, even though the last two were found on only a small number of the 18 pylons tested, whereas in contrast *A. capillaris* was found under nearly all pylons (Al-Hiyaly *et al.*, 1990)

Agrostis capillaris evolves As-tolerant populations. Such higher As-tolerance is related at least from one part to a reduction of As influx, by adaptation of the arsenate uptake system, i.e. in decreasing the Vmax of high and low-affinity uptake systems and by increasing the Km of the high-affinity uptake system (Meharg and Macnair, 1991). Based on genetics of As

tolerance in *A. capillaris*, As tolerance is a heritable character, the tolerant trait being dominant to the non-tolerant one and with more than one, but small number of gene loci involved. Watkins and MacNair (1991) suggested a single major gene is involved in As tolerance, with one or more minor genes modifying its control, allowing heritable variation in degree of tolerance among tolerant plants.

Over the years, some cultivars or populations highly tolerant have been identified and characterized such as **Cu-tolerant 'Parys'**, originated from Parys Mountain, Isle of Anglesey (Gartside and McNeilly, 1974; Walley *et al.*, 1974; Nicholls and Mc Neilly, 1979; Karataglis, 1980; Wu, 1981; Humphreys and Nicholls, 1984; Nicholls and McNeilly, 1985, Symeonidis *et al.*, 1985; Cotter-Howells, 1996; Boon *et al.*, 1998; Vogeler *et al.*, 2008); **Pb/Zn-tolerant 'Goginan'**, originated from a Lead/Zinc mine at Goginan, Dyfed (Bradshaw, 1960; Wu, 1981; Humphreys and Nicholls, 1984; Symeonidis *et al.*, 1985) or **Zn tolerant 'Trelogan'**, originated from a Zn mine at Trelogan, Flintshire (Turner, 1970; Karataglis, 1980; Walley *et al.*, 1974)

6.5. Salt and seawater tolerance

Both *A. capillaris* and *A. stolonifera* have potential to evolve salt tolerance. However, populations of *A. capillaris* are inhibited by seawater exposure whereas *A. stolonifera* is highly tolerant. The absence of evolution of a seashore ecotype has been related to a lack of Mg tolerance in *A. capillaris*, which root growth is almost completely inhibited (less than 5cm) in the presence of 170 mequiv.L^{-1} MgCl whereas *A. stolonifera* growth, even reduced, is high. Metal-tolerance couldn't be related to salt-tolerance, even if the Cu-tolerant population from Parys Mountain shows appreciable salt-tolerance compared to other *A. capillaris* populations (Wu, 1981). Although *A. capillaris* is absent from areas with high salinity, this species presents a significant response to salt selection but also a specific response to Mg excess. Other factors prevent this species from colonizing seashore soils and high Mg concentrations occurring together with the high salinity would be an explanation for the absence of *A. capillaris* ecotypes on seashore (Ashraf *et al.*, 1986; Ashraf *et al.*, 1989).

6.6. High tolerance inter- and intra-populations of *Agrostis* species

Several species from the genus *Agrostis*, including *A. capillaris*, can evolve populations with different tolerance to metals and other abiotic stresses. *A. stolonifera* displays Cu-tolerant populations, with increase in both the tolerance of individuals and the frequency of tolerant individuals in contaminated areas, as the population age increases. The Cu-tolerance evolves with the population age, even if the youngest population shows considerable tolerance (Wu *et al.*, 1975a). Based on Cu-uptake and impacts on roots of tolerant and non-tolerant populations,

A. stolonifera has an excluder phenotype and the tolerant population accumulates more Cu in roots than in shoots compared to the non-tolerant one (Wu *et al.*, 1975b). Ecotypes of *A. stolonifera* differ for their tolerance to salt stress, with some presenting high levels of tolerance (Ahmad and Wainwright, 1977; Ahmad *et al.*, 1981; Hodson *et al.*, 1981; Hodson *et al.*, 1985; Ashraf *et al.*, 1986; Ashraf *et al.*, 1989), and for heat-tolerance (Xu and Huang, 2008 and 2010). *A. castellana* and *A. delicatula* have been studied for their ability to evolve arsenate tolerant populations (De Koe and Jaques, 1993) and *A. scabra* for Cu-, Ni- and Zn-tolerant populations (Dudka *et al.*, 1995) or heat-tolerant populations (Tercek *et al.*, 2003; Xu and Huang, 2008 and 2010a).

6.7. Cases of multiple tolerance

Multiple metal tolerance has been investigated in *A. capillaris* by comparing root growth parameters of common pasture populations and Zn, Zn/Pb, Pb and Cu-mine populations on soils originated from each mine site. Pasture populations does not show any tolerance for any metal. Conversely, mine populations has marked tolerance to the particular metal highly concentrated in their original soil but this tolerance stayed specific to the concerned metal and is not measured for any other metal. Multiple tolerances only happen in populations originated from soil where more than one metal occur in phytotoxic concentrations (Gregory and Bradshaw, 1965). Similarly, populations originating from Parys Mountain Cu-contaminated soils show tolerance to both Cu and Zn, contaminants highly present in originated soil, whereas population from the Trelogan Zn-contaminated mine soil is only tolerant to Zn, showing no tolerance to either Cu or Pb (Karataglis, 1980).

Clones from a waste disposal site near a Pb-smelter are more tolerant to Pb, Zn, Cu and Cd than common soil populations, regarding to growth, dry weight and number of tillers, but each metal concentration in the contaminated soil exceeded its soil background level (Sudova *et al.*, 2008).

Germination and growth of Cu-tolerant seeds on Zn-contaminated soils reached intermediate levels between non-tolerant and Zn-tolerant populations (Walley *et al.*, 1974). Survivors had tolerance to both metals, suggesting that Cu-tolerance conferred some ability to survive on Zn-contaminated soil. Grown on Cu contaminated soil, Cu-tolerant population shows maximal survival, whereas Zn-tolerant population does not differ from non-tolerant. Same pattern occurred for Zn-tolerant population on Zn-contaminated soil but in mixed Cu-Zn contaminated soil, both populations behave similarly. Therefore, tolerance to Zn and Cu are independent, and as genes determining Cu and Zn resistance are not linked, occurrences of

individuals showing both Cu and Zn tolerance results the product of the frequencies of the occurrence of tolerance to each metal.

A clone of *Agrostis stolonifera* is tolerant to Cu and Zn, but Cu uptake is not affected by Zn exposure and vice versa, whereas strong interaction is found in toxic effects of both metals on root elongation (Wu and Antonovics, 1975). Additive Cu and Zn toxic effects occurs in Cu/Zn-tolerant genotype of *A. capillaris* from Parys Mountain, which, when exposed to both metals at doses inducing reduction of root length to 50% separately, shows 95% reduction of root growth (Karataglis, 1980).

More contrasted results have been obtained in comparing Cu-tolerant "Parys" and Pb/Zn-tolerant "Goginan" cultivars regarding to their tolerance to Cd, Cu, Pb, Ni and Zn. Respective tolerance to Cu and Pb/Zn was confirmed but tolerance to other metals was also observed. "Goginan" cultivar showed Cu-tolerance level intermediate between those from "Parys" and "Highland" (non-tolerant); marked Pb/Zn-tolerance compared to "Highland" was observed for "Parys" cultivar, despite low Cu and Pb/Zn levels were found in originated soil. To a lesser extent, both tolerant ecotypes, in particular 'Parys', showed higher Cd and Ni-tolerance than the non-tolerant Highland. Some non-specific general tolerance to metals would be conferred by tolerance to specific one (Symeonidis *et al.*, 1985).

Tolerance to Co, Cu, Ni and Zn highly varies in three clones of *A. gigantea* from a mine waste site; whereas one shows tolerance to Cu, Co and Ni, another is tolerant only to Ni and any is tolerant to Zn (Hogan and Rauser, 1979). This confirms that the high variability among plants within one population observed in *A. capillaris* is also observed in other *Agrostis* species.

For *A. capillaris*, the cell wall fraction influenced the Cu and Zn binding in roots of metal-tolerant plants at high metal exposure and at common metal supply this fraction contained more metal in metal-tolerant plants compared to non-tolerant (Turner, 1970; Turner and Marshall, 1971). Accumulation of Zn in cell wall fraction is correlated to the tolerance of populations, and was proposed as a mechanisms underlying tolerance to Zn in *A. capillaris* (Turner and Marshall, 1972).

6.8. Association with mycorrhizal fungi

Associations between mycorrhizal fungi and tolerant-populations of *A. capillaris* occur frequently on contaminated soils, e.g. Cu-, Zn/Cd-contaminated soils (Griffioen, 1994; Griffioen *et al.*, 1994). In three populations grown on various contaminated sites (Zn, Pb/Zn) and a common soil, most fungi belonged to *Glomus* genus. Infection is seasonal dependent and decreases in populations from contaminated areas compared to the common one, but it poorly

influences the ionome of plant tissues. Mycorrhizal infection and metal tolerance are not linked in *Agrostis capillaris* (Ietswaart *et al.*, 1992). Griffioen (1994) listed 26 species of mycorrhizal fungi found on contaminated soils (Mn, Zn, Cu, Pb, Ni or Cd) and 19 of them belonged to the genus *Glomus*. Spores found in Cu- or non-contaminated soils mainly belonged to *Glomus* species. The uncommon species *Scutellospora dipurpurescens* was present in the Zn/Cd soil.

Vesicular arbuscular mycorrhiza (VAM) infection of *A. capillaris* has been studied on populations from Cu-, Zn-mines or non-contaminated soil. On highly Cu-contaminated soil, infection is very low or absent, whereas it is much higher on uncontaminated or Zn/Cd-contaminated soil and increases in areas surrounding the copper mine, when Cu concentrations in soil decreased. This supports hypothesis of a higher toxicity of Cu compared to Zn/Cd and of more severe selection on mycorrhizal fungi in Cu-contaminated soil (Griffioen *et al.*, 1994).

Studies dealing with the effect of mycorrhizal fungi on metal uptake by host plants provide conflicting results among species, experimental conditions, types of substrates and contamination levels and types (Malcová *et al.*, 2003). Association with tolerant population of mycorrhizal fungi has been suggested to enhance metal-tolerance in plants (Griffioen, 1994; Hall, 2002), by storing metals in cells and then decreasing the translocation of metals in plant cells or by supplying nutrients and water to countepart the adverse soil conditions.

The work of Neagoe *et al.*, (2013) supported the last hypothesis, showing that on contaminated soil, the beneficial effect of inoculation with arbuscular mycorrhizal fungi is rather due to an improvement of P nutrition rather than to a reduction of transfer into plant cells.

Potential synergism between plant and fungal tolerance has been investigated in associating populations of *A. capillaris* from contaminated and normal sites with *Glomus intraradices* from contaminated and normal sites. Isolates of *G. intraradices* and populations of *A. capillaris* are more tolerant when originated from contaminated soil compared to those from uncontaminated soils. However, inoculation with each isolate decreases plant biomass (Malcová *et al.*, 2003) or does not confer any additional metal tolerance on either tolerant or non-tolerant plants when cultivated on contaminated substrates (Sudova *et al.*, 2008).

In the first work, effect of inoculation on metal uptake has been related to the intensity of contamination: At 0.01 mM Pb, root Pb concentrations increased for *Agrostis* plants inoculated with isolate from contaminated soil compared to non-inoculated and inoculated by the non-tolerant isolate. However, at a higher Pb level (0.1 mM), root and shoot Pb concentrations of inoculated and non-inoculated *A. capillaris* plants did not differ (Malcová *et al.*, 2003). In the second study, effect on plant growth and metal-uptake was dependent on both combination of plant population and fungal isolate, without clear differences between tolerant and non-tolerant

clones (Sudova *et al.*, 2008). These studies pointed out the absence of synergism between plant and fungal tolerance in case of association between *G. intraradices* and *A. capillaris*, although the low number of isolates studied and the large number of existing associations did not ruled out such synergism.

6.9. Influence on ecosystem services in metal(loid) and co-contaminated sites

Together with the development of sparse vegetation (Bes *et al.*, 2010), Cu excess in soil drastically reduces biological activity (Dickinson *et al.*, 1996). Restoring a vegetal cover notably using metallicolous *A. capillaris* cv. 'Parys' can counterpart Cu impacts by increasing pH, Dissolved Organic Carbon (DOC) and Ca in soil solution enough to reduce both the total dissolved Cu concentration and the free metal activity in Cu-contaminated soil (Römkens *et al.*, 1999). It also promotes conversion of ammonia to nitrate in soil (Römkens *et al.*, 1999), soil microbial activity, bacterial growth and presence of nematodes in Cu-contaminated soil (Boon *et al.*, 1998; Vogeler *et al.* 2008). With time, establishment of *A. capillaris* can decrease Cu concentration in upper layers of soil (Dahmani-Muller *et al.*, 2000) and enhance establishment of less-tolerant species (Bes, 2008).

Plant species diversity is low in highly metal(loid) contaminated soils. Persistence and high contribution to seed bank composition has been found for *A. capillaris* on contaminated soil indicated that implantation of tolerant populations may highly contribute to restore seed bank (Meerts and Grommesch, 2001).

6.10. Conclusion

The ability of *A. capillaris* to differentiate populations with distinct tolerance to various metals makes this species interesting for selection of highly tolerant populations called metallicolous populations. This perennial species is also adapted to adverse soil conditions which often occur in contaminated soil and presents an excluder phenotype for several metals. These characteristics and the ecosystem services of tolerant populations pointed out *A. capillaris* as a relevant candidate to phytostabilize metal-contaminated soils, in association with other species such as woody species. Incorporation of amendments into metal(loid) contaminated soils can limit metal(loid)-uptake in roots, leading to consideration of using this species for aided-phytostabilization trials. The existence of so many ecotypes makes also this species important for studying the mechanisms underlying metal tolerance in grassy plants. Using multiple-scale options with such species may help to elucidate both the mechanisms underlying the plant stress due to metal exposure and those related to the best metal tolerance of the metallicolous ecotype.

7. Hypothesis about better tolerance in metallicolous *A. capillaris*

Based on existing litterature, different hypotheses may be drawn about mechanisms of Cu tolerance in metallicolous populations of *A. capillaris*.

A reduced Cu-uptake, in decreasing number or affinity of transporters in roots, may limit Cu accumulation and toxicity in root tissues. This mechanism has been suggested for As tolerance in tolerant populations of *A. capillaris* L. and *Deschampsia cespitosa* (L.) Beauv., which shows adaptation of the arsenate uptake system, leading to reduced influx of arsenate in As-tolerant plants, by decreasing the Vmax of high-affinity system (Meharg and Macnair, 1991). Uptake limitation may also be achieved through rhizospheric mechanisms such as association with microorganisms (endophyte bacteria or mycorrhizal fungi) or exudation of root exudates. The precise role of association with microorganisms in Cu-tolerance remains unclear, a storage of Cu in the symbiotic organisms may protect plants from Cu toxicity but a positive effect on plant nutrition may also be involved.

A higher Cu accumulation or storage in roots of M populations, through a higher root production and/or a better ability to store Cu in existing tissues, may prevent translocation to shoots. Greater ability to accumulate Cu in roots of M populations has been suggested to be responsible for higher Cu tolerance in preventing Cu translocation into leaves (Bradshaw, 1965; Wu *et al.*, 1975b).

An **active limitation of root-to-shoot Cu-translocation** may also protect shoot from Cu toxicity in limiting oxidative stress and disruption photosynthesis processes. Tolerance of population of *A. capillaris* to antimony has been attributed to Sb exclusion, as concentrations of Sb in shoots of tolerant were three times lower than in the non-tolerant plants (Bech *et al.*, 2012).

A better ability to cope with Cu toxicity in leaves may also increase Cu tolerance in M plants. This ability may involve both a better Cu-storage in cells and a reinforcement of homeostasis and detoxification processes. The existence of a metal complexing system, just after entry of the metal in the cytoplasm may be involved in higher tolerance of *Agrostis* populations (Karataglis, 1980)

8. Origin of the study

An experimental phytoremediation platform, called "BIOGECO platform", has been created on a Cu-contaminated site, which purpose is outside-wood treatment, still in activity (St Médard d'Eyrans, 33; Bes, 2008). This factory first used copper sulfate to protect outside-wood again pathogens attack, but nowadays, main compound used is the CCA (chromated copper arsenate) resulting in multiple contamination, dominated by Cu (Solo-Gabriele and Townsend, 1999; Warner and Solomon, 1990). Cu-contamination ranges from 65 to 2600 mg Cu/kg soil (Bes, 2008; Bes and Mench, 2008; Mench and Bes, 2009) and several trials of phytoextraction and phytostabilization have been set up.

A biodiversity survey has been realized and pointed out several species as able to evolve Cu-tolerant populations, and this potential has thereafter been tested on pot experiments. Seeds of the metallicolous population have been collected on the P7 plot (Fig. 7) whereas the second population was sampled at a forest edge, free from any contamination (Belin-Beliet, 33; Bes, 2008).

Figure 7: Localization of the parcel for M seed sampling in the BIOGECO platform (St Médard d'Eyrans, 33; Bes, 2008).

9. Problematic, hypotheses and approach of the study

This project was designed to achieve several objectives and to answer several hypotheses.

First aim was to determine the response of *Agrostis capillaris* populations to an increasing Cu stress, using phenotypic, physiological and proteomic approaches. Proteomic was chosen to increase the knowledge about the molecular mechanisms underlying plant response to Cu stress. Will the exposition to the 1-50 µM Cu range affect *A. capillaris* population growth? What are the impacts of Cu exposure at phenotypic, physiological and proteomic levels? May the pattern of differential protein expression explain the symptoms reported at the plant scale?

Second aim was to evaluate the phenotypic plasticity for Cu-tolerance between two populations of *A. capillaris*, first originated from a Cu-contaminated soil (Metallicolous, M) and second one from an uncontaminated soil (Non-Metallicolous, NM). To what extent exists a phenotypic plasticity for Cu-tolerance between both M and NM populations in the 1-50 µM Cu range? Could the physiological and proteomic results explain this plasticity in Cu tolerance?

The higher Cu tolerance was reported for the M population during comparison on Cu-contaminated soils using a fading technic and hydro-culture in the 1-30 µM Cu range (Bes, 2008). Did a reduced accumulation of Cu in roots or translocation from root-to-shoot occur to protect leaves from Cu toxicity? Can an enhancement of Cu homeostasis and detoxification processes in roots and/or leaves be responsible of the higher Cu-tolerance of M plants?

Last objective concerned the potential of using metallicolous *A. capillaris* populations such as the one described here, for the phytostabilization of Cu-contaminated soils. Would the M population have ability to grow on Cu-contaminated soils without accumulating Cu amount high enough to injure herbivors throughgrazing of aerial parts?

To elucidate these questions, seeds from both contaminated (BIOGECO platform) and uncontaminated soils were grown on hydro-culture and exposed to increasing doses of Cu in the nutritive solution. After three months, plant growth of each population were characterized (length, biomass), then tissues were frozen in liquid nitrogen and stored at -80°C to perform analyses of soluble proteomes. Concentration elements were measured in tissues and proteins were extracted by Trichloroacetic acid (TCA)-Acetone procedure then separated with 2D-eletrophoresis. After images analysis, spots exhibiting variation in response to population origin and/or to Cu exposure were excised and submitted to LC-MS/MS for protein identification by bioinformatics procedures (Fig. 8).

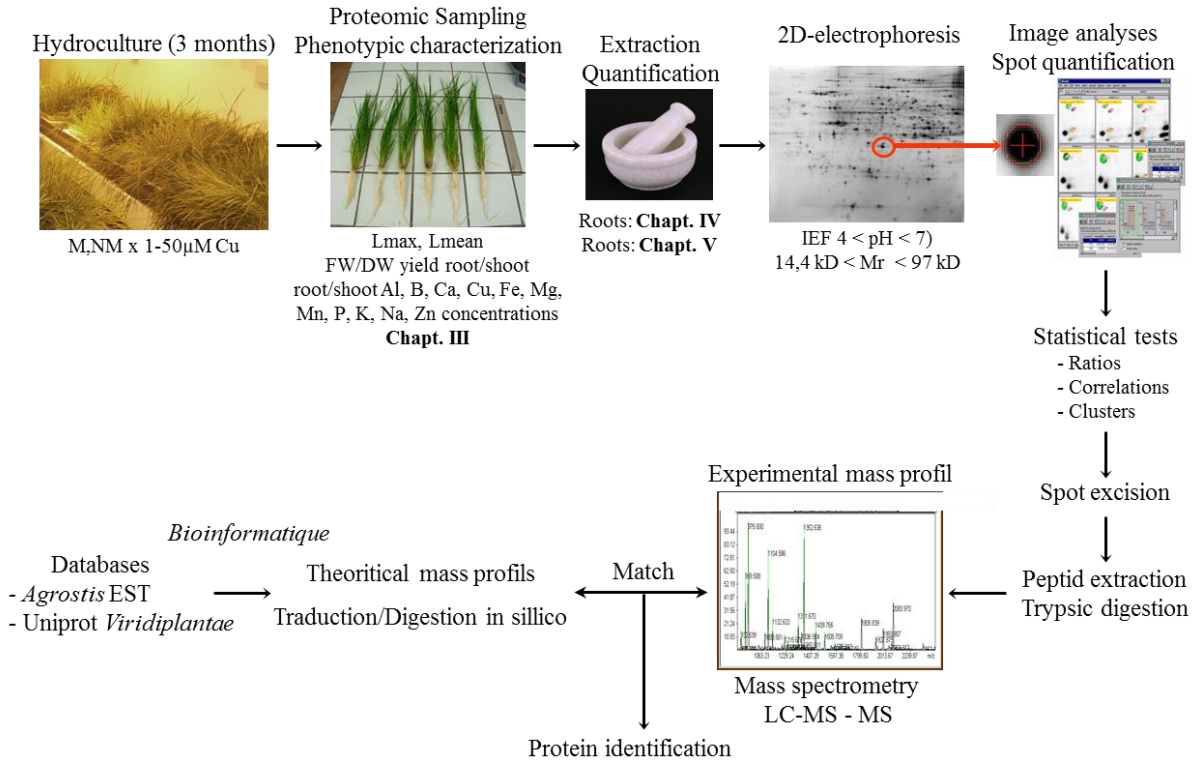

Figure 8: Summary of experimental procedure

CHAPTER II: Preliminary investigation of root soluble proteome

This part has been published in the journal 'Proteomics' as a peer-reviewed paper in August 2014 (*Proteomics* 2014, 14, 1746–1758; DOI 10.1002/pmic.201300168).

The corresponding bibliography list was presented at the end of this chapter to respect the article form. For the other chapters, the list was placed at the end of the manuscript, in a form of a general alphabetically-ordered list of publications.

Differential accumulation of soluble proteins in roots of metallicolous and nonmetallicolous populations of *Agrostis capillaris* L. exposed to Cu.

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Abbreviations: **Cu/Zn-SOD**, [Cu-Zn] superoxide dismutase; **DW**, dry weight; **FBP aldolase**, fructose bisphosphate aldolase; **FW**, fresh weight; **HNS**, Hoagland no. 2 nutrient solution; **M**, metallicolous; **MDH**, malate dehydrogenase; **MetE**, 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase; **NA**, nicotianamine; **NM**, non metallicolous; **PEP**, phosphoenolpyruvate; **SAMS**, S-adenosylmethionine synthase; **TCA**, trichloroacetic acid; **TIM**, triosephosphate isomerase cytosolic; **Tub α**, tubulin alpha; **UAM**, UDP-arabinopyranose mutase.

Key words: *Agrostis*, Cu-tolerance, Plant proteomics, Superoxide dismutase.

Abstract

Differential expression of soluble proteins was explored in roots of metallicolous (M) and non-metallicolous (NM) plants of *Agrostis capillaris* L. exposed to increasing Cu to partially identify molecular mechanisms underlying higher Cu tolerance in M plants. Plants were cultivated for 2 months on perlite with a $CuSO_4$ (1-30 µM) spiked-nutrient solution. Soluble proteins extracted by the trichloroacetic acid/acetone procedure were separated with 2-DE (linear 4-7 pH gradient). After Coomassie Blue staining and image analysis, 19 proteins differentially expressed were identified using LC-MS/MS and Expressed Sequence Tag (ESTs) databases. At supra-optimal Cu exposure (15-30 µM), glycolysis was likely altered in NM roots with increased production of glycerone-P and methylglyoxal based on over-expression of triosephosphate isomerase and fructose bisphosphate aldolase. Changes in tubulins and higher expressions of 5-methyltetrahydropteroyltriglutamatehomocysteine methyltransferase and S-adenosylmethionine synthase respectively underpinned impacts on the cytoskeleton and stimulation of ethylene metabolism. Increased L-methionine and S-Adenosylmethionine amounts may also facilitate production of nicotianamine, which complexes Cu, and of L-cysteine, needed for metallothioneins and GSH. In M roots, the increase of [Cu/Zn] Superoxide dismutase suggested a better detoxification of superoxide, when Cu exposure rose. Higher Cu-tolerance of M plants would rather result from simultaneous cooperation of various processes than from a specific mechanism.

1. Introduction

Many anthropogenic sources, for example Cu mining, metal (Cu) smelters, recycling of pig slurries and sewage sludge, Cu-based fungicides, waste incineration, releases from car engine wear, tire and brake pad wear, dust from urbanized and industrialized centres, and wood preservation, contribute to high soil Cu concentrations [1, 2]. Excessive root exposure to Cu in such soils, which often adds to other adverse soil conditions, can result in a sparse plant cover and low plant diversity with species belonging mostly from the *Poaceae* and *Asteraceae* families [3-5]. Even though Cu is an essential cofactor in many physiological processes, for example photosynthesis, mitochondrial respiration, oxidative stress responses, and transduction of ethylene signal, its presence in excess negatively impacts plant growth [6, 7].

Aided phytostabilization is one emerging option to sustainably minimize the dispersion and biological action of Cu and to restore a vegetation cover at wood preservation sites [8]. Soil

conditioners are incorporated into the Cu-contaminated soil to decrease the labile Cu pool and phytotoxicity by inducing various sorption and/or precipitation processes prior to planting tolerant plants with excluder phenotype [9, 10]. Plant candidates for aided phytostabilization of Cu-contaminated soils must have several characteristics, such as relative fast growth and perennial life cycle, high soil coverage, tolerance to abiotic/biotic stresses, low-input production (energy, costs), low nutrient/water requirements, and restricted uptake/accumulation of contaminants (excluder phenotype) [11-13]. *Agrostis capillaris* L. (Colonial bentgrass), also called *A. tenuis* Sibth, belongs to the genus *Agrostis* (*Poaceae*). This perennial grass, tolerant to partial shade and acid soil, is used for erosion control as well as on fairways and tees in golf courses [14, 15]. This pseudo-metallophyte has been recorded as dominant species on several Cu-contaminated sites (172 - 469 mg Cu/kg soil [16]; 305-2017 mg HNO_3-extractable Cu/kg soil [3]; 152-721 mg Cu/kg soil [5]). Grown on Cu-contaminated soils, *A. capillaris* accumulates more Cu in roots, with a shoot:root ratio of 0.3 typical of an excluder phenotype [16]. Several Cu-tolerant populations and cultivars of *A. capillaris* have been reported, such as "Parys" cultivar [17-20], and native populations collected at a Cu rod rolling factory [3] and at a wood preservation site [21]. The metallicolous (M) *A. capillaris* cv. Parys promotes the soil microbial activity, bacterial growth and presence of nematodes in a Cu-contaminated soil [20, 22]. A M population of *A. capillaris* from a wood preservation site well developed up to 1951 mg Cu/kg on a Cu-contaminated soil series (21-2600 mg Cu/kg) whereas a non-metallicolous (NM) one, from an uncontaminated forest edge was negatively impacted over 651 mg Cu/kg [21]. Similar results were obtained in perlite moistened with a nutrient solution (i.e. 1-30 µM Cu) [21].

There is a lack of knowledge on mechanisms underlying Cu-tolerance and low shoot:root ratio of Cu accumulation in grassy species such as *A. capillaris*, even though several processes have been suggested, for example root uptake limitation and efflux, differential accumulation between roots and aerial parts, and enzymatic and non-enzymatic systems to quench ROS damage. Complex network of homeostatic mechanisms exist to control metal uptake, trafficking and detoxification, involving transport, chelation, and sequestration processes [23]. This study aimed at preliminary investigating these molecular mechanisms for excess Cu at a proteomic level for a limited set of identified proteins.

A key option to investigate such tolerance mechanisms is to examine native populations adapted to stressful environment in comparison with non-adapted ones, since these plants may have evolved and retained molecular mechanisms enabling their survival [24]. Proteomic analysis can help in disclosing new aspects of plant tolerance to excess Cu, and has been used to study temporal plant responses to Cu exposure in shoots of *Oryza sativa* [25] and *Elsholtzia*

splendens [26], in seedlings of *Oryza sativa* [27] and *Phaseolus vulgaris* [28], and in roots of *Arabidopsis thaliana* [29], *Cannabis sativa* [30] and *E. splendens* [26]. Through regulation at the mRNA and protein levels, changes occur in the abundance and activity of proteins involved in redox homeostasis, energy metabolism, cell wall metabolism, cytoskeleton rearrangement, and cell defenses. Cell defenses include binding Cu to cell walls, sequestrating Cu into vacuoles, reducing mobile Cu ions, and secreting detoxifying peptides. These processes may work cooperatively to re-establish the cellular and redox homeostasis upon Cu stress [26, 30]. Most of these temporal studies, however, were carried out using short-term, high Cu exposures (e.g. 100 µM Cu, 3-6 days [26]; 601 µM Cu, 6 weeks [30]; 0.2-2 mM Cu, 4 days [27]), which are poorly mimicking plant germination and growth on Cu-contaminated soils.

Here, both NM and M populations of *A. capillaris* L. were chronically exposed to Cu in the 1-30 µM range for a 2-month period, then soluble proteins were extracted from roots, which are primary exposed and retained the highest Cu mass [21]. The objectives were to gain preliminary information on molecular mechanisms underlying the higher Cu tolerance in the M population, and to partially elucidate the differential expression of soluble proteins between NM and M roots in relation to the intensity of chronic Cu exposure.

2. Materials and Methods

2.1. Plants and Cu treatments

Seeds of M and NM populations were respectively collected from *A. capillaris* L. growing at a wood preservation site contaminated by Cu [2, 5, 9] and at a forest edge (RN10, Km 83, Belin Beliet, Gironde, France). Phenotypes of M and NM populations were previously characterized on a Cu-contaminated soil series obtained with the fading technique and on Cu-spiked perlite moistened with Hoagland nutrient solution in the 1-30 µM Cu range [21]. Seeds were sowed and plants cultivated for 2 months on perlite constantly bottom-imbibed with Hoagland no.2 nutrient solution (HNS) [31] containing 1, 5, 10, 15 and 30 µM Cu (added as $CuSO_4$, $7H_20$), weekly changed. Moistened perlite was preferred than hydroponics for maintaining root ultra-structure and Si nutrition more close to soil conditions [32]. All plastic pots (15 x 12 x 8 cm^3) were placed in a growth chamber with controlled environment (PAR 360 µMol/m^2/s; 14-h and 26°C day and 10-h and 18°C night regime, 47-55% relative humidity). For each Cu concentration in the HNS and population, ten replicates were carried out, divided in two sets of five pots. To avoid edges effects, sets and pots were weekly moved. Three types

of neon where used to cover a wide range of wavelength, «daylight» (400-700 nm), «Warmwhite» (620 nm), and «Fluora» (440/480 nm and 650/680 nm) [33].

After a 2-month period of growth all the plants were harvested. The perlite was removed with tap water and roots were rinsed in distilled water. For each Cu concentration in the HNS and population, root aliquots of 0.5 g fresh weight (FW), taken in the median part of root length, were collected in two pots of each set and pooled to constitute aliquots of 1 g FW (triplicates, n = 30). Then, these weighed aliquots were frozen in liquid nitrogen. Remaining roots and leaves were rinsed in distilled water weighed and oven dried (70°C). Dry weighed aliquots (0.5 g DW) were wet-digested in 14 M HNO_3 and 30% vol. H_2O_2 under microwaves (CEM Marsxpress) and elements determined by axial ICP-AES [5].

2.2. Protein extraction, quantification and separation

For all aliquots (1 g FW, n = 30), frozen root tissues were ground in a small mortar and pestle in liquid nitrogen. Total protein was extracted following the TCA (trichloroacetic acid)/acetone procedure described by [34] and modified by [35]. Soluble proteins were resolubilized in "TCT" buffer (i.e. urea 7 M, thiourea 2 M, Triton X-100 0.4% v/v, CHAPS detergent 4% w/v, DTT 10 mM, and IPG buffer 1% v/v) for 1 h at room temperature. Samples were then centrifuged (4 min, 2000 rpm, 20°C) and stored at -80°C. Protein content determination assay was triplicated for each extract using a modified Bradford assay [36]. Protein extracts were used for the subsequent 2-DE steps.

2-DE was used to analyze total soluble proteins from root samples. For IEF, 24 cm IPG strips (Immobiline DryStrip, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used with a linear pH gradient ranging from 4 to 7. A total of 300 µg of proteins were resuspended into 470 µL of "TCT" solution. Acidic strips were passively rehydrated with 470 µL of protein samples for 1 h at room temperature prior to the IEF run using the IPGphor system (Amersham Biosciences, Uppsala, Sweden) [35]. 2-DE procedure and Colloidal Coomassie Blue gel staining were performed as described in [35]. Triplicates were performed for the ten experimental conditions, resulting in a total of 30 gels.

2.3. Image analysis, spots detection and statistical analysis

Image acquisition of the stained gels was done as described in [35]. All scanned gel images were saved as tiff files and processed together. The alignment of 30 gel images, spot detection, quantification and pairing were carried out using the complete Melanie 7.0 software (GeneBio, Geneva, Switzerland) [37]. Protein spots (referred for ease thereafter as spots) were

automatically detected then manually corrected. For each detected spot in the gel, all intensity values inside the spot area are summed up to obtain the spot volume. The background estimation is obtained by fitting the pixel values located outside the spot area with a third-order polynomial function (automatic sub-routine in the Melanie software).

For each spot, the volume is corrected by subtracting out the respective background and the volume is then normalized according to the total spot volumes in the gel image, resulting in a percentage volume (%Vn). The 30 image gels were automatically aligned according to reference spots manually selected. Spots were matched and then manually corrected. Resulting matched spots were later visualized with the free and simplified version Melanie viewer 7.0. Even if this %Vn reflected the amount or accumulation of proteins, as the result of their synthesis, regulation and catabolism, it will therefore refer as protein expression in the results and discussion parts.

In this experiment, Cu was considered as a continuous variable so an ANCOVA model was preferentially chosen to an ANOVA to assess differences across Cu concentrations and between populations. First, the following model %Vn = Cu concentration (Cu) + Population (P) + Interaction (Concentration x Population, I) led to test existence of different ordinates between populations (P effect), existence of a slope different of zero when Cu exposure rises (Cu effect), and existence of different slopes for both populations across the Cu series (I effect).

Secondly, three sub-models (1) %Vn = Cu + P; (2) %Vn = Cu and (3) %Vn = P were used to test the independency of both variables and led to determine (i) existence of different ordinates and a slope different of zero but identical between populations; (ii) existence of a slope different of zero but identical for both populations and no P effect and, (iii) existence of different ordinates for the populations but no Cu effect on protein expression. For each ANCOVA test, when postulates were not validated, model was deleted.

To characterize the response of each population across the range of Cu exposures, each dataset was fitted with regression models using three options (Cu: Cu concentration in the nutrient solution, a, b, and c: constants): (iv) %Vn = a Cu + b, henceforth referred to linear model, (v) %Vn = a ln[Cu] + b, so-called logarithm model, and (vi) %Vn = a Cu^2 + b Cu + c, henceforth referred to polynomial model. Finally, protein expressions in M and NM roots were compared for each Cu concentration with a Student's test. Statistical analyses were conducted on R v2.11.1 (R Foundation for Statistical Computing; Vienna, Austria) and alpha error has been fixed at 0.1.

2.4. Protein identification by mass spectrometry (Liquid Chromatography coupled to tandem Mass Spectrometry: LC MS/MS)

Spots (n = 23) were manually excised, rinsed twice in ultrapure water, and shrunk in ACN for 10 min. After ACN removal, gel pieces were dried in a vacuum centrifuge, rehydrated in 10 ng/µL trypsin solution (Sigma-Aldrich) in 50 mM ammonium bicarbonate, and incubated overnight at 37°C. Hydrophilic peptides were extracted with 40 mM ammonium bicarbonate containing 10% ACN at room temperature for 10 min. Hydrophobic peptides were extracted with 47% v/v ACN and 5% v/v formic acid, and this extraction step was repeated twice. All three supernatants were pooled together, concentrated in a vacuum centrifuge, and acidified with 0.1% formic acid [35].

Peptide mixtures were analyzed by on-line capillary nano-HPLC (LC Packings, Amsterdam, The Netherlands) coupled to a nanospray LCQ Deca XP ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA). The peptide digests (10 µL) were separated using a 75 µm internal diameter x 15 cm C18 PepMapTM column (LC Packings, Amsterdam, The Netherlands) with a 5-40% linear gradient of solvent B in 30 min (solvent A was 0.1% formic acid in 5% ACN, and solvent B was 0.1% formic acid in 80% ACN). The separation flow rate was set at 200 nL/min. The mass spectrometer operated in positive-ion mode at a 2 kV needle voltage and a 3 V capillary voltage. Data acquisition was performed in a data-dependent mode alternating in a single run, a MS scan survey over the range m/z 150–2000, a zoom scan and a MS/MS scan of the most intense ion in survey scan. MS/MS spectra were acquired using a 2 *m/z* unit ion isolation window and a 35% relative collision energy [35]. Peptides were identified with SEQUEST algorithm through Proteome Discoverer 1.3 interface (Thermo-Finnigan, Torrence, CA, USA) against two constructed ESTs databases, translated in six reading frames by TRANSEQ software (http://www.ebi.ac.uk/Tools/emboss/transeq/). A first database was constructed on *Agrostis* spp. ESTs, including *A. capillaris*, *A. stolonifera*, *A. stolonifera* var. *palustris* and *A. scabra*, and resulted in 100 350 sequences (i.e. http://www.ncbi.nlm.nih.gov/, NCBI website). A second database was built using root ESTs of *Oryza sativa* L., a sequenced species from *Poaceae* genus to increase protein identification (232 476 sequences, http://compbio.dfci.harvard.edu/tgi/plant.html). Spectra from peptides higher than 5000 Da or lower than 350 Da were rejected. The search parameters were as follows: mass accuracy of the monoisotopic peptide precursor and peptide fragments was set to 2 Da and 1 Da respectively. Only b- and y-ions were considered for mass calculation. Methionine oxidation (+16 Da) was considered as variable modification and cysteine carbamidomethylation was considered as static modification. Two missed trypsin cleavages were allowed. Only “high confidence” peptides were retained corresponding to a 1% false positive rate at peptide level.

Additionally, a minimum of two different peptides was considered for protein validation. Functional information about peptides, Enzyme Code and Accessions numbers were obtained from the Swiss-Prot database (http://www.uniprot.org). All the spectra generated in this experiment and the peptide sequences identified were submitted to the proteomics identification database PRIDE [38, 39], accessions numbers inclusive (in submission).

3. Results

More than 1 000 out of 2 131 spots automatically delimited and paired by the software were manually validated as reproducible on at least 26 out of 30 gels. After a preliminary analysis, 23 spots with both a significant effect of either Cu exposure or population and a clear separation enabling manual excision were retained for LC-MS/MS analysis (Fig. 1). Unfortunately, further analysis was not practically possible due to material and resource limitations, leading to deterioration of the gels before any complementary excision. Therefore results consisted in a preliminary partial view of the response to Cu excess in roots of M and NM *A. capillaris* populations.

In NM plants, shoot and root DW yields peaked, respectively, at 1 and 5 µM Cu and then decreased (Table 1). In contrast, shoot and root DW yields of M plants increased, were the highest at 5 and 10 µM Cu, respectively, and then decreased. Shoot:root ratio of Cu concentrations (i.e. transfer factor, TF) increased between 1 and 5 µM Cu for M plants and then was reduced (Table 1). Conversely, TF value of Cu continuously decreased for NM plants as Cu exposure increased. The TF value of Cu was lower in NM plants for all Cu concentrations tested except 1 µM Cu (Table 1), which limited the hypothesis of a lower Cu translocation in M plants.

Table 1: Phenotypic traits (root and shoot DW yields; root and shoot Cu concentrations, and shoot and root Cu mineral masses) of M and NM plants of *Agrostis capillaris* L..

Populations	Cu concentrations in the nutrient solution imbibing perlite				
M	1 µM	5 µM	10 µM	15 µM	30 µM
Root DW yield (g/pot)	1.37 ± 0.12	1.44 ± 0.06	1.74 ± 0.24	1.33 ± 0.07	1.35 ± 0.05
Shoot DW yield (g/pot)	7.91 ± 1 [(b)]	11.23 ± 0.37 [(a)]	10.02 ± 1.34 [(ab)]	8.67 ± 0.27 [(ab)]	7.58 ± 0.55 [(b)]
Root Cu concentration (mg/kg DW)	25.7	17.7	31.4	64.5	209.6
Shoot Cu concentration (mg/kg DW)	7.09 ± 1.67	15.96 ± 0.38	19.66 ± 0.24	23.49 ± 1.61	25.34 ± 0.94
Transfer factor	0.28 ± 0.09	0.9 ± 0.03	0.63 ± 0.01	0.36 ± 0.04	0.12 ± 0.01
Root Cu mineral mass (µg Cu/pot)	35.26	25.55	54.69	85.80	282.98
Shoot Cu mineral mass (µg Cu/pot)	56.08	179.23	196.99	203.66	192.08
S:R ratio	1.59	7.01	3.60	2.37	0.68
NM	1 µM	5 µM	10 µM	15 µM	30 µM
Root DW yield (g/pot)	1.53 ± 0.04	1.84 ± 0.04	1.7 ± 0.05	1.66 ± 0.09	1.51 ± 0.07
Shoot DW yield (g/pot)	11.51 ± 1.52 [(a)]	9.67 ± 0.36 [(ab)]	8.82 ± 0.26 [(ab)]	9.12 ± 0.34 [(ab)]	7.45 ± 0.08 [(b)]
Root Cu concentration (mg/kg DW)	12.1	21.2	47.6	169.8	247.1
Shoot Cu concentration (mg/kg DW)	9.98 ± 0.29	16.2 ± 0	20.41 ± 0.99	21.56 ± 0.24	24.07 ± 0.73
Transfer factor	0.82 ± 0.03	0.76 ± 0	0.43 ± 0.03	0.13 ± 0	0.1 ± 0
Root Cu mineral mass (µg Cu/pot)	18.54	39.01	81.00	281.87	373.06
Shoot Cu mineral mass (µg Cu/pot)	114.87	156.65	180.02	196.63	179.32
S:R ratio	6.19	4.02	2.22	0.70	0.48

Transfer factor (TF), shoot Cu concentration versus root Cu concentration; S:R ratio, root Cu mineral mass versus shoot Cu mineral mass; letters indicated significant differences among Cu exposure for each population; data from p. 238 to 247 [21].

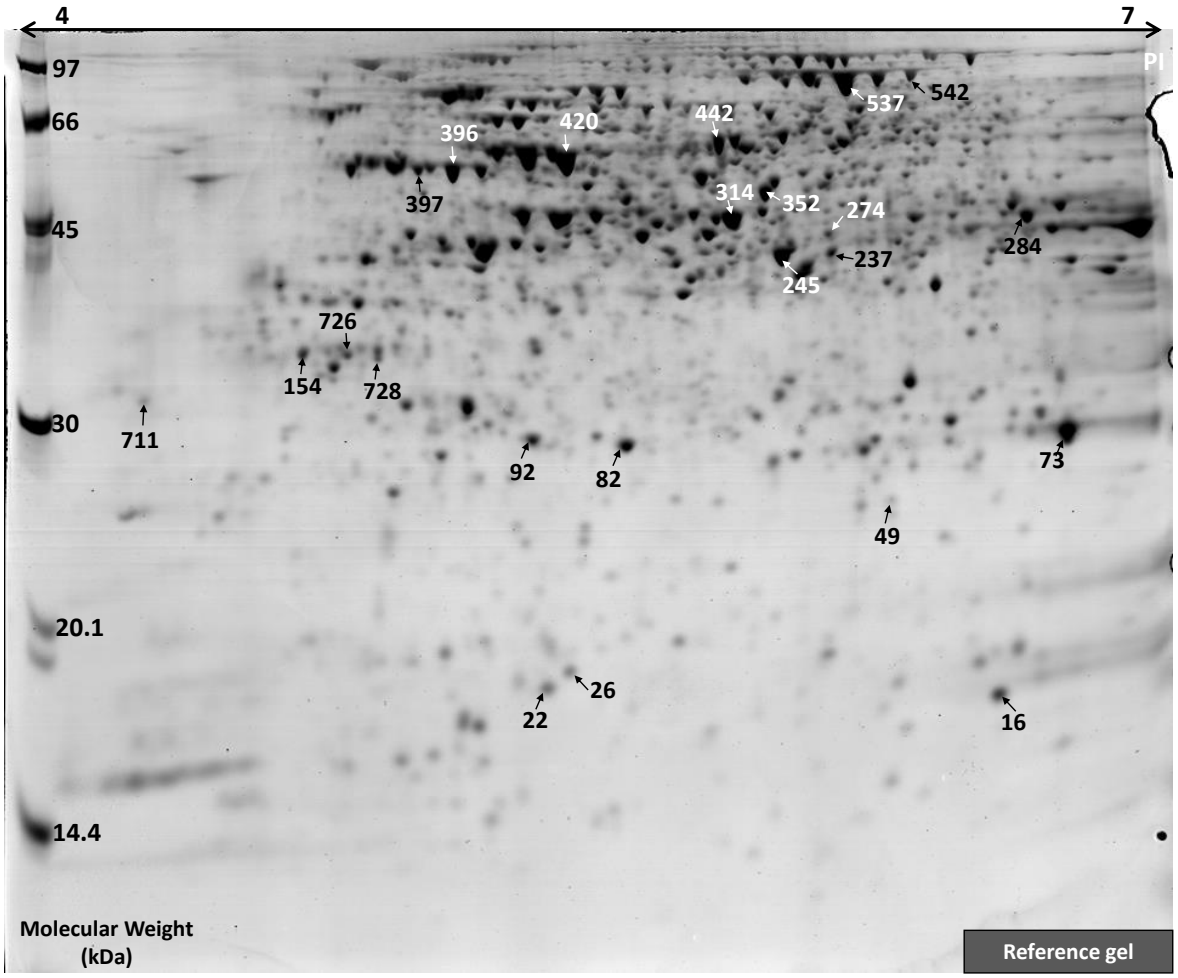

Figure 1. Reference gel showing the distribution of protein spots from *Agrostis capillaris* roots, and the location of the 40 spots selected for identification by MS. This master gel was realized with an equimolar protein extract from all experimental conditions, i.e. five Cu concentrations for both M and NM roots (Table 1).

3.1. Efficiency of database searching and protein identification.

Twenty-three spots showed a significant P or Cu effect, but four, 22, 73, 711 and 728 remained unidentified and were not further considered. The 19 others were identified and functionally grouped in six categories (Table 2). Most proteins belong to metabolic processes with seven spots involved in carbohydrate and energy metabolism, and five spots in nucleotide and amino-acid metabolism. Other main functions included cytoskeleton (three spots) and signal transduction (two spots). Three spots (i.e. 82, 92 and 237) matched only in the *Agrostis* database and four spots (i.e. 26, 274, 284 and 442) only in the *Oryza* database, whereas the twelve last (i.e. 16, 49, 154, 245, 314, 352, 396, 397, 420, 537, 542 and 726) matched in both databases. All these matches resulted in a unique or very similar identification.

Table 2: Proteins identified based on searching in *Agrostis* and Rice databases. Sp: Spot number, Dtb: Database used, Agr: *Agrostis*, Ory: *Oryza*; (pep): number of different peptides matched; cov: percentage of coverage for the peptides matched, Access: Uniprot accession; MW/pI: molecular weight (kDa) and calculated pI obtained from database searching. Peptides matched: list of peptides identified; x: non-specified isobaric amino acids Leucine or Isoleucine and lower case letters indicating residues with post-translational modifications (m: oxidation of a methionine residue, c: carbamidomethylation of a cysteine residue).

Sp/Dtb	(pep) cov	eval	Access	Protein identification (Enzyme Code)	EST, Contig or Gene Accession	MW	pI	Peptides matched
				Carbohydrate and Energy metabolism				
82/Agr	(4) 8.32	2e-78	P48494	Triosephosphate isomerase cytosolic: TIM (EC: 5.3.1.1)	tef4_a18.z1.abd	18.7	7.09	VIAcVGETLEQR ALLGESNEFVGDK ESGSTMDVVAAQTK VAYALAQGLK
92/Agr	(3) 0.23	2e-78	P48494	Triosephosphate isomerase cytosolic: TIM	tef4_a18.z1.abd	18.7	7.09	VIAcVGETLEQR ALLGESNEFVGDK VAYALAQGLK
284/Ory	(4) 4.65	1e-58	P17784	Fructose bisphosphate aldolase cytoplasmic isozymes (EC: 4.1.2.13)	C71711	13.7	7.28	GILAADESTGTIGK FASINVENVEDNR NAAYIGTPGK YKDELIK
	(2) 6.52	2e-60	P17784	Fructose bisphosphate aldolase cytoplasmic isozyme	AU094990	25.5	9.28	ANSEATLGTYKGDAVLGEGAAESLHVK KPWSLSFSFGR
420/Agr	(2) 9.71	5e-34	Q42971	Enolase (EC: 4.2.1.11)	Yan-SSH14-M13R_2009-02-11	7.4	9.91	LAmQEFmILPTGASSFK mGVEVYHNLK
	(2) 4.88	5e-71	P42895	Enolase 2	Yan-SSH42-M13R_2009-05-05	14.0	5.27	MTEEIGEQVQIVGDDLLVTNPTR SGETEDTFIADLAVGLSTGQIK
420/Ory	(5) 1.68	e-136	Q42971	Enolase	OSJNEe10C18.f	27.9	8.28	AAVPSGASTGVYEALELR YGQDATNVGDEGGFAPNIQENK AVDNVNSIIGPALIGK LAmQEFmILPTGASSFK mGVEVYHNLK
	(2) 6.31	2e-93	P42895	Enolase 2	AF53-pf_12_P20_T7_080.ab1	26.1	7.72	MTEEIGDQVQIVGDDLLVTNPTR VNQIGSVTESIEAVR
245/Agr	(5) 2.17	5e-99	Q08062	Malate dehydrogenase cytoplasmic (EC: 1.1.1.37)	EC01_d_2156	24.8	9.77	VLVVANPANTNALILK mELVDAAFPLLK ALGQISER EFAPSIPEK NVSIYK
245/Ory	(3) 0.71	6e-53	Q7XDC8	Malate dehydrogenase cytoplasmic	26686rsicef_2125.y1	15.0	5.97	VLVVANPANTNALILK SQASALEAHAAPNcK mELVDAAFPLLK
	(2) 4.66	3e-22	Q7XDC8	Malate dehydrogenase cytoplasmic	FLO--03-H02.g1	16.7	8.79	SFPVTcSGGEWTIVQGLPIDEFSR mDATAQELSEEK
352/Agr	(2) 8.52	3e-70	Q06197	Isocitrate dehydrogenase NADP: IDH (EC: 1.1.1.42)	EC04_d_1814	17.7	7.08	GGETSTNSIASIFAWTR TIEAEAAHGTVTR
	(4) 4.98	4e-98	Q40345	Isocitrate dehydrogenase NADP chloroplastic	Yan-SSH02-M13R_2008-12-16	30.0	7.44	TLEAEAAHGTVTR SEGGYVWAcK HAFGDQYR KWPLYLSTK
352/Ory	(9) 30.8	e-130	Q40345	Isocitrate dehydrogenase NADP chloroplastic	OSJNEc16H14.f	31.1	7.39	DATDDKVTVEAAEATLK VANPIVEmDGDEmTR DKLIFPFLDLDIK VTVEAAEATLK YYDLGVLHR LIFPFLDLDIK FKDIFQEVYEAGWK NIINGTVFR HAFGDQYR

	(7) 7.45	0.0	Q40345	Isocitrate dehydrogenase NADP chloroplastic	CT844156	52.1	7.94	GGETSTNSIASIFAWTR LIDDmVAYALK TIEAEAAHGTVTR SEGGYVWAcK FKDIFQEVYEAGWK NIINGTVFR HAFGDQYR
	(3) 6.59	3e-61	P50218	Isocitrate dehydrogenase NADP	BR060003B10A10.ab1	24.9	9.64	GGETSTNSIASIFAWTR TIEAEAAHGTVTR LLDFTQK
442/Ory	(4) 5.14	8e-69	P12862	ATP synthase subunit alpha mitochondrial	CI310078	15.8	4.84	mTNFYTNFQVDEIGR VVSVGDGIAR AAELTTLLESR TGSIVDVPAGKAmLGR
	(7) 8.57	1e-96	P12862	ATP synthase subunit alpha mitochondrial	CR278871	29.6	9.13	TAIAIDTILNQK VVDALGVPIDGK AVDSLVPIGR VVSVGDGIAR SVHEPmQTGLK TGSIVDVPAGKAmLGR APGIIER
	(2) 8.67	9e-89	P0C522	ATP synthase subunit alpha mitochondrial	MA_LYP9_09353	18.3	8.13	GIRPAINVGLSVSR LTEVLKQPQYEPLPIEK
				Nucleotide and Amino Acid metabolism				
16/Agr	(2) 8.93	3e-72	P93554	Nucleoside diphosphate kinase 1: NDK I (EC: 2.7.4.6)	EC04_d_1103	18.3	8.35	GDFAVDIGR KGFYLK
16/Ory	(3) 25	4e-40	P93554	Nucleoside diphosphate kinase 1	CI213465	16.2	7.01	IVSGPVVAmVWEGK NVIHGSDSVENAR GDFAVDIGR
274/Ory	(2) 3.02	e-112	Q6Z4G3	UDP-arabinopyranose mutase 3: OsUAM3 (EC: 5.4.99.30)	HDA1--05-L23.g1	21.6	6.38	YVDAVmTIPK GTLFPmcGmNLAFDR
	(2) 16.9	2e-82	Q8H8T0	UDP-arabinopyranose mutase 1: OsUAM1	AU184101	16.2	7.71	GTLFPmcGmNLAFDR ASNPFVNLK
314/Agr	(6) 26.82	e-156	Q0DKY4	S-adenosylmethionine synthase 1, AdoMet synthase 1 (EC: 2.5.1.6)	EC02_d_2744	37.6	6.61	VHTVLISTQHDETVTNDEIAADLK FVIGGPHGDAGLTGR TNmVmVFGEITTK VLVNIEQQSPDIAQGVHGHFTK TQVTVEYR TIFHLNPSGR
314/Ory	(5) 33.5	e-103	P93438	S-adenosylmethionine synthase 2	60317rsicek_3090.y1	21.6	8.88	VHTVLISTQHDETVTNDEIAADLK FVIGGPHGDAGLTGR SIVASGLAR TIFHLNPSGR TAAYGHFGR
	(5)28.21	e-143	Q0DKY4	S-adenosylmethionine synthase 1	OSJNEd05G24.f	30.5	6.28	VHTVLISTQHDETVTNDEIAADLK TNmVmVFGEITTK VLVNIEQQSPDIAQGVHGHFTKTQVTVEYR TIFHLNPSGR
537/Agr	(4) 40.36	1e-72	P93263	5-methyltetrahydropteroyltriglutamatehomocysteine methyltransferase = Methionine synthase, MetE (EC: 2.1.1.14)	Yan-SSH11-M13R_2009-02-11	17.9	9.07	KLNLPILPTTTIGSFPQTVELR DEAYFAANAAALASR VLEVNALAK LVVSTScSLmHTAVDLVNETK
	(2) 26.75	2e-74	Q42662	Methionine synthase	npl2_b188.b1.abi	17.0	5.10	ALGVDTVPVLVGPVSYLLLSKPAK WFDTNYHFIVPELGPNTK

537/Ory	(4) 19.75	e-103	Q42699	Methionine synthase	OSIIEb07G15.f	25.9	5.06	DEAYFAANAAAxASR SFALLSLLSSILPVYKYLFAGVVDGR EVIAELK
	(4) 55.56	4e-54	Q42662	Methionine synthase	35282rsiceg_3210.y1	12.8	9.17	SFALLSLLSSILPVYK ALGVDTVPVLVGPVSYLLLSKPAK WFDTNYHFIVPELGPNTK EVIAELK
	(2) 17.59	4e-45	Q42699	Methionine synthase	RZ109.F	11.3	5.10	YLFAGVVDGR xVEVNALAK
542/Agr	(7) 25.33	e-151	Q42699	Methionine synthase	EC02_d_3041	49.5	9.63	GmLTGPVTILNWSFVR DEAYFAANAAAQASR FETcYQIALAIK IQEELDIDVLVHGEPER KLNLPVLPTTTIGSFPQTVELR SWLAFAAQK cVKPPIIYGDVSRPNPmTVFWSK
	(5) 46.32	e-106	Q42662	Methionine synthase	Yan-SSH30-M13R_2009-04-14	21.5	5.57	KEVEDLEAGGIQVIQIDEAALR SEHAFYLDWAVHSFR GmLTGPVTILNWSFVR FETcYQIALAIK cVKPPIIYGDVSRPNPmTVFWSK
	(4) 32.53	1e-72	P93263	Methionine synthase	Yan-SSH11-M13R_2009-02-11	17.9	9.07	LVVSTScSLmHTAVDLVNETK SWLAFAAQK VLEVNALAK DEAYFAANAAALASR
542/Ory	(9) 21.84	0	Q42699	Methionine synthase	RECm0509	74.4	7.34	KEVEDLEAGGIQVIQIDEAALR GmLTGPVTILNWSFVR DEAYFAANAAAQASR IQEELDIDVLVHGEPER FETcYQIALAIK YLFAGVVDGR LVVSTScSLmHTAVDLVNETK SWLAFAAQK cVKPPIIYGDVSRPNPmTVFWSK
	(6) 36.1	e-156	Q42662	Methionine synthase	OSIIEa01E09.f	31.5	5.54	KEVEDLEAGGIQVIQIDEAALR GmLTGPVTILNWSFVR IQEELDIDVLVHGEPER FETcYQIALAIK IPSTEEIADR cVKPPIIYGDVSRPNPmTVFWSK
	(2) 20.53	2e-16	Q42662	Methionine synthase	CI618640	16.1	11.55	WFDTNYHFIVPELGPTPSS GNATVPAmEmTK
				Antioxidant system				
26/Ory	(2) 13.33	9e-64	P93407	Superoxide dismutase [Cu-Zn] chloroplastic (EC: 1.15.1.1)	CI255518	22.4	8.35	AFVVHELEDDLGK GAHELSLSTGNAGGR
				Signal transduction				
154/Agr	(2) 10.71	2e-89	Q2R2W2	14-3-3-like protein GF14-D	kml3_a164.b1.abi	18.6	5.85	DSTLImQLLR IISSIEQK
	(2) 14.02	1e-56	Q6ZKC0	14-3-3like protein GF14-C	Yan-SSH31-M13R_2009-04-14	12.2	7.43	IcDGILK NLLSVAYK
154/Ory	(3) 21.13	e-104	Q6EUP4	14-3-3like protein GF14-E	83172rsicen_28845.y1	23.9	6.02	AAQDIALAELAPTHPIR TVDSEELTVEER LLDSHLVPSSTAPESK
	(3) 9.97	e-151	Q7XTE8	14-3-3like protein GF14-B	RECm1010	43.7	5.21	TVDSEELTVEER AAQDIALAELPPTHPIR DSTLImQLLR
	(2) 4.94	e-145	Q6ZKC0	14-3-3like protein GF14-C	CT845125	45.8	7.81	DSTLImQLLR YEEmVEYmEK

726/Agr	(6) 44.64	2e-89	Q2R2W2	14-3-3-like protein GF14-D	kml3_a164.b1.abi	18.6	5.85	IcDGILALLDSHLVPSAGAAESK AAQDIALADLAPTHPIR DSTLImQLLR EAAESTmNAYK YLAEFK IISSIEQK
	(2) 14.02	1e-56	Q6ZKC0	14-3-3like protein GF14-C	Yan-SSH31-M13R_2009-04-14	12.2	7.43	NLLSVAYK IcDGILK
726/Ory	(4) 30.05	e-110	Q2R2W2	14-3-3-like protein GF14-D	ABF--04-H10.b1	21.5	5.91	IcDGILALLDSHLVPSAGAAESK AAQDIALADLAPTHPIR DSTLImQLLR IISSIEQK
	(3) 24.39	6e-96	Q2R2W2	14-3-3-like protein GF14-D	67654rsicem_8114.y1	22.6	6.57	IcDGILALLDSHLVPSAGAAESK AAQDIALADLAPTHPIR YEEmVEYMER
	(4) 23.18	e-127	Q06967	14-3-3-like protein GF14-F	80187rsicen_4592.y1	25.0	5.19	LLDSHLVPSATAAESK DSTLImQLLR SAQDIALADLPTTHPIR IISSIEQK
	(3) 23.81	e-105	Q06967	14-3-3-like protein GF14-F	MA_PA64s_01101	21.4	5.71	TADVGELTVEER LLDSHLVPSATAAESK SAQDIALADLPTTHPIR
				Cytoskeleton				
49/Agr	(2) 7.54	0	O22347	Tubulin alpha 1 chain	EC04_d_3297	39.2	6.96	LVSQVISSLTASLR TIQFVDWcPTGFK
49/Ory	(2) 9.7	e-158	O22347	Tubulin alpha 1 chain	OSJNEb15P22.r	32.7	5.77	LVSQVISSLTASLR AVFVDLEPTVIDEVR
	(2) 15.43	e-110	O22347	Tubulin alpha 1 chain	60306rsicek_3078.y1	19.6	6.67	LVSQVISSLTASLR TIQFVDWcPTGFK
396/Agr	(12) 47.21	0	O22347	Tubulin alpha 1 chain	EC04_d_3297	39.2	6.96	IHFmLSSYAPVISAEK LVSQVISSLTASLR AYHEQLSVAEITNSAFEPSSmmAK TIQFVDWcPTGFK cGINYQPPSVVPGGDLAK SLDIERPTYTNLNR EIVDLcLDR QLFHPEQLISGK FDGALNVDVNEFQTNLVPYPR DVNAAVATIK YmAccLmYR EDAANNFAR
	(9) 49.55	e-117	O22349	Tubulin alpha 3 chain	EC04_d_265	24.8	8.35	IHFmLSSYAPVISAEK LISQIISSLTTSLR AVcmISNNTAVAEVFSR cGINYQPPSVVPGGDLAK SLDIERPTYTNLNR IDHKFDLmYAK DVNAAVATIK YmAccLmYR FDLmYAK
	(6) 19.84	e-172	P33627	Tubulin alpha-6 chain	EC01_d_2987	41.6	6.33	IHFmLSSYAPVISAEK AIFVDLEPTVIDEVR SLDIERPTYTNLNR EIVDLcLDR QLFHPEQLISGK EDAANNFAR

396/Ory	(9) 43.97	e-155	Q53M52	Tubulin alpha-2 chain	OC01F02	38.7	6.24	IHFmLSSYAPVISAEK AVcmISNSTSVVEVFSR LVSQVISSLTASLR AYHEQLSVAEITNSAFEPSSmmAK TIQFVDWcPTGFK cGINYQPPSVVPGGDLAK AFVHWYVGEGmEEGEFSEAR FDGALNVDVNEFQTNLVPYPR DVNAAVATIK
	(6) 33.11	e-158	O22347	Tubulin alpha 1 chain	OSJNEb15P22.r	32.7	5.77	IHFmLSSYAPVISAEK AVFVDLEPTVIDEVR LVSQVISSLTASLR AYHEQLSVAEITNSAFEPSSmmAK FDGALNVDVNEFQTNLVPYPR EIVDLcLDR
	(9) 61.51	e-113	Q53M52	Tubulin alpha-2 chain	AU164469	26.6	4.75	IHFmLSSYAPVISAEK AVcmISNSTSVVEVFSR AYHEQLSVAEITNSAFEPSSmmAK TIQFVDWcPTGFK cGINYQPPSVVPGGDLAK AFVHWYVGEGmEEGEFSEAR FDGALNVDVNEFQTNLVPYPR EDLAALEK DVNAAVATIK
	(5) 23.74	e-142	P28752	Tubulin alpha-1 chain	Plate14-C8-T7promoter	30.5	6.10	IHFmLSSYAPVISAEK LISQIISSLTTSLR AIFVDLEPTVIDEVR QLFHPEQLISGK EIVDLcLDR
397/Agr	(5) 30.45	e-117	O22349	Tubulin alpha 3 chain	EC04_d_265	24.8	8.35	IHFmLSSYAPVISAEK AFVHWYVGEGMREGESQRPx SLDIERPTYTNLNR DVNAAVATIK FDLmYAK
	(5) 16.14	e-172	P33627	Tubulin alpha-6 chain	EC01_d_2987	41.6	6.33	IHFmLSSYAPVISAEK EIVDLcLDR AIFVDLEPTVIDEVR SLDIERPTYTNLNR LSVDYGK
397/Ory	(3) 13.38	e-158	O22347	Tubulin alpha-1 chain	OSJNEb15P22.r	32.7	5.77	IHFmLSSYAPVISAEK AVFVDLEPTVIDEVR EIVDLcLDR
	(3) 22.18	e-113	Q53M52	Tubulin alpha-2 chain	AU164469	26.6	4.75	AVcmISNSTSVVEVFSR IHFmLSSYAPVISAEK AFVHWYVGEGmEEGEFSEAR
	(2) 10.66	e-124	Q53M52	Tubulin alpha-2 chain	OF05G09	35.4	8.57	IHFmLSSYAPVISAEK cGINYQPPSGRPGGDLAK
				Other proteins				
237/Agr	(2) 9.09	1e-09	P09802	Legumin A	65996rsicem_11355.y1	25.4	5.05	LVSSQPASGIVK EVGLGADLVR

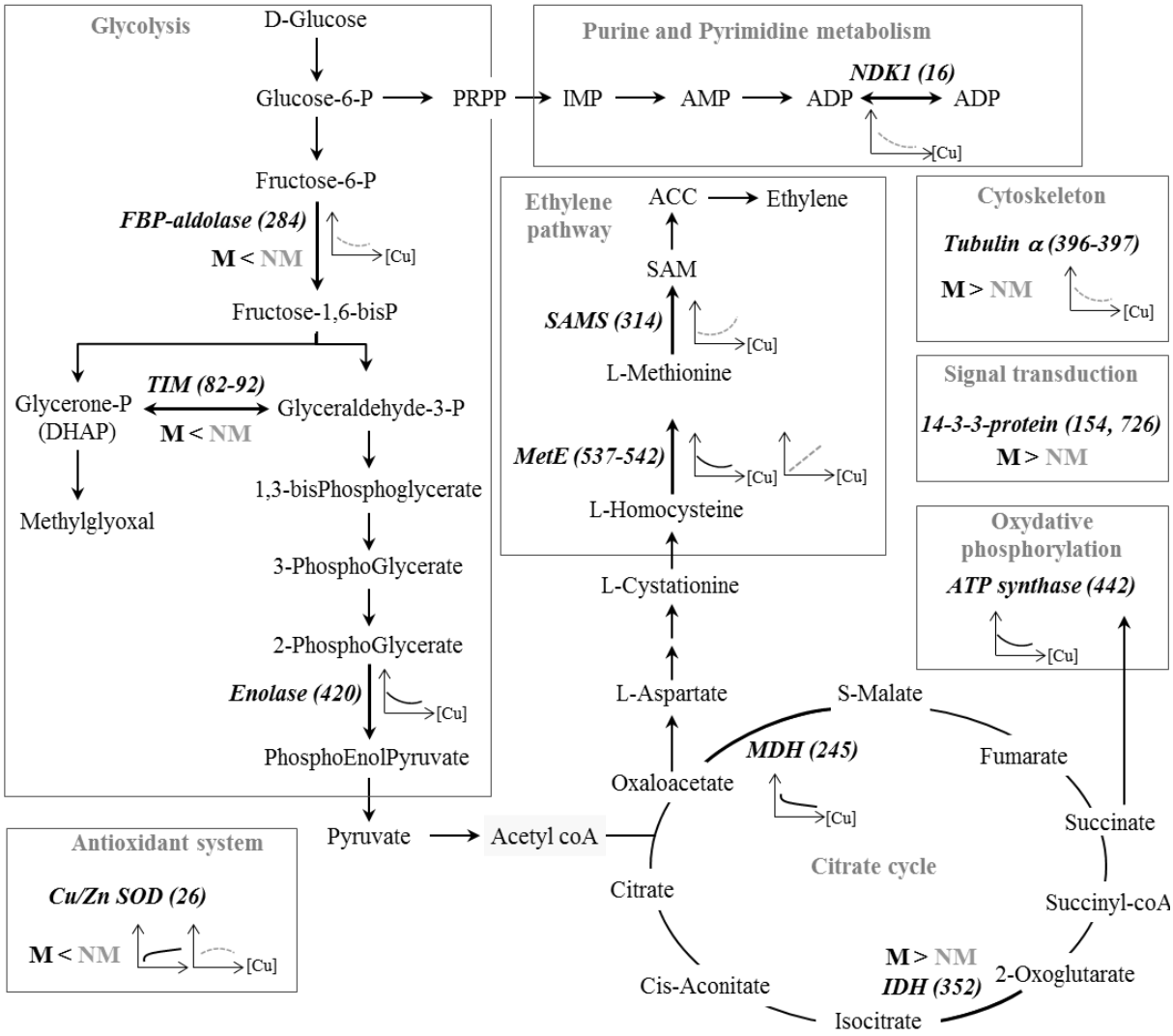

Figure 2: Functions of the identified enzymes in metabolic processes of plants. Enzymes are represented by their short name and spot number, referring to Table 2. M: metallicolous (Cu-tolerant) population of *Agrostis capillaris* L. NM: non-metallicolous population of *Agrostis capillaris.*

3.2. Quantification of protein spots and statistical results

Significant results and best models of ANCOVA are presented only for the 19 spots successfully identified by MS/MS (Tables 2 and 3). For spots 26, 82 and 274, the data were well fitted by the complete model (Table 3a), with an interaction Cu concentration x Population (I), and different but not significant responses to Cu exposure. The (I) effect reflected slight differences of protein expression between populations in response to Cu exposure. For spots 92, 154, 237, 314, 352, 396 and 397, highest *p*-values were obtained with the additive model (Table 3b), indicating a similar response to Cu exposure for both populations. Based on ANCOVA, protein expression did not differ for the nine other spots (data not shown).

Table 3: Significant changes in protein expressions between *A. capillaris* populations and across increasing Cu concentrations (ANCOVA analysis, α = 10%).

(a) Complete ANCOVA model

Spot	P effect	S.g.	Differences in ordinates	Cu effect	S.g.	Estimated M/NM slopes	I effect	R^2
26	**0.036**	*	NM > M	**0.061**	§	0.0008/ 0.0002	0.211	0.29
82	**0.023**	*	NM > M	0.963	Ns	−0.001 / 0.002	0.212	0.22
274	**0.034**	*	M > NM	0.279	Ns	0.001 / -0.0002	0.174	0.24

(b) Additive ANCOVA model

Spot	P effect	S.g.	Differences in ordinates	Cu effect	S.g.	Estimated common slope	Direction variation	R^2
92	**0.043**	*	M > NM	0.171	Ns	−0.0009	↓	0.19
154	**0.004**	**	M > NM	0.591	Ns	−0.0004	↓	0.28
237	**0.007**	**	M > NM	0.808	Ns	0.0002	↑	0.24
314	0.135	Ns	M > NM	**0.028**	*	0.015	↑	0.22
352	**0.068**	§	M > NM	0.482	Ns	0.001	↑	0.13
396	**0.046**	*	M > NM	**0.040**	*	−0.004	↓	0.25
397	**0.004**	**	M > NM	**0.046**	*	−0.0016	↓	0.35

Data for (a) complete model (different slopes and ordinates for each population) and (b) additive model (common slope but different ordinates). Spot, spot number. *p*-values (significant values in bold) are shown for the population effect (P effect: difference between M and NM ordinates), Cu effect (effect of Cu concentration on M slope in the complete model and on a common [M/NM] slope in the additive model), and I effect (interaction P × Cu, difference between M and NM slopes). S.g., significance levels: 0.001 < ** < 0.01 < * < 0.05 < § < 0.1 < Ns < 1. R^2, determination coefficient for the model used.

Eight spots were over-expressed in one population at only one Cu concentration (Student's test, Table 4): (i) five in M roots: 352, and 726 at 1 µM Cu; 154 at 5 µM Cu; 397 at 15 µM Cu; 274 at 30 µM; and (ii) three in NM roots: 49 at 5 µM Cu, 82 and 284 at 30 µM Cu.

Only 26 was overexpressed in NM roots at two Cu concentrations, 1 and 10 µM Cu (Table 4). For the ten other spots and all Cu concentrations, protein expression in roots did not differ between populations, based on Student's test.

Table 4: Significant differences in protein expression between M and NM roots at each Cu concentration (1, 5, 10, 15, and 30 µM Cu).

Spot	1 µM	S.g.	Ratio	5 µM	S.g.	Ratio	10 µM	S.g.	Ratio	15 µM	S.g.	Ratio	30 µM	S.g.	Ratio
26	**0.085**	§	82	0.343	Ns	88	**0.024**	*	72	0.752	Ns	95	0.876	Ns	103
726	**0.036**	*	123	0.126	Ns	124	0.455	Ns	125	0.420	Ns	116	0.770	Ns	107
352	**0.075**	§	138	0.915	Ns	103	0.682	Ns	108	0.378	Ns	117	0.250	Ns	114
154	0.166	Ns	133	**0.029**	*	168	0.412	Ns	124	0.206	Ns	152	0.6360	Ns	119
49	0.135	Ns	148	**0.083**	§	75	0.595	Ns	91	0.628	Ns	132			
397	0.189	Ns	115	0.133	Ns	118	0.205	Ns	122	**0.095**	§	142	0.632	Ns	110
274	0.332	Ns	136	0.666	Ns	93	0.409	Ns	126	0.928	Ns	102	**0.055**	§	165
82	0.451	Ns	87	0.212	Ns	82	0.349	Ns	89	0.940	Ns	97	**0.081**	§	63
284	0.338	Ns	64	0.999	Ns	100	0.706	Ns	118	0.236	Ns	80	**0.073**	§	47

For each Cu concentration, *p*-values (significant values in bold) of the Student's test are associated to their significance levels, S.g.: 0.001 < ** < 0.01 < * < 0.05 < § < 0.1 < ns < 1, and to the ratio: %Vn in M roots / %Vn in NM roots, Ratio. Spot, spot number.

Well-fitting regression models between %Vn and Cu concentration are listed in Table 5. Spots 26 and 397 were Cu-responsive in both populations but direction variation increased for 26 and decreased for 397. In M roots, spots 245, 420, 442, and 542 decreased while 274 increased as Cu exposure rose. In NM roots, spots 16 and 396 significantly decreased whereas 314 and 537 rose as Cu concentration increased.

Table 5: Well-fitting regression models for the relationship between spot %Vn in soluble root proteome and Cu exposure for each spot and *A. capillaris* population.

Spot	Pop.	*p*-Values	Variat.	Model equation	R^2
397	M	**0.082**§	↓	%Vn = – 0.017 ln(Cu) + 0.312	0.22
	NM	0.46 / **0.024** *	↓	%Vn = 0.0002 Cu^2 – 0.009 Cu + 0.28	0.38
26	M	**0.04***	↑	%Vn = 0.008 ln(Cu) + 0.065	0.29
	NM	0.961 / **0.051**§	↑	%Vn = – 0.00007 Cu^2 + 0.002 Cu + 0.08	0.28
245	M	**0.014***	↓	%Vn = – 0.061 ln(Cu) + 1.232	0.38
420	M	0.854 / **0.055**§	↓	%Vn = 0.0008 Cu^2 – 0.028 Cu + 1.895	0.28
442	M	0.668 / **0.041***	↓	%Vn = 0.001 Cu^2 – 0.045 Cu + 0.9	0.31
542	M	0.765 / **0.073**§	↓	%Vn = 0.0004 Cu^2 – 0.013 Cu + 0.26	0.25
274	M	**0.007** ** / 0.123	↑	%Vn = 0.0001 Cu^2 – 0.003 Cu + 0.134	0.53
16	NM	0.976 / **0.031** *	↓	%Vn = 0.0003 Cu^2 – 0.01 Cu + 0.254	0.33
396	NM	**0.055**§	↓	%Vn = – 0.056 ln(Cu) + 0.868	0.25
314	NM	**0.033*** / 0.517	↑	%Vn = 0.001 Cu^2 – 0.02 Cu + 1.062	0.34
537	NM	**0.087**§	↑	%Vn = 0.013 Cu + 1.51	0.21

Spot, spot number; Pop., population; *p*-values in bold were significant (0.001 < ** < 0.01 < * < 0.05 < § < 0.1). For the polynomial model, first *p*-value refers to Cu^2 and the second to Cu. Cu, Cu concentration in the nutrient solution. Variat., arrows indicate the direction variation. R^2, determination coefficient for the model used.

3.3. Variations of protein expression

Based on statistical analyses, these spots were classed in three main groups (Fig. 3): (i) spots differentially expressed between populations but non-responsive to Cu exposure, (ii) spots overexpressed in one population and Cu-responsive, and (iii) spots only responsive to Cu.

3.3.1. Protein expression only influenced by population effect

Three spots showed a population effect based on both ANCOVA and Student's tests (Tables 3, 4 and Fig. 3a): one triose phosphate isomerase spot (TIM, 82) was overexpressed in NM roots, notably at 50 µM; one isocitrate dehydrogenase (IDH, 352) and one 14-3-3-like protein spot (154) were overexpressed in M roots, significantly at 1 and 5 µM, respectively. The second TIM (92) and one legumin A (237) showed a population effect only based on ANCOVA and were overexpressed in M roots. Three spots showed a population effect at only one concentration, based on Student's tests; one tubulin alpha (tub α, 49) and one fructose-bisphosphate aldolase (FBP aldolase, 284) were overexpressed in NM roots at 5 and 50 µM Cu, respectively, whereas the second 14-3-3-like protein spot (726) was overexpressed at 1 µM Cu in M roots.

3.3.2. Spots responsive to population and Cu effects

Four spots, i.e. 26, 274, 396, and 397 were differentially expressed between populations and across the series of Cu exposures in at least one of the statistical tests (Fig. 3b). Tub α (396 and 397, ANCOVA Table 3, and Student's test for 397 at 15 µM Cu, Table 4) was overexpressed in M roots and reduced as Cu exposure rose with a well-fitted regression model for at least one population, i.e. 396 in NM roots, 397 in M and NM roots (Table 5). UDP-arabinopyranose mutase (274, ANCOVA, Table 3, and Student's test at 30 µM Cu, Table 4)

and [Cu/Zn] Superoxide dismutase (Cu/Zn-SOD, 26, ANCOVA, Table 3, and Student's test at 1 and 10 µM Cu, Table 4) were respectively overexpressed in M and NM roots and increased in response to Cu exposure in at least one population with well-fitted regression model, i.e. 26 in M and NM roots, 274 in M roots (Table 5).

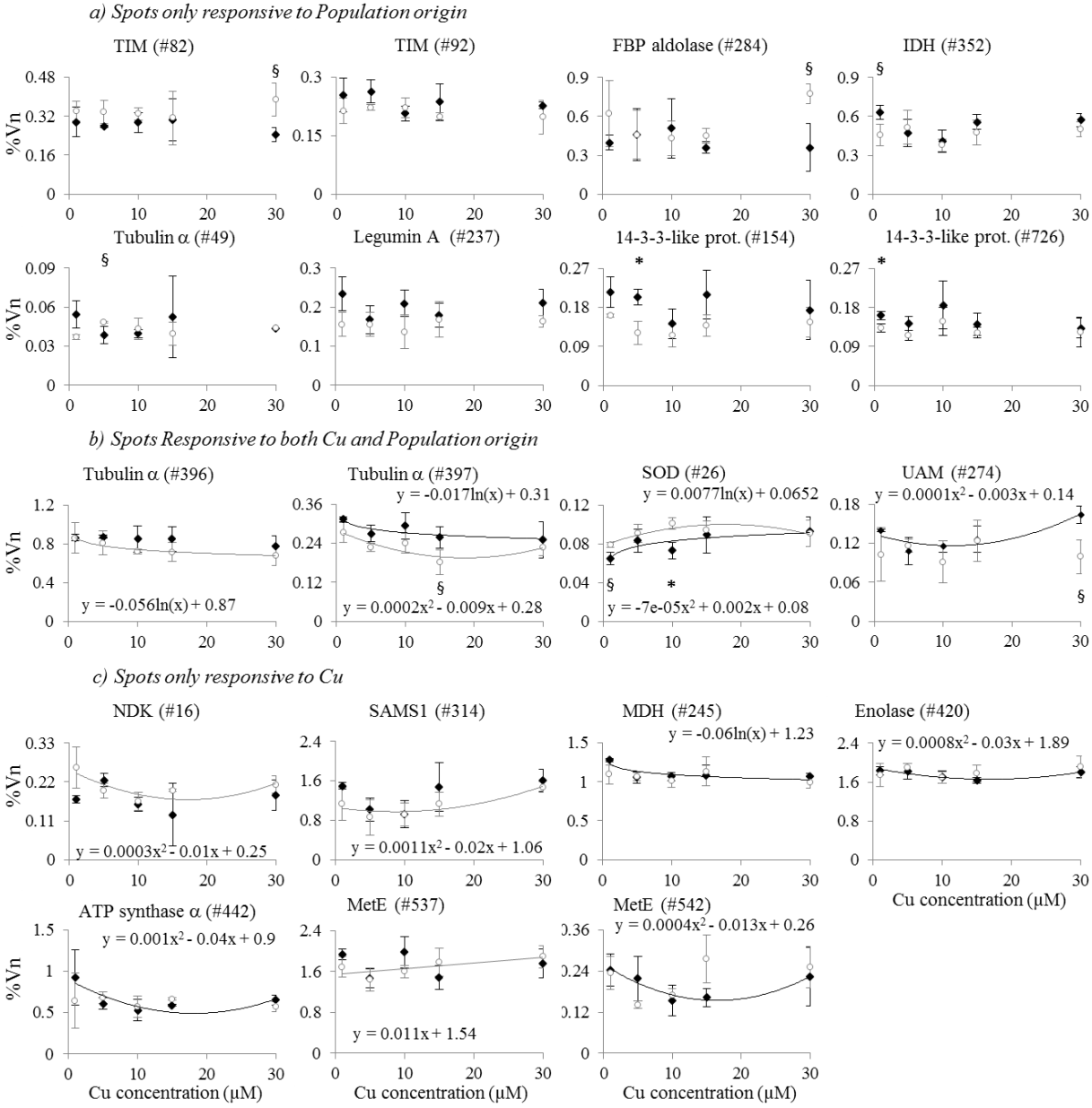

Figure 3: Changes in protein expression (%Vn, *n* = 3, Melanie 7.0) when Cu exposure increased in the 1-30 µM Cu range, for protein spots a) only influenced by the population origin (P effect), b) responsive to both Cu and population origin, and c) only responsive to Cu (Cu effect). M roots: black; NM roots: open. Significant differences (Student's test, Table 4) referred to 0.001 < ** < 0.01 < * < 0.05 < § < 0.1. Well-fitted regression models were displayed for M (upper part, black line) and NM (lower part, grey line) roots (Table 5). Abbreviated protein names refer to Table 2, TIM: triosephosphate isomerase (82 and 92); 14-3-3-like prot.: 14-3-3-like protein GF14 (154 and 726); FBP aldolase: fructose-bisphosphate aldolase (284); IDH: isocitrate dehydrogenase (352); SOD: superoxide dismutase (26); UAM: UDP-arabinopyranose mutase (274); NDK: nucleoside diphosphate kinase 1 (16); MDH: malate dehydrogenase (245); SAMS1: S-adenosylmethionine synthetase 1 (314); MetE: 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (537 and 542).

3.3.3. Spots only responsive to Cu exposure

In M roots, malate dehydrogenase (MDH, 245), enolase (420), ATP (adenosine triphosphate) synthase α (442) and the second spot of 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (MetE, 542) were reduced in response to increasing Cu concentrations (Table 5 and Fig. 3c). In NM roots, nucleoside diphosphate kinase (NDK, 16), was reduced as Cu exposure rose (Table 5 and Fig. 3c). Copper effect was significant in ANCOVA for S-adenosylmethionine synthetase 1 (SAMS1, 314, Table 3). SAMS1 (314) and one MetE spot (537) were increased in NM roots (Table 5 and Fig. 3c).

4. Discussion

Elucidation of mechanisms underlying greater Cu tolerance in M populations is one option to promote plant selection for phytoremediation of Cu-contaminated soils. Having some Cu-tolerant populations, *A. capillaris* is a candidate for studying differential responses of grassy populations to chronic Cu exposure. Plants were cultivated on imbibed perlite, notably as Si can alleviate Cu toxicity and hydroponics alters root ultrastructure [32, 40].

Proteomic profiles in roots of M and NM populations of *A. capillaris* exposed to increasing Cu concentrations from 1 to 30 µM were compared to identify potential soluble proteins involved in tolerance to Cu excess. However, this experiment constituted a preliminary work, as only a partial snapshot of 23 spots was achieved due to material and resource limitations. The functions and accumulation of the 19 identified protein spots were discussed for their possible implication in Cu-tolerance, without forgetting that the results remained partial and incomplete. This work will be followed by complementary experiments to increase these partial results.

More than 1 000 spots were reproducibly recorded (Fig. 1) which exceeded the 300 spots determined in *Cannabis sativa* roots exposed to 601 µM Cu [30] and was similar to spot number recorded in root proteome of *E. splendens* exposed to 100 µM Cu [26]. Studies on the proteomic responses to abiotic stresses in *Agrostis* spp. are scarce,but exist for Cu [41], As [42], and heat stress [24]. Consequently, few EST sequences of *Agrostis* spp. are available online and the additional use of rice database was required to identify proteins (Table 2). Identified proteins are involved in several metabolic processes, i.e. defense, energy and carbon metabolism, ethylene metabolism, signaling molecules and cytoskeleton (Table 2, Fig. 2).

Overall, these partial results agreed with the general scheme for plant responses to excessive metal(loid) exposure, with differential expression of several proteins involved in

signaling pathways, detoxification processes, and changes in primary metabolism [43]. Several enzymes identified in this study interact with Cu in *A. thaliana* roots [29]. This included enzymes with metal ions as cofactors, for example enolase, SAMS and IDH, and enzymes interacting with Cu by direct binding, for example metE.

4.1. Proteins involved in oxidative response

As a redox-active metal, Cu catalyzes formation of hydroxyl radicals via Haber-Weiss and Fenton-like reactions, generating oxidative stress in cells [44]. Accordingly, lipid peroxidation and other cellular impacts caused by reactive oxygen species (ROS) should be accompanied by changes in antioxidative and defense mechanisms [43].

One Cu/Zn-SOD (26) was differently expressed in roots depending on either *A. capillaris* populations or Cu concentration in the nutrient solutions (Tables 2-5). SODs are converting superoxide anion radicals ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) and molecular oxygen [40, 45]. Chloroplastic Cu/Zn-SOD (26) increased with a biphasic response in NM roots but with a constant rise in M roots (Fig. 3), and was overexpressed in NM roots at 1 and 10 µM Cu (Table 4). This suggested that oxidative stress was higher in NM roots, especially at 10 µM Cu but also at the lower Cu exposure tested, together with a slight reduction in Cu/Zn-SOD expression over 15 µM Cu, compared to M roots. Increases in Cu/Zn-SODs are reported in roots of *Poaceae*, for example *Zea mays* [46] and *Festuca arundinacea* [47]. SOD expression did not change in Cu-stressed (0.6 mM Cu) *C. sativa* roots [30], however this harmful Cu exposure is not common in soil pore water of Cu-contaminated soils [9]. In leaves of Cu-stressed *Hordeum vulgare*, Mn-SOD decreased whereas Cu/Zn-SODs increased [48]. Here, Cu stress could induce chloroplastic Cu/Zn-SOD expression to quench ROS production in roots. Alternatively, as Cu uptake reduced leaf Fe concentration more in NM than in M *A. capillaris* [21], Cu-stress may affect Fe homeostasis and Fe-SOD expression, promoting Cu/Zn- and Mn-SOD expressions.

4.2. Proteins involved in signaling pathways

Proteins involved in signaling pathways are expected to be differentially expressed in metal (Cu) stressed plants for perception and transmission of stress signals [43]. L-homocysteine is converted into L-methionine by MetE (537 and 542, Fig. 2) which is then transformed by SAMS1 (314) into S-adenosyl methionine (SAM), a direct precursor of ethylene (Fig. 2), which is involved in growth, development, and stress signaling notably during senescence. For MetE, spot 542 decreased in M roots, whereas 537 increased in NM roots but regarding to the expression level, the increase of 537 in NM was dominant compared to the decrease of 542 in M (Fig. 3). The SAMS spot (314) was Cu-responsive and increased in NM

roots (Fig. 3). In As-stressed rice roots [49] and Cu-stressed resistant and sensitive strains of the brown algae *Ectocarpus siliculosus* [50], SAMS increased whereas it slightly decreased in Cd-stressed *B. juncea* [51]. Increase in SAMS and MetE abundances in Cu-stressed NM roots could stimulate ethylene production [52], and may reflect a higher Cu-induced senescence in NM than in M roots. In parallel, SAM acts as GSH precursor through its conversion to cysteine via the trans-sulphuration pathway [53]. It may contribute to enhance levels of cellular GSH level and related metabolites, for maintaining Cu-binding, transport, and storage in NM roots. SAM and L-methionine are also direct precursors of nicotianamine (NA), which complexes Cu [50, 54]. Its role is controversial as NA may be only implied in Cu transport from roots to shoots in case of deficiency [55] whereas a Cu-induced rise in NA may reflect interspecies variations concerning Cu impacts [56].

Signal transduction in plant cells can be either a direct process where reverse (de)phosphorylation regulates target enzymes activity or a multistep process involving 14-3-3 proteins [57]. 14-3-3 proteins contribute to regulate H^+-ATPase that governs the electrochemical gradient across the plasmic membrane and is essential to control ion transport and cytosolic pH [58]. The 14-3-3 proteins are also involved in regulating signal transduction pathways, hormone signaling, transcription factors, metabolism, apoptosis, adhesion, cellular proliferation, differentiation, and survival, and ion homeostasis by being positive regulators of plasma membrane H^+-ATPase and ions channels [59-62]. 14-3-3 proteins interact with several proteins involved in ethylene biosynthesis, for example ACC (1-aminocyclopropane-1-carboxylate) synthase, ETO-like protein, and SAMS. In this study, spots identified as 14-3-3-like proteins (154 and 726) were overexpressed in M roots, especially at 5 µM Cu for 154 and 1 µM Cu for 726 (Tables 3-4, Fig. 3), but did not continuously vary with Cu exposure. This suggested a difference in signal transduction between the M and NM populations of *A. capillaris* and potential enrolment of 14-3-3-like proteins to explain their behavior.

4.3. Proteins involved in energy and carbohydrate (primary) metabolisms

To maintain correct cell functioning under Cu stress, an increasing demand for ATP, NADH (nicotinamide adenine dinucleotide), NADPH (nicotinamide adenine dinucleotide phosphate), and reducing molecules may occur, leading to changes in expression of enzymes involved in energy provision [63]. Four energy processes were addressed in our study, i.e. glycolysis/gluconeogenesis, Tricarboxylic Acid Cycle (TCA cycle), respiratory chain in mitochondrion (oxidative phosphorylation) and purine/pyrimidine metabolism (Table 2, Fig. 2). All identified enzymes belonging to energy metabolism were highly expressed in *A. capillaris* roots (Table 2, Fig. 1).

4.3.1. Glycolysis

Glucose degradation by dehydrogenation during glycolysis produces pyruvate, and high-energy compounds, i.e. ATP and NADH (Fig. 2). The five enzymes involved in glycolytic reactions, TIM (82 and 92), FBP aldolase (284), and enolase (420), were highly expressed in *A. capillaris* roots (Table 2). TIM spots 82) and 92 were respectively overexpressed in NM roots, markedly at 30 µM Cu, and in M roots (Fig. 3). Spot 82 was more expressed than 92 and more influenced by population effect, indicating a global TIM over-expression in NM roots when both spots are combined. Additionally, FBP aldolase (284) was overexpressed in NM roots at 30 µM Cu (Fig. 3). Taken together, this suggested a higher production of glycerone-1-phosphate in Cu-stressed NM roots, leading to a higher production of methylglyoxal. Less methylglyoxal production may contribute to the higher Cu-tolerance observed in M population of *A. capillaris*. Enolase catalyzes the intermediate step of the conversion of glyceraldehyde-3-phosphate to phosphoenolpyruvate in glycolysis (Fig. 2). Expression of enolase (420) did not depend on populations but decreased in M roots as Cu exposure rose (Fig. 3). It was the most expressed soluble protein in *Agrostis* roots, suggesting high phosphoenolpyruvate and pyruvate productions but enolase has a relatively low enzymatic efficiency so a lot of protein is needed just to keep the pace of the other enzymes. Two spots of enolase occurred in Cu-stressed *C. sativa* roots: one was non-responsive to Cu while the expression of the second was halved [30]. In Cu-stressed rice roots, enolase accumulation was also halved [27]. However, Cu exposure was 100-fold higher in these studies compared to our experiment.

4.3.2. TCA cycle

MDH (245) and IDH (352), which respectively catalyze in the TCA cycle the conversion of malate into oxaloacetate (and vice versa) using NAD^+/NADH and the oxidative decarboxylation of isocitrate, producing α-ketoglutarate and CO_2 using NAD^+/NADH (Fig. 2) [64], were highly expressed in *A. capillaris* roots. In M roots, 245 was reduced as Cu exposure rose (Fig. 3). At the lowest Cu concentration (1 µM Cu), IDH (352) and MDH (245) were overexpressed in M roots (Table 3, Fig. 3), suggesting a sub-optimal Cu supply and higher synthesis of malic and citric acids, which are potential ligands for free Cu^{2+} and may optimize Cu distribution and use in cells [23, 65].

4.3.3. Oxidative phosphorylation

The ATP synthase subunit α (442, Fig. 2) catalyzes ATP synthesis in the last step of oxidative phosphorylation [66]. Expression did not differ between M and NM roots, but decreased in M roots between 1 and 10 µM Cu as MDH and MetE (Fig. 3).

4.3.4. Purine and pyrimidine metabolism

Expression of NDK (16, Fig. 2) decreased in NM roots between 1 and 10 µM Cu (Fig. 3), which may indicate a slowing of cellular processes as Cu rose. This suggested a higher energy production in Cu-stressed M roots that may confer a better ability to maintain cellular processes.

4.4. Other functions

Three spots identified as tub α (49, 396, and 397), one of the two basal components of microtubules, were over-expressed in M roots (Tables 3 and 4) and only 49 was not Cu-responsive (Fig. 3). Spot 396 decreased only in NM roots but 397 in both M and NM roots. However, due to the respective expression rate of these spots, the decrease in NM was the dominant effect (Table 4, Fig. 3). Cytoskeleton would be negatively impacted by excessive Cu exposure, markedly in NM roots, confirming previous findings in *Allium sativum* [67].

5. Conclusion

The soluble proteome was partially analyzed in roots of 2-month-old M and NM *A. capillaris* plants cultivated on perlite and exposed to Cu (1-30 µM range) since their sowing to investigate (i) differential expression of soluble proteins in NM and M roots when Cu stress is increasing, and (ii) molecular mechanisms underlying higher tolerance to excess Cu in M plants.

Some insights were gained into mechanisms underlying Cu tolerance in both *A. capillaris* populations, but a complete model of such mechanisms could not be drawn, due to the low number of selected spots. Only 19 out of the 23 spots selected for differential expression were identified as databases are limited for this non-model plant. Based on these preliminary results, M plants of *A. capillaris* did not evolve a specific mechanism in roots explaining their higher Cu-tolerance in the range 17.7-210 mg Cu/kg root DW, and it would merely result from simultaneous cooperation of various processes. Main functions in line with differential responses of M and NM roots at low (1-5 µM Cu) and high (15-30 µM Cu) Cu exposure concerned antioxidative mechanisms, carbohydrate and energy metabolism, and signal transduction.

At supra-optimal Cu exposure (15-30 µM), glycolysis was likely altered in NM roots with increased production of glycerone-P and methylglyoxal based on over-expression of TIM and FBP-aldolase. Higher superoxide detoxification would occur in M roots, in line with the increase of chloroplastic Cu/Zn-SOD. Changes in tubulins and higher MetE and SAMS

abundances, respectively underpinned impacts on the cytoskeleton and stimulation of ethylene metabolism in NM root cells, which may reflect a higher Cu-induced senescence. Increased L-methionine and SAM amounts in NM roots may also facilitate production of NA, which complexes Cu, and of L-cysteine, which is needed for metallothioneins and GSH production. At low Cu exposure (1-5 µM), soluble root proteomes differed between populations, suggesting a suboptimal Cu supply in M at 1 µM. Over-expression of 14-3-3 proteins in M roots at 1-5 µM Cu and of IDH at 5 µM Cu suggested, respectively, a higher signal transduction and higher synthesis of Cu^{2+} ligands such as citric acids. Over-expression of SOD in NM roots at 1 µM Cu may indicate a higher oxidative stress in NM plants even at the lower Cu exposure. This preliminary work will initiate further characterization of soluble proteome in Cu-stressed roots and leaves of both *Agrostis* populations.

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CHAPTER III: Phenotypic description

Influence of increasing Cu exposure on the growth of Cu-tolerant and sensitive populations of *Agrostis capillaris* L.

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Abstract

A. capillaris L. is a pseudo-metallophyte known for its plasticity regarding metal(loid) tolerance, including Cu. Two populations differing by their Cu tolerance were compared under increasing Cu excess (1, 5, 10, 15, 20, 25, 30, 40 and 50 µM) to investigate plant response to Cu stress. Seeds of the tolerant (Metallicolous, M) and the non-tolerant (Non-Metallicolous, NM) populations were respectively collected on a Cu-contaminated soil and an unpolluted forest edge. After a 3-month period of growth on perlite moistened with a $CuSO_4$ spiked-nutrient solution, plants were harvested. Maximal Length (L_{max}) and Mean Length (L_{mean}) of shoots were measured and root/shoot Fresh and Dry Weight yields (FW, DW) determined.

Cu impacted plant growth, disturbed root architecture and induced chlorotic symptoms in both populations, more intensively in NM, indicating a higher tolerance of the M population in this range of Cu exposure. Shoot length, fresh and dry weight yields decreased sharply in NM but did not vary or slightly decreased in M plants. Shoot/roots ratios of Cu concentrations confirmed the "excluder" phenotype of *A. capillaris* and indicated limitation of Cu transport to aerial parts. Root Cu concentrations refuted the possibility of a reduced Cu accumulation in M roots at low and high Cu exposure but at intermediate (25-30 µM Cu), lower Cu concentrations and higher biomass of M plants suggested a similar uptake but a dilution of Cu in tissues through an increase of root biomass. A better efficiency to cope with Cu toxicity and to maintain root growth and functions deserved further investigations. Foliar Cu concentrations excluded a reduced Cu translocation in M plants, as they were either similar or higher in M leaves. On the contrary, this supported the existence of a better efficiency of M leaves to cope with the deleterious effects of Cu excess, and even more suggested a high need for Cu in this population. Foliar Fe concentrations decreased with Cu excess in shoots of both populations, while Zn concentrations increased, so chlorosis symptoms were rather attributed to Fe than Zn

deficiency. Maintaining of roots K concentrations and regulation of Ca, Na and Al foliar concentrations appeared to be involved in the enhanced Cu-tolerance of the M population.

1. Introduction

Some plant species, called “full metallophytes”, have only been observed in naturally metal-enriched areas, such as Cu-rich soils in Africa, and present growth reduction when cultivated in low metal supply. In case of Cu, these species have been named absolute cuprophytes (Faucon *et al.*, 2008). Some others, called “pseudo-metallophytes”, exhibit phenotypic plasticity for metal-tolerance and may evolve populations on both metal-free and metal-contaminated soils. These species constitute a relevant tool to examine tolerance (including resistance) mechanisms, as populations grown on contaminated soil may have evolved molecular mechanisms enabling their survival.

A tolerant (Metallicolous, M) population of *Agrostis capillaris* L. (Colonial bentgrass) has been recorded as dominant species at a French wood preservation site with Cu-contaminated soils (65 - 2600 mg Cu/kg soil, Bes, 2008; Bes *et al.*, 2010). This pseudo-metallophyte has long-time been studied for evolving metal-tolerant populations, including Cu (Gregory and Bradshaw, 1965; Nicholls and McNeilly, 1985; Symeonidis *et al.*, 1985 a and b; Lepp *et al.*, 1997; Vogeler *et al.*, 2008) and present interesting characteristics for aided phytostabilization of Cu-contaminated soils, i.e. relative fast growth and perennial life cycle, high soil coverage, tolerance to abiotic/biotic stresses, low-input production (energy, costs), low nutrient/water requirements, and restricted uptake/accumulation of contaminants in shoots, with a shoot:root ratio of 0.3 typical of an excluder phenotype (Dahmani-Muller *et al.*, 2000; Padmavathiamma and Li, 2007; Vangronsveld *et al.*, 2009).

There is a lack of knowledge on mechanisms underlying Cu-tolerance to excess Cu and low shoot:root ratio in grassy species such as *A. capillaris*. At the plant level, several processes have been suggested, e.g. limitation of root Cu uptake, accumulation in roots and limitation of Cu translocation into aerial parts and better ability to cope with Cu in both root and leaf cells.

In previous work, this M population has been compared to another non-tolerant one, called non-metallicolous (NM), and collected on the uncontaminated soil of a forest edge. Response to Cu exposure has been evaluated on a Cu-contaminated soil series obtained with the fading technique and indicates a higher tolerance for the M population under increasing Cu excess (Bes, 2008). A second experiment on Cu-spiked perlite moistened with Hoagland nutrient solution in the 1-30 µM Cu range for a 2-month period, has confirmed the higher tolerance of the M population and indicated differential accumulation of soluble proteins

depending on both the Cu exposure and the population origin (Bes, 2008; Hego *et al.*, 2014, Chapt. II).

Here, the M and NM populations of *A. capillaris* L. were chronically exposed to Cu in the 1-50 µM range for a 3-month period, to confirm the better tolerance in the M population under Cu-contaminated conditions and identify the mechanisms underlying the enhanced Cu tolerance of the M population. Did M plants avoid Cu accumulation or possess a better ability to cope with Cu excess in cells?

2. Materials and Methods

2.1. Plants and Cu treatments

Seeds of metallicolous (M) and non-metallicolous (NM) populations were respectively collected from *A. capillaris* L. growing at a wood preservation site contaminated by Cu (Bes and Mench, 2009; Mench and Bes, 2009; Bes *et al.*, 2010) and at a forest edge (RN10, Km 83, Belin Beliet, Gironde, France) in August-September 2011. Phenotypes of M and NM populations were previously characterized on a Cu-contaminated soil series obtained with the fading technique and on Cu-spiked perlite moistened with Hoagland n°2 nutrient solution in the 1-30 µM Cu range (Bes, 2008). Seeds were sowed and plants cultivated for three months on perlite constantly bottom moistened with Hoagland n°2 nutrient solution (Hewitt, 1966) containing 1, 5, 10, 15, 20, 25, 30, 40 and 50 µM Cu (added as $CuSO_4$, $7H_20$), weekly changed. Moistened perlite was preferred than hydroponics for maintaining root ultra-structure and Si nutrition closer to soil conditions (Lux, 2010). Seeds were germinated under natural light in plastic pots (15 x 12 x 8 cm). After 28 days, plants were transferred in a growth chamber with a 14h, 27°C day and a 10h, 22°C night regime, with 220-240 µmol photons $m^{-2}.s^{-1}$ light intensity and 65-75% relative humidity.

For each experimental condition (i.e. Population x Cu concentration), 6 replicates were carried out, divided in two sets of three pots, leading to 3 replicates for both populations in each set. To avoid edge effects, sets and pots were moved every three days.

2.2. Morphological parameters and concentrations in elements

After a 3-month period of growth all plants were harvested by removing perlite from roots with milliQ water. Maximal length (L_{max}) and mean length (L_{mean}) were measured only on shoots for the experiment with a high plant density because of the partial damage of the root apical parts during the harvest, notably the longest roots. After sampling roots and leaves for

proteomic experiments (see chapters IV and V), remaining tissues were rinsed in milliQ water, weighed and oven dried (one week, 70°C) to calculate fresh and dry weight yields (FW, DW). Aliquots of root and leaf dry matter (0.5 g DW) were wet-digested in 14 M HNO_3 and 30% vol. H_2O_2 under microwaves (CEM Marsxpress) and elements determined by axial ICP-AES at the INRA USRAVE laboratory, Villenave d'Ornon, France.

2.3. Statistical analyses

In this experiment, Cu was considered as a continuous variable to include the "dose" notion in the analysis. To characterize the response of each population across the range of Cu exposures, Pearson's correlation was used between each population dataset (M and NM) and Cu exposure (1-50 µM). Datasets were also fitted with regression models using three options (Cu: Cu concentration in the nutrient solution, a, b, c and d: constants):

(1) $Param_{M/NM} = a\ Cu + b$, henceforth referred as Linear model,

(2) $Param_{M/NM} = a\ \ln[Cu] + b$, referred as Logarithm model,

(3) $Param_{M/NM} = a\ \sqrt{Cu} + b$, referred as Square root model

(4) $Param_{M/NM} = a\ Cu^2 + b$ referred as Square model

(5) $Param_{M/NM} = a\ Cu^2 + b\ Cu + c$, referred as Polynomial 2 model

(6) $Param_{M/NM} = a\ Cu^3 + b\ Cu^2 + c\ Cu + d$, referred as Polynomial 3 model.

To characterize differences between M and NM populations for each parameter, Student's tests were applied at each Cu exposure (n = 6). Alpha error has been fixed at 0.1 because of inter-replicates variability. Statistical analyses were conducted on R v2.11.1 (R Foundation for Statistical Computing; Vienna, Austria). Graphical figures were obtained on R then modified with Power Point.

3. Results

3.1. Phenotype and growth parameters

For the 18 experimental conditions (2 populations x 9 Cu exposure levels), phenotypes of the 6 replicates after the 3-month-growth period (Fig. 1) were characterized by 4 parameters in roots and 6 in shoots (Fig. 2). These parameters included mean and maximal shoot length (Lmean and Lmax), mean fresh and dry weight yield of shoot and roots (FWr, FWs, DWr and DWs yield per plant). Table of mean values (+/- standard deviation), Student's test results and Pearson's correlations for all growth parameters and ionome are given in Annex 4, 5 and 6 respectively.

As described below, high growth variability occurred among plants composing replicates (intra-replicate), among replicates of a selected population and Cu exposure (inter-replicates), among the mean replicate of a population upon Cu exposure (inter-Cu exposures + intra-population) and between population at each Cu exposure (inter-populations + intra-Cu exposure).

i) Intra-replicate variability (i.e. among individuals inside the same replicate of one population). This variability was not quantified because the comparison was made at the replicate level (one pot consisted in a small population of 30-40 individuals), but noticed because it was highly visible in M population at high Cu exposure (30-50 µM, Fig. 3). At low and moderate exposures, this variability was either not observed or low in both populations. At 50 µM, such variability was also observed for NM plants but three groups of M plants were clearly discriminated for Cu-tolerance: i.e. no or low, intermediate and high tolerance. For all analyses, mean value of each replicate (among all individuals) was used to make comparisons.

ii) Inter-replicate variability (i.e. among the 6 replicates of one experimental condition). This variability limited application of linear regressions in particular for the FWs and DWs yield parameters in M population (Fig. 2).

iii) Variability including inter-Cu exposure + intra-population (i.e. among Cu exposure conditions of the same population (Cu Effect + Interaction Cu x Pop, Regressions and Correlations)

iv) Variability including inter-populations + intra-Cu exposure: i.e. between populations at each experimental condition (Pop Effect + Interaction Cu x Pop, Student's test).

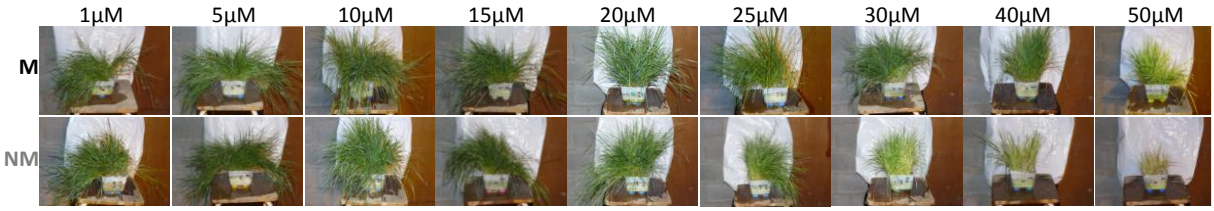

Figure 1: Pots with high population density (30-40 plants per pot) from M and NM populations of *Agrostis capillaris* exposed to increasing Cu exposures (1-50 µM Cu)

Growth of both populations was visibly impacted by Cu exposure (Fig. 1-2, Annex 2 and 3), but reduction was more drastic for the NM one, whatever the plant parameter observed. Both datasets for shoot length, i.e. Lmean and Lmax, were negatively correlated with Cu exposure in M plants ($r = -0.52$ and -0.36, p-values < 0.0001 and $= 0.007$ respectively) and NM plants ($r = -0.91$ and -0.84, p-values < 0.0001, respectively). For each population, these datasets could be fitted by the same type of linear model: i.e. Polynomial 3 for M ($R^2 = 0.37$ and 0.26, respectively) and Linear for NM ($R^2 = 0.84$ and 0.71, respectively; Fig. 2), indicating that for both parameters these populations had different behavior across this Cu exposure range. For the M population, an increase of Lmean and Lmax between 1 and 15 µM Cu was followed by a decrease from to 20 to 40 µM, and a slight increase at 50 µM Cu. For the NM population, Lmean and Lmax linearly decreased between 1 and 50 µM Cu.

Between 1 and 50 µM, fresh weight yield of roots (FWr yield) and shoots (FWs yield) were correlated with Cu exposure, positively in M ($r = 0.36$ and 0.23, p-values $= 0.007$ and 0.09, respectively), but negatively in NM ($r = -0.66$ and -0.75, p-values < 0.0001 respectively, Tab. 1). These results were confirmed for FWr yield by data-fitted models: a Square model ($R^2 = 0.46$) indicated a decrease in NM whereas a Square Root model ($R^2 = 0.14$) pointed out an increase for the M population (Fig. 2). For FWs yield, datasets could only be fitted in NM population due to the high variability inter-replicates measured in M population, and a Logarithmic model ($R^2 = 0.35$) showed a decrease as Cu exposure raised.

Correlation between both Dry Weight yield of roots (DWr yield) and shoots (DWs yield) and Cu exposure was negative in NM plants ($r = -0.56$ and -86, p-values < 0.0001) and non-significant in M plants ($r = 0.22$ and -0.02, p-values $= 0.11$ and 0.90). For DWr yield, data could not be fitted in any population because of a high inter-replicate variability among experimental conditions, which in M population increased as Cu exposure raised and peaked at 50 µM. For DWs yield, data was only fitted for NM population and a Square model ($R^2 = 0.67$) showed a decrease on 1-50 µM Cu range (Fig. 2, Tab. 1).

Table 1. Coefficient of correlations (rM/NM) between growth parameters and Cu exposure in roots and shoots of M and NM populations and results of Student's tests between M and NM at 1, 5, 10, 15, 20, 25, 30, 40 and 50 µM Cu (alpha = 10%), with significant symbols referring to *** < 0.001 < ** < 0.01 < * < 0.05 < # < 0.1 < ns < 1 and M/NM indicating the population with higher mean value. FWr/FWs: Fresh Weight yield in roots and shoots in g; DWr/DWs: Dry Weight yield in g; Lmean: Mean length of shoots in cm; Lmax: Maximal length of shoots in cm. Details available in Annex 4, 5 and 6.

	rM	rNM	1	5	10	15	20	25	30	40	50
FWr	0.36** ↗	-0.66*** ↘	=	=	M*	M**	M*	M**	M**	M**	M**
FWs	0.23 # ↗	-0.75*** ↘	=	=	M#	=	M#	M*	M***	M*	M**
DWr	0.22 ns -	-0.56*** ↘	=	M#	M*	M*	M*	M*	M**	M*	M**
DWs	-0.02 ns -	-0.86*** ↘	=	=	M*	M*	M**	M**	M***	M**	M**
Lmean	-0.52*** ↘	-0.91*** ↘	NM**	=	=	M*	M#	M*	M**	M***	M***
Lmax	-0.36** ↘	-0.84*** ↘	=	=	=	M#	M*	M#	M**	M#	M***

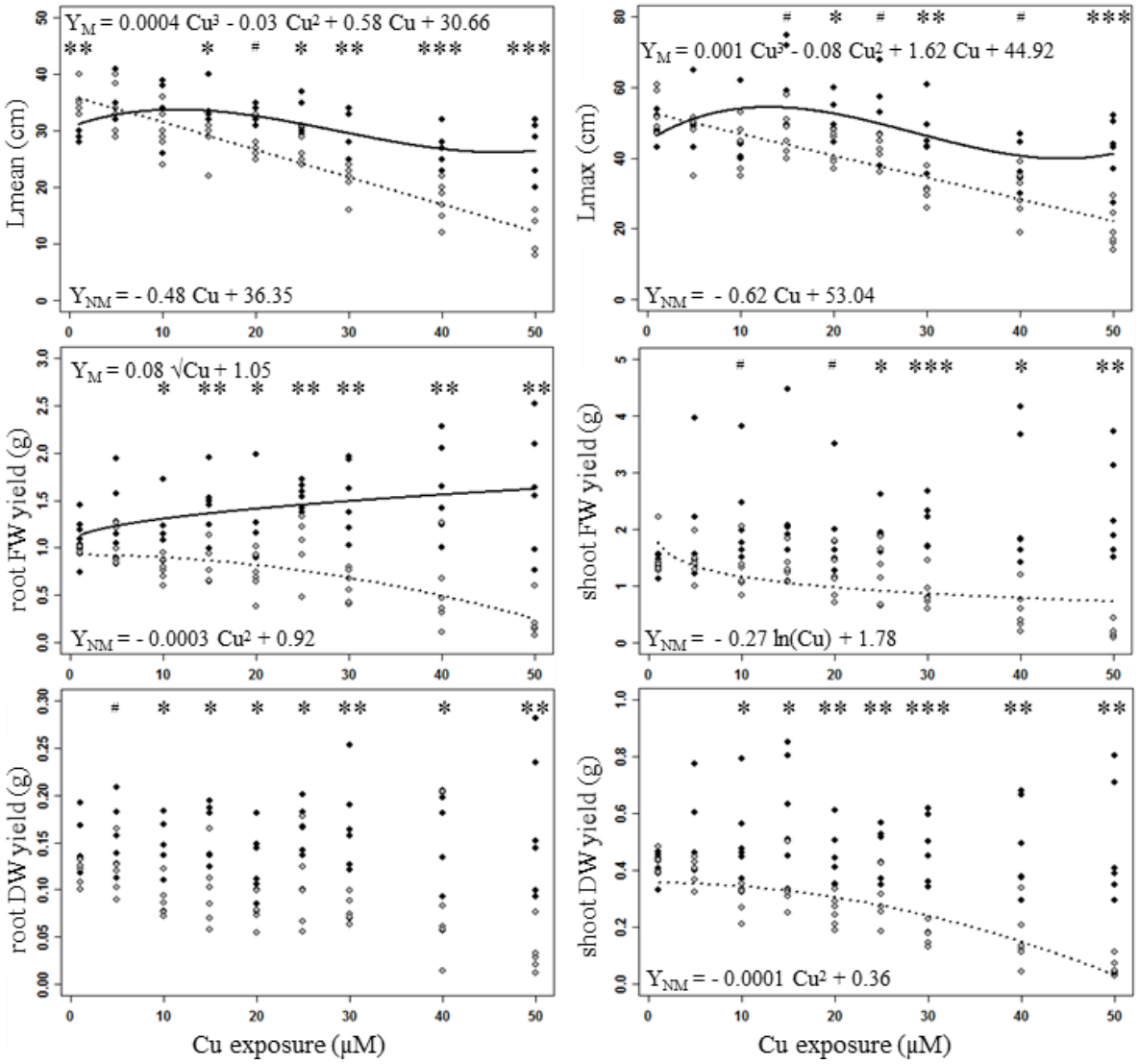

Figure 2: Growth parameters after 3-months growth of M and NM populations of *Agrostis capillaris* exposed to increasing doses of Cu exposure (1-50 µM Cu)

From 20 µM to 50 µM Cu, all plant parameters were significantly higher in M population compared to the NM one but more contrasted results occurred at lower Cu exposure (Student's tests, n = 6, Fig. 2, Tab. 1). At 1 µM, only Lmean was higher in the M population and at 5 µM, only DWr yield was higher in M population, the other parameters did not differ between populations at these exposures. At 10 µM, only Lmean and Lmax did not differ between populations, FWr, FWs, DWr and DWs yields were higher in M population. At 15 µM, only FWs yield did not differ between populations, all others parameters were significantly higher in M population.

No toxicity symptom was observed on aerial parts of both plant populations between 1 and 20 µM Cu, but chlorophyll degradation was observed at exposure higher than 25 µM Cu (Fig. 1 and 2, Annex 2 and 3). Shoot morphological pattern varied from an abundant, dark green biomass with numerous leaves by stem, distant from few cm, to a small biomass, with few leaves by stem (2-3) very close to each other's. Leaves were discolored and thinner than at low exposure and shoots exhibited a color varying between white, yellow and brown. Root system architecture was progressively modified in response to Cu exposure, changing from a long, abundant, highly ramified, fibrous and fasciculate white-yellow system to a short one, atrophied, blistered, coralloid-like, with low secondary ramifications and a color varying from yellow to brown-black for the most impacted plants (Fig. 3, Annex 3).

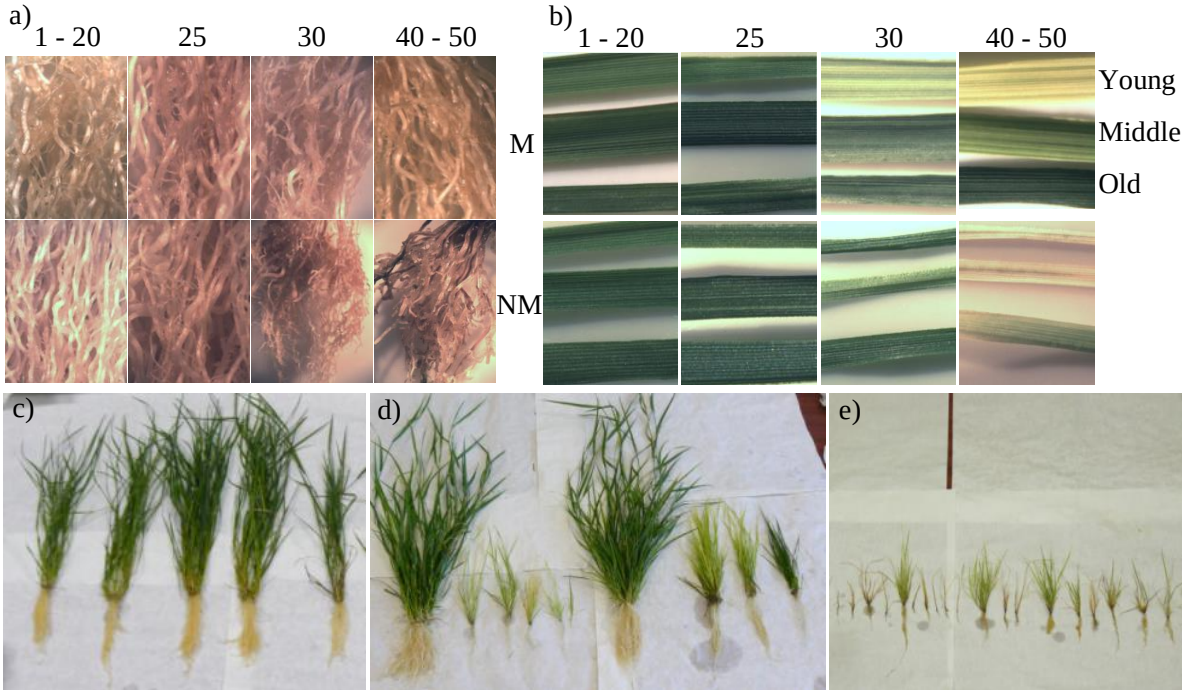

Figure 3: Impacts of Cu exposure on a) roots and b) in leaves from M and NM populations of *Agrostis capillaris* exposed to increasing Cu exposure (1-50 µM Cu) and variability intra-population of plant growth at c) 1-20 µM (M and NM not visibly different) and at 50 µM in d) M and e) NM populations.

At high Cu (30-50 µM), 4 phenotypes of Cu-tolerance were distinguished (Fig. 3):

i) Sensible individuals, with brown-black coralloid small roots, less than 1cm, and thin shoots of few centimeters (4-8 cm), with color varying from white-yellow/green- brown, with old leaves burned-like (Fig. 3e).

ii) Individuals with **low Cu-tolerance**, which presented small roots (less than 3 cm) with coralloid aspect and a yellow-brown-black color. Shoot length was slightly higher than non-tolerant (5-15cm) and leaves were more pigmented, with a coloration depending on leaf age: young leaves varied from white to yellow whereas intermediate were green and old ones were brown (Fig. 3d-e).

iii) Individuals with **intermediate Cu-tolerance**, which exhibited visible but less marked symptoms on roots and shoots and a growth significantly higher than individuals with low-tolerance (3-6 cm for roots and 15-25 cm for shoots). Roots exhibited low coralloid symptoms but were shorter and less abundant. Chlorosis symptoms varied from poorly marked (intense green) to a patchwork of yellow and green for more severely impacted plants. Once again, shoot colorations were not homogeneous reflecting leaf age: young leaves were yellow whereas intermediate were green and old were dark green and sometimes purple (Fig. 3d).

iv) Individuals **highly tolerant**, which were able to grow without any visible symptoms of toxicity, neither on roots or shoots, to sizes similar or higher than those measured at low Cu exposure (Fig. 3d). Unlike plants from the three first groups, these tolerant individuals produced some stolons, which occurred currently at low Cu exposure, and had a dense, deep root system, with any symptom, and abundant long green shoots, similar or higher than plants at low Cu exposure (Fig. 3c).

Individual variability was visible in the 25-30 µM Cu range for the NM population with a mixed stand exhibiting these four Cu-tolerance phenotypes, but at 40-50 µM Cu, most individuals presented no or low Cu-tolerance and only some had intermediate tolerance (Fig. 3a, b, e). In the M population, individual variability was detected at 30 µM Cu and increased drastically at 40-50 µM Cu. Although most plants exhibited intermediate Cu-tolerance, some displayed one of the three other phenotypes (Fig. 3a, b, d).

3.2. Shoot and root ionomes

3.2.1. Copper

Mean Cu concentrations ranged from 12 to 543 mg kg^{-1} DW in M roots and from 12 to 840 mg kg^{-1} DW in NM ones (Fig. 4) and were positively correlated with Cu exposure (r = 0.81 and 0.85, p-values < 0.0001 for M and NM, respectively). Both populations showed similar behavior with a visible increase but no model can be applied due to the concomitant increase of inter-replicate variability (non-respect of homoscedasticity). Student's test showed that root Cu concentration was significantly higher in NM plants at 25 and 30 µM Cu (p-values = 0.015 and 0.02) but did not differ between populations at other exposures.

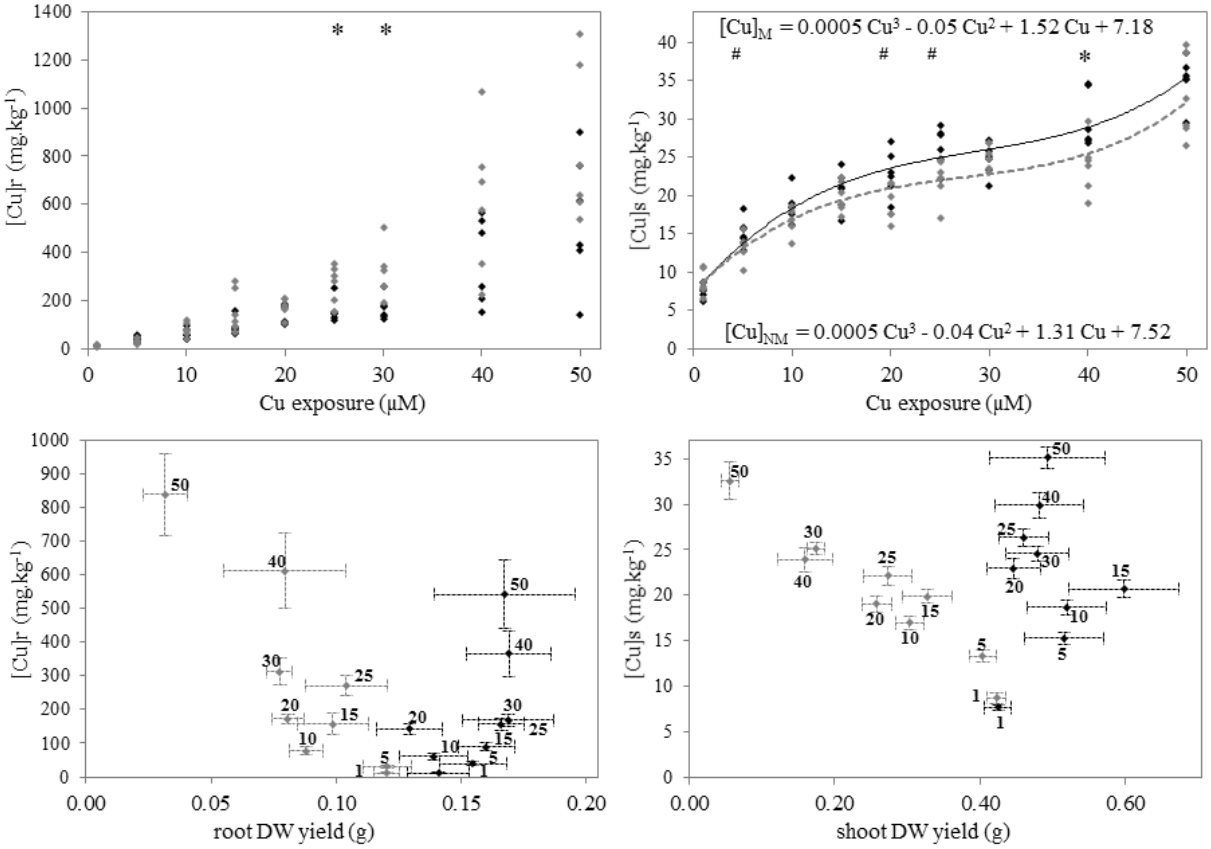

Figure 4: Root and shoot Cu concentrations of M and NM plants of *Agrostis capillaris* exposed to increasing doses of Cu exposure (1-50 µM Cu)

Shoot Cu concentrations ([Cu]s) ranged from 7.7 to 35 mg kg^{-1} DW for M plants and from 8.7 to 33 mg kg^{-1} DW for M ones (Fig. 4) and were positively correlated with Cu exposure (r = 0.92 and 0.89, p-values < 0.0001 for M and NM, respectively). Increases in [Cu]s were fitted by a Polynomial 3 model for both populations (Fig. 4, Tab. 2) and mean [Cu]s were significantly higher in M at 5, 20, 25 and 40 µM (Student's test, p-values = 0.097, 0.031, 0.021 and 0.017, respectively). Cu concentrations were higher in roots compared to shoots for both populations and mean shoot/roots ratio decreased with Cu exposure, ranging from 0.64 at 1 µM to 0.06 at 50 µM for M plants and from 0.72 to 0.04 for NM plants.

However, when examined in function of biomass production (DW yield), patterns of Cu concentrations in tissues were opposed for both populations but similar in shoot and roots. In NM, Cu concentrations increased sharply with the decrease of biomass, while in M, biomass remained constant or slightly increased when Cu concentrations increased. Mean Cu mineralomass by plant (mg. plant^{-1}) was computed for root and shoot from Cu concentrations (mg.kg^{-1} DW) and DW yield (g.plant^{-1}). Cu mineralomass was higher in M roots at 5 and 50 µM (p-values = 0.01 and 0.001), and in shoots at Cu exposure higher or equal to 10 µM (p-values < 0.05, data not shown).

3.2.2. Other Elements

All mineral concentrations are expressed in mg.kg^{-1} DW and results presented refer to figures 5 and 6 and to table 2. To avoid repetitions, concentrations values will be used directly without repetition of units and no reference to figures or table will be inserted in the text.

Aluminum concentrations ([Al]) ranged from 46 to 256 in M roots and from 64 to 179 in NM ones and were negatively correlated with Cu exposure in both populations (r = -0.36 and -0.27, p-values = 0.008 and 0.05 for M and NM, respectively) but data were fitted only for NM by a Logarithmic model (R^2 = 0.07). In M shoots, [Al] ranged from 13 to 36, were positively correlated with Cu exposure (r = 0.4 and p-val = 0.002) and fitted by a Polynomial 2 model (R^2 = 0.36). In NM shoots, [Al] ranged from 10 to 123 and no correlation was found with Cu exposure. Mean [Al] was higher in NM roots at 50 µM (p-val = 0.098) and in NM shoots at 30 µM (p-val = 0.02) did not differ between populations at other Cu exposure. Shoot/root ratio ranged from 0.4 to 0.13 in both populations.

Boron concentrations ([B]) ranged in M plants from 1.4 to 20 in roots and from 11 to 76 in shoots, whereas in NM ones, they varied from 1.5 to 39 in roots and from 12 to 154 in shoots, resulting in shoot/root ratios from 3 to 5.7 in M plants and from 2.3 to 6.2 in NM ones. [B] were positively correlated with Cu exposure in roots (r = 0.42 and 0.34, p-values = 0.002 and 0.012 for M and NM) and shoots (r = 0.70 and 0.69, p-values < 0.0001 for M and NM respectively) of both populations, and higher at 50 µM Cu in NM plants (p-values = 0.066 and 0.03 for roots and shoots).

In M plants, Calcium concentrations ([Ca]) ranged from 1600 to 3 900 in roots and from 2 940 to 8 200 in shoots, whereas in NM plants, it varied from 1700 to 12 000 in roots and from 3 550 to 18 800 in shoots. [Ca] were 1.6 to 2.7 higher in shoots compared to roots for both populations, with lower shoots/roots ratios at low exposures (1 to 10 µM Cu) and higher ones at high exposure (30-40 µM Cu). For both populations, [Ca] were positively correlated with Cu

exposure in roots (r = 0.34 and 0.58, p-values = 0.011 and < 0.0001 for M and NM respectively) and in shoots (r = 0.81 and 0.82, p-values < 0.0001). For the M population, [Ca] were fitted by a Linear model in roots and shoots (R^2 = 0.12 and 0.66), but only in NM shoots (R^2 = 0.66). Higher [Ca] were measured in NM roots at 50 µM Cu (p-values = 0.016) and in shoots at 1, 10, 20, 30, 40 and 50 µM Cu (p-values = 0.038, 0.004, 0.099, 0.0096, 0.073 and 0.004 respectively).

In M plants, Iron concentration ([Fe]) ranged from 34 to 143 in roots and from 35 to 112 in shoots, while it varied in NM plants from 36 to 296 in roots and from 24 to 158 in shoots. Shoot/root ratios ranged from 0.81 to 0.6 in M plants and 0.91 to 0.41 in NM ones, with a decrease in NM plants after 15 µM Cu from 0.9 to 0.4 and in M plants after 30 µM from 0.8 to 0.6. [Fe] were higher in NM roots at 50 µM Cu (p-val = 0.094) but were not correlated with Cu exposure for any population despite a Logarithmic model fitted on the M dataset (R^2 = 0.08). [Fe] did not differ between population shoots and were negatively correlated with Cu exposure for M and NM (r = -0.41 and -0.49, p-values = 0.002 and 0.0001, respectively).

Root magnesium concentration ([Mg]) ranged from 757 to 3110 in M plants and from 500 to 3600 in NM plants, while shoot [Mg] varied from 2100 to 5660 in M and from 1985 to 9120 in NM. Shoot/root ratios ranged from 1.7 at 1 µM Cu for both population to 2.89 for NM plants (regular increase) and 2.29 for M plants (slight increase). Indeed, [Mg] were significantly higher in NM shoots at 30 and 50 µM Cu (p-values = 0.029 and 0.002) whereas it did not differ between M and NM roots. Mg concentrations were positively correlated with Cu exposure in roots (r = 0.44 and 0.50, p-values = 0.0008 and 0.0001 for M and NM, respectively) and shoots (r = 0.78 and 0.83, p-values < 0.0001). Root datasets were fitted by a Square (M, R^2 = 0.61) and a Polynomial 2 (NM, R^2 = 0.32) model, and shoot datasets by linear models (R^2 = 0.61 and 0.68).

Root Mn concentration ([Mn]) ranged from 6.4 to 200 in M plants and from 8.7 to 790 mg kg^{-1} in NM ones. Shoot Mn concentration ([Mn]) varied from 36 in both M and NM plants to 226 mg kg^{-1} DW for M plants and to 412 for NM plants. [Mn] were significantly higher in NM shoots, at 25, 30 and 50 µM (p-values = 0.078, 0.064 and 0.051) but did not differ between populations in roots. [Mn] were positively correlated with Cu exposure in roots (r = 0.38 and 0.52, p-values = 0.004 and 0.0001) and in shoots (r = 0.62 and 0.74, p-values < 0.0001 for M and NM plants, respectively) but no model was validated due to the non-respect of homoscedasticity. Shoot/root ratios ranged from 1 to 3.1 in NM plants and from 1.9 to 2.8 in M plants, with lower ratios at low (1 µM Cu) and high exposures (40-50 µM Cu for NM and 50 µM Cu for M).

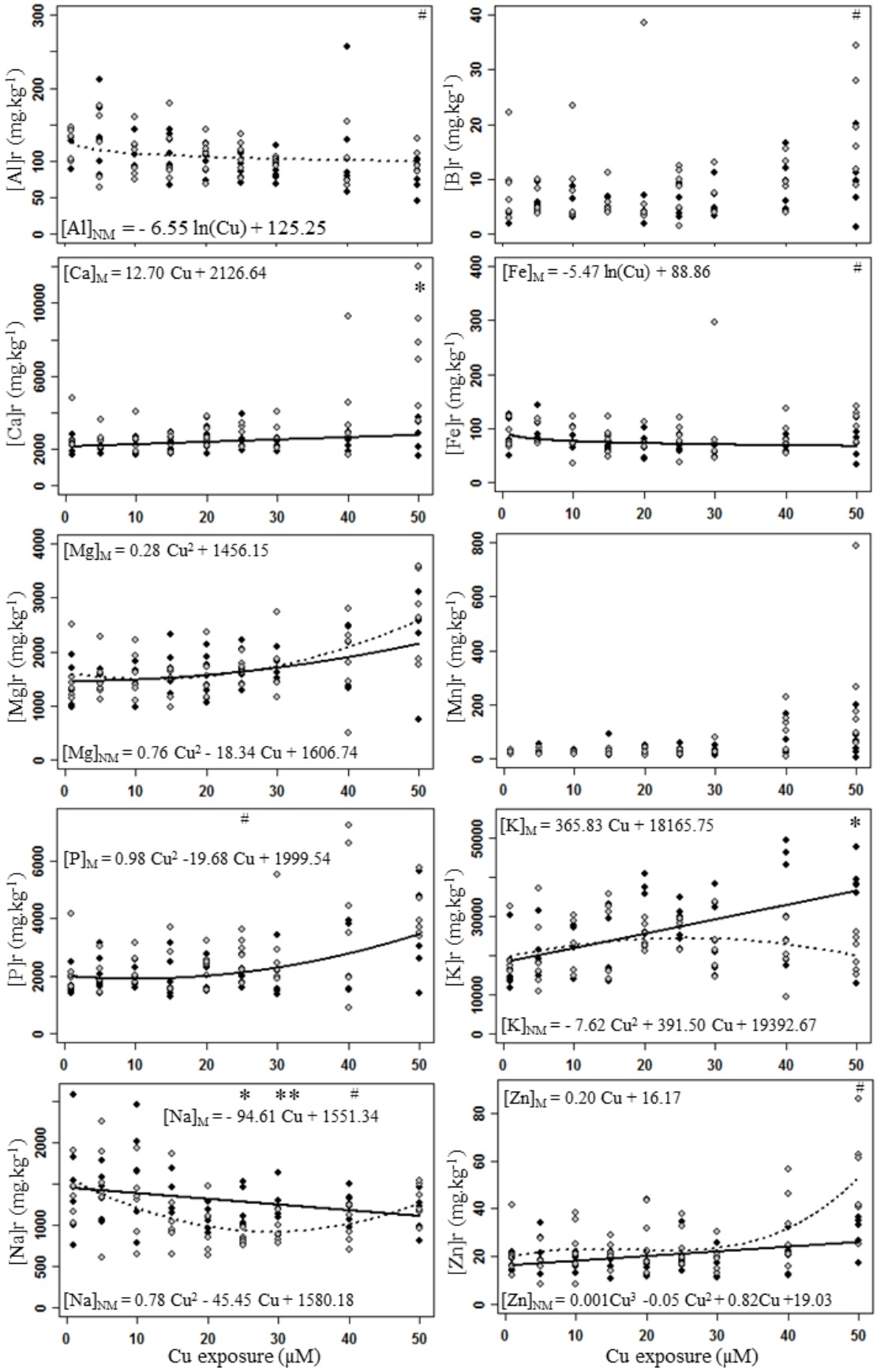

Fig. 5: Variations of Al, B, Ca, Fe, Mg, Mn, P, K, Na and Zn concentrations in roots of M and NM plants of *Agrostis capillaris* in response to increasing Cu supply in nutrient solution (1-50 µM Cu).

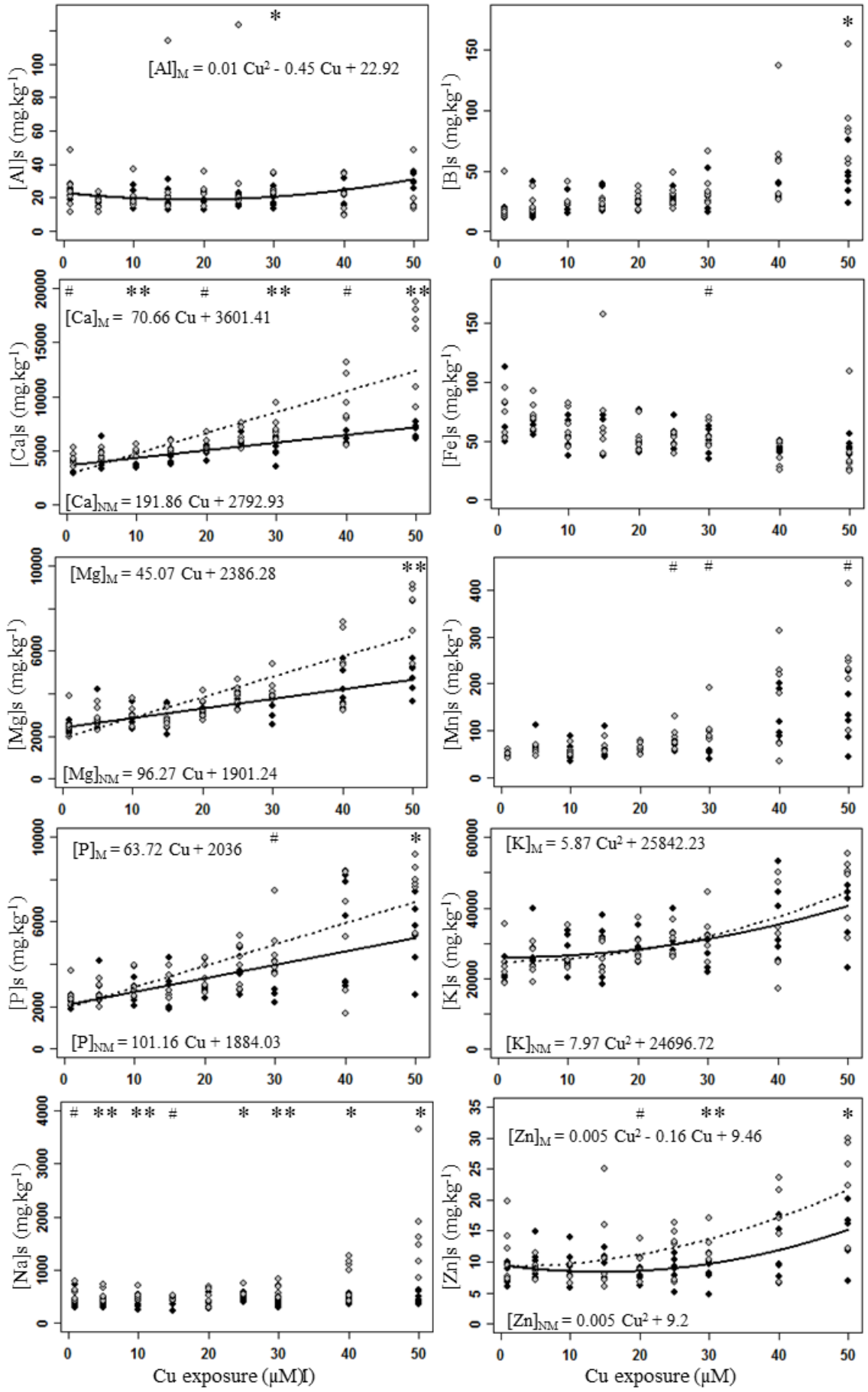

Fig. 6: Variations of Al, B, Ca, Fe, Mg, Mn, P, K, Na and Zn concentrations in shoots of M and NM plants of *Agrostis capillaris* in response to increasing Cu supply in nutrient solution (1-50 µM Cu).

Table 2. Coefficient of correlations (rM/NM) between growth parameters and Cu exposure in roots and shoots of M and NM populations and results of Student's tests between M and NM at 1, 5, 10, 15, 20, 25, 30, 40 and 50 µM Cu (alpha = 10%), with significant symbols referring to *** < 0.001 < ** < 0.01 < * < 0.05 < # < 0.1 < ns < 1 and M/NM indicating the population with higher mean value.

	rM	rNM	1	5	10	15	20	25	30	40	50
[Al]r	-0.36 ** ↘	-0.27 * ↘	=	=	=	=	=	=	=	=	NM#
[B]r	0.42 ** ↗	0.34 * ↗	=	=	=	=	=	=	=	=	NM#
[Ca]r	0.34 * ↗	0.58 *** ↗	=	=	=	=	=	=	=	=	NM*
[Fe]r	-0.2 ns -	0.15 ns -	=	=	=	=	=	=	=	=	NM#
[Mg]r	0.44 *** ↗	0.5 *** ↗	=	=	=	=	=	=	=	=	=
[Mn]r	0.38 ** ↗	0.52 *** ↗	=	=	=	=	=	=	=	=	=
[P]r	0.49 *** ↗	0.54 *** ↗	=	=	=	=	=	NM#	=	=	=
[K]r	0.55 *** ↗	0.03 ns -	=	=	=	=	=	=	=	=	M*
[Na]r	-0.29 * ↘	-0.26 # ↘	=	=	=	=	=	M*	M**	M#	=
[Zn]r	0.39 ** ↗	0.54 *** ↗	=	=	=	=	=	=	=	=	NM#
[Al]s	0.40 ** ↗	-0.02 ns -	=	=	=	=	=	=	NM*	=	=
[B]s	0.7 *** ↗	0.69 *** ↗	=	=	=	=	=	=	=	=	NM*
[Ca]s	0.81 *** ↗	0.82 *** ↗	NM*	=	NM**	=	NM#	=	NM**	NM#	NM**
[Fe]s	-0.41 ** ↘	-0.49 *** ↘	=	=	=	=	=	=	=	=	=
[Mg]s	0.78 *** ↗	0.83 *** ↗	=	=	=	=	=	=	NM*	=	NM**
[Mn]s	0.62 *** ↗	0.74 *** ↗	=	=	=	=	=	NM#	NM#	=	NM#
[P]s	0.64 *** ↗	0.78 *** ↗	=	=	=	=	=	=	NM#	=	NM*
[K]s	0.58 *** ↗	0.67 *** ↗	=	=	=	=	=	=	=	=	=
[Na]s	0.36 ** ↗	0.6 *** ↗	NM#	NM**	NM**	NM#	=	NM*	NM**	NM*	NM*
[Zn]s	0.47 *** ↗	0.58 *** ↗	=	=	=	=	NM#	=	NM**	=	NM*

FW: Fresh Weight in g; DW: Dry Weight in g; Lmean: Mean length of shoots in cm; Lmax: Maximal length of shoots in cm; [X]: Concentration of X in tissues in $mg.kg^{-1}$ DW, Cu: Copper, Al: Aluminum; B: Bore; Ca: Calcium; Fe: Iron; Mg: Magnesium; Mn: Manganese; P: Phosphorus; K: Potassium; Na: Sodium; Zn: Zinc; r: roots and s: shoots.

Phosphorus concentrations ([P]) varied in M roots from 1280 to 5630 and in NM ones from 890 to 7200, while it varied from 1850 to 8190 in M shoots and from 1660 to 9200 in NM ones. [P] was significantly higher in NM roots at 25 µM Cu (p-val = 0.066) and in NM shoots at 30 and 50 µM Cu (p-val = 0.052 and 0.022). [P] were positively correlated with Cu exposure in roots (r = 0.49 and 0.54, p-values = 0.0002 and < 0.0001 for M and NM plants, respectively) and shoots (r = 0.64 and 0.78, p-values < 0.0001). Datasets were fitted by a Polynomial model in M roots (R^2 = 0.3) and by a Linear model in M (R^2 = 0.41) and NM (R^2 = 0.61) shoots. Shoot/root ratios ranged from 1.3 to 1.9 in NM plants and from 1.1 to 1.9 in M ones.

Potassium concentrations, [K], ranged from 11830 to 49400 in M and from 9500 to 37100 in NM roots; from 19300 to 53220 in M shoots and from 1730 to 55400 in NM ones. [K] were higher in M roots at 50 µM Cu (p-val = 0.026) but did not differ between populations in shoots.

[K] were positively correlated with Cu exposure in M roots (r = 0.55 and p-val < 0.0001) and well fitted by a Linear model (R^2 = 0.3), but not in NM roots, for which a Polynomial 3 model (R^2 = 0.07) showed a slight increase at intermediate Cu exposure, followed by a decrease to initial levels, resulting in an absence of correlation (r = 0.03) on this exposure range. [K]s were positively correlated with Cu exposure in M and NM plants (r = 0.58 and 0.67, p-values < 0.0001 respectively) and increases were fitted by square models (R^2 = 0.34 and 0.5 respectively).

Sodium concentrations ([Na]) ranged from 763 to 2580 in M roots, from 610 to 2250 in NM ones, and from 231 to 720 in M, from 300 to 3655 in NM shoot plants (Fig. 6). [Na] were higher in M roots at 25, 30 and 40 µM Cu (p-values = 0.012, 0.008 and 0.079) but in NM shoots at 1, 5, 10, 15, 25, 30, 40 and 50 µM (p-values = 0.091, 0.007, 0.004, 0.054, 0.018, 0.003, 0.019 and 0.024, respectively). [Na] were correlated with Cu exposure, negatively for roots (r = -0.29 and -0.26, p-values = 0.03 and 0.055 for M and NM) and positively in shoots (r = 0.36 and 0.6, p-values = 0.007 and < 0.0001). Root datasets were fitted by Linear (M, R^2 = 0.09) and polynomial 2 (NM, R^2 = 0.29) models. Shoot/root ratios ranged from 0.42 to 0.2 in M plants and 1.39 to 0.39 in NM plants, with a marked increase in NM at the high Cu exposures (increase from 0.4 between 1 to 15 µM up to 1.39 at 50 µM) and mean ratio was significantly higher in NM plants.

Zinc concentrations, [Zn], ranged from 10.9 to 44.1 in M roots, from 8.5 to 86 mg kg^{-1} in NM ones; from 4.7 to 21.6 in M shoots and from 6 to 29.9 in NM ones. [Zn] were higher in NM roots at 50 µM Cu (p-val = 0.061) and shoots at 20, 30 and 50 µM Cu (p-val = 0.062, 0.0097 and 0.023). [Zn] were positively correlated with Cu exposure in roots (r = 0.39 and 0.54, p-values = 0.004 and < 0.0001 for M and NM) and shoots (r = 0.47 and 0.58, p-values = 0.0003 and < 0.0001 for M and NM). Increases in roots were fitted by a Linear (M, R^2 = 0.15) and a Polynomial 3 (NM, R^2 = 0.44) models and in shoots by a Polynomial 2 (M, R^2 = 0.33) and a Square model (NM, R^2 = 0.44). Shoot/root ratios ranged from 0.37 to 0.66 in M plants and from 0.36 to 0.63 in NM plant and mean ratio did not differ between populations.

4. Discussion

Seeds of *A. capillaris* populations, collected on a Cu-contaminated and a normal soil, were cultivated on perlite with increasing Cu exposure (1-50 µM Cu added as CuSO4, in Hoagland solution) In order to study mechanisms underlying Cu-tolerance and to characterize variability in Cu-tolerance between these populations. Cu impacts were quantified by measuring shoot length and root/shoot biomass production.

4.1. Cu effects on morphological parameters

Growth parameters measured in this experiment were coherent with those found in previous works (Bes, 2008). Growth indicators (Maximal Shoots Length, Dry Matter) indicated that increasing Cu exposure impacted both populations in the range of concentrations tested (1-50 µM). However, they also confirmed the higher tolerance of the M population, which was able to evolve tolerant individuals until an exposure of 50 µM, while individuals from NM population were not able to survive at Cu exposure higher than 30 µM.

Higher growth in M population was significant in roots at Cu exposure higher or equal to 10 µM. In shoots, patterns between 5 and 15 µM differed among parameters. At Cu higher or equal to 20 µM Cu, all shoot parameters pointed out a better fitness of M plants. Lmean and Lmax indicated better fitness at Cu higher or equal to 15 µM Cu, while yield parameters indicated significant difference at 10 µM Cu. This suggested that roots were impacted by lower Cu exposure and may have a buffering effect to protect shoots from Cu toxicity. For a comparison, Cu concentration in Hoagland solution (hydroponic culture) higher than 0.5 µM has a deleterious effect on *Nicotiana plumbaginifolia* growth and 15 µM induces mortality of all plants (EC100, 100% effective concentration), while the cuprophyte *Haumaniastrum katangense* exhibites maximal growth at 12 µM Cu and an EC100 of 100 µM Cu, indicating that individuals are able to survive at high Cu exposure (Chipeng *et al.*, 2010).

Cu impacted sharply root growth and structure in both populations, as well as photosynthetic apparatus, shown by the yellow coloration of leaves at Cu higher than 25-30 µM. At 40 and 50 µM Cu, the several highly tolerant individuals from the M population exhibited higher and deeper root systems than individuals cultivated at low Cu exposure. Their shoot length was slightly reduced and plants presented larger tufts and no symptom of phytotoxicity (data not shown). It appeared that plants changed matter allocation, in favoring root development, which may permit to conserve portions of functional roots, and then to

maintain proper nutrients uptake. Additionally, by increasing root biomass, plants may store higher Cu quantities in tissues, protecting shoots from Cu translocation. These phytotoxic symptoms have been reported in rice leaf segments exposed to 250 µM Cu (Hajduch *et al.*, 2001). The progressive brown coloration exhibited by plant roots under increasing Cu has previously been observed in *Solanum melongena* L. and may be the symptom of an increasing accumulation of suberin, which restricts water absorption by roots (Körpe and Aras, 2011).

The high variability within populations and replicates was probably due to the wild origin of seed tested, which were collected in the field. Large variability between individuals of the same population is usual in natural environment and frequently observed in studies about wild populations. High variability within populations has also been found in Pb-tolerant populations of *A. capillaris* with large differences in Pb or Zn contents of shoot for a given Pb or Zn concentration in soil (Barry and Clark, 1978). High variability in tolerance to Co, Cu, Ni and Zn has been also identified among three clones of *A. gigantea* originated from a mine waste site; whereas one shows tolerance to Cu, Co and Ni, another is tolerant only to Ni and any is tolerant to Zn (Haugan and Rauser 1979).

The observation of strong differentiation among populations regarding Cu tolerance, with higher performance of plants from Cu-contaminated soil when cultivated in the same abiotic conditions, indicated that Cu-tolerance acquisition was a heritable trait, due to physiological adaptation and not to environmental acclimation (Wu and Kruckeberg, 1985). Regarding this fact, populations may be called ecotypes.

4.2. Cu concentrations in tissues

Root Cu concentrations were consistent with those found in previous studies for these populations (Bes, 2008), in *A. capillaris* spontaneously occurring on antimony mine soils (178-196 mg $Cu.kg^{-1}$ DW; Bech *et al.*, 2012). Similarly, shoot Cu concentrations were in the same range as those found in *A. capillaris* spontaneously occurring on soils of a Cu/Pb mine (<10 to 85 mg $Cu.kg^{-1}$ DW, with a mean of 33; Thompson and Proctor, 1983), of an antimony mine (24-28 mg $Cu.kg^{-1}$ DW; Bech *et al.*, 2012), or of lysimeters built on contaminated soil (8-20 mg $Cu.kg^{-1}$ DW; Ruttens *et al.*, 2006).

Shoot/root ratios of Cu concentrations indicated storage of Cu in roots and limitation of Cu transport to aerial parts and confirmed the “excluder” phenotype for both populations. Cu retention in roots became stricter with increase of Cu exposure, shoot/root ratio of [Cu] decreased with Cu exposure in both populations, ranging from 0.64 and 0.72 at 1 µM to 0.06 and 0.04 at 50 µM for M and NM respectively. A similar observation has been made in

Cannabis sativa plants exposed to 150ppm $CuSO_4$ for six weeks, which exhibit eight-fold increase of Cu concentrations in roots but only a two-fold increase in shoots (Bona *et al.*, 2007). Early study on localization of Cu in plant tissues has pointed out the root cell wall as a major storage target in *A. capillaris* (Turner, 1970). Manceau *et al.* (2008) have suggested that plants limit the incorporation of excessive metal in photosynthetic tissues by limiting their transport through the root endoderm and in compartmenting them in root cortex.

Cu concentrations in shoots increased in response to the rise of Cu exposure in the nutrient solution. Between 1 and 30 µM Cu, Cu concentrations in shoots stayed around the usual values measured in plants which range between 1 and 30 mg/kg MS (Marschner, 1995; Kabata-Pendias et Pendias, 1992). Maximal values measured at 40 and 50 µM were above the toxic level established for domestic herbivores (20 mg/kg MS, from Kabata-Pendias and Pendias, 1992). This indicated that *Agrostis capillaris* may present a risk for Cu transfer into food chain, when used for phytostabilization of soils with high Cu contamination, but may be suitable for soils with intermediate Cu levels.

Cu concentrations in tissues were lower than those measured in tolerant and non-tolerant populations of *Agrostis stolonifera*, exposed to similar range of Cu exposure for 8 days, and uptake strategies obviously differ for Cu uptake between both species. At 1 and 2 µM Cu, both populations exhibited similar Cu content, but at 5, 10 and 50 µM, roots of tolerant plants reach twice the concentration of non-tolerant ones, leading to mean concentration around 3 200 mg $Cu.kg^{-1}$ DW in roots of the tolerant population and around 1 700 in non-tolerant ones. Shoot concentrations exhibited also differentiation after 5 µM, but with an opposite pattern, they increased sharply in the non-tolerant population from around 30 to 110 mg $Cu.kg^{-1}$ DW at 50 µM but slowly in shoots of the tolerant one, from around 30 to 50 mg $Cu.kg^{-1}$ DW. It appeared that in *A. stolonifera*, Cu-tolerance is related to a higher storage in roots and a limitation of root-to-shoot translocation (Wu *et al.*, 1975b). The Cu-tolerance of an *A. capillaris* population originated from the antimony mine has also been attributed to restriction of both uptake of Cu in roots and translocation to shoots, as plants exhibit lower Cu concentration but also lower concentrations of potentially toxic trace elements like Sb, Pb, Zn or As, in roots and shoots, compared to *Agrostis* plants from commercial seeds (Bech *et al.*, 2012).

Here, Cu concentrations were similar in roots of both *A. capillaris* populations at low and high exposure but higher in NM at 25 and 30 µM, which may be due to a limitation of Cu uptake or to a dilution effect through an increase of biomass. The latter possibility was strongly suggested by the higher biomass of M roots but the similar mineralomass. Together with the observations made on highly tolerant M plants this confirmed that Cu-tolerance involved an

increase of root growth to store Cu and maintain portions of functional roots. In producing new tissues, plants may be able to maintain nutrients and water uptake. This implied a better ability for M roots to cope with intracellular Cu toxicity, probably by enhancing Cu chelation, storage and detoxification. The enhanced root growth in tolerant plants may also be an active avoidance mechanism. In exploring more soil surface, plants could find less contaminated areas, more favorable for nutrients uptake. Hypothesis of a limitation of root-to-shoot Cu-translocation must also be excluded, as Cu-concentrations were either similar in both populations or higher in M shoots at 5, 20, 25 and 40 µM. However, it suggested the existence of a better Cu homeostasis in M leaf cells.

4.3. TE concentrations in tissues

Typical elemental concentrations of metals and metalloids in plant shoots have been established around 1.5 µg/g for Ni, 50 µg/g for Zn, 0.05 µg/g for Cd, 1 µg/g for Pb, 10 µg/g for Cu, 0.2 µg/g for Co, 1.5 µg/g for Cr, 200 µg/g for Mn, 0.02 µg/g for Tl, 0.1 µg/g for As and 0.02 µg/g for Se (Van der Ent *et al.*, 2013). Increasing Cu exposure altered root and shoot ionomes, with differences observed among both tissues and population origin. Although all elements displayed differences in mineral patterns between populations, main changes concerned Ca, Fe, K, Al, Na and Zn.

Cu exposure induced an increasing Ca uptake and translocation to shoots, resulting in increasing Ca concentrations in tissues. Increase in Ca concentrations has also been reported in seedlings of Hassawi wheat plants grown in soil under increasing Cu exposure (Azooz *et al.*, 2012). Calcium is involved in cell membranes formation and plasticity, in protein synthesis as activator of enzyme systems, in transport of other nutrients, in photosynthesis and acts as a detoxifying agent by neutralizing organic acids (Uchida, 2000). As storage in cell walls by binding to pectates has been suggested to be a major mechanism of Cu-tolerance, increase in Ca contents may increase pectins content and increase ability to store Cu. The increase was more marked in NM roots but similar in shoots of both populations, indicating that the lower Ca concentrations in M leaves were rather due to a limitation of Ca uptake by roots than to a limitation of Ca translocation. Cu is known to modify stability of Ca channels, and induce increasing Ca flux into cells (Manara, 2012), so a better regulation of Ca uptake by roots may participate to enhance Cu tolerance in M plants.

Na concentrations decreased in roots of both populations, were higher in M between 25 and 40 µM, but increased more intensively in NM shoots compared to M ones, resulting in higher concentrations in NM shoots at almost all Cu exposure tested. This suggested that

reduction of Na uptake was a common mechanism for both populations in response to Cu excess. On the opposite, two mechanisms specific of the M population permitted reduction of foliar concentrations. The higher [Na] in M roots between 25 and 40 µM indicated a better ability to accumulate Na in M roots at intermediate Cu excess, while smaller foliar concentrations indicated lower root-to-shoot translocation in M plants even at low Cu exposure.

[Fe] did not vary in roots but decreased in shoots of both populations in response to Cu exposure. Cu excess (100 µM Cu) altered Fe uptake in roots of *Cucumis sativus* during short-term treatment (72h) and indicated different accumulation among root parts (Song *et al.*, 2014). As the Fe measure was realized on the global root biomass, a Fe deficiency occurring only in some parts of the rhizosphere, for example only in young roots, may be masked by the procedure. Further investigations on Fe distribution in the root system would help to elucidate the possible Fe deficiency in *A. capillaris* roots under Cu excess. Shoot/root ratios indicated limitation of Fe translocation from roots to shoots, which became stricter at the end of the Cu gradient (decrease of ratios in NM after 15 µM from 0.9 to 0.4 and in M after 30 µM, from 0.8 to 0.6). Fe is essential for chlorophyll synthesis, is part of heme enzyme system in many enzymes, like catalase, peroxidase, or cytochrome oxidase, and of protein ferredoxin (Uchida, 2000). Zinc and iron deficiency are known to induce interveinal chlorosis in younger leaves (Uchida, 2000). In this experiment, Cu excess induced Fe deficiency in leaves, Zn concentrations increased, indicating that the interveinal chlorosis observed at exposure higher than 25 µM may be attributed to Fe deficiency. In *Becium homblei*, appearance of chlorosis was related to Cu interference in Fe accumulation in chloroplast, rather than limitation of Fe uptake and soil addition of Fe was able to alleviate phytotoxic symptoms in reducing Cu uptake and restoring levels of Fe in chloroplasts (Reilly and Reilly, 1973). Similar decreases of foliar Fe concentrations was reported in white lupin and soybean plants subjected to 192 µM Cu treatment (Sanchez *et al.*, 2014).

The lower decrease of Fe concentrations in M shoots may explain, at least partially, the lower chlorotic symptoms observed in M plants. Additionally, as Fe is poorly mobile in plant tissues, this deficiency may be dependent on the development stage of the tissues; young leaves exhibiting stronger chlorotic symptoms than old ones. Age of plant tissues affects their content in metals and nutrients, with mature leaves having higher metal-contents than young leaves (Barry and Clark, 1978). Because concentrations were measured on all materials, it will be necessary to measure nutrient concentrations in regard to leaf age to determine whether or not Fe deficiency was stronger in young leaves.

Patterns of K concentrations in roots differed sharply between populations, with an increase in M roots but any change in NM ones, leading to higher [K] in M roots at 50 µM Cu. More precisely, K concentrations increased in NM between 1 and 25 µM but decreased at Cu exposure higher than 25 µM. K was the most abundant cation and the only element more concentrated in M roots at 50 µM. In shoots, [K] increased in both populations, more sharply in NM, which may increase the deficiency in roots. As a major plant nutrient, K regulates opening and closure of stomata and is involved in protein synthesis and photosynthesis (Uchida, 2000). The increasing K content in M plants can reduce water loss from leaves by maintaining correct stomata functioning and correct photosynthesis. On the opposite, the limitation of K uptake and concentrations in NM roots may contribute to the growth reduction of the NM population at Cu exposure superior to 25 µM. Whereas in this study K increases in roots of both populations but only in M shoots, K decreases in shoot and roots of *Matricaria chamomilla* cultivars exposed to 20 µM Cu (Kováčik *et al.*, 2011), which suggest that different strategies exist among species concerning K uptake.

Zn concentrations increased in roots of both populations, but more sharply in NM one resulting in higher values in NM shoots at high Cu exposure. This indicated a lower of both Zn uptake and translocation in M plants, suggesting a better regulation of Zn. Al concentrations decreased in roots of both populations, probably due to Cu/Al competition for root uptake. In shoots, Al concentrations increased in M but did not vary in NM, indicating an enhanced translocation in M plants, which may be involved in the higher tolerance of the M population. An Al deficiency in NM shoots may contribute to impair the photosynthetic process.

Concentration of phosphorus, another major macronutrient needed for plant growth, increased in roots and shoots of both populations, probably reflecting a higher need to maintain correct cell functioning. However, this increase was higher in NM shoots, suggesting a higher need to maintain cell growth and functioning. P is needed in large quantities in young cells, during first stages of cell division and has numerous roles in cell functioning, e.g. in energy storage and transfer (ATP/ADP; NADP/NADPH), in RNA and DNA structures, in phosphorylation/dephosphorylation or cell signaling and as component of nucleic acid, phospholipids, nucleoprotein, and a number of co-enzymes (Uchida, 2000; Vance *et al.* 2003).

Mg is a major part of the chlorophyll molecule and is a cofactor for many enzymatic systems (Uchida, 2000). [Mg] increased in roots and shoots of both *A. capillaris* populations, but were significantly higher in NM shoots at 30 and 50 µM, which indicated a higher uptake and translocation of Mg when Cu exposure increased, more marked in NM at the end of the Cu gradient tested. Increase in Mg concentrations has also been reported in seedling of Hassawi

wheat plants grown on soil under increasing Cu exposure (Azooz *et al.*, 2012). The enhanced accumulation of Mg may aim to counterpart the deleterious effect of Cu on chlorophyll biosynthesis. Mn is involved in the oxidation-reduction process in photosynthesis, in enzyme structure and in photolysis (Uchida, 2000). [Mn] increased in roots and shoots, more markedly in NM plants and were significantly higher in NM shoot, at 25, 30 and 50 µM. Shoot/root ratios ranged from 1 to 3.1 in NM and from 1.9 to 2.8 in M, with lower ratios at low (1 µM) and high exposure (40-50 µM for NM and 50 µM for M), indicating a higher uptake and translocation when Cu exposure increased, particularly in NM, followed by a decrease after a threshold, 30 µM for NM, 40 for M. As for Mg, enhanced accumulation of Mn may aim to restore photosynthesis processes.

5. Conclusion

Cu impacted plant growth, disturbed root architecture and induced chlorotic symptoms in both populations. However, these symptoms were more marked in NM plants, indicating a higher tolerance of the M population in this range of Cu exposure. The higher tolerance of M plants was confirmed by the response of growth parameters, i.e. shoot length and fresh and dry weight yields, which all decreased sharply in NM but did not vary or slightly decreased in M plants.

Shoot/roots ratios of Cu concentrations indicated Cu storage in roots and limitation of Cu transport to aerial parts, confirming the "excluder" phenotype for both populations of *A. capillaris*, but also a reduction of this translocation as Cu exposure rose.

Based on the evaluation of Cu concentrations in both populations, some mechanisms potentially supporting the higher Cu tolerance of the M population may be suggested. The measure of Cu concentrations in root tissues pointed out a triphasic response depending on intensity of Cu supply, low (1-20 µM) intermediate (25-30 µM) and high Cu exposure (40-50 µM). The possibility of a reduced cu accumulation in M roots was refuted at low (1-20 µM) and high (40-50 µM) Cu exposure by the determination of Cu concentrations in roots, which did not differ between populations. However, at intermediate Cu exposure (25-30 µM Cu), lower Cu concentrations and higher biomass of M plants resulted in similar mineralomasses between populations. This suggested a similar uptake but a dilution of Cu in tissues through an increase of root biomass production at intermediate Cu excess. These results also suggested a better efficiency to cope with Cu toxicity and to maintain root growth and functions.

Existence of a reduced Cu translocation from roots to shoots was excluded in case of the higher Cu tolerance of the M population, as Cu concentrations were either similar or higher in M leaves. On the contrary, this supported the existence of a better efficiency of M leaves to cope with the deleterious effects of Cu excess, and even more suggested a high need for Cu in this population.

Cu altered root and shoot ionomes of both populations. In particular, Fe concentrations in roots did not vary among the Cu exposure but decreased in shoot of both populations, indicating Fe deficiency in shoots under Cu excess but also a probable deficiency in roots as an increasing need in Fe may not be satisfied without an increase of Fe uptake. As Zn increased in roots and shoots of both populations, the chlorotic effect was rather attributed to Fe than Zn deficiency. Regulation of Ca, Na and Al foliar concentrations appeared to be involved in the enhanced Cu-tolerance of the M population. The increasing Ca uptake was lower in M roots, enabling a lower root-to-shoot translocation and lower Ca concentrations in shoots.

Na uptake was reduced in both M and NM roots, but a better ability to accumulate Na in roots at intermediate Cu excess and a smaller root-to-shoot translocation were specifically observed in M plants. Al translocation increased in M plants but did not vary in NM ones, which may induce Al deficiency in this population. K concentrations increased in NM roots between 1 and 25 µM but decreased at Cu exposure superior to 25 µM, while they increased linearly in M roots, indicating a limitation of K uptake in NM roots at Cu higher than 25 µM. The probable K deficiency in roots was confirmed by the higher K translocation from roots to shoots. As a major plant nutrient, such limitation of K uptake may contribute to growth reduction in the NM population at Cu exposure higher than 25 µM, while the higher translocation may reflect a higher need of P.

CHAPTER IV: Characterization of root soluble proteome

Characterization of root soluble proteome in Metallicolous and Non-Metallicolous populations of *Agrostis capillaris* L. exposed to Cu.

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Abstract

Both metallicolous (M) and non-metallicolous (NM) populations of *Agrostis capillaris* L. were used to deeply investigate the differential accumulation of root soluble proteins in response to increasing Cu stress. Plants were germinated and cultivated 3 months on perlite moistened with a $CuSO_4$ spiked-nutrient solution to obtain a Cu exposure series (1, 5, 10, 15, 20, 25, 30, 40 and 50 µM Cu). Root soluble proteins extracted by the trichloroacetic acid/acetone procedure were separated using 2-DE (linear 4-7 pH gradient). Gels were CCB-stained, image analysis performed using PDQuest, and proteins identified by LC-MS/MS.

Some proteins did respond to Cu in both populations, but most proteins indicated higher Cu-induced damages in NM roots. In both populations, energy metabolism was altered, as shown by the up-regulation of a G3PDH and several formate dehydrogenases, but the down-regulation of ATP synthase subunit alpha. This indicated a higher need in reducing power (NADH); a reduced ATP production/ H^+ transport and an increased cellular respiration.

In NM roots, limitation of G3PDH accumulation at high Cu concentrations in nutrient solution (30-50 µM Cu), in line with the over-expression of phosphoglucomutase only at low and intermediate concentrations (1-25 µM Cu), indicated a limited glycolysis process at Cu concentrations higher than 25 µM. Additionally, higher alteration of mitochondrial activity and protein metabolism in NM roots were respectively suggested by the strong down-regulation of

proteins involved in the Krebs cycle, i.e. aconitases, succinate dehydrogenase, NADH dehydrogenase Fe/S protein and V-type proton ATPase, and the up-regulation of several protein chaperones, i.e. CPN60-1, CPN60-2 and PDI.

On the opposite, M roots did not exhibit any limitation of G3PDH accumulation at high Cu exposure, which may provide a constant source for NADH production. Additionally, the up-regulation of an alpha-galactosidase together with the over-expression of a sucrose:sucrose 1-fructosyltransferase and a 6-phosphofructokinase pyrophosphate-dependent at intermediate Cu exposure suggested that several carbohydrate-related enzymes cooperated together to maintain the supply of glycolysis and Krebs cycle under Cu stress. Potential accumulations of malic and citric acids were pointed out by the up-regulation of MDH and IDH only in M roots, which may contribute to chelate free Cu in cells. Moreover, over-expression of a HSP70 at intermediate and high Cu exposures may be a key player in Cu-tolerance in protecting protein metabolism, while induction of two proteasome subunits and a Phytepsin, together with the over-expression of a peptidase at almost all Cu exposure, supported a better proteolysis process.

The induction of S-adenosylmethionine synthetase (SAMS) by Cu stress in both populations suggested increasing SAM accumulation. SAM may have a pivotal role in plants stress response in stimulating nicotianamine (NA) and glutathione (GSH) production, but also ethylene synthesis. Down-regulation of methionine synthase only in NM roots, leading to higher accumulation in M roots at high Cu level, may reflect a better ability of Cu-stressed M root cells to maintain methionine biosynthesis. Cysteine synthase was specifically induced in NM roots, indicating a higher need for cysteine to process chelation mechanisms including binding of free Cu. Over-expression of ascorbate peroxidase and glutathione-S-transferase may contribute to enhance antioxidative and detoxification mechanisms in M roots, while increase in aldehyde dehydrogenase accumulation only in M roots may allow a better degradation of potentially toxic aldehydes.

1. Introduction

Previous work on two media, i.e. a Cu-contaminated soil series and Cu-spiked perlite series (1-30 µM Cu), have indicated that Cu-stressed M plants have higher fitness and lower chlorotic symptoms (Hego *et al.*, 2014). In a preliminary proteomic experiment, accumulation of root soluble proteins has depend on both the Cu exposure (in the 1-30 µM Cu range) and the population origin (Bes, 2008; Hego *et al.* 2014, Chapt. II). As the M population originated from the Cu-contaminated soil may have evolved molecular mechanisms enabling their survival, these populations represent a relevant tool to examine the mechanisms underlying Cu-tolerance. There is a lack of knowledge on these mechanisms in grassy species with 'excluder' phenotypes such as *A. capillaris*. At the plant level, a limitation of Cu uptake and accumulation by roots is not clearly identified and may depend on the level of Cu exposure, but a higher ability to cope with Cu toxicity in tissues is strongly suggested.

Cu, as essential micronutrient with redox properties, is a cofactor for several metallo-enzymes and needs to be strictly controlled for proper uptake, delivery and storage (Burkhead *et al.*, 2009). Plants have evolved several mechanisms to deal with metal toxicity, including reduction of metal influx in cells, exclusion, compartmentation, and chelation by organic ligands, such as organic acids, amino acids, proteins, and peptides (Cobbett and Goldsbrough 2002; Yruela 2009), as well as more efficient quenching of ROS, and better detoxification and repair mechanisms (Yruela, 2005). As differences in efficiency of Cu homeostasis and detoxification processes may explain the higher Cu tolerance of metallicolous individuals, proteomic tools could give new pieces of evidence to better understand the molecular mechanisms underlying Cu tolerance in plant roots.

Temporal root responses to Cu exposure are reported at a proteomic level, e.g. in four-week-old *Elsholtzia splendens* plants exposed to 100 µM Cu for 3 or 6 days (Li *et al.*, 2009), in 10-day old seedlings of *Phaseolus vulgaris* exposed to 15 or 50 µM Cu for 7 days (Cuypers *et al.*, 2005) and in pre-germinated seedlings of *Oryza sativa*, grown for 7 days in common nutrient solution (0.32 µM Cu) and then exposed to 8 µM Cu for 3 days (Song *et al.*, 2013). However, these studies did focus on plants grown in common conditions and then short-time exposed to Cu. Few data exist for long term Cu exposure and chronic exposure from germination to harvest. Long-term Cu exposure has been studied in roots of *Cannabis sativa* seedlings, exposed to 150 mg/L $CuSO_4$ for six weeks, after germination in metal-free solution (Bona *et al.*, 2007), but this experiment has included only two conditions, Cu-free and one Cu exposure. In roots of *P. vulgaris* seedlings, five protein spots varying in response to Cu treatment belong to the PR-10 family (Cuypers *et al.*, 2005), whereas in *E. splendens* roots, the

45 protein spots, either down- or up-regulated by Cu stress, are involved in many cellular processes such as energy metabolism signal transduction, regulation of transcription, translation, redox homeostasis and cell defense (Li *et al.*, 2009).

Data are available on proteomic characterization of *A. capillaris* shoot response to arsenic and arsenate, in plants grown for one month in As-free conditions and then short-term exposed for 8 days (Duquesnoy *et al.*, 2009), and on proteomic analysis of differential heat-response between heat-tolerant *Agrostis scabra* and heat-sensitive *Agrostis stolonifera* (Xu and Huang, 2008, 2010a). However, to our knowledge, *Agrostis capillaris* response to Cu exposure has not yet been characterized at a proteomic level. Proteomic characterization of metal-stress in *Agrostis* populations differing by their metal tolerance has only been explored by Hego et al (2014). However, a similar approach has compared roots of populations, genotypes and cultivars exhibiting large difference in metal tolerance: e.g. in *O. sativa* varieties exposed to 8 µM Cu for 3 days (Song *et al.*, 2013), in *Glycine max* cultivars exposed to 10 µM Al for 6, 51 or 72 hours (Duressa *et al.*, 2011), and in *Hordeum vulgare* cultivars and genotypes exposed to 0, 50 or 200 µM Al for 3 days (Dai *et al.*, 2013). Most findings indicate implication of proteins related to carbohydrate/energy metabolism, sulfur metabolism, mainly GSH, and antioxidative enzymes.

In this work, long-term Cu exposure was chosen preferentially to short-term Cu exposure. Plant exposure started from germination to harvest and a series of nine Cu exposure levels was tested. This aimed at investigating, using proteomic approach, changes in the soluble root proteome of *A. capillaris* M and NM plants in response to chronic Cu-exposure in the 1-50 µM range for a 3-month period, notably to unravel molecular mechanisms underlying higher Cu tolerance in the M population.

2. Materials and Methods

2.1. Plants and Cu treatments

Seeds of metallicolous (M) and non-metallicolous (NM) populations were respectively collected from *A. capillaris* L. growing at a wood preservation site contaminated by Cu (Bes and Mench 2009; Mench and Bes 2009; Bes *et al.*, 2010) and at a forest edge (RN10, Km 83, Belin Beliet, Gironde, France) in August-September 2011. Phenotypes of M and NM populations have previously characterized on a Cu-contaminated soil series obtained with the fading technique and on Cu-spiked perlite moistened with Hoagland nutrient solution in the 1-30 µM Cu range (Bes, 2008).

Seeds were sowed and plants cultivated for three months on perlite constantly bottom moistened with Hoagland n°2 nutrient solution (Hewitt, 1966) containing 1, 5, 10, 15, 20, 25, 30, 40 and 50 µM Cu (added as $CuSO_4$), weekly changed. Moistened perlite was preferred to hydroponics for maintaining root ultra-structure and Si nutrition closer to soil conditions (Lux, 2010). Seeds were germinated under natural light in plastic pots (15 x 12 x 8 cm). After 28 days, plants were transferred in a growth chamber with a 14h, 27°C day and a 10h, 22°C night regime, with 220-240µmol photons $m^{-2}.s^{-1}$ light intensity and 65-75% relative humidity. After a 3-month period of growth plants were harvested by removing perlite from roots with milliQ water. For each experimental condition (i.e. Population x Cu concentration), 3 replicates were selected randomly out of a set of 6 (previously phenotypically characterized) for the proteomic experiment. For each replicate, several root aliquots (1g FW) were composed by mixing samples taken in the median part of plant roots, frozen in liquid nitrogen and stored at -80°C.

2.2. Protein extraction, quantification and separation

For all aliquots (1g FW, n = 54), frozen tissues were ground in a small mortar and pestle in liquid nitrogen. Total protein was extracted following the trichloroacetic acid/acetone procedure described by Damerval *et al.*, (1986) and modified by Gion *et al.*, (2005). Soluble proteins were re-solubilized in "TCT" buffer (i.e. 7 M urea, 2 M thiourea, 0.4% v/v Triton X-100, 4% w/v CHAPS detergent, 10 mM DTT, and 1% v/v IPG buffer) for one hour at room temperature. Samples were then centrifuged (4 min, 2 000rpm, 20°C) and stored at -80°C. Protein content was determined in triplicates for each extract using a modified Bradford assay (Ramagli *et al.*, 1985). Protein extracts were stored at -80°C for the subsequent 2-DE steps.

For the isoelectric focusing step (IEF), 24 cm immobilized pH gradients (IPG) strips (Immobiline DryStrip, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used with a linear pH gradient ranging from 4 to 7. A mix containing 450 µg of total soluble proteins, re-suspended into 470 µL of "TCT" solution, was used to rehydrate passively acidic strips for 1h at room temperature prior to the IEF run. The IPGphor system (Amersham Biosciences, Uppsala, Sweden) was programmed at 30 (12 h), 500 (1 h), 1000 (1 h) and finally, at 8000 V/h to achieve a total of 64 000 V/h. Strips were equilibrated in two steps with an equilibration solution (50 mM TRIS-HCl, 6 M urea, 2% SDS, 30% glycerol, bromophenol blue) and Dithiothreitol (DTT, 50 mM) and stirred for 15 min. Iodoacetamide (125 mM) was added and the mixture was stirred for additional 15min. SDS-PAGE was carried out on batches of six or twelve gels per stage of development in a buffer (25 mM Tris, 0.2 M glycine, 0.1% SDS) at 30 W for 30 min, then at 90 W. The gels were then stained with colloidal blue (Coomassie Blue G-250). Triplicates were performed for the 18 conditions, resulting in a total of 54 gels.

2.3. Image analysis and spots detection

2D-gels were scanned (GS-800 Imaging densitometer; Bio-Rad). The alignment of 30 gel images, spot detection, quantification and pairing were carried out using PDQuest Advanced (v 8.0.1). Protein spots (referred for ease thereafter as spots) were automatically detected and manually corrected if necessary. For each spot, the volume was computed with background subtraction, normalized to the total volume in the gel image and expressed in %Vn. The 30 image gels were automatically aligned according to landmark spots manually selected. Spots were matched and manually corrected if necessary (Vilain *et al.*, 2004).

2.4. Statistical analysis

In this experiment, Cu was considered as a continuous variable to include the "dose" notion in the analysis. To characterize the response of each population across the range of Cu exposures, Pearson's correlation was used between spot dataset of each population (M and NM) and Cu exposure (1-50 µM). Statistical analyses were conducted on R v2.11.1 (R Foundation for Statistical Computing; Vienna, Austria) and alpha error was fixed at 0.1 because of inter-replicates variability. A clustering analysis of spot volumes was conducted on GENESIS software (v. 1.7.6).

As replicate number was too low to perform Student's tests, differential expression between M and NM populations at each Cu exposure (1-50 µM) was estimated using ratios between mean values of each population. Protein spots from M and NM populations, cultivated at the same Cu exposure (1-50 µM), were considered to display significant differences if they fulfilled the following criteria:

(i) over-expression in M population compared to NM one:

$(M_{mean} + SE_M) / (NM_{mean} - SE_{NM}) < 0.7$ and $(M_{mean} – SE_M) / (NM_{mean} + SE_{NM}) < 1.5$

(ii) over-expression in NM population compared to M one:

$(M_{mean} + SE_M) / (NM_{mean} - SE_{NM}) > 0.7$ and $(M_{mean} – SE_M) / (NM_{mean} + SE_{NM}) > 1.5$

In which M_{mean} and NM_{mean} represent average spot volumes (n = 2 or n = 3) and SE_M and SE_{NM} are standard errors on the M_{mean} and NM_{mean} respectively. The 1.5-fold ratio for significant spot alteration have been arbitrarily chosen from comparison with other proteomic studies on Cu-tolerance (Li *et al.*, 2009; Ritter *et al.*, 2010; Song *et al.*, 2013). Ratios were calculated using Excel (Word), graphical figures were obtained on R then modified with Power Point (Word).

2.5. Protein identification by mass spectrometry

Most spots were automatically excised using "Spotcutter" (EXQuest, Bio-Rad pieces of 0.5 mm Ø and with three pieces maximum for large spots). Few ones not present in the gel part automatically cut were manually excised. Spots were rinsed twice in ultrapure water, and shrunk in Acetonitrile (ACN) for 10 min. After ACN removal, gel pieces were dried at room temperature, rehydrated in 10 ng/µL trypsin solution (T6567, Sigma-Aldrich) in 50 mM ammonium bicarbonate, and incubated overnight at 37°C. Hydrophilic peptides were extracted with 40 mM ammonium bicarbonate containing 10% ACN at room temperature for 10 min. Hydrophobic peptides were extracted with 47% v/v ACN and 5% v/v formic acid, and this extraction step was repeated twice. All three supernatants were pooled together, concentrated in a vacuum centrifuge, and acidified with 0.1% formic acid before nanoLC-MS/MS analysis (Gion *et al.*, 2005).

Peptide mixtures were analyzed by on-line capillary nanoHPLC (LC Packings, Amsterdam, The Netherlands) coupled to a nanospray LCQ Deca XP ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA). Ten microliters of each peptide extract were loaded on a 300 µm ID x 5 mm PepMap C_{18} precolumn (LC Packings, Dionex, USA) at a flow rate of 20 µL/min. After 5 min desalting, peptides were online separated on a 75 µm internal diameter x 15 cm C18 PepMapTM column (LC Packings, Amsterdam, The Netherlands) with a 5-40% linear gradient of solvent B in 48 min (solvent A was 0.1% formic acid in 5% ACN, and solvent B was 0.1% formic acid in 80% ACN). The separation flow rate was set at 200 nL/min. The mass spectrometer operated in positive ion mode at a 1.8kV needle voltage and a 34V capillary voltage. Data acquisition was performed in a data-dependent mode alternating in a single run, a MS scan survey over the range m/z 300–1700 and three MS/MS scans with Collision Induced Dissociation (CID) as activation mode. MS/MS spectra were acquired using a 2 m/z unit ion isolation window, a 35% relative collision energy, and a 0.5 min dynamic exclusion duration (Gion *et al.*, 2005).

Mascot and Sequest algorithms through Proteome Discoverer 1.4 Software (Thermo Fisher Scientific Inc.) were used for protein identification in batch mode by searching against two constructed databases. The first was constructed with ESTs from NCBI (http://www.ncbi.nlm.nih.gov/) from *Agrostis* spp., including *A. capillaris*, *A. stolonifera*, *A. stolonifera* var. *palustris* and *A. scabra,* and resulted in 123,605 sequences translated in six reading frames by TRANSEQ software (http://www.ebi.ac.uk/Tools/emboss/transeq/). The second database contained all protein sequences from *Viridiplantae* UniProt Database (31,395 entries, release 2013_09, http://www.uniprot.org/).

Two missed enzyme cleavages were allowed. Mass tolerances in MS and MS/MS were set to 2 Da and 1 Da. Oxidation of methionine was searched as variable modifications and carbamidomethylation on cysteine was searched as fixed modification. Peptide validation was performed using Percolator algorithm (Käll *et al.*, 2007) and only "high confidence" peptides were retained corresponding to a 1% False Positive Rate at peptide level. A minimum of two different peptides was considered for protein validation. EST annotations were identified by searching with a protein Viridiplantae index from Swiss-Prot (BLASTX) and TrEMBL (BLASTX) database using UniProtKB (http://www.uniprot.org).

3. Results

For convenience and to shorten the text 'M roots' was abbreviated in this chapter by M and 'NM root's by NM, if no additional indication is provided

3.1. Spot detection on 2D-gels and statistical analyzes

Due to the high number of experimental conditions (18) the image analysis was made on 54 2D-gels (triplicates), and only 419 spots were accurately delimited (Fig. 1, all gel images are available in the Annex 8). To characterize the differential expression of protein spots across experimental conditions, a global hierarchical clustering (Fig. 2) was first applied on total data then Pearson's Correlations were computed for each population to focus on the Cu effect, i.e. effect of Cu exposure on protein expression. To study the population's origin effect, i.e. differential expression between M and NM populations, ratios were calculated between M and NM mean values. Summary of statistical tests for the 419 spots are shown in Tab.1 and more data are available in Annex 9 (graphs: Variation of protein expression among Cu exposure for M and NM plants; table of mean values ± sd; summary of identification and statistical tests).

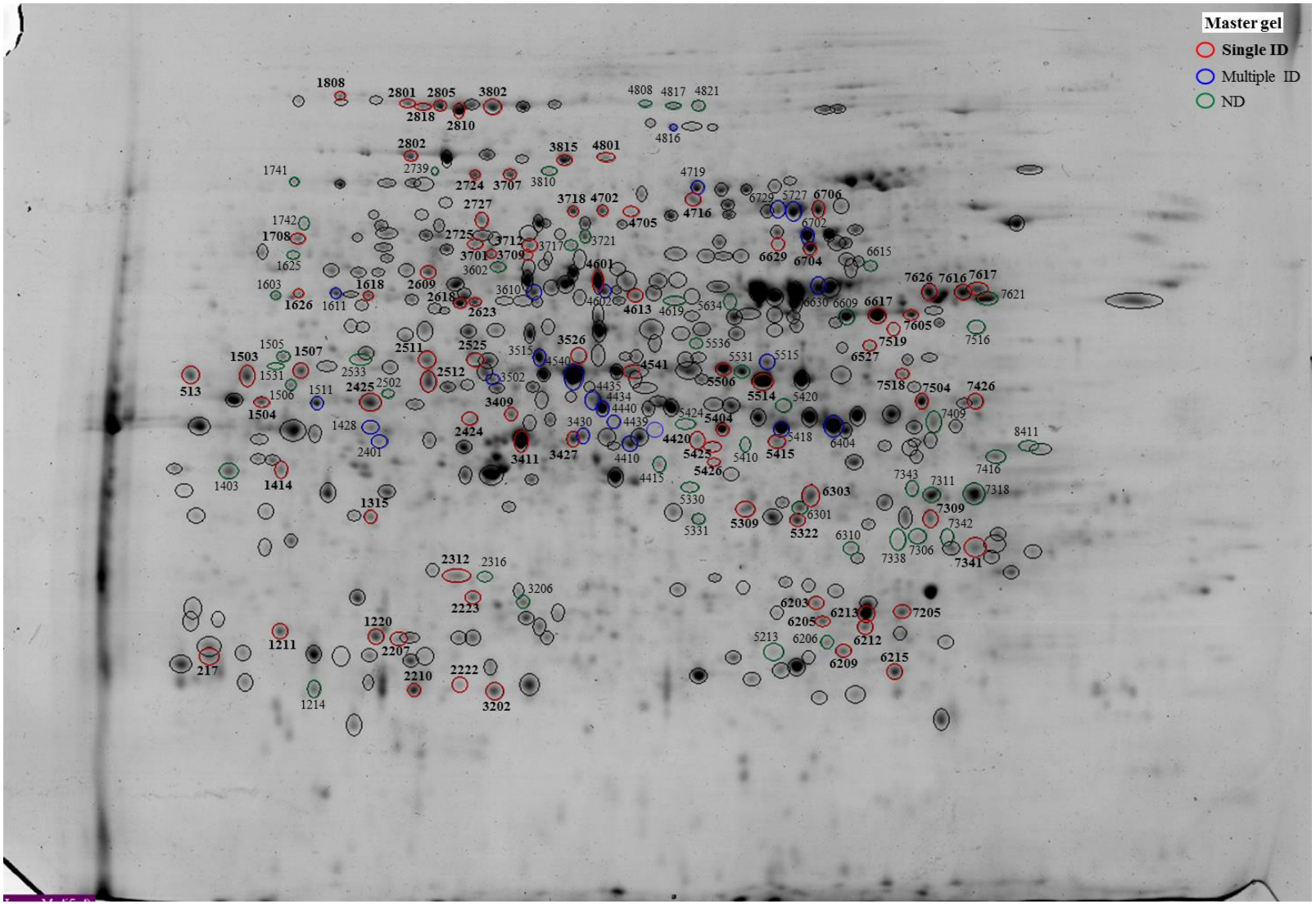


Figure 1: Reference gel (10%) showing the distribution of protein spots from *Agrostis capillaris* roots, with location of the 87 spots selected for identification by mass spectrometry. Spots circled in green remained unidentified, those in purple matched to 2 or 3 different identifications, and those in red corresponded only to one or very similar identification (#3427 and 3707).

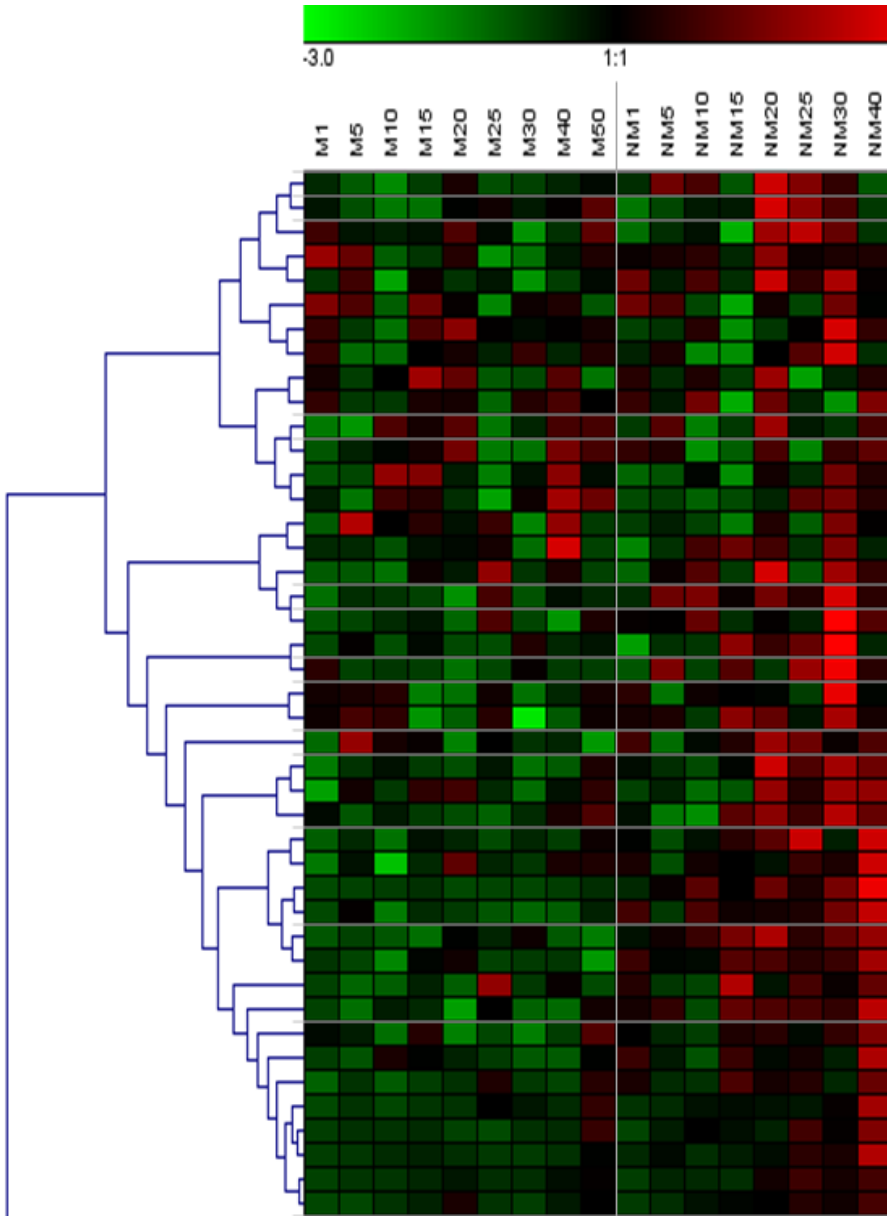
Table 1. Results of statistical tests for the 419 accurately quantified spots. Sp: spots number; rM/rNM: significance level of the Pearson's correlation for population referring to p-val = 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; R1-50: significance of comparative ratio between populations values at each Cu exposure, -: no difference, M/NM indicated the population with higher values based on ratio > 1.5.

Sp	rM	rNM	R1	R5	R10	R15	R20	R25	R30	R40	R50	Sp	rM	rNM	R1	R5	R10	R15	R20	R25	R30	R40	R50
214	-	↗	-	-	-	-	-	-	-	-	-	4435	-	↗↗	-	-	-	-	-	-	-	-	-
215	-	-	-	-	-	-	-	-	-	-	-	4439	↘↘	-	NM	NM	NM	NM	NM	NM	NM	-	NM
217	↘	-	M	M	M	M	-	M	M	M	-	4440	↗↗↗↗	↗↗↗↗	-	-	-	NM	-	-	-	-	-
218	-	-	-	-	-	-	-	-	-	-	-	4504	-	-	-	-	-	-	-	-	-	-	-
220	-	-	-	-	-	-	-	-	-	-	-	4505	-	-	-	-	-	-	-	-	-	-	-
314	-	-	-	-	-	-	-	-	M	-	-	4508	-	-	-	NM	-	-	-	-	-	-	-
322	-	-	-	-	-	-	-	-	-	-	-	4510	-	-	-	-	-	-	-	-	-	-	-
412	-	-	-	-	-	-	-	-	-	-	-	4512	-	-	-	-	-	-	-	-	-	-	-
414	-	-	-	-	-	M	-	-	-	-	-	4514	-	-	-	-	-	-	-	-	-	-	-
513	↗↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-	4516	-	-	-	-	-	-	-	-	-	-	-
1206	-	-	-	-	-	-	-	-	-	-	-	4518	↘	-	-	-	-	-	-	-	-	-	-
1207	-	-	-	-	-	-	-	-	-	-	-	4521	↗	-	-	-	-	NM	-	-	-	-	-
1211	↘↘↘↘	↘↘↘↘	-	-	-	-	-	-	-	-	-	4526	-	↘	-	-	-	-	-	-	-	-	-
1213	-	-	-	-	-	-	-	-	-	-	-	4527	-	-	-	-	-	-	-	-	-	-	-
1214	↘↘	-	-	-	-	M	-	-	-	-	-	4528	↗	-	-	-	-	-	-	-	-	-	-
1215	-	-	-	-	-	-	-	-	-	-	-	4533	-	-	-	-	-	-	-	-	-	-	-
1216	-	-	-	-	-	-	-	-	-	M	-	4538	-	-	-	-	-	-	-	-	-	-	-
1218	-	-	-	-	-	-	-	-	-	-	-	4540	↗↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-
1220	↘↘↘	↘↘↘↘	-	-	-	-	-	-	-	-	-	4541	↗	↗↗	-	-	-	-	-	-	-	-	-
1227	-	-	-	-	-	-	-	-	-	-	-	4601	↘↘	-	-	-	-	-	-	-	-	-	-
1229	-	-	-	-	-	-	-	-	-	-	-	4602	↗↗↗	-	-	-	-	-	-	-	-	-	-
1302	-	-	-	-	-	-	-	-	-	-	-	4607	-	-	-	-	-	-	-	-	-	-	NM
1306	-	-	-	-	-	-	-	-	-	-	-	4608	-	↘	-	-	-	-	-	-	-	-	-
1309	-	-	-	-	-	-	-	-	-	-	-	4610	-	-	-	-	-	-	-	-	-	-	-
1311	-	-	-	-	-	-	-	-	-	-	-	4613	-	↘↘	-	-	-	-	-	-	-	-	-
1315	↗↗	-	-	-	-	-	-	-	-	-	-	4614	↗	-	-	-	-	-	-	-	-	-	-
1328	-	↘	-	-	-	-	-	-	-	-	-	4615	-	-	-	-	-	-	-	-	-	-	-
1403	-	-	-	-	-	-	-	-	-	M	-	4619	-	-	-	-	-	-	M	-	-	-	-
1408	-	-	-	-	-	-	-	-	-	-	-	4621	-	-	-	-	-	-	-	-	-	-	-
1410	-	-	-	-	-	-	-	-	-	-	-	4630	-	-	-	-	-	-	-	-	-	-	-
1413	-	-	-	-	-	-	-	-	-	-	-	4631	-	-	-	-	-	-	-	-	-	-	-
1414	-	↘	-	-	-	-	-	-	-	M	-	4632	-	-	-	-	-	-	-	-	-	-	-
1415	-	↗	-	-	-	-	-	-	-	-	-	4702	-	↘↘↘↘	-	-	-	-	-	-	-	-	-
1416	-	-	-	-	-	-	-	-	-	-	-	4704	↗	-	-	-	-	-	-	-	-	-	-
1428	↗↗	-	-	-	-	-	-	-	-	-	-	4705	-	↘↘	NM	NM	-	NM	-	NM	-	-	-
1502	-	-	-	-	-	-	-	-	-	-	-	4709	-	-	-	-	-	-	-	-	-	-	-
1503	↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-	4714	-	-	-	-	-	-	-	-	-	-	-
1504	-	↗↗	-	-	-	-	-	-	-	-	-	4715	-	-	-	-	-	-	-	-	-	-	-
1505	-	↘↘↘	-	-	-	-	-	-	-	-	-	4716	-	-	-	-	M	-	-	M	M	M	-
1506	↘↘	-	-	-	-	-	-	-	-	-	NM	4719	-	↘↘↘↘	-	-	-	-	-	-	-	-	-
1507	-	↗↗↗↗	-	-	-	-	-	-	-	-	-	4801	-	↘↘↘	-	-	-	-	-	-	-	-	-
1511	-	↗↗↗↗	M	M	-	-	M	-	-	-	-	4808	↘↘	↘↘↘↘	-	-	-	-	-	M	-	-	NM
1513	-	-	NM	-	-	-	-	-	-	-	-	4809	-	-	-	-	-	-	-	-	-	-	-
1519	-	-	-	-	-	-	-	-	-	-	-	4816	↗↗	-	-	-	-	-	-	-	-	-	-
1521	↘	-	-	-	-	-	-	-	-	-	-	4817	-	↘↘↘↘	-	-	-	-	-	M	-	-	-
1522	-	-	-	-	-	-	-	-	-	-	-	4820	-	-	NM	-	-	-	-	-	-	-	-
1531	-	-	-	M	-	-	-	-	-	-	-	4821	-	↘↘	-	-	-	-	-	M	-	-	-
1603	-	-	-	-	NM	-	-	-	-	-	-	5205	-	↗	-	-	-	-	-	-	-	-	-

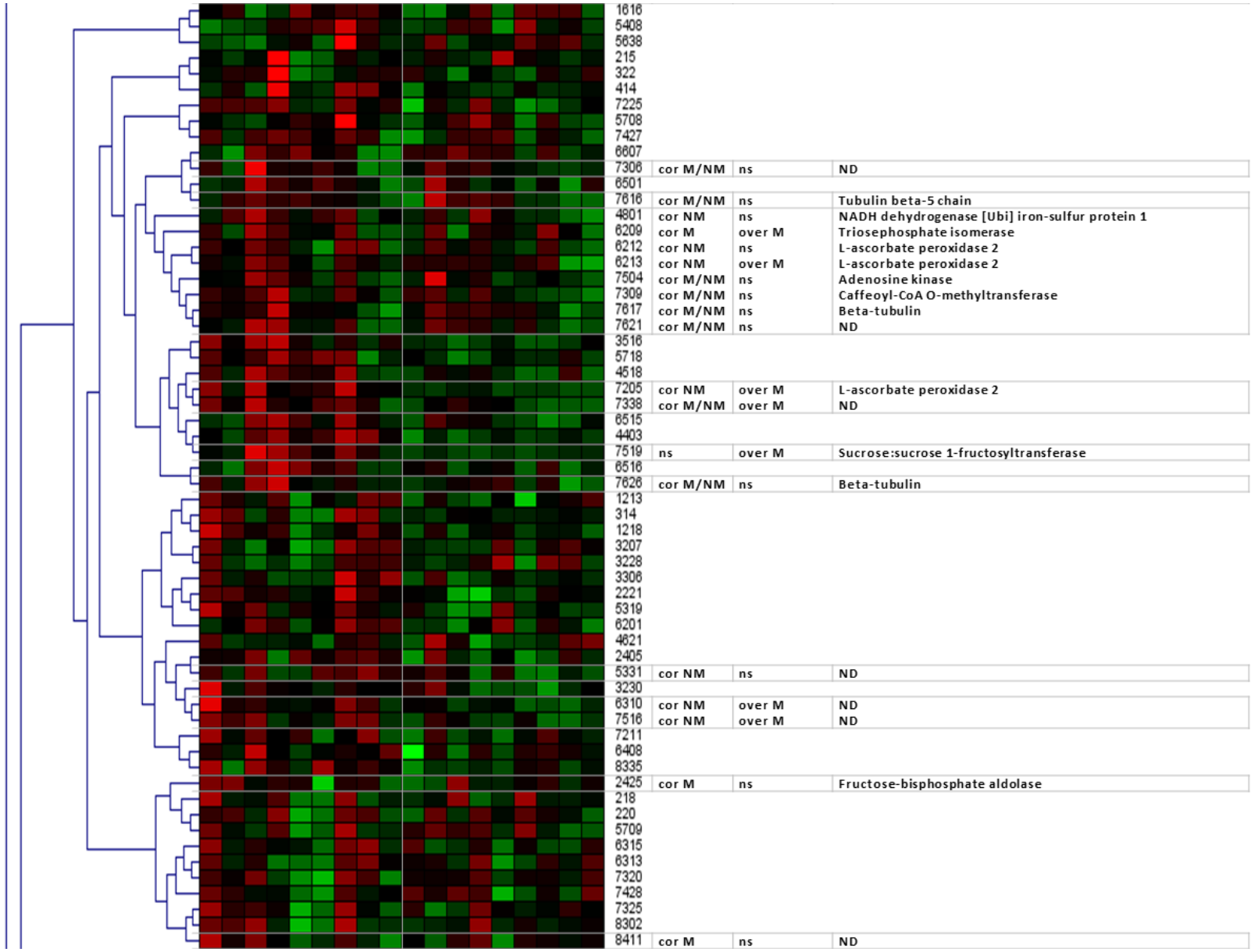
1610	-	-	-	-	-	-	-	-	-	-	-	5208	-	-	-	-	-	-	-	-	-	-	-	-	-
1611	-	↘↘	-	-	-	-	-	-	-	-	-	5213	-	-	-	-	-	-	-	M	-	-	-	-	-
1615	-	-	-	-	-	-	-	-	-	-	-	5217	-	↗	-	-	-	-	-	-	-	-	-	-	-
1616	-	-	-	-	-	-	-	-	-	-	-	5221	-	-	-	-	-	-	-	-	-	-	-	-	-
1617	-	-	-	-	-	-	-	-	-	-	-	5222	-	↗	-	-	-	-	-	-	-	-	-	-	-
1618	-	-	M	M	M	M	M	M	-	M	M	5301	-	-	-	-	-	-	-	-	-	-	-	-	-
1625	↘↘↘	↘	-	-	-	-	-	-	-	-	-	5309	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
1626	↘↘↘	-	-	-	-	-	M	-	-	-	-	5316	-	-	-	-	-	-	-	-	-	-	-	-	-
1703	-	-	-	-	-	-	M	-	-	M	-	5318	-	-	-	-	-	-	-	-	-	-	-	-	-
1708	↘	-	-	M	-	-	-	M	M	-	-	5319	-	-	-	-	-	-	-	-	-	-	-	-	-
1716	-	-	-	-	-	-	-	-	-	-	-	5322	↘↘↘	↘	-	-	-	-	-	-	-	-	-	-	-
1719	-	-	-	-	-	-	-	-	-	-	-	5330	↗↗	↗↗	-	-	-	-	-	NM	-	NM	-		
1725	-	-	-	-	-	-	-	-	-	-	-	5331	-	↘↘	-	-	-	-	-	-	-	-	-	-	-
1741	-	-	-	M	-	-	M	M	-	-	-	5403	-	-	-	-	-	-	-	-	-	-	-	-	-
1742	-	↘↘	-	-	-	-	-	-	-	-	-	5404	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
1803	↘	-	-		-	-	-	-	NM	-	-	5407	↘	-	-	-	-	-	-	-	-	-	-	-	-
1808	↘↘	-	-	M	-	-	-	-	-	-	-	5408	-	-	-	-	-	-	-	-	-	-	-	-	-
1813	↘	-	-	M	-	-	-	-	-	-	-	5410	-	↘↘↘	-	-	-	-	-	-	-	-	-	-	-
1817	-	-	-		-	-	-	-	-	-	-	5412	-	-	-	-	-	-	-	-	-	-	-	-	-
2207	-	-	-	-	-	-	-	-	-	-	M	5415	↘↘↘↘	-	-	-	-	-	-	-	-	-	-	-	-
2208	-	-	-	-	-	-	-	-	-	-	-	5418	-	↗↗	-	-	NM	-	-	-	-	NM	-		
2209	-	-	-	-	-	-	-	-	-	-	-	5420	↗↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	-
2210	↗	↗↗↗	-	-	-	-	-	-	-	-	-	5424	-	↘↘	NM	-	-	-	-	NM	NM	-	-		
2213	↗	-	-	-	NM	-	-	-	-	-	-	5425	-	↗↗↗	-	-	M	-	-	-	-	NM	-		
2221	-	-	-	-	M	M	-	-	-	-	-	5426	↗	↗↗↗↗	-	-	M	-	-	-	NM	-	NM		
2222	↗↗	-	-	-	-	-	-	-	-	-	-	5506	↗↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	-
2223	↗↗↗	↗↗	-	-	-	-	-	-	-	-	-	5508	-	-	-	-	-	-	-	-	-	-	-	-	-
2224	-	-	-	-	-	-	-	-	-	-	-	5514	-	↘↘	-	-	-	-	-	-	-	-	-	-	-
2232	-	-	-	-	M	-	-	-	-	-	-	5515	↗↗	↗↗	NM	-	-	-	-	NM	-	-	NM		
2307	-	-	-	-	-	-	-	-	-	-	-	5531	↗↗	-	-	-	-	-	-	-	-	-	-	-	-
2312	-	↗↗↗↗	-	-	-	-	-	-	-	-	-	5535	-	-	-	-	-	-	-	-	-	-	-	-	-
2316	↗	-	-	-	-	NM	-	-	-	-	M	5536	↗↗↗↗	↗↗↗↗	-	-	-	NM	-	-	-	-	NM		
2319	-	-	-	-	-	-	-	-	-	-	-	5537	-	-	-	-	-	-	-	-	-	-	-	-	-
2401	↗↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-	5603	-	-	-	-	-	-	-	-	-	-	-	-	-
2405	-	-	-	-	-	-	-	-	-	-	-	5607	-	-	-	-	-	-	-	-	-	-	-	-	-
2407	-	-	-	NM	-	-	-	-	-	-	-	5610	-	↘	-	-	-	-	-	-	-	-	-	-	-
2412	-	-	-	-	-	-	-	-	-	-	-	5616	-	↘	-	-	NM	-	-	-	-	-	-	-	-
2413	-	-	-	-	-	-	-	-	-	-	-	5622	↘	-	-	-	-	-	-	-	-	-	-	-	-
2424	↗↗	↗↗↗	-	-	-	-	-	-	-	-	-	5631	↗	-	-	-	-	-	-	-	-	-	-	-	-
2425	↘↘	-	-	-	-	-	-	-	-	-	-	5633	-	↘	-	-	-	-	-	-	-	-	-	-	-
2502	-	-	-	NM	-	-	-	-	-	-	-	5634	-	-	-	-	-	-	NM	-	NM	-	-		
2511	-	↗↗	-	-	-	-	-	-	-	-	-	5637	-	-	-	-	-	-	-	-	-	-	-	-	-
2512	-	↘↘↘↘	-	-	NM	-	-	-	-	-	-	5638	-	-	-	-	-	-	-	-	-	-	-	-	-
2515	-	-	-	-	-	-	-	-	-	-	-	5639	-	-	-	-	-	-	-	-	-	-	-	-	-
2522	-	-	-	-	-	-	-	-	-	-	-	5702	-	-	-	-	-	-	-	-	-	-	-	-	-
2523	↗	-	-	-	-	-	-	-	-	-	-	5703	-	-	-	-	-	-	-	-	-	-	-	-	-
2525	↗↗	↗	-	-	-	-	-	-	-	-	-	5705	-	-	-	-	-	-	-	-	-	-	-	-	-
2532	-	↗	-	-	-	-	-	-	-	-	-	5707	-	-	-	-	-	-	-	-	-	-	-	-	-
2533	↘↘	-	-	-	-	-	M	-	-	-	-	5708	-	-	-	-	-	-	-	-	-	-	-	-	-
2534	-	-	-	-	-	-	-	-	-	-	-	5709	-	↘	-	-	-	-	-	-	-	-	-	-	-
2535	-	-	-	M	-	-	-	-	-	-	-	5712	-	-	-	-	-	-	-	-	-	-	-	-	-
2601	-	-	-	-	-	-	-	-	-	-	-	5716	-	-	-	-	-	-	-	-	-	-	-	-	-
2602	-	-	-	-	-	-	-	-	-	-	-	5718	-	-	-	-	-	-	-	-	-	-	-	-	-
2606	-	-	-	-	-	-	-	-	-	-	-	5719	-	-	-	-	-	-	-	-	-	-	-	-	-
2607	-	-	-	-	-	-	-	-	-	-	-	5727	↗↗	↘↘	-	-	-	-	-	-	-	-	-	-	-

2609	↗↗↗	-	-	-	-	-	-	-	-	-	-	5812	↗	-	-	-	-	-	-	-	-	-	-
2614	-	-	-	-	-	-	-	-	-	-	-	6201	-	-	-	-	M	-	-	-	-	-	-
2617	↗	-	-	-	-	-	-	-	-	-	-	6203	-	-	M	-	-	-	-	-	-	-	M
2618	-	↗↗	-	-	-	-	-	-	-	-	-	6204	-	-	-	-	-	-	-	-	-	-	-
2623	-	-	-	-	-	-	-	-	-	-	M	6205	↘↘	-	-	-	-	-	-	-	-	-	-
2627	-	-	-	-	-	-	-	-	-	-	-	6206	↗↗	↗↗	-	-	-	-	-	-	-	-	-
2628	↗	-	-	-	-	-	-	-	-	-	-	6209	↘↘	-	-	-	M	-	-	-	-	-	-
2629	-	-	-	-	-	-	-	-	-	-	-	6211	-	-	-	NM	-	-	-	-	-	-	-
2701	-	-	-	-	-	-	-	-	-	-	-	6212	-	↘↘	-	-	-	-	-	-	-	-	-
2702	-	-	-	-	-	-	M	-	-	-	-	6213	-	↘↘↘↘	-	-	-	-	-	-	-	M	-
2703	-	-	-	-	-	-	-	-	-	-	-	6215	↗↗↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-
2708	-	-	-	-	-	-	-	-	-	-	-	6219	-	-	-	-	-	-	-	-	-	-	-
2709	-	-	-	-	-	-	-	-	-	-	-	6220	-	-	-	-	-	-	-	-	-	-	-
2710	-	↗	-	-	-	-	-	-	-	-	-	6301	↘↘	↘	-	-	-	-	-	-	-	-	-
2711	-	-	-	-	-	-	-	-	-	-	-	6302	-	-	-	-	-	-	-	-	-	-	-
2716	-	-	-	-	-	-	-	-	-	-	-	6303	-	↗↗↗	-	-	-	-	-	-	-	-	-
2717	-	↗	-	-	-	-	-	-	-	-	-	6308	-	↗	-	-	-	-	-	-	-	-	-
2724	↘↘	↘↘	-	-	-	-	-	-	-	-	-	6310	-	↘↘	-	-	-	-	-	-	M	M	-
2725	↗	-	-	-	NM	-	-	-	-	-	-	6313	-	-	-	-	-	-	-	-	-	-	-
2727	-	-	-	-	-	-	-	-	-	-	M	6315	-	-	-	-	-	-	-	-	-	-	-
2728	-	-	-	M	-	-	-	-	-	-	-	6316	-	-	-	-	-	-	-	-	-	-	-
2739	↘↘	↘↘↘	-	-	-	-	-	-	-	-	-	6401	-	-	-	-	-	-	-	-	-	-	-
2740	-	-	-	M	NM	-	M	-	-	-	-	6404	-	↘↘↘↘	-	-	-	-	-	-	-	-	-
2801	-	↘↘↘	-	-	-	-	-	-	-	-	-	6408	-	-	-	-	-	-	-	-	-	-	-
2802	↘	↘↘↘↘	-	-	-	-	-	-	-	M	-	6409	-	-	-	-	-	-	-	-	-	-	-
2805	-	↘↘↘	-	-	-	-	-	-	-	-	M	6411	-	-	-	-	-	-	-	-	-	-	-
2807	-	-	-	-	-	-	-	-	-	-	-	6415	-	-	-	-	-	-	-	-	-	-	-
2810	↘	↘↘	-	-	-	-	-	-	-	-	-	6501	-	-	-	-	-	-	-	-	-	-	-
2813	-	↘	-	-	-	-	-	-	-	-	-	6515	-	-	-	-	-	-	-	-	-	-	-
2818	-	↘↘	-	M	-	-	-	-	-	-	-	6516	-	-	-	-	-	-	-	-	-	-	-
3202	↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-	6517	-	-	-	-	-	-	-	-	-	-	-
3206	-	↗↗	-	-	-	-	M	-	-	-	-	6527	↘↘	↘	-	-	-	-	-	-	-	-	-
3207	-	-	-	-	-	-	-	-	-	-	-	6535	-	-	-	M	-	-	-	-	-	-	-
3208	-	-	-	-	-	-	-	-	-	-	-	6536	↘	-	-	-	-	-	-	-	-	-	-
3211	-	↗	-	-	-	-	-	-	-	-	-	6537	↘	↘	-	-	-	-	-	-	-	-	-
3228	-	-	-	-	-	-	-	-	-	-	-	6607	-	-	-	-	-	-	-	-	-	-	-
3229	-	-	-	-	-	-	-	-	-	-	-	6609	-	↘↘↘	-	-	-	-	-	-	-	-	-
3230	-	-	-	-	-	-	-	-	-	-	-	6610	-	-	-	-	-	-	-	-	-	-	-
3303	-	-	-	-	M	-	-	-	-	-	-	6612	-	-	-	-	-	-	-	-	-	-	-
3306	-	-	-	-	-	-	-	-	-	-	-	6613	-	↗	-	-	-	-	-	-	-	-	-
3320	↗	-	-	-	-	-	-	-	-	-	-	6615	↘↘↘	-	-	-	-	-	-	-	-	-	-
3403	-	-	-	-	-	-	-	-	-	-	-	6617	-	↘↘↘	-	-	-	-	-	-	-	-	-
3409	↗↗	-	-	-	-	-	-	-	-	-	-	6627	-	-	-	M	-	-	-	-	-	-	-
3411	↗↗	-	-	-	-	-	-	-	-	-	-	6629	-	↗↗↗	-	-	-	-	-	-	-	-	-
3413	-	-	-	-	-	-	-	-	-	-	-	6630	↘↘	-	-	-	-	-	-	-	-	-	-
3418	-	-	-	-	-	-	-	-	-	-	-	6702	↗↗	-	-	-	-	-	-	-	-	-	-
3427	↘↘	↘↘↘	-	-	-	-	-	-	-	-	-	6704	-	↗↗	-	-	-	-	-	-	-	-	-
3430	↘↘↘	↘	-	-	M	M	-	-	-	-	M	6706	-	↘↘↘	-	-	-	-	-	-	-	-	-
3501	-	↗	-	-	-	-	-	-	-	-	-	6710	-	↗	-	-	-	-	-	-	-	-	-
3502	↗↗↗↗	-	-	-	NM	-	-	-	-	-	-	6712	-	-	-	-	-	-	M	-	-	-	-
3504	-	-	-	-	M	-	-	-	-	-	-	6713	-	-	-	-	-	-	-	-	-	-	-
3505	-	-	-	-	-	-	-	-	-	-	-	6715	↘	-	-	-	-	-	-	-	-	-	-
3512	-	-	-	-	-	-	-	-	-	-	-	6729	-	↘↘	-	-	-	-	-	-	-	-	-
3514	-	-	-	-	-	-	-	-	-	-	-	6730	-	-	-	-	-	-	-	-	M	-	M
3515	↘↘	-	-	-	-	-	-	-	-	-	-	6807	-	-	-	-	-	-	-	-	-	-	-

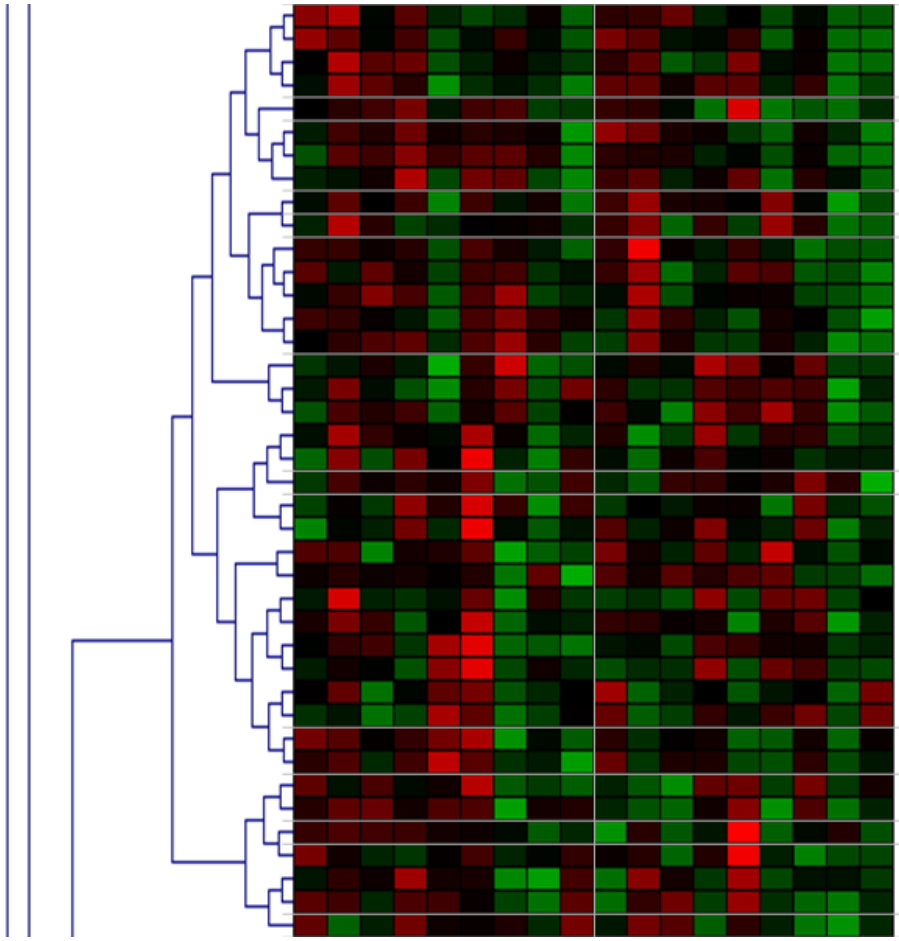
3516	↘	-	-	-	M	M	-	-	-	-	-	6809	-	-	-	-	-	-	-	-	-	-	-
3518	-	-	-	-	-	-	-	-	-	-	-	7205	-	↘↘	-	-	M	-	-	-	M	-	-
3521	-	-	-	-	-	-	-	-	-	-	-	7211	-	-	-	-	-	-	-	-	-	-	-
3524	-	-	-	-	-	-	-	-	-	-	-	7212	↘	-	-	-	-	-	-	-	-	-	-
3526	↗↗	-	-	-	-	-	-	-	-	-	-	7220	-	↘	-	-	-	-	-	-	-	-	-
3528	-	-	-	-	-	-	-	-	-	-	-	7225	-	-	-	-	-	-	-	-	-	-	-
3538	-	-	-	-	-	-	-	-	-	-	-	7303	-	-	-	-	-	-	-	-	-	-	-
3602	↗↗	-	-	-	-	-	-	-	-	-	-	7306	↘↘↘	↘↘↘	-	-	-	-	-	-	-	-	-
3605	-	↗	-	-	-	-	-	-	-	-	-	7309	↘	↘↘↘↘	-	-	-	-	-	-	-	-	-
3607	-	-	-	-	-	-	-	-	-	-	-	7311	↗↗	↗↗↗↗	-	M	-	-	-	-	-	-	-
3609	-	-	-	-	-	-	-	-	-	-	-	7314	-	↘	-	-	-	-	M	-	-	-	-
3610	↗↗	-	-	-	-	-	-	-	-	-	-	7318	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
3611	-	↗	-	-	-	-	-	-	-	-	-	7320	-	-	-	-	-	-	-	-	-	-	-
3613	-	-	-	-	-	-	-	-	-	-	-	7321	-	-	-	-	-	-	-	-	-	-	-
3614	↗	-	-	-	-	-	-	-	-	-	-	7325	-	-	-	-	-	-	-	-	-	-	-
3615	-	-	-	-	-	-	-	-	-	-	-	7338	↘↘	↘↘↘	M	-	-	-	-	-	-	-	-
3620	-	-	-	-	M	-	-	-	-	-	-	7341	↗↗↗	↗	-	-	M	-	-	-	-	-	-
3632	-	-	-	-	-	-	-	-	-	-	-	7342	↘↘↘	↘↘↘↘	-	-	-	-	-	-	-	-	-
3634	-	↗	-	-	-	-	-	-	-	-	-	7343	↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-
3701	↗	↗↗	-	-	-	-	-	-	-	-	-	7403	-	-	-	-	-	-	-	-	-	-	-
3707	-	↘↘	-	-	M	-	-	-	-	-	-	7405	-	-	-	-	-	-	-	-	-	-	-
3709	↗↗↗↗	-	-	-	-	-	-	-	-	M	-	7408	-	-	-	-	-	-	-	-	-	-	-
3712	↗↗	-	-	-	-	-	-	-	-	-	-	7409	-	↗↗↗	-	-	-	-	-	-	-	-	-
3714	-	-	-	-	-	-	-	-	-	-	-	7411	-	-	-	-	-	-	-	-	-	-	-
3716	-	-	-	-	-	-	-	-	-	-	-	7416	↘	-	-	-	-	-	-	-	-	-	NM
3717	↗↗↗	↗↗↗	-	-	-	-	-	-	-	-	-	7425	↘	-	-	-	-	-	-	-	-	-	-
3718	↘	↘↘↘	-	-	-	-	-	-	-	-	-	7426	↘↘	-	-	-	-	-	-	-	-	-	-
3721	↗↗	-	-	-	-	-	-	-	-	-	-	7427	↘	-	-	-	-	-	-	-	-	-	-
3722	-	-	-	-	-	-	-	-	-	-	-	7428	-	-	-	-	-	-	-	-	-	-	-
3736	-	-	-	-	-	-	-	-	-	-	-	7429	↘	-	-	-	-	-	-	-	-	-	-
3738	-	↘	-	-	-	-	-	-	-	-	-	7502	-	-	-	-	-	-	-	-	-	-	-
3739	-	-	-	-	-	-	-	-	-	-	-	7503	-	-	-	-	-	-	-	-	-	-	-
3801	-	-	-	-	-	-	-	-	-	-	-	7504	↘↘	↘↘	-	-	-	-	-	-	-	-	-
3802	-	↘↘↘	-	-	-	-	-	-	-	-	-	7506	-	-	-	-	-	-	-	-	-	-	-
3806	-	-	-	NM	-	-	-	-	-	-	-	7516	-	↘↘	-	-	-	-	-	-	M	M	-
3807	-	-	-	-	-	-	-	-	-	-	-	7518	↘↘	-	-	-	-	-	-	-	-	-	-
3810	-	↘↘↘	-	-	M	-	-	-	-	-	M	7519	-	-	-	-	M	M	M	-	-	-	-
3812	-	-	-	-	-	-	-	-	-	NM	-	7521	-	-	M	-	-	-	-	-	-	-	-
3815	-	↘↘↘↘	-	-	-	-	-	-	-	-	-	7605	-	↘↘↘	-	-	-	-	-	-	-	-	-
4216	-	-	-	-	-	-	-	-	-	-	-	7610	-	-	-	-	-	-	-	-	-	-	-
4316	-	-	-	-	-	-	-	-	-	-	-	7616	↘↘	↘↘↘	-	-	-	-	-	-	-	-	-
4403	-	-	-	-	-	-	-	-	-	-	-	7617	↘↘↘	↘↘↘	-	-	-	-	-	-	-	-	-
4405	-	-	-	-	-	-	-	-	-	-	-	7621	↘↘	↘↘↘	-	-	-	-	-	-	-	-	-
4407	↘	-	-	-	-	-	-	-	-	-	-	7626	↘↘	↘	-	-	-	-	-	-	-	-	-
4410	↘↘	↘↘↘	-	-	-	-	-	-	-	-	-	8302	-	↗	-	-	-	-	-	-	-	-	-
4412	-	↘	-	-	-	-	-	M	-	-	M	8335	-	-	-	-	-	-	-	-	-	-	-
4413	-	-	-	-	-	-	-	-	-	-	-	8403	-	-	-	-	-	-	-	-	-	-	NM
4415	-	↗↗	-	-	-	-	-	-	-	-	-	8411	↘↘	-	-	-	-	-	-	-	-	-	-
4417	-	-	-	-	-	-	-	-	-	-	-	8602	-	-	-	-	-	-	-	-	-	-	-
4420	↘↘↘	↘↘↘↘	-	NM	-	-	-	-	-	-	-	8711	-	-	-	-	-	-	-	-	-	-	-
4429	-	-	-	-	-	-	-	-	-	-	-	8802	↘	-	-	-	-	-	-	-	-	-	-



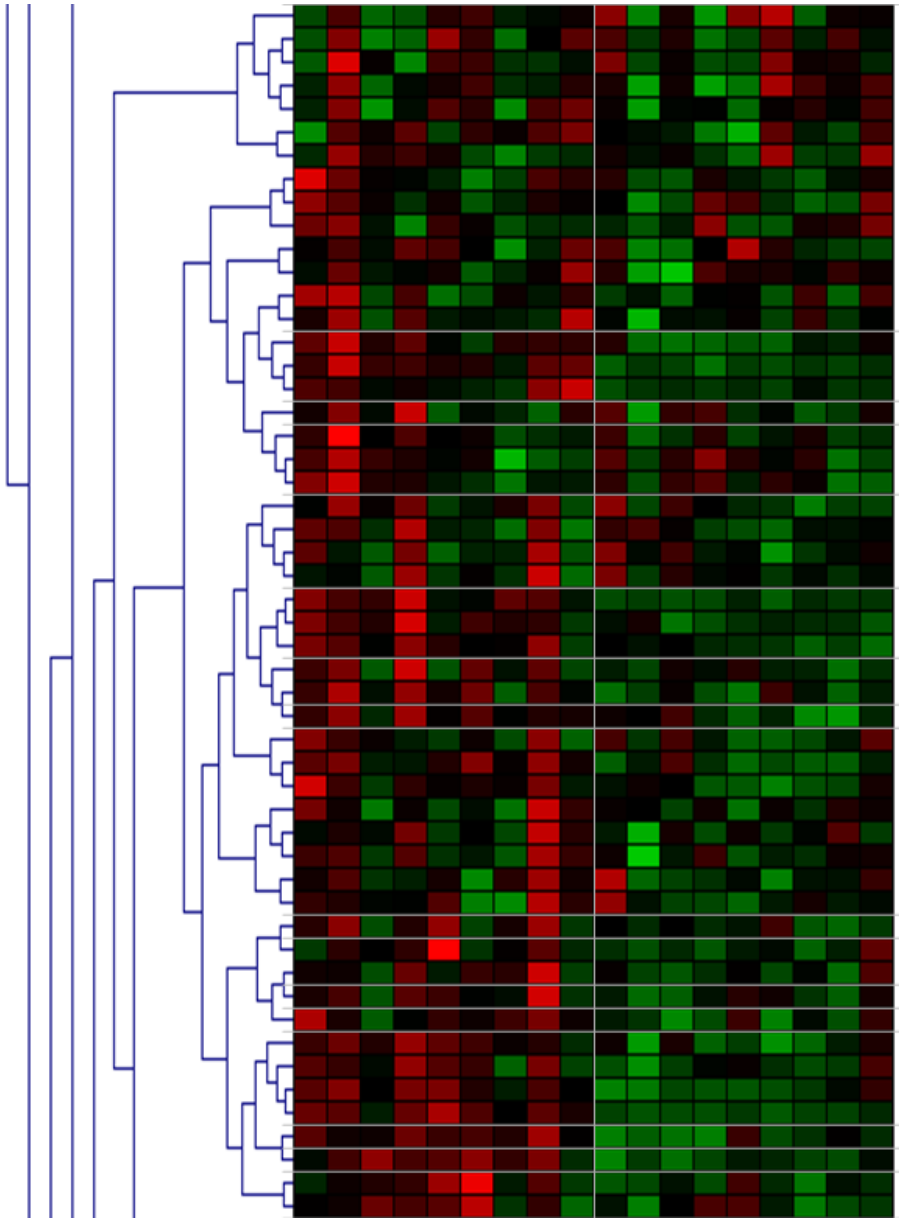
Sp	cor	ratio	ID
2412			
3409	cor M	ns	Alpha-galactosidase
3418			
5316			
5705			
1215			
5712			
6308			
1416			
2307			
2222	cor M	ns	Proteasome subunit beta type
2232			
5222			
6316			
4527			
5537			
5603			
5634	ns	over NM	ND
5637			
214			
2502	ns	over NM	ND
1719			
3521			
3515	cor M	ns	MID
3605			
3611			
5205			
5330	cor M/NM	over NM	ND
6702	cor M	ns	MID
4439	cor M	over NM	MID
5418	cor NM	over NM	MID
4216			
6710			
2703			
3211			
6704	cor NM	ns	Chaperonin CPN60-2
5425	cor NM	over M/NM	Methylthioribose-1-phosphate isomerase
4541	cor M/NM	ns	S-adenosylmethionine synthase
4440	cor M/NM	over NM	MID
5420	cor M/NM	ns	ND
5426	cor M/NM	over M/NM	Methylthioribose-1-phosphate isomerase
5536	cor M/NM	over NM	ND
7318	cor NM	ns	ND



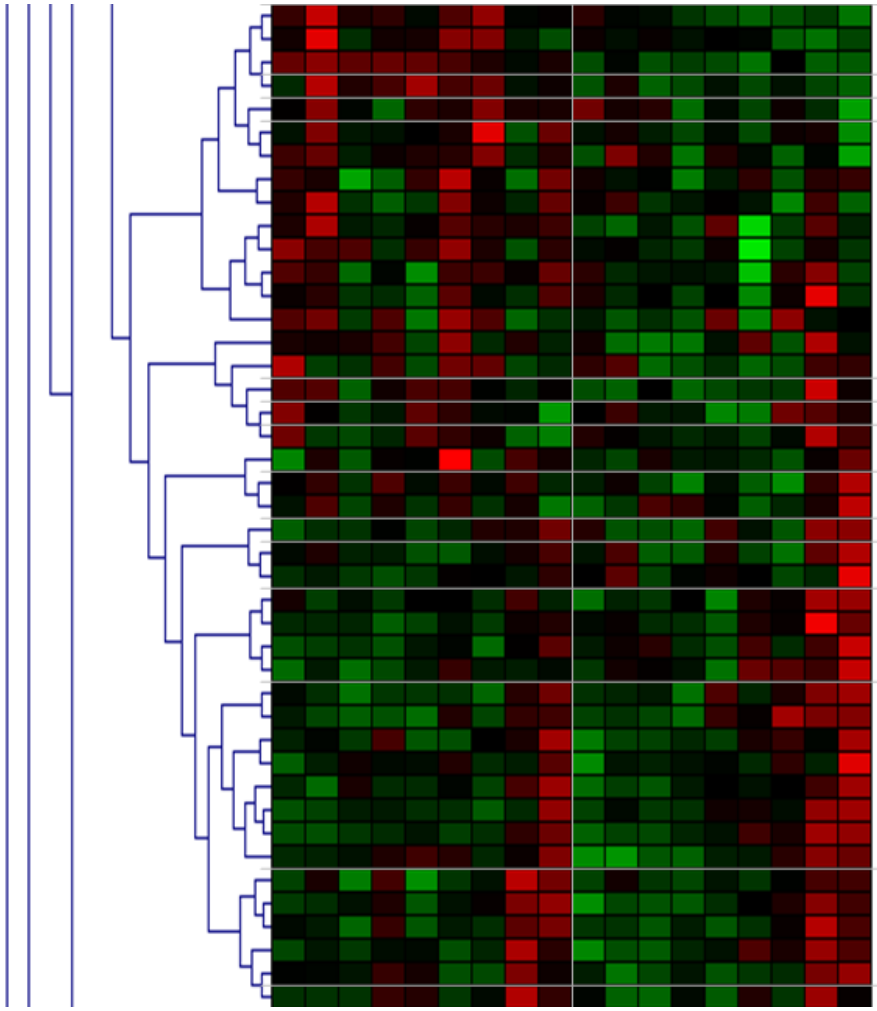
1616			
5408			
5638			
215			
322			
414			
7225			
5708			
7427			
6607			
7306	cor M/NM	ns	ND
6501			
7616	cor M/NM	ns	Tubulin beta-5 chain
4801	cor NM	ns	NADH dehydrogenase [Ubi] iron-sulfur protein 1
6209	cor M	over M	Triosephosphate isomerase
6212	cor NM	ns	L-ascorbate peroxidase 2
6213	cor NM	over M	L-ascorbate peroxidase 2
7504	cor M/NM	ns	Adenosine kinase
7309	cor M/NM	ns	Caffeoyl-CoA O-methyltransferase
7617	cor M/NM	ns	Beta-tubulin
7621	cor M/NM	ns	ND
3516			
5718			
4518			
7205	cor NM	over M	L-ascorbate peroxidase 2
7338	cor M/NM	over M	ND
6515			
4403			
7519	ns	over M	Sucrose:sucrose 1-fructosyltransferase
6516			
7626	cor M/NM	ns	Beta-tubulin
1213			
314			
1218			
3207			
3228			
3306			
2221			
5319			
6201			
4621			
2405			
5331	cor NM	ns	ND
3230			
6310	cor NM	over M	ND
7516	cor NM	over M	ND
7211			
6408			
8335			
2425	cor M	ns	Fructose-bisphosphate aldolase
218			
220			
5709			
6315			
6313			
7320			
7428			
7325			
8302			
8411	cor M	ns	ND



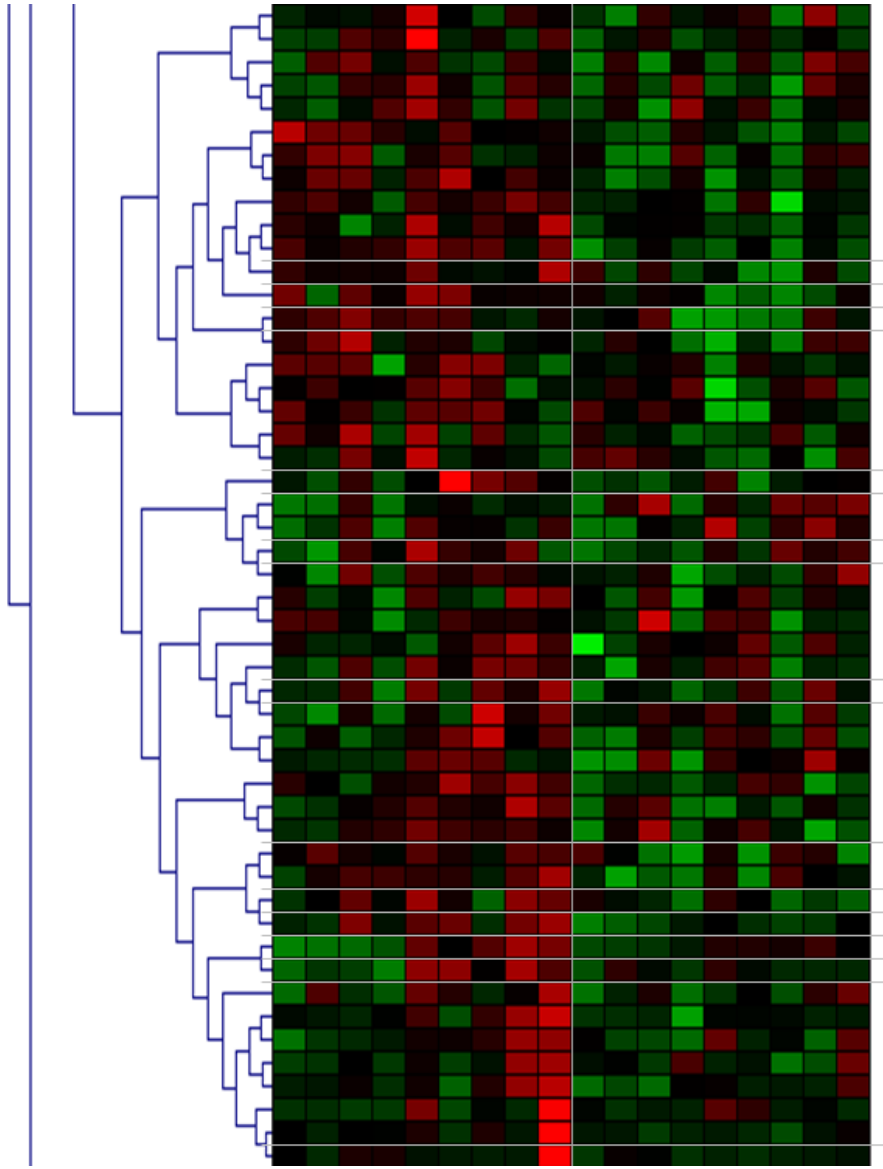
1211	cor M/NM	ns	L-ascorbate peroxidase 1
1220	cor M/NM	ns	L-ascorbate peroxidase 1
2724	cor M/NM	ns	Phenylalanine - Phenylalanine/tyrosine ammonia-lyase
2739	cor M/NM	ns	ND
2224			
4808	cor M/NM	over M/NM	ND
4817	cor NM	over M	ND
4821	cor NM	over M	ND
2802	cor M/NM	over M	Methionine synthase
2813			
3427	cor M/NM	ns	Flavone 3'-O-methyltransferase 1
3707	cor NM	over M	Phenylalanine / Phenylalanine/tyrosine ammonia-lyase
3810	cor NM	over M	ND
6609	cor NM	ns	ND
6617	cor NM	ns	ATP synthase subunit alpha
3801			
2807			
7220			
1813			
2602			
2727	ns	over M	D-3-phosphoglycerate dehydrogenase
3716			
4810			
1803			
5610			
1817			
3632			
4407			
4533			
1716			
2708			
1626	cor M	over M	Mitochondrial processing peptidase alpha-chain precursor
7518	cor M	ns	Glutamine synthetase
4615			
6535			
4601	cor M	ns	ATP synthase subunit alpha
6730			
3413			
3609			
7605	cor NM	ns	Alpha tubulin



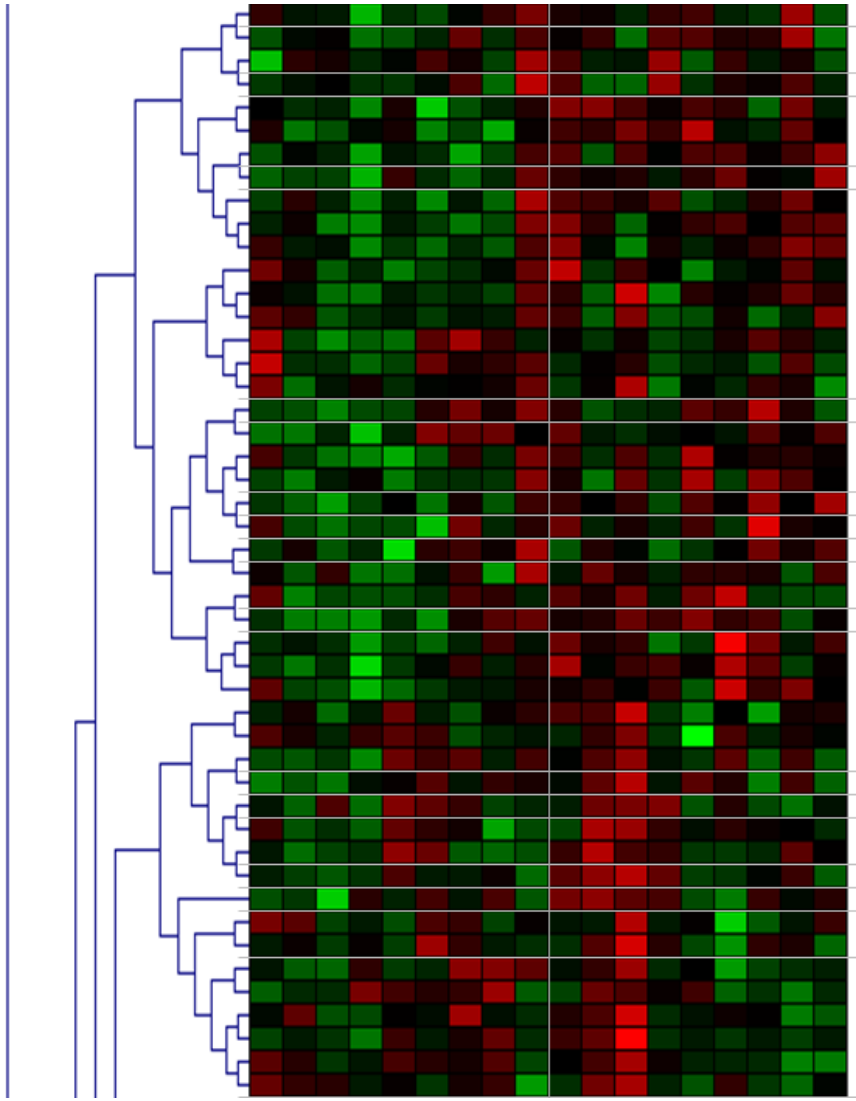
2627				
2702				
2728				
2711				
2740				
2617				
7425				
2208				
2535				
4429				
3714				
4538				
412				
2701				
1531	ns	over M	ND	
1618	ns	over M	Mitochondrial-processing peptidase subunit alpha	
6203	ns	over M	L-ascorbate peroxidase 2	
1725				
1808	cor M	over M	Glycine dehydrogenase [decarboxylating]	
5322	cor M/NM	ns	Remorin	
6301	cor M/NM	ns	ND	
1328				
1306				
1408				
1413				
217	cor M	over M	Glutathione S-transferase	
1214	cor M	over M	ND	
1505	cor NM	ns	ND	
1502				
1703				
1742	cor NM	ns	ND	
1410				
1610				
3208				
1522				
6807				
6809				
1519				
7303				
1414	cor NM	over M	Probable voltage-gated potassium channel subunit beta	
1415				
1302				
1403	ns	over M	ND	
2209				
1708	cor M	over M	6-phosphofructokinase, pyrophosphate dependent	
1504	cor NM	ns	Protein disulfide isomerase-like 2-1	
1511	cor NM	over M	MID	
1741	ns	over M	ND	
1207				
4716	ns	over M	Heat shock 70 kDa protein 10	
4632				
7403				



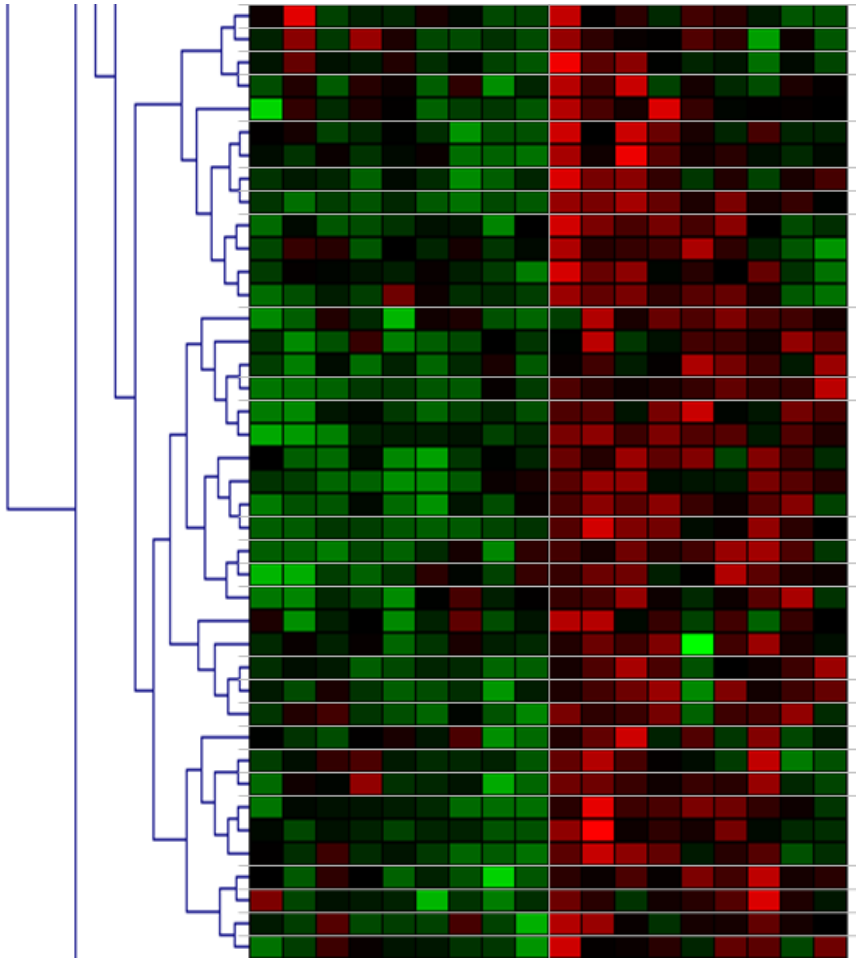
2801	cor NM	ns	Aconitate hydratase
2818	cor NM	over M	Aconitate hydratase
3430	cor M/NM	over M	MID
4412			
2805	cor NM	over M	Aconitate hydratase
3807			
4608			
3514			
5702			
3518			
4413			
1311			
5719			
6536			
1229			
3528			
5404	cor NM	ns	Glutamine synthetase
2533	cor M	over M	ND
6715			
2532			
2511	cor NM	ns	Probable cinnamyl alcohol dehydrogenase
4435	cor NM	ns	MID
2424	cor M/NM	ns	UDP-arabinopyranose mutase 1
3501			
3634			
2312	cor NM	ns	Probable L-ascorbate peroxidase 6
4415	cor NM	ns	ND
3717	cor M/NM	ns	ND
6629	cor NM	ns	Chaperonin CPN60-2
2210	cor M/NM	ns	Superoxide dismutase [Mn]
3202	cor M/NM	ns	Superoxide dismutase [Mn]
513	cor M/NM	ns	Formate dehydrogenase
5309	cor NM	ns	Cysteine synthase
2401	cor M/NM	ns	MID
4540	cor M/NM	ns	MID
6215	cor M/NM	ns	Adenine phosphoribosyltransferase 1
7311	cor M/NM	over M	ND
1315	cor M	ns	26S proteasome non-ATPase regulatory subunit 14
5506	cor M/NM	ns	S-adenosylmethionine synthase
7343	cor M/NM	ns	ND
1503	cor M/NM	ns	Formate dehydrogenase
1507	cor NM	ns	Formate dehydrogenase
7502			



3512			
4316			
2710			
6220			
7411			
1521			
3303			
3504			
1617			
2319			
2606			
2623	ns	over M	Alanine aminotransferase 2
4512			
4619	ns	over M	ND
6612			
5639			
5403			
6712			
7429			
7503			
5213	ns	over M	ND
3524			
3607			
6303	cor NM	ns	Cysteine synthase
6613			
2523			
2614			
4704			
2628			
3712	cor M	ns	Ketol-acid reductoisomerase
3736			
3722			
5217			
1216			
4528			
4714			
2207	ns	over M	Cysteine proteinase inhibitor 12
3411	cor M	ns	Malate dehydrogenase
2413			
7341	cor M/NM	over M	Phytepsin
2223	cor M/NM	ns	Glyceraldehyde-3-phosphate dehydrogenase 1
3320			
3206	cor NM	over M	ND
2609	cor M	ns	Aldehyde dehydrogenase family 2 member B7
3709	cor M	over M	Ketol-acid reductoisomerase
3526	cor M	ns	S-adenosylmethionine synthase
6206	cor M/NM	ns	ND
5531	cor M	ns	ND
3602	cor M	ns	ND
7610			



2316	cor M	over NM/M	ND
4505			
4809			
4816	cor M	ns	MID
3739			
4631			
2717			
3610	cor M	ns	MID
3613			
2709			
5208			
2629			
2601			
3505			
5301			
3229			
5318			
4434	cor M	ns	MID
4521			
5508			
5631			
2725	cor M	over NM	Ketol-acid reductoisomerase
6219			
2525	cor M/NM	ns	Isocitrate dehydrogenase [NADP]
6302			
2534			
3502	cor M	over NM	MID
4510			
4630			
6411			
2515			
5607			
4614			
5727	cor M/NM	ns	MID
4613	cor NM	ns	Succinate-semialdehyde dehydrogenase
5616			
5412			
6706	cor NM	ns	Vacuolar proton ATPase catalytic subunit A
1603	ns	over NM	ND
1309			
5716			
1428	cor M	ns	MID
5514	cor NM	ns	Actin
2512	cor NM	over NM	Alcohol dehydrogenase
6729	cor NM	ns	MID
4702	cor NM	ns	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1
7426	cor M	ns	40S ribosomal protein SA



2810	cor M/NM	ns	Aconitate hydratase
4526			
3718	cor M/NM	ns	Succinate dehydrogenase [ubi] flavoprotein subunit 1
6401			
1513			
1611	cor NM	ns	MID
1625	cor M/NM	ns	ND
4405			
4705	cor NM	over NM	Phosphoglucomutase, cytoplasmic
3802	cor NM	ns	Aconitate hydratase
3815	cor NM	ns	NADH dehydrogenase [Ubi] iron-sulfur protein 1
4719	cor NM	ns	MID
6404	cor NM	ns	MID
3806			
4508			
2407			
5515	cor M/NM	over NM	MID
5221			
5707			
1206			
2213			
1227			
5424	cor NM	over NM	ND
4417			
4602	cor M	ns	MID
5812			
5703			
6409			
1506	cor M	over NM	ND
2716			
6615	cor M	ns	ND
5633			
5410	cor NM	ns	ND
6537			
4410	cor M/NM	ns	MID
4420	cor M/NM	over NM	Tricetin 3',4',5'-O-trimethyltransferase
7342	cor M/NM	ns	ND
5622			
6205	cor M	ns	Glutathione S-transferase GSTZ5
6630	cor M	ns	MID
8403			

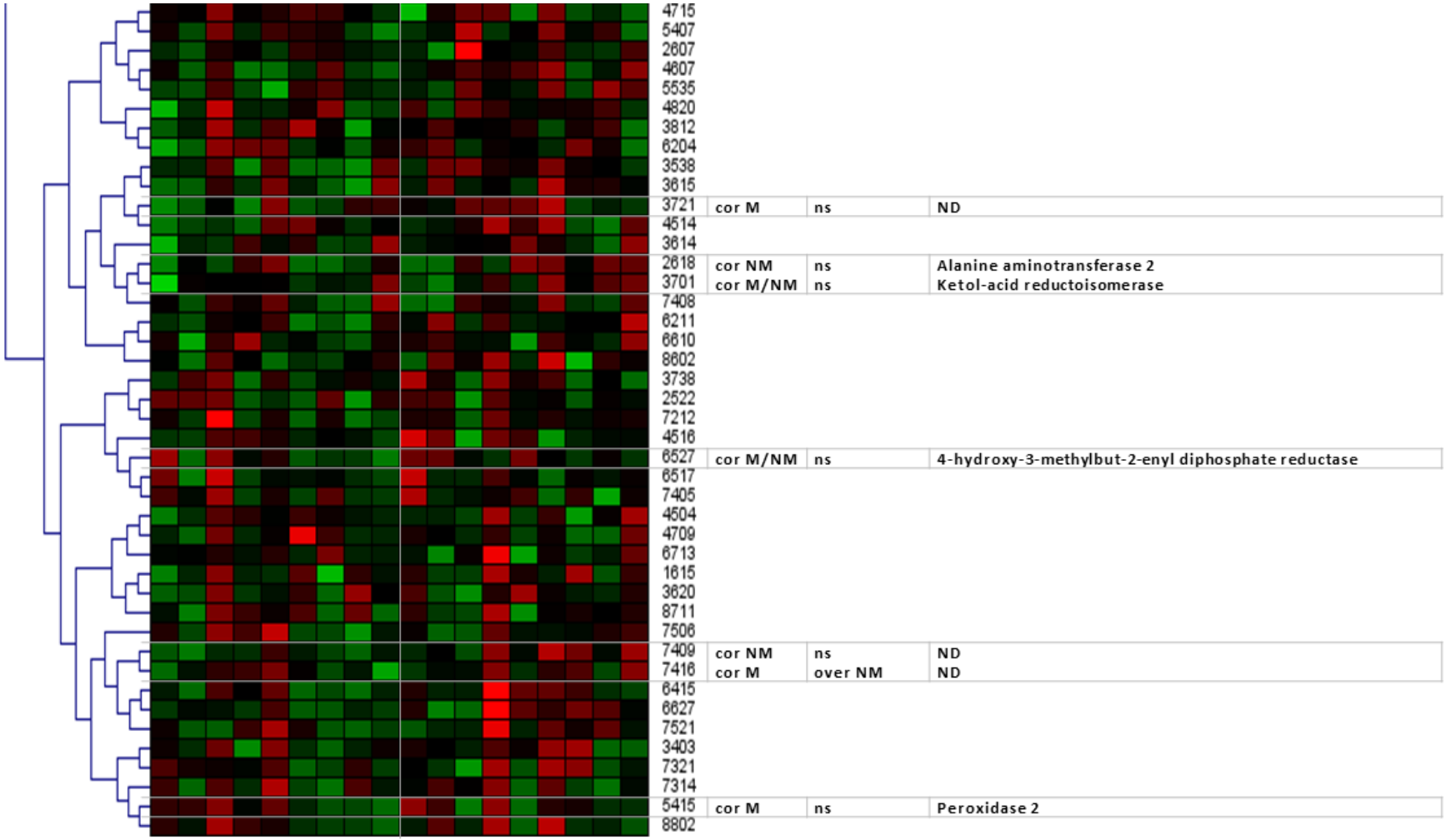


Figure 2: Cluster of protein spots variation for the 419 accurately delimited spots (PDQuest) and identification of the 157 excised spots analyzed by LC-MS/MS. ID: most probable protein identity based on MS analysis, ND: Not Determined, MID: Multiple Identifications. Cor: Pearson's correlation; cor M, NM or M/NM: significant correlation of spot expression with Cu exposure only in M, only in NM or in both populations. Ratio: results of ratio between M and NM; over M, NM or M/NM: over-expression of spot in M, NM or both populations.

3.1.1. Cu effect

The expression of 199 spots was correlated with Cu exposure in at least one population (P < 0.1, Tab. 1, Fig. 4, Annexes 10-12):

- 51 spots were correlated with Cu exposure in both populations (Annex 10): 1 spot increased in M roots but decreased in NM ones, 24 spots increased with Cu exposure (7 similarly in both populations, 3 more sharply in M roots, and 14 more sharply in NM ones) and 26 spots decreased (6 similarly in both populations, 6 more sharply in M roots, and 14 more sharply in NM ones).
- 67 spots were correlated with Cu exposure only in M roots: 32 increased and 35 decreased (Annex 11)
- 81 spots were correlated with Cu exposure only in NM roots: 35 increased and 46 decreased (Annex 12)

The expression of 220 spots did not exhibit any correlation with Cu exposure.

3.1.2. Population effect

95 spots were over-expressed in one population (ratio of 1.5) at least for one Cu exposure (Annex 13); 60 were over-expressed in M, 30 in NM, and 5 were first over-expressed in one and then in the other population (Fig. 4; Tab. 1).

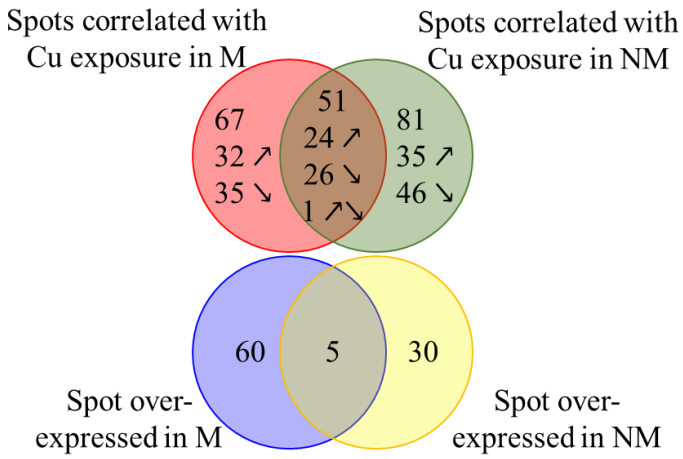


Figure 4: Venn diagram of spots which respond to Cu treatment or population origin. Red: spots which expression was correlated with Cu exposure in M roots; Green: spots which expression was correlated with Cu exposure in NM roots; ↗: positive correlation; ↘: negative correlation; Blue: spots over-expressed in M roots; Yellow: spots over-expressed in NM roots.

3.1.3. Integration of both effects

After both Cu and Population effects were examined separately, information about variation of root spots was integrated and synthesized in Fig. 5.

Expression of 108 spots was correlated with Cu exposure in only one population and did not differ significantly between populations:

- 48 in M (26 increased, 22 decreased)
- 60 in NM (31 increased, 29 decreased)

Expression of 39 spots was correlated with Cu exposure in both populations and did not differ significantly between populations:

- 17 increased in M and NM
- 21 decreased in M and NM
- 1 increased in M and decreased in NM

43 spots were over-expressed in one population and did not respond to Cu exposure:

- 30 over-expressed only in M
- 12 over-expressed only NM
- 1 over-expressed in M at 5 and 20 µM Cu and in NM at 10 µM Cu

52 spots were over-expressed and correlated with Cu in at least one population (Annex 14)

- 10 over-expressed in M and correlated with Cu only in M (1 increased, 9 decreased)
- 15 over-expressed in M and correlated with Cu only in NM (2 increased, 13 decreased)
- 5 over-expressed in M and correlated with Cu in M and NM (2 increased, 3 decreased)
- 8 over-expressed in NM and correlated with Cu only in M (3 increased, 5 decreased)
- 5 over-expressed in NM and correlated with Cu only in NM (1 increased, 4 decreased)
- 5 over-expressed in NM and correlated with Cu in M and NM (4 increased, 1 decreased)
- 1 over-expressed in M at 50 µM Cu, in NM at 15 µM Cu and increased only in M
- 1 over-expressed in M at 10 µM Cu, in NM at 40 µM Cu and increased only in NM
- 1 over-expressed in M at 25 µM Cu, in NM at 40 µM Cu and decreased in M and NM
- 1 over-expressed in M at 10 µM Cu, in NM at 30, 50 µM Cu and increased in M and NM

177 spots did not respond to Cu- or Population in roots (Annex 15).

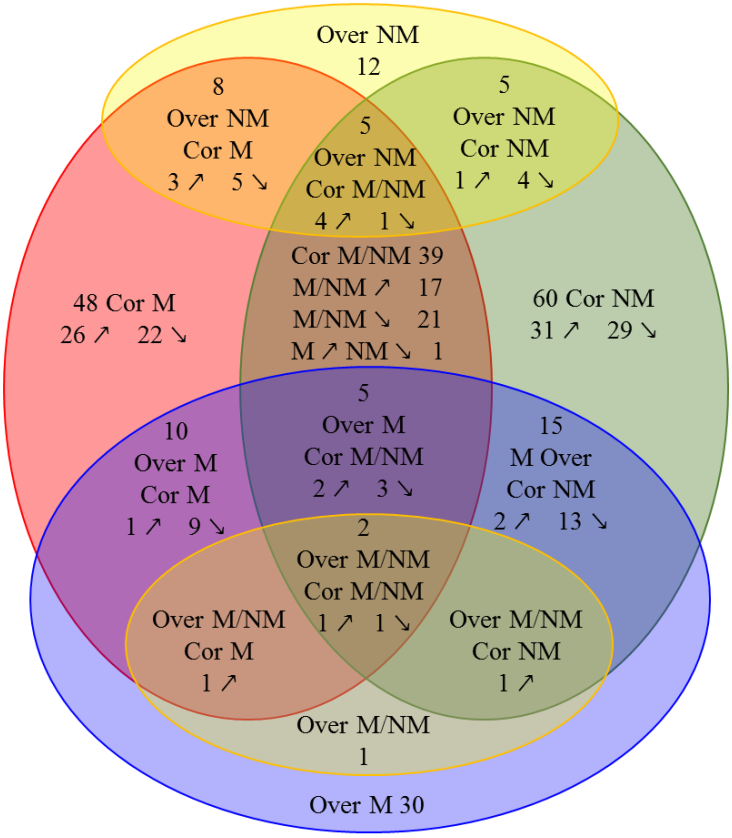


Figure 5: Adapted Venn diagram for the 242 spots wich vary among either Cu treatment or population origins. Red, cor M: spots which expression was correlated with Cu exposure in M roots; Green, cor NM: spots which expression was correlated with Cu exposure in NM roots; Blue, Over M: spots over-expressed in M roots; Yellow, Over NM: spots over-expressed in NM roots; cor M/NM: spots which expression was correlated with Cu exposure in M and NM roots; Over M/NM: spots over-expressed in one population then in the other one. ↗: positive correlation; ↘: negative correlation.

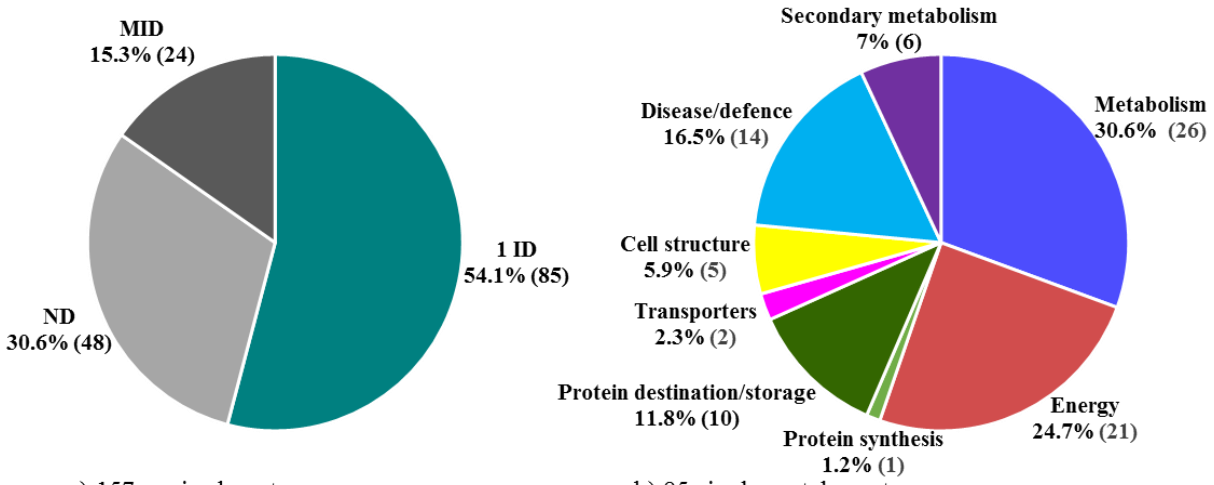
3.2. Protein spots excision and identification

157 of the 419 accurately delimited spots in roots, were selected for excision (Tab. 1-2, Fig. 3) as being correlated with Cu exposure in at least one population ($P < 0.05$, Pearson's correlations) and/or over-expressed significantly in one population for at least one Cu exposure (population ratio higher than 1.5). As shown in Fig. 6a, 48 (31%) out of the 157 excised spots characterized by LC-MS/MS remained unidentified after "*Agrostis* EST" and "Viridiplantae proteins" databases searching (ND, circled in green color on the master gel picture in Fig. 1, Tab. 2, Fig. 2).

The other 59 spot led at least to one match in one database: 24 (15%) matched with two or three different proteins (MID, circled in purple, Fig. 1, Tab. 2, Fig. 2 and complete identification available in Annex 17) and 85 (54%) matched to a single protein identification or two very similar identifications in case of #3427 and #3707 (1ID, in red, Fig. 1, Tab. 2-4, Fig. 2 and complete identification available in Annex 17).

The 85 single-match protein spots were assigned according identifications to functional categories (Fig. 6b) described in Bevan *et al.* (1998), i.e. 26 spots (30.6%) belonged to category 1: Metabolism, 21 (24.7%) to category 2: Energy, 1 (1.2%) to category 5: Protein synthesis, 10 (11.8%) to category 6: Protein destination and storage, 2 (2.3%) to category 7: Transporters, 5 (5.9%) to category 9: Cell structure, 14 (16.5%) to category 11: Disease/defense and 6 spots (7%) to category 20: Secondary metabolism (Tab. 4). Statistical results for the 46 single-match protein spots were consigned in Tab. 3, identifications in Tab. 4, and their functions and variations illustrated in Fig. 7.

Although all 157 excised spots were shown on heat map (Fig. 2), in Tab. 2 and in pie chart (Fig. 6a), the 72 spots with no or multiple identifications were not further described in results and considered for the discussion. To remember, complete identification data for MID spots are available in Annex 17.



a) 157 excised spots b) 85 single-match spots

Figure 6: a) Results of protein spot identification for the 157 excised root spots, ND: not determined, MID: multiple identifications and 1ID: single-match identification. b) Assignment of the 85 single-match spots in functional categories defined by Bevan *et al.* (1998).

Table 2. List of the 157 spots selected for excision, with results of protein identification and statistical tests. Sp: spots number; ID: results of protein identification (ND = non identified, MID: multiple protein identity); rM/rNM: r coefficient of Pearson's correlation for either the M or NM population, p-val = 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; Ratio 1 to ratio 50: comparative ratio between population values at each Cu exposure, -: no difference, >/>>: intensity of the difference (> indicated ratio higher than x1.5 but lower than x2, >> indicated ratio superior to x2) and M/NM indicated the population with higher values.

SSP	ID	rM	pval	signif	rNM	pval	signif	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
217	Glutathione S-transferase	-0.34	0.082	↘	-0.06	0.77	-	M >	M >>	M >>	M >>	=	M >	M >	M >	=
513	Formate dehydrogenase	0.52	0.006	↗↗↗	0.77	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
1211	L-ascorbate peroxidase 1	-0.66	<0.0001	↘↘↘↘	-0.76	<0.0001	↘↘↘↘	=	=	=	=	=	=	=	=	=
1214	ND	-0.38	0.047	↘↘	-0.25	0.21	-	=	=	=	M >	=	=	=	=	=
1220	L-ascorbate peroxidase 1	-0.55	0.003	↘↘↘	-0.66	0.0002	↘↘↘↘	=	=	=	=	=	=	=	=	=
1315	26S proteasome non-ATPase regulatory subunit 14	0.41	0.032	↗↗	0.30	0.13	-	=	=	=	=	=	=	=	=	=
1403	ND	0.05	0.79	-	0.22	0.27	-	=	=	=	=	=	=	=	M >>	=
1414	Probable voltage-gated potassium channel subunit beta	-0.11	0.58	-	-0.32	0.099	↘	=	=	=	=	=	=	=	M >>	=
1428	MID	0.45	0.019	↗↗	-0.27	0.17	-	=	=	=	=	=	=	=	=	=
1503	Formate dehydrogenase	0.40	0.036	↗↗	0.73	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
1504	Protein disulfide isomerase-like 2-1	-0.21	0.29	-	0.42	0.029	↗↗	=	=	=	=	=	=	=	=	=
1505	ND	-0.26	0.19	-	-0.51	0.007	↘↘↘	=	=	=	=	=	=	=	=	=
1506	ND	-0.39	0.045	↘↘	0.20	0.32	-	=	=	=	=	=	=	=	=	NM >
1507	Formate dehydrogenase	0.16	0.41	-	0.61	0.0008	↗↗↗↗	=	=	=	=	=	=	=	=	=
1511	MID	-0.29	0.15	-	0.65	0.0003	↗↗↗↗	M >	M >	=	=	M >	=	=	=	=
1531	ND	-0.24	0.22	-	0.21	0.30	-	=	M >>	=	=	=	=	=	=	=
1603	ND	0.19	0.37	-	-0.26	0.19	-	=	=	NM >>	=	=	=	=	=	=
1611	MID	-0.30	0.13	-	-0.39	0.043	↘↘	=	=	=	=	=	=	=	=	=
1618	Mitochondrial-processing peptidase subunit alpha	-0.04	0.82	-	0.04	0.84	-	M >>	M >>	M >>	M >>	M >>	M >>	=	M >	M >
1625	ND	-0.52	0.005	↘↘↘	-0.37	0.054	↘	=	=	=	=	=	=	=	=	=
1626	mitochondrial processing peptidase alpha-chain	-0.54	0.004	↘↘↘	-0.16	0.43	-	=	=	=	=	M >	=	=	=	=
1708	6-phosphofructokinase, pyrophosphate dependent	-0.32	0.098	↘	0.14	0.47	-	=	M >>	=	=	=	M >	M >	=	=
1741	ND	-0.09	0.65	-	0.01	0.97	-	=	M >>	=	=	M >	M >>	=	=	=
1742	ND	-0.11	0.59	-	-0.46	0.015	↘↘	=	=	=	=	=	=	=	=	=
1808	Glycine dehydrogenase [decarboxylating]	-0.48	0.012	↘↘	-0.15	0.46	-	=	M >>	=	=	=	=	=	=	=
2207	Cysteine proteinase inhibitor 12	0.10	0.62	-	-0.20	0.32	-	=	=	=	=	=	=	=	=	M >>
2210	superoxide dismutase [Mn]	0.34	0.080	↗	0.53	0.005	↗↗↗	=	=	=	=	=	=	=	=	=

2222	Proteasome subunit beta type	0.41	0.033	↗↗	0.22	0.27	-	=	=	=	=	=	=	=	=	=
2223	Glyceraldehyde-3-phosphate dehydrogenase 1	0.59	0.001	↗↗↗	0.40	0.037	↗↗	=	=	=	=	=	=	=	=	=
2312	Probable L-ascorbate peroxidase 6	0.05	0.80	-	0.69	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
2316	ND	0.38	0.052	↗	-0.07	0.72	-	=	=	=	NM >>	=	=	=	=	M >
2401	MID	0.58	0.001	↗↗↗	0.81	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
2424	UDP-arabinopyranose mutase 1	0.48	0.012	↗↗	0.51	0.006	↗↗↗	=	=	=	=	=	=	=	=	=
2425	Fructose-bisphosphate aldolase	-0.40	0.037	↘↘	0.10	0.62	-	=	=	=	=	=	=	=	=	=
2502	ND	-0.21	0.29	-	0.23	0.24	-	=	NM >	=	=	=	=	=	=	=
2511	Probable cinnamyl alcohol dehydrogenase	-0.08	0.69	-	0.45	0.018	↗↗	=	=	=	=	=	=	=	=	=
2512	Alcohol dehydrogenase	-0.06	0.78	-	-0.61	0.0008	↘↘↘↘	=	=	NM >	=	=	=	=	=	=
2525	Isocitrate dehydrogenase [NADP]	0.39	0.042	↗↗	0.37	0.060	↗	=	=	=	=	=	=	=	=	=
2533	ND	-0.47	0.016	↘↘	0.16	0.42	-	=	=	=	=	M >>	=	=	=	=
2609	Aldehyde dehydrogenase family 2 member B7	0.56	0.002	↗↗↗	0.08	0.69	-	=	=	=	=	=	=	=	=	=
2618	Alanine aminotransferase 2	0.09	0.66	-	0.48	0.011	↗↗	=	=	=	=	=	=	=	=	=
2623	Alanine aminotransferase 2	0.22	0.28	-	-0.30	0.12	-	=	=	=	=	=	=	=	=	M >
2724	Phenylalanine / Phenylalanine/tyrosine ammonia-lyase	-0.40	0.040	↘↘	-0.39	0.043	↘↘	=	=	=	=	=	=	=	=	=
2725	Ketol-acid reductoisomerase	0.35	0.075	↗	0.25	0.20	-	=	=	NM >>	=	=	=	=	=	=
2727	D-3-phosphoglycerate dehydrogenase	-0.03	0.87	-	-0.19	0.34	-	=	=	=	=	=	=	=	=	M >>
2739	ND	-0.47	0.014	↘↘	-0.49	0.009	↘↘↘	=	=	=	=	=	=	=	=	=
2801	Aconitate hydratase	-0.20	0.31	-	-0.53	0.004	↘↘↘	=	=	=	=	=	=	=	=	=
2802	Methionine synthase	-0.33	0.089	↘	-0.60	0.001	↘↘↘↘	=	=	=	=	=	=	=	M >	=
2805	Aconitate hydratase	0.03	0.87	-	-0.51	0.007	↘↘↘	=	=	=	=	=	=	=	=	M >
2810	Aconitate hydratase	-0.37	0.058	↘	-0.43	0.025	↘↘	=	=	=	=	=	=	=	=	=
2818	Aconitate hydratase	-0.32	0.11	-	-0.46	0.016	↘↘	=	M >	=	=	=	=	=	=	=
3202	superoxide dismutase [Mn]	0.46	0.015	↗↗	0.61	0.0008	↗↗↗↗	=	=	=	=	=	=	=	=	=
3206	ND	0.04	0.39	-	0.46	0.017	↗↗	=	=	=	=	M >	=	=	=	=
3409	Alpha-galactosidase	0.48	0.011	↗↗	0.30	0.13	-	=	=	=	=	=	=	=	=	=
3411	Malate dehydrogenase	0.39	0.044	↗↗	0.29	0.14	-	=	=	=	=	=	=	=	=	=
3427	Flavone 3'-O-methyltransferase 1	-0.38	0.048	↘↘	-0.57	0.002	↘↘↘	=	=	=	=	=	=	=	=	=
3430	MID	-0.52	0.006	↘↘↘	-0.35	0.071	↘	=	=	M >	M >	=	=	=	=	M >>
3502	MID	0.61	0.0008	↗↗↗↗	-0.21	0.28	-	=	=	NM >	=	=	=	=	=	=
3515	MID	-0.42	0.031	↘↘	0.15	0.47	-	=	=	=	=	=	=	=	=	=
3526	S-adenosylmethionine synthase	0.44	0.023	↗↗	0.11	0.59	-	=	=	=	=	=	=	=	=	=

3602	ND	0.46	0.015	↗↗	0.10	0.61	-	=	=	=	=	=	=	=	=	=
3610	MID	0.44	0.021	↗↗	0.26	0.19	-	=	=	=	=	=	=	=	=	=
3701	Ketol-acid reductoisomerase	0.35	0.075	↗	0.46	0.017	↗↗	=	=	=	=	=	=	=	=	=
3707	Phenylalanine / Phenylalanine/tyrosine ammonia-lyase	-0.25	0.21	-	-0.48	0.011	↘↘	=	=	M >	=	=	=	=	=	=
3709	Ketol-acid reductoisomerase	0.65	0.0003	↗↗↗↗	0.19	0.35	-	=	=	=	=	=	=	=	M >>	=
3712	Ketol-acid reductoisomerase	0.39	0.043	↗↗	0.30	0.13	-	=	=	=	=	=	=	=	=	=
3717	ND	0.57	0.002	↗↗↗	0.54	0.004	↗↗↗	=	=	=	=	=	=	=	=	=
3718	Succinate dehydrogenase [ubi] flavoprotein subunit 1	-0.36	0.062	↘	-0.60	0.001	↘↘↘	=	=	=	=	=	=	=	=	=
3721	ND	0.38	0.048	↗↗	-0.31	0.12	-	=	=	=	=	=	=	=	=	=
3802	Aconitate hydratase	0.06	0.78	-	-0.56	0.002	↘↘↘	=	=	=	=	=	=	=	=	=
3810	ND	-0.25	0.20	-	-0.57	0.002	↘↘↘	=	=	M >	=	=	=	=	=	M >
3815	NADH dehydrogenase [Ubi] iron-sulfur protein 1	-0.05	0.79	-	-0.63	0.0005	↘↘↘↘	=	=	=	=	=	=	=	=	=
4410	MID	-0.42	0.028	↘↘	-0.50	0.008	↘↘↘	=	=	=	=	=	=	=	=	=
4415	ND	0.26	0.19	-	0.42	0.028	↗↗	=	=	=	=	=	=	=	=	=
4420	Tricetin 3',4',5'-O-trimethyltransferase	-0.54	0.004	↘↘↘	-0.61	0.0008	↘↘↘↘	=	NM >>	=	=	=	=	=	=	=
4434	MID	0.39	0.043	↗↗	0.00	0.98	-	=	=	=	=	=	=	=	=	=
4435	MID	-0.26	0.19	-	0.41	0.033	↗↗	=	=	=	=	=	=	=	=	=
4439	MID	-0.41	0.033	↘↘	0.30	0.13	-	NM >>	NM >>	NM >>	NM >>	NM >>	NM >>	NM >>	=	NM >>
4440	MID	0.68	<0.0001	↗↗↗↗	0.71	<0.0001	↗↗↗↗	=	=	=	NM >	=	=	=	=	=
4540	MID	0.55	0.003	↗↗↗	0.71	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
4541	S-adenosylmethionine synthase	0.33	0.097	↗	0.47	0.012	↗↗	=	=	=	=	=	=	=	=	=
4601	ATP synthase subunit alpha	-0.48	0.012	↘↘	-0.01	0.96	-	=	=	=	=	=	=	=	=	=
4602	MID	0.58	0.002	↗↗↗	-0.11	0.58	-	=	=	=	=	=	=	=	=	=
4613	Succinate-semialdehyde dehydrogenase	-0.04	0.85	-	-0.38	0.049	↘↘	=	=	=	=	=	=	=	=	=
4619	ND	-0.28	0.16	-	-0.02	0.90	-	=	=	=	=	M >>	=	=	=	=
4702	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1	-0.15	0.46	-	-0.63	0.0004	↘↘↘↘	=	=	=	=	=	=	=	=	=
4705	Phosphoglucomutase, cytoplasmic	-0.28	0.15	-	-0.42	0.033	↘↘	NM >>	NM >>	=	NM >>	=	NM >	=	=	=
4716	Heat shock 70 kDa protein 10	-0.13	0.50	-	0.31	0.11	-	=	=	M >>	=	=	M >	M >	M >>	=
4719	MID	-0.31	0.12	-	-0.61	0.0006	↘↘↘↘	=	=	=	=	=	=	=	=	=
4801	NADH dehydrogenase [Ubi] iron-sulfur protein 1	-0.24	0.23	-	-0.56	0.003	↘↘↘	=	=	=	=	=	=	=	=	=
4808	ND	-0.46	0.017	↘↘	-0.73	<0.0001	↘↘↘↘	=	=	=	=	=	M >	=	=	NM >>
4816	MID	0.38	0.049	↗↗	0.05	0.80	-	=	=	=	=	=	=	=	=	=
4817	ND	-0.29	0.15	-	-0.64	0.0005	↘↘↘↘	=	=	=	=	=	M >	=	=	=

4821	ND	-0.27	0.18	-	-0.49	0.017	↘↘	=	=	=	=	=	M >>	=	=	=
5213	ND	0.31	0.12	-	0.22	0.28	-	=	=	=	=	=	M >>	=	=	=
5309	Cysteine synthase	0.29	0.15	-	0.55	0.003	↗↗↗	=	=	=	=	=	=	=	=	=
5322	Remorin	-0.51	0.006	↘↘↘	-0.35	0.076	↘	=	=	=	=	=	=	=	=	=
5330	ND	0.39	0.045	↗↗	0.45	0.019	↗↗	=	=	=	=	=	NM >>	=	NM >>	=
5331	ND	0.18	0.36	-	-0.40	0.040	↘↘	=	=	=	=	=	=	=	=	=
5404	Glutamine synthetase	-0.15	0.46	-	0.49	0.010	↗↗↗	=	=	=	=	=	=	=	=	=
5410	ND	-0.25	0.21	-	-0.49	0.009	↘↘↘	=	=	=	=	=	=	=	=	=
5415	Peroxidase 2	-0.63	0.0004	↘↘↘↘	-0.26	0.20	-	=	=	=	=	=	=	=	=	=
5418	MID	-0.12	0.55	-	0.48	0.012	↗↗	=	=	NM >	=	=	=	=	NM >>	=
5420	ND	0.56	0.003	↗↗↗	0.69	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
5424	ND	0.14	0.48	-	-0.39	0.047	↘↘	NM >>	=	=	=	=	NM >>	NM >>	=	=
5425	Methylthioribose-1-phosphate isomerase	-0.09	0.67	-	0.59	0.001	↗↗↗	=	=	M >>	=	=	=	=	NM >>	=
5426	Methylthioribose-1-phosphate isomerase	0.37	0.055	↗	0.72	<0.0001	↗↗↗↗	=	=	M >	=	=	=	NM >>	=	NM >
5506	S-adenosylmethionine synthase	0.57	0.002	↗↗↗	0.68	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
5514	Actin	0.13	0.52	-	-0.39	0.043	↘↘	=	=	=	=	=	=	=	=	=
5515	MID	0.42	0.031	↗↗	0.46	0.016	↗↗	NM >>	=	=	=	=	NM >	=	=	NM >>
5531	ND	0.47	0.014	↗↗	-0.10	0.64	-	=	=	=	=	=	=	=	=	=
5536	ND	0.64	<0.0001	↗↗↗↗	0.84	<0.0001	↗↗↗↗				NM >>	=	=	=	=	NM >>
5634	ND	0.19	0.34	-	-0.24	0.24	-	=	=	=	=	NM >>	=	NM >	=	=
5727	MID	0.44	0.021	↗↗	-0.40	0.039	↘↘	=	=	=	=	=	=	=	=	=
6203	L-ascorbate peroxidase 2	0.32	0.10	-	-0.09	0.67	-	M >>	=	=	=	=	=	=	=	M >>
6205	Glutathione S-transferase GSTZ5	-0.40	0.037	↘↘	-0.04	0.84	-	=	=	=	=	=	=	=	=	=
6206	ND	0.46	0.015	↗↗	0.48	0.011	↗↗	=	=	=	=	=	=	=	=	=
6209	Triosephosphate isomerase	-0.41	0.035	↘↘	-0.19	0.35	-	=	=	M >	=	=	=	=	=	=
6212	L-ascorbate peroxidase 2	-0.21	0.30	-	-0.45	0.019	↘↘	=	=	=	=	=	=	=	=	=
6213	L-ascorbate peroxidase 2	-0.24	0.22	-	-0.69	<0.0001	↘↘↘↘	=	=	=	=	=	=	=	M >	=
6215	Adenine phosphoribosyltransferase 1	0.65	<0.0001	↗↗↗↗	0.84	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
6301	ND	-0.40	0.041	↘↘	-0.34	0.087	↘	=	=	=	=	=	=	=	=	=
6303	Cysteine synthase	0.11	0.60	-	0.53	0.005	↗↗↗	=	=	=	=	=	=	=	=	=
6310	ND	-0.32	0.10	-	-0.40	0.036	↘↘	=	=	=	=	=	=	M >	M >>	=
6404	MID	0.05	0.80	-	-0.67	0.0001	↘↘↘↘	=	=	=	=	=	=	=	=	=
6527	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	-0.41	0.032	↘↘	-0.35	0.073	↘	=	=	=	=	=	=	=	=	=

6609	ND	0.04	0.85	-	-0.51	0.006	↘↘↘	=	=	=	=	=	=	=	=	=
6615	ND	-0.53	0.004	↘↘↘	-0.14	0.50	-	=	=	=	=	=	=	=	=	=
6617	ATP synthase subunit alpha	-0.15	0.45	-	-0.57	0.002	↘↘↘	=	=	=	=	=	=	=	=	=
6629	Chaperonin CPN60-2	0.25	0.20	-	0.49	0.009	↗↗↗	=	=	=	=	=	=	=	=	=
6630	MID	-0.39	0.046	↘↘	-0.26	0.20	-	=	=	=	=	=	=	=	=	=
6702	MID	0.39	0.043	↗↗	0.28	0.15	-	=	=	=	=	=	=	=	=	=
6704	Chaperonin CPN60-2	0.14	0.47	-	0.47	0.014	↗↗	=	=	=	=	=	=	=	=	=
6706	Vacuolar proton ATPase catalytic subunit A	-0.08	0.69	-	-0.51	0.007	↘↘↘	=	=	=	=	=	=	=	=	=
6729	MID	0.23	0.25	-	-0.42	0.028	↘↘	=	=	=	=	=	=	=	=	=
7205	L-ascorbate peroxidase 2	-0.16	0.43	-	-0.40	0.038	↘↘	=	=	M >	=	=	=	M >>	=	=
7306	ND	-0.55	0.003	↘↘↘	-0.49	0.009	↘↘↘	=	=	=	=	=	=	=	=	=
7309	Caffeoyl-CoA O-methyltransferase	-0.33	0.096	↘	-0.65	0.0003	↘↘↘↘	=	=	=	=	=	=	=	=	=
7311	ND	0.40	0.039	↗↗	0.84	<0.0001	↗↗↗↗	=	M >>	=	=	=	=	=	=	=
7318	ND	0.31	0.12	-	0.72	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
7338	ND	-0.40	0.038	↘↘	-0.55	0.003	↘↘↘	M >	=	=	=	=	=	=	=	=
7341	Phytepsin	0.51	0.007	↗↗↗	0.32	0.100	↗	=	=	M >>	=	=	=	=	=	=
7342	ND	-0.54	0.004	↘↘↘	-0.62	0.0005	↘↘↘↘	=	=	=	=	=	=	=	=	=
7343	ND	0.43	0.026	↗↗	0.63	0.0005	↗↗↗↗	=	=	=	=	=	=	=	=	=
7409	ND	0.08	0.70	-	0.52	0.005	↗↗↗	=	=	=	=	=	=	=	=	=
7416	ND	-0.33	0.093	↘	0.26	0.18	-	=	=	=	=	=	=	=	=	NM >>
7426	40S ribosomal protein SA	-0.46	0.016	↘↘	-0.30	0.13	-	=	=	=	=	=	=	=	=	=
7504	Adenosine kinase	-0.39	0.042	↘↘	-0.41	0.033	↘↘	=	=	=	=	=	=	=	=	=
7516	ND	-0.25	0.22	-	-0.47	0.013	↘↘	=	=	=	=	=	=	M >>	M >>	=
7518	Glutamine synthetase	-0.41	0.035	↘↘	-0.32	0.12	-	=	=	=	=	=	=	=	=	=
7519	Sucrose:sucrose 1-fructosyltransferase	-0.18	0.36	-	0.00	0.99	-	=	=	M >>	M >>	M >>	=	=	=	=
7605	Alpha tubulin	0.13	0.53	-	-0.53	0.004	↘↘↘	=	=	=	=	=	=	=	=	=
7616	Tubulin beta-5 chain	-0.39	0.043	↘↘	-0.53	0.005	↘↘↘	=	=	=	=	=	=	=	=	=
7617	Beta-tubulin	-0.59	0.001	↘↘↘	-0.52	0.007	↘↘↘	=	=	=	=	=	=	=	=	=
7621	ND	-0.46	0.015	↘↘	-0.55	0.003	↘↘↘	=	=	=	=	=	=	=	=	=
7626	Beta-tubulin	-0.39	0.047	↘↘	-0.34	0.090	↘	=	=	=	=	=	=	=	=	=
8411	ND	-0.46	0.017	↘↘	0.08	0.68	-	=	=	=	=	=	=	=	=	=

Table 3. Results of statistical tests for the 85 protein spots matched with a single protein identity. Sp: spots number; ID: results of protein identification after LC/MS/MS (ND = not determined); rM/rNM: r from Pearson's correlation for either M or NM population, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations values at each Cu exposure, from1 to 50 µM Cu, =: no difference, >/>>: intensity of the difference (> indicated ratio higher than x1.5 but lower than x2, >> indicated ratio superior to x2) and M/NM indicated the population with higher values.

Sp	ID	rM	pval	Sign.	rNM	pval	Sign.	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
Functional category 1: Metabolism																
1808	Glycine dehydrogenase [decarboxylating]	-0.48	0.012	↘↘	-0.15	0.46	-	=	M >>	=	=	=	=	=	=	=
2727	D-3-phosphoglycerate dehydrogenase	-0.03	0.87	-	-0.19	0.34	-	=	=	=	=	=	=	=	=	M >>
2618	Alanine aminotransferase 2	0.09	0.66	-	0.48	0.011	↗↗	=	=	=	=	=	=	=	=	=
2623	Alanine aminotransferase 2	0.22	0.28	-	-0.30	0.12	-	=	=	=	=	=	=	=	=	M >
4613	Succinate-semialdehyde dehydrogenase	-0.04	0.85	-	-0.38	0.049	↘↘	=	=	=	=	=	=	=	=	=
5404	Glutamine synthetase	-0.15	0.46	-	0.49	0.010	↗↗↗	=	=	=	=	=	=	=	=	=
7518	Glutamine synthetase	-0.41	0.035	↘↘	-0.32	0.12	-	=	=	=	=	=	=	=	=	=
5309	Cysteine synthase	0.29	0.15	-	0.55	0.003	↗↗↗	=	=	=	=	=	=	=	=	=
6303	Cysteine synthase	0.11	0.60	-	0.53	0.005	↗↗↗	=	=	=	=	=	=	=	=	=
2802	Methionine synthase	-0.33	0.089	↘	-0.60	0.0010	↘↘↘↘	=	=	=	=	=	=	=	M >	=
3526	S-adenosylmethionine synthase	0.44	0.023	↗↗	0.11	0.59	-	=	=	=	=	=	=	=	=	=
4541	S-adenosylmethionine synthase	0.33	0.097	↗	0.47	0.012	↗↗	=	=	=	=	=	=	=	=	=
5506	S-adenosylmethionine synthase	0.57	0.002	↗↗↗	0.68	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
5425	Methylthioribose-1-phosphate isomerase	-0.09	0.67	-	0.59	0.001	↗↗↗	=	=	M >>	=	=	=	=	NM >>	=
5426	Methylthioribose-1-phosphate isomerase	0.37	0.055	↗	0.72	<0.001	↗↗↗↗	=	=	M >	=	=	=	NM >>	=	NM >
2725	Ketol-acid reductoisomerase	0.35	0.075	↗	0.25	0.20	-	=	=	NM >>	=	=	=	=	=	=
3701	Ketol-acid reductoisomerase	0.35	0.075	↗	0.46	0.017	↗↗	=	=	=	=	=	=	=	=	=
3709	Ketol-acid reductoisomerase	0.65	0.0003	↗↗↗↗	0.19	0.35	-	=	=	=	=	=	=	=	M >>	=
3712	Ketol-acid reductoisomerase	0.39	0.043	↗↗	0.30	0.13	-	=	=	=	=	=	=	=	=	=
2724	Phenylalanine/tyrosine ammonia-lyase	-0.40	0.040	↘↘	-0.39	0.043	↘↘	=	=	=	=	=	=	=	=	=
3707	Phenylalanine/tyrosine ammonia-lyase	-0.25	0.21	-	-0.48	0.011	↘↘	=	=	M >	=	=	=	=	=	=
6215	Adenine phosphoribosyltransferase 1	0.65	<0.0001	↗↗↗↗	0.84	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
7504	Adenosine kinase	-0.39	0.042	↘↘	-0.41	0.033	↘↘	=	=	=	=	=	=	=	=	=
7519	Sucrose:sucrose 1-fructosyltransferase	-0.18	0.36	-	0.00	0.99	-	=	=	M >>	M >>	M >>	=	=	=	=
3409	Alpha-galactosidase	0.48	0.011	↗↗	0.30	0.13	-	=	=	=	=	=	=	=	=	=
1708	6-phosphofructokinase, pyrophosphate dependent	-0.32	0.098	↘	0.14	0.47	-	=	M >>	=	=	=	M >	M >	=	=

Functional category 2: Energy																
4705	Phosphoglucomutase, cytoplasmic	-0.28	0.15	-	-0.42	0.033	↘↘	NM >>	NM >>	=	NM >>	=	NM >	=	=	=
2425	Fructose-bisphosphate aldolase	-0.40	0.037	↘↘	0.10	0.62	-	=	=	=	=	=	=	=	=	=
6209	Triosephosphate isomerase	-0.41	0.035	↘↘	-0.19	0.35	-	=	=	M >	=	=	=	=	=	=
2223	Glyceraldehyde-3-phosphate dehydrogenase 1	0.59	0.001	↗↗↗	0.40	0.037	↗↗	=	=	=	=	=	=	=	=	=
2801	Aconitate hydratase	-0.20	0.31	-	-0.53	0.004	↘↘↘	=	=	=	=	=	=	=	=	=
2805	Aconitate hydratase	0.03	0.87	-	-0.51	0.007	↘↘↘	=	=	=	=	=	=	=	=	M >
2810	Aconitate hydratase	-0.37	0.058	↘	-0.43	0.025	↘↘	=	=	=	=	=	=	=	=	=
2818	Aconitate hydratase	-0.32	0.11	-	-0.46	0.016	↘↘	=	M >	=	=	=	=	=	=	=
3802	Aconitate hydratase	0.06	0.78	-	-0.56	0.002	↘↘↘	=	=	=	=	=	=	=	=	=
2525	Isocitrate dehydrogenase [NADP]	0.39	0.042	↗↗	0.37	0.060	↗	=	=	=	=	=	=	=	=	=
3411	Malate dehydrogenase	0.39	0.044	↗↗	0.29	0.14	-	=	=	=	=	=	=	=	=	=
3718	Succinate dehydrogenase [Ubi] flavoprotein	-0.36	0.062	↘	-0.60	0.001	↘↘↘	=	=	=	=	=	=	=	=	=
4702	Succinate dehydrogenase [Ubi] flavoprotein	-0.15	0.46	-	-0.63	0.0004	↘↘↘↘	=	=	=	=	=	=	=	=	=
3815	NADH dehydrogenase [Ubi] iron-sulfur protein 1	-0.05	0.79	-	-0.63	0.0005	↘↘↘↘	=	=	=	=	=	=	=	=	=
4801	NADH dehydrogenase [Ubi] iron-sulfur protein 1	-0.24	0.23	-	-0.56	0.003	↘↘↘	=	=	=	=	=	=	=	=	=
4601	ATP synthase subunit alpha	-0.48	0.012	↘↘	-0.01	0.96	-	=	=	=	=	=	=	=	=	=
6617	ATP synthase subunit alpha	-0.15	0.45	-	-0.57	0.002	↘↘↘	=	=	=	=	=	=	=	=	=
6706	Vacuolar proton ATPase catalytic subunit A	-0.08	0.69	-	-0.51	0.007	↘↘↘	=	=	=	=	=	=	=	=	=
513	Formate dehydrogenase	0.52	0.006	↗↗↗	0.77	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
1503	Formate dehydrogenase	0.40	0.036	↗↗	0.73	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
1507	Formate dehydrogenase	0.16	0.41	-	0.61	0.0008	↗↗↗↗	=	=	=	=	=	=	=	=	=
Functional category 5: Protein synthesis																
7426	40S ribosomal protein SA	-0.46	0.016	↘↘	-0.30	0.13	-	=	=	=	=	=	=	=	=	=
Functional category 6: Protein destination and storage																
4716	Heat shock 70 kDa protein 10	-0.13	0.50	-	0.31	0.11	-	=	=	M >>	=	=	M >	M >	M >>	=
6704	Chaperonin CPN60-1	0.14	0.47	-	0.47	0.014	↗↗	=	=	=	=	=	=	=	=	=
6629	Chaperonin CPN60-2	0.25	0.20	-	0.49	0.009	↗↗↗	=	=	=	=	=	=	=	=	=
1504	Protein disulfide isomerase-like 2-1	-0.21	0.29	-	0.42	0.029	↗↗	=	=	=	=	=	=	=	=	=
2207	Cysteine proteinase inhibitor 12	0.10	0.62	-	-0.20	0.32	-	=	=	=	=	=	=	=	=	M >>
1618	Mitochondrial-processing peptidase subunit alpha	-0.04	0.82	-	0.04	0.84	-	M >>	M >>	M >>	M >>	M >>	M >>	=	M >	M >
1626	Mitochondrial-processing peptidase subunit alpha	-0.54	0.004	↘↘↘	-0.16	0.43	-	=	=	=	=	M >	=	=	=	=
7341	Phytepsin	0.51	0.007	↗↗↗	0.32	0.100	↗	=	=	M >>	=	=	=	=	=	=

1315	26S proteasome non-ATPase regulatory subunit	0.41	0.032	↗↗	0.30	0.13	-	=	=	=	=	=	=	=	=	=
2222	20S Proteasome subunit beta type	0.41	0.033	↗↗	0.22	0.27	-	=	=	=	=	=	=	=	=	=
Functional category 7: Transporters																
1414	Probable voltage-gated potassium channel	-0.11	0.58	-	-0.32	0.099	↘	=	=	=	=	=	=	=	M >>	=
5322	Remorin	-0.51	0.006	↘↘↘	-0.35	0.076	↘	=	=	=	=	=	=	=	=	=
Functional category 9: Cell structure																
5514	Actin	0.13	0.52	-	-0.39	0.043	↘↘	=	=	=	=	=	=	=	=	=
7605	Alpha tubulin	0.13	0.53	-	-0.53	0.004	↘↘↘	=	=	=	=	=	=	=	=	=
7616	Tubulin beta-5 chain	-0.39	0.043	↘↘	-0.53	0.005	↘↘↘	=	=	=	=	=	=	=	=	=
7617	Beta-tubulin	-0.59	0.001	↘↘↘	-0.52	0.007	↘↘↘	=	=	=	=	=	=	=	=	=
7626	Beta-tubulin	-0.39	0.047	↘↘	-0.34	0.090	↘	=	=	=	=	=	=	=	=	=
Functional category 11: Disease/defense																
1211	L-ascorbate peroxidase 1	-0.66	<0.0001	↘↘↘↘	-0.76	<0.0001	↘↘↘↘	=	=	=	=	=	=	=	=	=
1220	L-ascorbate peroxidase 1	-0.55	0.003	↘↘↘	-0.66	0.0002	↘↘↘↘	=	=	=	=	=	=	=	=	=
2312	Probable L-ascorbate peroxidase 6	0.05	0.80	-	0.69	<0.0001	↗↗↗↗	=	=	=	=	=	=	=	=	=
6203	L-ascorbate peroxidase 2	0.32	0.10	-	-0.09	0.67	-	M >>	=	=	=	=	=	=	=	M >>
6212	L-ascorbate peroxidase 2	-0.21	0.30	-	-0.45	0.019	↘↘	=	=	=	=	=	=	=	=	=
6213	L-ascorbate peroxidase 2	-0.24	0.22	-	-0.69	<0.0001	↘↘↘↘	=	=	=	=	=	=	=	M >	=
7205	L-ascorbate peroxidase 2	-0.16	0.43	-	-0.40	0.038	↘↘	=	=	M >	=	=	=	M >>	=	=
5415	Peroxidase 2	-0.63	0.0004	↘↘↘↘	-0.26	0.20	-	=	=	=	=	=	=	=	=	=
217	Glutathione S-transferase	-0.34	0.082	↘	-0.06	0.77	-	M >	M >>	M >>	M >>	=	M >	M >	M >	=
6205	Glutathione S-transferase GSTZ5	-0.40	0.037	↘↘	-0.04	0.84	-	=	=	=	=	=	=	=	=	=
2210	Superoxide dismutase [Mn]	0.34	0.080	↗	0.53	0.005	↗↗↗	=	=	=	=	=	=	=	=	=
3202	Superoxide dismutase [Mn]	0.46	0.015	↗↗	0.61	0.0008	↗↗↗↗	=	=	=	=	=	=	=	=	=
2609	Aldehyde dehydrogenase family 2 member B7	0.56	0.002	↗↗↗	0.08	0.69	-	=	=	=	=	=	=	=	=	=
2512	Alcohol dehydrogenase	-0.06	0.78	-	-0.61	0.0008	↘↘↘↘	=	=	NM >	=	=	=	=	=	=
Functional category 20: Secondary metabolism																
3427	Flavone 3'-O-methyltransferase 1	-0.38	0.048	↘↘	-0.57	0.002	↘↘↘	=	=	=	=	=	=	=	=	=
4420	Tricetin 3',4',5'-O-trimethyltransferase	-0.54	0.004	↘↘↘	-0.61	0.0008	↘↘↘↘	=	NM >>	=	=	=	=	=	=	=
7309	Caffeoyl-CoA O-methyltransferase	-0.33	0.096	↘	-0.65	0.0003	↘↘↘↘	=	=	=	=	=	=	=	=	=
2511	Probable cinnamyl alcohol dehydrogenase	-0.08	0.69	-	0.45	0.018	↗↗	=	=	=	=	=	=	=	=	=
2424	UDP-arabinopyranose mutase 1	0.48	0.012	↗↗	0.51	0.006	↗↗↗	=	=	=	=	=	=	=	=	=
6527	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	-0.41	0.032	↘↘	-0.35	0.073	↘	=	=	=	=	=	=	=	=	=

Table 4. Identification of the 85 protein spots matched with a single protein identity; only the best match between both databases is shown. Sp: spot number; Db: consulted database, V: *Viridiplantae* of Uniprot and A: *Agrostis* spp. EST database; ID: Protein identity; Uniprot: Uniprot Accession; gb Access: Genbank Accession; eval: e-value of NCBI blastx; Cov: % of sequence coverage between experimental and database; (nb): number of peptides matched between both sequences; peptides: list of matched peptides. Complete identification is available in Annex 16.

Sp	Db	ID	Uniprot	cov (nb)	gb Access / eval
		Functional category 1: Metabolism			
1808	A	Glycine dehydrogenase [decarboxylating] EC = 1.4.4.2	O49852	17.5 (4)	DV857616 / 4E-177
2727	V	D-3-phosphoglycerate dehydrogenase EC = 1.1.1.95	O04130	4.8 (3)	
2618	V	Alanine aminotransferase 2 EC = 2.6.1.2	P52894	21.6 (6)	
2623	V	Alanine aminotransferase 2	P52894	19.5 (6)	
4613	V	Succinate-semialdehyde dehydrogenase, mitochondrial EC = 1.2.1.24	P51649	20.5 (8)	
5404	A	Glutamine synthetase EC = 6.3.1.2	C5IW59	5.2 (3)	GR282200_2 / 1e-124
7518	A	Glutamine synthetase	I1J2T4	16.5 (2)	GR278149_5 / 5e-105
5309	V	Cysteine synthase EC = 2.5.1.47	P38076	24.6 (6)	
6303	V	Cysteine synthase	P38076	37.5 (8)	
2802	V	Methionine synthase : MetE EC = 2.1.1.14	P93263	7.3 (5)	
3526	V	S-adenosylmethionine synthase EC = 2.5.1.6	B0LXM0	33.8 (9)	
4541	V	S-adenosylmethionine synthase 3	Q4LB22	35.5 (8)	
5506	V	S-adenosylmethionine synthase 1	A2Y053	27.5 (7)	
5425	V	Methylthioribose-1-phosphate isomerase EC = 5.3.1.23	Q9AYT7	8.5 (3)	
5426	V	Methylthioribose-1-phosphate isomerase	Q9AYT7	14.7 (4)	
2725	V	Ketol-acid reductoisomerase EC = 1.1.1.86	Q65XK0	11.3 (5)	
3701	V	Ketol-acid reductoisomerase, chloroplastic	Q65XK0	4.5 (2)	
3709	V	Ketol-acid reductoisomerase, chloroplastic	Q65XK0	4.5 (2)	
3712	V	Ketol-acid reductoisomerase, chloroplastic	Q01292	5.4 (2)	
2724	V	Phenylalanine ammonia-lyase EC = 4.3.1.24	P14717	15.4 (11)	
		Phenylalanine/tyrosine ammonia-lyase EC = 4.3.1.25	Q8VXG7	13.1 (9)	
3707	V	Phenylalanine ammonia-lyase	P14717	19.5 (11)	
		Phenylalanine/tyrosine ammonia-lyase	Q8VXG7	14.2 (9)	
6215	V	Adenine phosphoribosyltransferase 1 EC = 2.4.2.7	Q43199	30.9 (5)	
7504	A	Adenosine kinase EC = 2.7.1.20	Q8L5P6	22.5 (4)	DV866906_3 / 5e-65
7519	A	Sucrose:sucrose 1-fructosyltransferase EC = 2.4.1.99	Q9FSV7	15.4 (3)	GR279352 / 4E-63
3409	V	Alpha-galactosidase EC = 3.2.1.22	Q9FXT4	17 (6)	
1708	V	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase sub. beta EC = 2.7.1.90	Q41141	9.4 (6)	

		Functional category 2: Energy			
4705	V	Phosphoglucomutase, cytoplasmic EC = 5.4.2.2	Q9SNX2	23.8 (10)	
2425	A	Fructose-biphosphate aldolase EC = 4.1.2.13	Q9XGH5	33.6 (7)	DV853997_1 / 5e-142
6209	V	Triosephosphate isomerase, chloroplastic : TIM EC = 5.3.1.1	P46225	45.6 (13)	
2223	A	Glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic EC = 1.2.1.12	P26517	28 (6)	DV857802 / 8E-155
2801	A	Putative aconitate hydratase, cytoplasmic EC = 4.2.1.3	Q6YZX6	36.6 (8)	GR280935 / 9E-167
2805	A	Putative aconitate hydratase, cytoplasmic	Q6YZX6	29.8 (6)	GR280935 / 9E-167
2810	A	Putative aconitate hydratase, cytoplasmic	M8CZ57	14.9 (2)	FD932947_3 / 3e-60
2818	V	Aconitate hydratase (Fragment)	Q42669	4.19 (2)	
3802	A	Putative aconitate hydratase, cytoplasmic	Q6YZX6	15.1 (3)	GR280935 / 1E-163
2525	V	Isocitrate dehydrogenase [NADP], chloro. EC = 1.1.1.42	Q40345	9 (3)	
3411	V	Malate dehydrogenase EC = 1.1.1.37	Q9FSF0	34.6 (7)	
3718	V	Succinate dehydrogenase [Ubi] flavoprotein subunit 1, mito. EC = 1.3.5.1	O82663	16.4 (7)	
4702	V	Succinate dehydrogenase [Ubi] flavoprotein subunit 1, mito.	O82663	27.4 (11)	
3815	A	NADH dehydrogenase [Ubi] iron-sulfur protein 1, mito. EC = 1.6.5.3 - 1.6.99.3	Q9FGI6	37.8 (6)	DV868571 / 4E-87
4801	V	NADH dehydrogenase [Ubi] iron-sulfur protein 1, mito.	Q9FGI6	9.8 (4)	
4601	V	ATP synthase subunit alpha, mito. EC = 3.6.3.14	P0C520	36.5 (14)	
6617	V	ATP synthase subunit alpha, mito.	P0C520	28.3 (9)	
6706	V	Vacuolar proton ATPase catalytic subunit alpha EC = 3.6.3.14	Q40002	34.3 (15)	
513	V	Formate dehydrogenase 1, mitochondrial EC = 1.2.1.2	Q9SXP2	21.3 (7)	
1503	V	Formate dehydrogenase 1, mitochondrial	Q9SXP2	30.9 (11)	
1507	V	Formate dehydrogenase, mitochondrial	Q9ZRI8	6.4 (3)	
		Functional category 5: Protein synthesis			
7426	V	40S ribosomal protein SA	O80377	9.1 (3)	
		Functional category 6: Protein destination and storage			
4716	V	Heat shock 70 kDa protein 10, mitochondrial	Q9LDZ0	3.7 (2)	
6704	V	Chaperonin CPN60-1, mitochondrial	P29185	19.4 (13)	
6629	V	Chaperonin CPN60-2, mitochondrial	Q05046	6.4 (3)	
1504	V	Protein disulfide isomerase-like 2-1 : PDI EC = 5.3.4.1	Q75M08	12.6 (4)	
2207	V	Cysteine proteinase inhibitor 12	Q0JNR2	10.4 (3)	
1618	A	Mitochondrial-processing peptidase subunit alpha EC = 3.4.24.64	P29677	19.1 (4)	DV855540 / 3E-41
1626	A	Mitochondrial-processing peptidase alpha-chain	Q9FNU9	21.1 (4)	DV855540_3 / 4e-77
7341	V	Phytepsin EC = 3.4.23.40	P42210	13.8 (6)	
1315	A	26S Proteasome non-ATPase regulatory subunit 14 EC = 3.4.19.-	G0Z6F1	16.9 (2)	DV857892_2 / 2e-142
2222	A	20S Proteasome subunit beta type EC = 3.4.25.1	I1H1Q7	23.1 (4)	DV860130_6 / 3e-122

		Functional category 7: Transporters			
1414	V	Probable voltage-gated K(+) channel subunit beta	Q40648	11.6 (3)	
5322	A	Remorin : DNA-binding protein	B4G1B0	17.3 (4)	DV856161_3 / 2e-37
		Functional category 9: Cell structure			
5514	V	Actin-1	A2XLF2	33.4 (10)	
7605	V	Tubulin alpha-1 chain	O22347	37.7 (13)	
7616	V	Tubulin beta-4 chain	Q9ZRA8	41.4 (15)	
7617	V	Tubulin beta-5 chain	Q9ZRA8	51 (17)	
7626	V	Tubulin beta-2 chain	P18026	48.2 (15)	
		Functional category 11: Disease/defense			
1211	A	L-ascorbate peroxidase 1: APX EC = 1.11.1.11	Q10N21	28.7 (7)	DV857848 / 2E-135
1220	A	L-ascorbate peroxidase 1, cytosolic	M7ZQM4	15.8 (4)	DV857848_1 / 2e-141
2312	V	Probable L-ascorbate peroxidase 6, chloroplastic	P0C0L1	23.6 (6)	
6203	A	L-ascorbate peroxidase 2, cytosolic	Q9FE01	24.8 (5)	GR281667 / 4E-108
6212	V	L-ascorbate peroxidase 2, cytosolic	Q9FE01	20.7 (3)	
6213	A	L-ascorbate peroxidase 2, cytosolic	M8C1W9	30.4 (6)	GR281667_1 / 9e-118
7205	A	L-ascorbate peroxidase 2, cytosolic	Q9FE01	34.1 (7)	GR281667 / 4E-108
5415	V	Peroxidase 2 (Fragment) EC = 1.11.1.7	Q01548	9.4 (2)	
217	A	Glutathione S-transferase: GST EC = 2.5.1.18	P12653	11.7 (3)	DV862008 / 2E-46
6205	A	Protein IN2-1 homolog B = GSTZ5	Q8H8U5	20.5 (7)	DV854188 / 1E-103
2210	A	Superoxide dismutase [Mn] : Mn-SOD EC = 1.15.1.1	I1HKJ7	47.8 (7)	DV859502_4 / 2e-105
3202	A	Superoxide dismutase [Mn]	I1HKJ7	33.3 (6)	DV859502_4 / 2e-105
2609	A	Aldehyde dehydrogenase family 2 member B7, mitochondrial EC = 1.2.1.3	Q8S528	25.8 (5)	DY543427 / 2E-91
2512	V	Alcohol dehydrogenase 3 EC = 1.1.1.1	P10848	21.1 (6)	
		Functional category 20: Secondary metabolism			
3427	V	Flavone O-methyltransferase 1 EC = 2.1.1.42	Q84N28	38.6 (11)	
4420	V	Tricetin 3',4',5'-O-trimethyltransferase	Q38J50	8.7 (4)	
7309	A	Caffeoyl-CoA O-methyltransferase EC = 2.1.1.104	M4GQ75	32.4 (8)	DV856154_2 / 5e-163
2511	V	Probable cinnamyl alcohol dehydrogenase EC = 1.1.1.195	O22380	22.4 (7)	
2424	V	UDP-arabinopyranose mutase 1 EC = 5.4.99.30	Q9SRT9	6.4 (2)	
6527	V	4-hydroxy-3-methylbut-2-enyl diphosphate reductase, chloroplastic EC = 1.17.1.2	Q94B35	9 (4)	

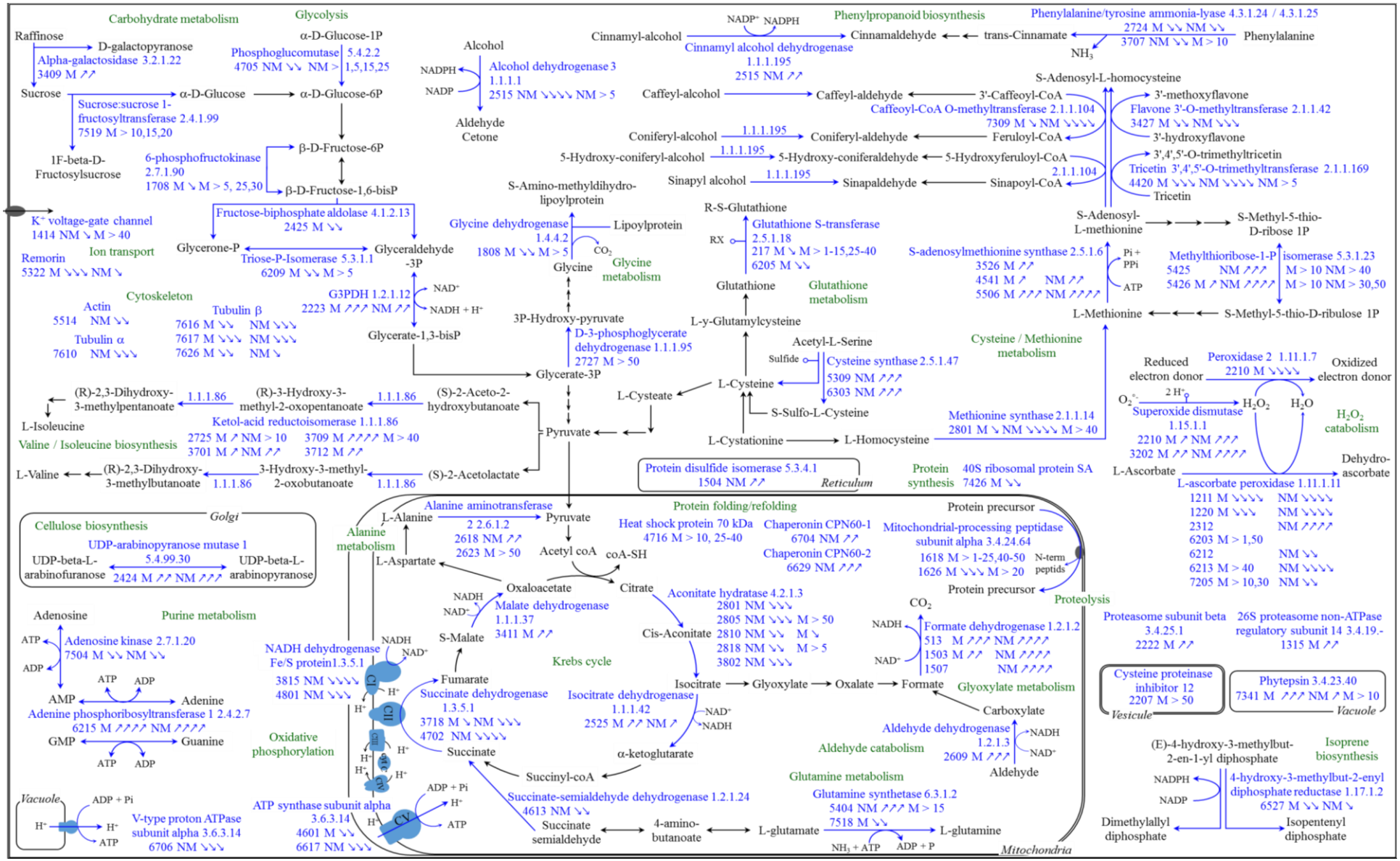


Figure 7: Functions of the identified proteins (in blue) in plant metabolic processes. Enzymes are represented by their name and EC. Spot numbers and identifications are listed in Tab. 2. Variation of root spots refers to Tab. 3. M / NM: Metallicolous / Non-Metallicolous population of *A. capillaris*. ↗ / ↘: positive / negative correlation (Pearson); p-val: 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; M/NM > 1-50: population with higher expression at 1-50 µM Cu (ratio > x1.5).

3.3. Pattern of protein accumulation

Description of protein spot expression and identification was made according to the functional categories presented in Fig. 6b and referred to Tab. 3-4 and Fig. 7, so no further reference to these tables were cited in the text. Even if the correlations with p-value comprised between 0.05 and 0. 1 were indicated in the figure 7, these variations were considered as non-significant and not considered in the following parts.

To simplify reading, 'protein spot expression' were sometimes abbreviated by 'expression', if no additional indication is provided. To shorten the text, 'protein spot matched as XX' or 'protein spot identified as XX' formula were not used and protein identities were cited directly (Tab. 4). Additionally, 'positively/negatively correlated with Cu exposure' were replaced by 'increased/decreased' or 'down-/up-regulated'.

3.3.1. Functional category 1: Metabolism

Enzymes belonging to the metabolism of amino-acids, i.e. Glycine, Alanine, Glutamine, Cysteine/Methionine, Valine/Leucine and Phenylalanine, were differentially expressed depending on Cu exposure and populations.

A glycine dehydrogenase (#1808) and a D-3-phosphoglycerate dehydrogenase (#2727) were over-expressed in M at 5 and 50 µM Cu respectively (ratio > 2). Expression of #2727 decreased significantly under Cu exposure only in M ($r = -0.48$, p-val = 0.012).

Expression of one alanine aminotransferase 2, #2618 increased with Cu only in NM ($r = 0.48$; p-val = 0.011), while #2623 expression was higher in M at 50 µM ($1.5 < \text{ratio} < 2$) but not significantly correlated with Cu. Expression of a succinate-semialdehyde dehydrogenase (#4613) and one glutamine synthetase (#7518) decreased respectively in NM ($r = -0.38$, p-val = 0.049) and M roots ($r = -0.41$, p-val = 0.035), while expression of the second glutamine (#5404) increased in NM ($r = 0.49$; p-val = 0.01). None of these three spots differed significantly between populations on this range of Cu exposure.

Three spots involved in cysteine and methionine biosynthesis did respond to Cu only in NM. Both cysteine synthases (#5309 and 6303) were up-regulated ($r = 0.55$ and 0.53; p-values = 0.003 and 0.005), while a methionine synthase (#5309) was down-regulated ($r = -0.60$, p-val = 0.001), resulting in a higher expression in M at 40 µM Cu ($1.5 < \text{ratio} < 2$). Expression of the three S-adenosylmethionine synthases (#3526, 4541 and 5506) was significantly up-regulated by Cu exposure in at least one population, #3526 increased only in M ($r = 0.44$ and p-val = 0.023), #4541 only in NM ($r = 0.47$, p-val = 0.012) and #5506 in both M ($r = 0.57$, p-val =

0.002) and NM (r = 0.68, p-val < 0.0001). Both methylthioribose-1-phosphate isomerases (#5425 and 5426) were significantly up-regulated only in NM roots (r = 0.59 and 0.72, p-values = 0.001 and < 0.0001 respectively), over-expressed in M at 10 µM (ratio > 2 for #5425 and 1.5 < ratio < 2 for #5426) but in NM at high Cu exposure (1.5 < ratio < 2 at 40 µM Cu for #5425 and at 30 and 50 µM Cu for #5426).

Three out of four ketol-acid reductoisomerases were up-regulated by Cu exposure, #3701 only in NM (r = 0.46, p-val = 0.017), while #3709 and 3712 only in M (r = 0.65 and 0.39, p-values = 0.0003 and 0.043), leading to over-expression of #3709 in M at 40 µM Cu (ratio > 2). One additional spot (#2725) was over-expressed in NM at 10 µM (ratio > 2) but did respond significantly to Cu exposure.

Expression of two phenylalanine/phenylalanine-tyrosine ammonia-lyase (#2724 and 3707), decreased in NM (r = -0.39 and -0.48; p-values = 0.043 and 0.011), but only #2724 decreased also in M roots (r = -0.40; p-val = 0.04) and expression of #3707 was higher in M at 10 µM (1.5 < ratio < 2).

Two enzymes involved in purine metabolism did respond to Cu in both populations, an adenine phosphoribosyltransferase 1 (#6215) was up-regulated (r = 0.65 and 0.84, p-val < 0.0001), while an adenosine kinase (#7504) was down-regulated (r = -0.39 and -0.41; p-values = 0.042 and 0.033 for M and NM respectively).

Among the three enzymes belonging to carbohydrate metabolism, a sucrose:sucrose 1-fructosyltransferase (#7519) and a 6-phosphofructokinase (#1708) were over-expressed in M at intermediate Cu exposure, #7519 between 10 and 20 µM (ratio > 2) and #1708 at 5 (ratio > 2), 25 and 30 µM Cu (1.5 < ratio < 2). An alpha-galactosidase (#3409) was up-regulated by Cu exposure only in M roots (r = 0.48, p-val = 0.011).

3.3.2. Functional category 2: Energy

Among the four enzymes involved in glycolysis, only the glyceraldehyde-3-phosphate dehydrogenase 1 (#2223) was up-regulated in both populations, more markedly in M roots (r = 0.59 and 0.4, p-values = 0.001 and 0.03). Phosphoglucomutase (#4705) expression was higher in NM at low and intermediate exposures (1, 5, 15 and 25 µM, ratio > 2), but decreased only in this population (r = -0.42, p-val = 0.033), leading to non-significant difference at higher Cu exposure. On the opposite, expression of a fructose-bisphosphate aldolase (#2425) and a triosephosphate isomerase (#6209) decreased only in M (r = -0.40 and -0.41, p-values = 0.037 and 0.035 respectively). #6209 was also over-expressed in M at 10 µM (1.5 < ratio < 2).

Among the seven enzymes belonging to the Krebs cycle/Oxidative phosphorylation only isocitrate (IDH, #2525) and malate (MDH, #3411) dehydrogenases were up-regulated only in M ($r = 0.39$ and 0.39, p-values = 0.042 and 0.044) and all other were down-regulated. Mitochondrial ATP synthase subunit alpha spots (#4601 and 6617) were respectively down-regulated in M ($r = -0.48$, p-val = 0.012) and NM ($r = -0.57$, p-val = 0.002).

Five aconitate hydratases, (#2801, 2805, 2810, 2818 and 3802), were down-regulated only in NM ($r = -0.53$, -0.51, -0.43, -0.46 and -0.56, p-values = 0.004, 0.007, 0.025, 0.016 and 0.002), as well as two succinate dehydrogenases [ubiquinone] flavoprotein subunit spots (#3718 and 4702, $r = -0.60$ and -0.63, p-values = 0.001 and 0.0004), two NADH dehydrogenase Fe/S protein (#3815 and 4801, $r = -0.63$ and -0.56, p-values = 0.0005 and 0.003) and a V-type ATP synthase subunit alpha (#6706, $r = -0.51$, p-val = 0.007). Among these last ten spots, two aconitases, #2805 and 2818 were over-expressed in M at 50 and 5 µM Cu respectively ($1.5 < \text{ratio} < 2$).

Expression of three formate dehydrogenase spots (#513, 1503 and 1507) increased sharply in NM ($r = 0.77$, 0.73 and 0.61, p-values < 0.0001, < 0.0001 and $= 0.0008$ respectively) but only two, #513 and 1503, increased also to a lesser extent in M ($r = 0.52$ and 0.40, p-values = 0.006 and 0.036 respectively).

3.3.3. Functional category 5: Protein synthesis

Expression of a 40S ribosomal protein SA decreased only in M ($r = -0.46$, p-val = 0.016) but no significant difference between populations was indicated by ratios.

3.3.4. Functional category 6: Protein destination and storage

A 70kDa heat shock protein (#4716) was over-expressed in M at 10 (ratio > 2), 25, 30 ($1.5 < \text{ratio} < 2$) and 40 µM Cu (ratio > 2), while expression of two chaperonins (CPN60-1, #6704 and CPN60-2, #6629) and a protein disulfide isomerase (#1504) increased only in NM ($r = 0.47$, 0.49 and 0.42, p-values = 0.014 and 0.009 respectively) but did not differ between populations, according to ratios.

Among the six proteins related to proteolysis, two, a cysteine proteinase inhibitor 12 (#2207) and a mitochondrial-processing peptidase subunit alpha (#1618) did not vary among Cu exposure but were respectively over-expressed in M at 50 µM Cu (ratio > 2) and at all tested Cu exposures except 30 µM (1-25 µM Cu: ratio > 2, 40-50 µM Cu: $1.5 < \text{ratio} < 2$). Another mitochondrial-processing peptidase subunit alpha (#1626) and a phytepsin (#7341) were over-expressed in M at 20 µM ($1.5 < \text{ratio} < 2$) and 10 µM Cu (ratio > 2) but also respectively down- and up-regulated only in M ($r = -0.54$ and 0.51, p-values = 0.004 and 0.007). The last two, 26S

proteasome non-ATPase regulatory subunit 14 (#1315) and 20S proteasome subunit beta type (#2222), increased only in M roots (r = 0.41 and 0.41, p-values = 0.032 and 0.033 respectively) but did not differ between populations according to ratios.

3.3.5. Functional category 7: Transporters

A voltage-gated potassium channel (#1414) was over-expressed in M at 40 µM Cu (ratio > 2) and expression of a remorin decreased only in M (r = -0.51, p-val = 0.006) but did not differ between populations.

3.3.6. Functional category 9: Cell structure

Five cytoskeleton proteins were down-regulated by Cu exposure in at least one population, one actin (#5514) and one tubulin alpha (#7605) only in NM (r = -0.39 and -0.53, p-values = 0.043 and 0.004), one tubulin beta (#7626) only in M (r = -0.39, p-val = 0.047) and two other tubulins beta (#7616 and 7617) in both M (r = -0.39 and -0.59, p-values = 0.043 and 0.001) and NM (r = -0.53 and -0.52, p-values = 0.005 and 0.007).

3.3.7. Functional category 11: Disease/defense

Among the seven spots identified as L-ascorbate peroxidases, one (#6203) was over-expressed in M at 1 and 50 µM Cu (ratio > 2) but did not differ among Cu exposure, while two other were over-expressed in M at 10 (#7205, 1.5 < ratio < 2), 25 (#7205, ratio > 2) and 30 µM Cu (#6213, ratio > 2) but also down-regulated in NM roots (r = -0.69 and -0.40, p-values < 0.0001 and = 0.038 for #6213 and 7205 respectively). Expression of two L-ascorbate peroxidases 1 (#1211 and 1220) were decreased in both M (r = -0.66 and -0.55, p-values < 0.0001 and = 0.003) and NM (r = -0.76 and -0.66, p-values < 0.0001 and = 0.0002), while an L-ascorbate peroxidase 2 (#6212) was down-regulated only in NM (r = -0.45, p-val = 0.019). Only one, a probable L-ascorbate peroxidase 6 (#2312), was up-regulated by Cu exposure, only in NM roots (r = 0.69, p-val < 0.0001).

Expression of a peroxidase 2 (#5415) and a glutathione S-transferase (GST, #6205) decreased only in M roots (r = -0.63 and -0.40, p-values = 0.0004 and 0.037 respectively) but did not differ significantly in NM or between populations. Another GST (#217) was over-expressed in M at 1 (1.5 < ratio < 2), 5-15 (ratio > 2) and 25-40 µM Cu (1.5 < ratio < 2).

Two Mn-superoxide dismutases (#2210 and 3202) were up-regulated in NM roots (r = 0.53 and 0.61, p-values = 0.005 and 0.0008) but only one, #3202 was also up-regulated in M(r = 0.46, p-val = 0.015). Two dehydrogenases, i.e. aldehyde dehydrogenase (#2609) and alcohol dehydrogenase (#2512) were respectively up-regulated in M (r = 0.56, p-val = 0.002) and down-

regulated in NM (r = -0.61, p-val = 0.0008). Spot #2512 was also over-expressed in NM at 10 µM Cu (1.5 < ratio < 2).

3.3.8. Functional category 20: Secondary metabolism

Two methyltransferases, flavone 3'-O-methyltransferase 1 (# 3427) and tricetin 3',4',5'-O-trimethyltransferase (#4420) were down-regulated by Cu exposure in both M (r = -0.38 and -0.54, p-values = 0.048 and 0.004) and NM (r = -0.57 and -0.61, p-values = 0.002 and 0.0008), while one UDP-arabinopyranose mutase 1 (#2424) was up-regulated in both M (r = 0.48, p-val = 0.012) and NM roots (r = 0.51, p-val = 0.006). Only #4420 was also over-expressed in NM at 5 µM Cu (ratio > 2).

Another methyltransferase, caffeoyl-CoA O-methyltransferase (#7309), was down-regulated significantly (r = -0.65, p-val = 0.0003) and a probable cinnamyl alcohol dehydrogenase up-regulated (r = 0.45, p-val = 0.018) only in NM roots.

4. Discussion

4.1. General comments

Comparing metallicolous and non-metallicolous populations from pseudo-metallophyte species is one option to unravel mechanisms underlying metal-tolerance in plants. To examine mechanisms of Cu-tolerance in roots, a metallicolous population of *A. capillaris*, native from a wood preservation site with Cu-contaminated soils, was compared to a non-metallicolous population collected on an uncontaminated soil, on the 1-50 µM Cu range.

Around 420 spots were reproducibly recorded in roots of *A. capillaris* (Fig. 1). This exceeded the amount of 300 spots determined in roots of *A. stolonifera* cultivars exposed to salt-stress for 28 days (Xu *et al.*, 2010), and in roots of *Cannabis sativa* plants exposed to 150 mg Cu/L (Bona *et al.*, 2007). However, it was lower than the 900 and 1 000 spots respectively recorded in roots of *Oryza sativa* seedlings exposed to 8 µM Cu for 3 days (Song *et al.*, 2013) and of *E. splendens* exposed to 100 µM Cu (Li *et al.*, 2009). Nevertheless, more spots did proportionally respond to Cu treatment in this study, as around half of the 419 quantified spots did respond to Cuwhereas only 34 out of 900 and 45 out of 1 000 spots respectively detected in *Oryza sativa* (Song *et al.*, 2013) and *E. splendens* (Li *et al.*, 2009) roots exhibited more than 1.5-fold change under Cu stress compared to control.

After running Pearson's correlations, 157 spots were excised and submitted to LC MS/MS, for been significantly correlated with Cu exposure in at least one population (p-val < 0.05) or over-expressed at least for one concentration with a ratio higher than two. In this experiment, the choice of Pearson's correlations permit to evaluate the pattern on the global range of Cu exposure but did not permit to identify the biphasic-type responses, which may result in a non-significant correlation. To get a precise overview of change in protein accumulations, additional time and statistical analyses will be necessary. It would be interesting to separate Cu exposure in two or three groups, i.e. low, intermediate and high, to obtain a better knowledge about nonlinear patterns of protein accumulation.

4.2. Involvement of proteins in metabolic pathways

In overall, our results agreed with the scheme for plant responses to excessive metal(loid) exposure, with differential expression of proteins involved in a large range of cellular processes including energy metabolism, amino-acid and protein metabolism, antioxidative and detoxification processes (Ahsan *et al.*, 2009, Hossain *et al.*, 2013).

4.2.1. Energy metabolism

Seven enzymes involved in Glycolysis/Carbohydrate metabolism reactions were addressed in this study (Tab. 3 and 4, Fig. 7) and exhibited great difference between populations in accumulation pattern. To maintain correct cell functioning under Cu stress, an increasing demand for ATP, NADH, NADPH, and reductive molecules occurs, leading to changes in expression of enzymes involved in energy provision (Cuypers *et al.*, 2011). Only the glyceraldehyde-3-phosphate dehydrogenase (G3PDH, EC = 1.2.1.12, #2223) was up-regulated by Cu exposure in both populations, although more sharply in M roots, which may promote both the production of pyruvate, which enters the Krebs cycle once converted into acetyl-coA, and the production of NADH, providing an increased source of reductive power for quenching the oxidative stress. Induction of G3PDH by Cu excess (100 µM) has been also reported in roots of four-week-old *E. splendens* plants, which expression increases 2.4 and 4.3-fold after 3 and 6 days of exposure respectively (Li *et al.*, 2009).

Accumulation of a G3PDH is induced by heat stress in roots of both heat-tolerant *A. scabra* and heat-sensitive *A. stolonifera*, while a second is induced only in the heat-tolerant species (Xu and Huang, 2008). A G3PDH is also induced by salt stress in roots of a NaCl-tolerant *A. stolonifera* cultivar but not in the sensitive one (10 $dS.m^{-1}$ for 28 days, Xu *et al.*, 2010). Induction of G3PDH in tolerant cultivar may contribute to this tolerance by promoting production of NADH. As G3PDH is also induced by Al in *A. comosus* roots (300 µM for 4

weeks; Chen and Lin, 2010) but repressed by As in *O. sativa* (50 and 100 µM for 4 days, Ahsan *et al.*, 2008), its role in tolerance to abiotic stress including metal(loid) excess, may vary depending on the stress.

Although over-expressed at low and intermediate exposure (1-25 µM Cu), a phosphoglucomutase (#4705) was down-regulated only in NM roots, and, together with the limitation of G3PDH accumulation, which reached a plateau at high Cu exposure (40-50 µM Cu), this indicated a strong limitation of energy metabolism in NM at Cu exposure higher than 25 µM. When exposed to 8 µM Cu, both Cu-tolerant and sensitive varieties of rice exhibit an induction of phosphoglucomutase (2.5/3-fold decrease; Song *et al.*, 2013), indicating that maintaining phosphoglucomutase accumulation may participate to the higher tolerance of the *Agrostis* population.

In M population, a sucrose:sucrose 1-fructosyltransferase (#7519) and a 6-phosphofructokinase pyrophosphate-dependent (#1708) were over-expressed at intermediate Cu exposure (10-20 and 25-30 µM Cu respectively) but decreased at higher Cu exposure (40-50 µM Cu). Together with the up-regulation of alpha-galactosidase (#3409), these proteins could contribute to the higher Cu tolerance in M at intermediate Cu exposure, by regulating sucrose metabolism to support glycolysis flow. In the same way, the linear increase of G3PDH accumulation, even at 40-50 µM Cu, may promote accumulation of NADH but also of pyruvate for Krebs cycle supply. Results suggested that M roots required more energy (ATP) and organic acids to maintain cell homeostasis under Cu stress, leading to consumption of stored carbohydrates and increased accumulation of Krebs-involved enzymes to provide more organic acids and ATP.

Surprisingly, accumulation of fructose bisphosphate aldolase (FBP aldolase, #2425) and triose phosphate isomerase (#6209) was down-regulated by Cu exposure only in M roots. However, this can stimulate the pentose phosphate pathway in favoring accumulation of β-D-fructose-6P. Various patterns of FBP aldolase accumulation were reported under abiotic stresses. In roots of *H. vulgare* genotypes exposed to Al (50 and 200 µM for 24 hours, Dai *et al.*, 2013) and of *A. stolonifera* cultivars exposed to salt stress (10 $dS.m^{-1}$ for 28 days, Xu *et al.*, 2010), FBP aldolase was induced only in the tolerant cultivar, but not in the sensitive one. Similarly, two FBP aldolase spots are induced by heat stress in roots of a tolerant *A. scabra* population but not in a heat-sensitive *A. stolonifera* one; however, a third one decreases in both species (Xu and Huang, 2008). Under Al excess, FBP aldolase is repressed in *A. comosus* roots (300 µM for 4 weeks; Chen and Lin, 2010).

While several enzymes involved in Krebs cycle and oxidative phosphorylation were differentially regulated by Cu exposure, most were repressed only in NM roots. Two mitochondrial ATP synthase subunit alpha (#4601 and 6617) were respectively down-regulated in M and NM roots, indicating that oxidative phosphorylation was disturbed by Cu toxicity in both populations. Higher Cu-induced damages on mitochondria in NM roots were shown by the sharp down-regulation of all other enzymes involved in Krebs cycle/Oxidative phosphorylation, i.e. aconitate hydratase (#2801, 2805, 2810, 2818 and 3802), succinate dehydrogenase [Ubi] flavoprotein (#3718 and 4702), NADH dehydrogenase [Ubi] Fe/S protein 1 (#3815 and 4801) and V-type proton ATPase subunit alpha (#6706) only in NM roots. Down-regulation of a NADH-ubiquinone dehydrogenase by Cu excess has previously been recorded in roots of four-week-old *E splendens* plants exposed to 100 µM Cu for 3 or 6 days (2-fold decrease, Li *et al.*, 2009). One aconitase is strongly repressed by Cd excess in roots of *Kandelia candel* exposed to 100 – 800 µM Cd for 3 days (Weng *et al.*, 2013), while another is down-regulated only in roots of Al-sensitive genotype of *Hordeum vulgare* exposed to 50 µM Al for 24 hours (Dai *et al.*, 2013).

Two additional enzymes involved in Krebs cycle, malate (MDH, #3411) and isocitrate (IDH, #2525) dehydrogenases were significantly up-regulated only in M roots ($p < 0.05$) and may provide an increasing amount of NADH but also of malic acid, which can chelate Cu and then maintain mitochondria integrity under Cu stress. Additionally, over-expression of alanine aminotransferase 2 (#2623) in M roots at 50 µM may enhance pyruvate supply for Krebs cycle and maintain a better energy supply in highly Cu-stressed M roots (50 µM Cu). Taken together, these results suggested a better protection of mitochondria and maintaining of energy metabolism in M roots for this Cu exposure range.

Four MDH spots are up-regulated by Cd stress in roots of the Cd-tolerant mangrove-like species *K. candel* (300 µM for 28 days; Chen and Lin, 2010), while another MDH spot is down-regulated by heat stress in roots of a thermal *A. scabra* population (Xu and Huang, 2008). Over-expression of two IDH spots has been recorded under salt stress in roots of *A. stolonifera* salt-tolerant cultivar compared to sensitive one (10 $dS.m^{-1}$ for 28 days; Xu *et al.*, 2010) and another one is induced in roots of *Ananas comosus* Al-tolerant cultivar in response to Al stress (300 µM for 28 days; Chen and Lin, 2010), indicating that increase of these enzymes may participate to enhance plant tolerance in response to different metal(loid) stresses.

4.2.2. Methionine/Cysteine metabolism

L-homocysteine is converted by methionine synthase into L-methionine, which is transformed by S-adenosylmethionine synthase into S-adenosyl methionine. Under increasing

Cu exposure, accumulation of a methionine synthase (#2801) was down-regulated only in NM roots while three S-adenosylmethionine synthases (#3526, 4541 and 5506) were up-regulated in one or both populations. Two methylthioribose-1-phosphate isomerases (#5425 and 5426), which catalyze the interconversion of S-methyl-5-thio-D-ribose-1-phosphate into S-methyl-5-thio-D-ribulose-1-phosphate, were over-expressed in M at low Cu exposure, then in NM at high Cu, due to a strong up-regulation only in NM roots. These results indicated that methionine metabolism was affected by Cu excess in *Agrostis* roots, confirming previous findings. In the preliminary experiment (Chapt II, section 4.2), two SAMS spots increased in Cu-stressed roots of both populations, more strictly in NM, while a third one increased only in M roots. In both experiments, although spots were differently regulated among population, any significant difference was recorded between populations.

Different patterns of protein accumulation have been reported for SAMS under various abiotic stresses, including Cu. Under low Cu exposure (8 µM Cu for 3 days), SAMS accumulation is up-regulated in roots of a Cu-tolerant (x2.1) and a sensitive (x1.6) varieties of *O. sativa* (Song *et al.*, 2013), while it is down-regulated in roots of *E. splendens* under high Cu exposure (1.5 and 2.4-fold decrease after 3 and 6 days at 100 µM Cu; Li *et al.*, 2009). SAMS accumulation is also down-regulated by Cd stress in roots of *K. candel* (100-800 µM for 3 days, Weng *et al.*, 2013) and *B. juncea* (250 mM; Alvarez *et al.*, 2009); by Al exposure in roots of *L. corniculatus* (10 and 20 µM for 14 days; Navascués *et al.*, 2012) and by heat stress in roots of tolerant *A. scabra* and heat-sensitive *A. stolonifera* (Xu and Huang, 2008). On the opposite, SAMS accumulation is gradually up-regulated in rice roots under increasing As exposure (50 and 100 µM; Ahsan *et al.*, 2008). In Al-resistant XN1 rice cultivar, two SAMS isoforms react differently, SAMS1 decreases while SAMS2 increases (2 mM for 3 days; Yang *et al.*, 2007).

However, the precise role of SAMS in tolerance remains unclear as its product, S-adenosyl methionine (SAM), is involved in three key metabolic pathways: trans-methylation, trans-sulfuration and polyamine synthesis. SAM is the main biological donor of methyl groups, which are transferred by methyl-transferases to a large variety of acceptors, such as DNA, phospholipids and proteins (Lu, 2000). Such methyl-transferases were found in our experiment, tricetin 3',4',5'-O-trimethyltransferase (#4420), flavone 3'-O-methyltransferase (#3427) and caffeoyl-CoA O-methyltransferase (#7309), which decreased in both populations, more sharply in NM.

SAM can provide a higher supply of methyl groups for methylation reactions, which may induce changes in membrane properties. However, such trans-methylation reactions were down-regulated by Cu-induced reduction of methyltransferase accumulation in both

populations, more markedly in NM roots, indicating a regulation of methylation to protect membrane integrity. Increase in cysteine synthase occurred only in NM roots, indicating an increasing need in sulfur-containing cysteine to process chelation mechanisms. Increasing accumulation of methyl donors may promote activity of methyltransferase, in order to compensate their reduced accumulation. However, increase in SAM content may also increase phospholipid methylation, leading to changes in membrane fluidity, so decrease in methyltransferase accumulation may reduce the negative impacts of methylation on membrane properties.

SAM also acts as direct precursor for nicotianamine (NA), trough nicotianamine synthase (Shojima *et al.*, 1990; Higuchi *et al.*, 1994) and indirect precursor for glutathione (GSH) through its conversion to cysteine via the trans-sulfuration pathway (Lu, 2000; Brosnan and Brosnan, 2006). NA is a key player in Cu homeostasis, for Cu transport, distribution, and accumulation (Pich *et al.*, 1996) but its role in Cu-tolerance remains controversial. It may be only involved in Cu transport from roots to shoots in case of deficiency (Irtelli *et al.*, 2009) whereas a Cu-induced NA accumulation may reflect interspecies variations of Cu impacts (Pich *et al.*, 1996). As NA is the precursor for mugineic acids biosynthesis (Haydon *et al.*, 2007), an increased production of NA may aim to increase Fe uptake trough exsudation and Fe-complexation in the rhizosphere. SAM is also a direct precursor of ethylene (Brosnan and Brosnan, 2006), which is involved in growth, development, and stress signaling notably during senescence, so increase in SAMS expression more marked in Cu-stressed NM roots could stimulate ethylene production (Maksymiec, 2007), inducing a higher Cu-induced senescence in NM than in M roots.

The down-regulation of methionine synthase in NM, together with up-regulation of cysteine synthase (5309 and 6303) indicated that thiol groups were mainly used for biosynthesis of cysteine and its derived compounds GSH, MTs and PCs, which are involved in Cu homeostasis and tolerance (Van Hoof *et al.*, 2001). In *O. sativa* roots, accumulation of cysteine synthases increases in response to Cu (8 µM for 3 days; Song *et al.*, 2013), Al (2 mM for 3 days; Yang *et al.*, 2007) and As (50 and 100 µM; Ahsan *et al.*, 2008). Induction of a glutamine synthetase (#5404) suggested an increased production of GSH in NM roots under Cu excess.

4.2.3. Stress response and detoxification

Glutathione S-transferases (GST, EC = 2.5.1.18) catalyze the conjugation of GSH with a large variety of substrates. Among the two GST spots, #217 was significantly over-expressed in M at all Cu exposures except for 20 and 50 µM and #6205 decreased with Cu exposure only in M. Two GST are more expressed in roots of a Cu-tolerant variety of *O. sativa* compared to

a sensitive one, when exposed to 8 µM Cu. The first increases in both varieties compared to Cu-free conditions, more intensively in the tolerant one (x5.2 and x1.9 respectively) while the second increases only in the tolerant variety and is not detected in the sensitive one at any experiment condition (Song *et al.*, 2013). Taken together, these results suggest that GST plays a role in higher Cu-tolerance of tolerant plant population, by increasing conjugation of various hydrophobic or electrophilic compounds, including free Cu.

Additionally, the expression of two GST spots is induced by heat stress in roots of heat-tolerant *Agrostis scabra* and heat-sensitive *Agrostis stolonifera* Penncross cultivar, while a third one is specifically induced in the heat-tolerant population (Xu and Huang, 2008). Induction of GST spots have been also recorded in response to Cd in roots of *K. candel* (100-800 µM for 3 days, Weng *et al.*, 2013) and *B. juncea* (250 mM; Alvarez *et al.*, 2009); to Al exposure in roots of *L. corniculatus* (10 and 20 µM for 14 days; Navascués *et al.*, 2012) and *O. sativa* (100/250 µM for 12 or 36 hours; Yang *et al.*, 2007) and to As also in *O. sativa* roots (50 and 100 µM; Ahsan *et al.*, 2008). All these results point out that GST induction may occur in response to a large range of abiotic stress, including metal(loid) excess and that over-expression of such proteins in tolerant ecotypes may underlay this tolerance.

Three types of ascorbate peroxidases were identified in roots, APx1 (#1211 and 1220), APx2 (#6203, 6212, 6213 and 7205) and APx6 (#2312). Accumulation of both APx1 spots sharply decreased in both M and NM roots. Three of the four APx2 spots were down-regulated, while the APx6 one was up-regulated only in NM roots. Three APx2 spots were also over-expressed in M at low or high Cu exposure. Free Cu in cells may increase accumulation of H_2O_2, through Fenton reactions, which levels are controlled by cells by adapting redox homeostasis. Ascorbic acid (AsA) is an important reducing substrate for H_2O_2 detoxification in photosynthetic organisms such as plants and algae. Ascorbic acid is used as electron donor by APx to reduce H_2O_2 into H_2O, resulting in the formation of monodehydroascorbate (MDHA). AsA is then regenerated by the action of MDHA reductase (MDHAR) or by the spontaneous disproportionation of MDAsA in AsA and dehydroascorbate (DHA). DHA may also be reduced by DHA reductase (DHAR) to regenerate AsA using GSH as electron donor, participating to AsA-GSH cycle (see Fig. 4 section 6.1.6). As APx are instable in case of AsA deprivation and degraded to an inactive form by 10nM H_2O_2, the decreasing accumulation of APx may indicate a decrease of AsA and/or an accumulation of H_2O_2 (Shigeoka *et al.*, 2002). However, the over-expression of several APx in M roots at high Cu exposure (30-50 µM) might confer an additional protection against H_2O_2 accumulation.

In roots of *O. sativa* seedlings exposed to 8 µM Cu for 3 days, three cytosolic APx spots increase in both Cu-tolerant and sensitive varieties but two raise more intensively in the tolerant one (Song *et al.*, 2013). Induction of APx spots have also been recorded in *K. candel* roots in response to Cd (100-800 µM for 3 days; Weng *et al.*, 2013) but as plants have first been grown on unspiked nutrient conditions and then short-term exposed to metal(loid) stress, it can be assumed that different mechanisms are involved and that response of ascorbate peroxidases may be metal, species, time or dose dependent. However, the sharper increased measure in the Cu-tolerant rice variety (Song *et al.*, 2013) and over-expression of three out of seven APx spots (#5230, 6213 and 7205) in the Cu-tolerant population of *A. capillaris* point out the probable involvement of these antioxidative enzymes in the improvement of Cu-tolerance.

Expression of aldehyde dehydrogenase spot (#2609) increased markedly in M roots with Cu exposure but did not differ significantly between populations. This increase may provide a better detoxification of toxic aldehyde in mitochondria of M roots cells. Another aldehyde dehydrogenase spot is up-regulated by long term heat stress in roots of a heat-tolerant *A. scabra* population (30 or 40°C for 10 days; Xu and Huang, 2008). Accumulation of a peroxidase 2 was down-regulated only in M roots. In *C. sativa* long-term exposed to Cu exposure a similar decrease of peroxidase accumulation has been reported (Bona *et al.*, 2007)

4.2.4. Protein synthesis, folding and degradation

In NM roots, two mitochondrial chaperones, i.e. chaperonin CPN60-1 (#6704) and CPN60-2 (#6629) and a protein disulfide isomerase (PDI, #1504) were induced by Cu excess, indicating a higher need for protection of protein folding. Together with decreases of enzymes involved in mitochondrial Krebs cycle / Oxidative phosphorylation more marked in NM, this increase in mitochondrial chaperone probably reflected higher damages on mitochondria in NM roots and reduced energy production.

In M roots, a third mitochondrial chaperone, heat shock 70 kDa protein 10 (#4716) did not respond to Cu exposure but over-expressed at intermediate and high Cu exposures (10, 25, 30 and 40 µM Cu) compared to NM, which may provide a better protection of protein metabolism in M roots. HSPs belong to a large family of proteins involved in protein folding/unfolding processes and alteration of HSPs accumulation has been recorded under Cu exposure in various organisms; some are induced, i.e. a Heat shock 8 1-2, belonging to the Hsp90 family, in roots of *O. sativa* (8 µM; Song *et al.*, 2013), a DnaJ-class molecular chaperone in *Pseudomonas spp.* bacteria (4 mM; Li *et al.*, 2012), three Hsp88, six Hsp70 and one Hsp60 in *Rhodotorula mucilaginosa* yeasts (0.5 mM; Irazusta *et al.*, 2012), but a HSP70 protein 1 is down-regulated in roots of *E. splendens* (100 µM; Li *et al.*, 2009). Alteration also occurs under

Cd excess, for example, two low molecular mass HSPs class I and II spots are induced in *K. candel* roots (100-800 µM for 3 days; Weng *et al.*, 2013), while a HSP90 is repressed in roots of *O. sativa* (1 mM for 8 days; Zhao *et al.*, 2012) and four HSP70 and one HSP90 are repressed by Al excess in roots of *L. corniculatus* (10 and 20 µM for 14 days; Navascués *et al.*, 2012). Down-regulation of HSPs occurring only or more sharply in sensitive populations/cultivars/genotypes of plant species compared to tolerant one occurs in Al stressed *H. vulgare* roots (50 or 200 µM for 24 hours; Dai *et al.*, 2013) and in salt-stressed *A. stolonifera* roots (10 $dS.m^{-1}$; Xu *et al.*, 2010), indicating that HSPs participate to the enhanced tolerance to different abiotic stresses.

Several proteolysis-related proteins were regulated by Cu excess only in M roots. Two proteasome subunits, i.e. proteasome subunit beta type (#2222) and 26S proteasome non-ATPase regulatory subunit 14 (#1315) and a phytepsin (#7341) were induced by Cu exposure only in M roots. In the same way, two mitochondrial-processing peptidase subunit alpha (#1618 and 1626) and a cysteine proteinase inhibitor 12 (also called cystatin, #2207) were over-expressed in M roots, #1618 at all Cu exposure except 30 µM, #1626 at 20 µM and #2207 at 50 µM Cu. These results suggested a better proteolysis process in M roots, which might counteract the toxic effect of Cu on protein metabolism by avoiding accumulation of damaged proteins. Other proteolysis-related proteins are regulated by abiotic stresses. In response to Cd stress, two proteasome subunit beta type and one proteasome subunit alpha type spots are up-regulated in *K. candel* roots (100-800 Cd for 3 days; Weng *et al.*, 2013) but a 26S proteasome AAA-ATPase subunit RPT5a is repressed in *B. juncea* roots (250 mM; Alvarez *et al.*, 2009).

4.2.5. Cell structure

All cytoskeleton proteins were down-regulated by Cu excess in one or both population. As found in the preliminary experiment (see section 3.3 and 4.4 from Chapt. II), a tubulin alpha spot (#7610) decreased only in NM roots. An actin spot (#5514) was also down-regulated only in NM, while two tubulin beta spots (#7616 and 7617) decreased in both M and NM and a third one (#7626) only in M. Globally, accumulation of cytoskeleton proteins decreased only or more sharply in NM roots, indicating higher impacts on cell integrity in this population. A down-regulation of tubulins alpha by Cu exposure also occurs in rice roots exposed to 8 µM Cu, with a decrease more intense in roots of the sensitive cultivar compared to the tolerant one (Song *et al.*, 2013). An opposite pattern is found in roots of four-week-old *E. splendens* plants, where actin tubulin alpha spots two-fold increases when plants are exposed to 100 µM Cu for 3 or 6 days (Li *et al.*, 2009), and in roots of *C. sativa*, where actin is up-regulated after 6 weeks of Cu excess (Bona *et al.*, 2007).

4.2.6. Other functions

Enzymes belonging to amino-acids metabolism, i.e. Glycine (glycine dehydrogenase #1808, D-3-phosphoglycerate dehydrogenase #2727), Alanine (alanine aminotransferase 2 #2623), Valine/Leucine (ketol-acid reductoisomerase #2725 and 3709), and Phenylalanine (phenylalanine / phenylalanine-tyrosine ammonia-lyase #3707), were either regulated by Cu-exposure or differentially expressed between M and NM roots. Glycine dehydrogenase (#1808), which catalyzes the degradation of glycine, was over-expressed in M at 5 µM and decreased with Cu exposure in M, while D-3-phosphoglycerate dehydrogenase (#2727), was over-expressed in M at 50 µM but did not respond to Cu exposure. Globally, accumulation of ketol-acid reductoisomerases (#2725, 3701, 3709 and 3712) increased under Cu treatment in both populations, more intensively in M roots. #3701 increased only in NM, #3709 and 3712 only in M. #2725 over-expressed in NM at 10 µM while #3709, due to its up-regulation, was over-expressed in M at 40 µM Cu. This indicated that Cu excess induced valine and isoleucine biosynthesis from pyruvate by increasing accumulation of ketol-acid reducto-isomerases.

Accumulation of two phenylalanine ammonia-lyases (PAL) decreased under Cu excess, #2724 in both populations and #3707 only in NM roots. Strong repression of a PAL spot by Cu excess has also been recorded in 28-day-old *E. splendens* plants exposed to 100 µM Cu, after both 3 and 6 days of exposure (Li *et al.*, 2009). PAL catalyzes the biosynthesis of trans-cinnamate from L-phenylalanine to initiate the synthesis of a wide range of compounds based on phenylpropane skeleton, including lignin. Decreasing PAL accumulation could then lead to reduced production of lignin or to alteration of lignin composition. Together with the respective down- and up-regulation of caffeoyl-coA O-methyltransferase and Cinnamyl alcohol dehydrogenase only in NM, the decrease of a second PAL only in NM roots may indicate a stronger alteration of lignin biosynthesis in this population. Accumulation of two PAL spots is also down-regulated by heat stress in roots of heat-tolerant *A. scabra* and heat-sensitive *A. stolonifera* (Xu and Huang, 2008), indicating that PAL accumulation may be altered by different abiotic stresses.

An adenine phosphoribosyltransferase 1 (#6215) was sharply induced, while an adenosine kinase (#7504) was repressed by increasing Cu exposure in M and NM roots, indicating that purine metabolism was altered by Cu excess in both populations.

As one of the three primary macronutrients, K^+ has various functions in plants so over-expression of a K^+ voltage-gated channel (#1414) in M roots at 40 µM Cu probably conferred an advantage for this population, in permitting a higher K^+ uptake at high Cu excess.

5. Conclusions

These investigations on soluble root proteome of *A. capillaris* populations indicated that increasing Cu exposure resulted in complex changes on a large range of cellular processes including energy metabolism, antioxidative and detoxification processes, protein metabolism and sulfur metabolism. Changes in protein accumulation patterns occurred in both metallicolous and non-metallicolous populations, but results showed that some cellular processes were more affected in NM roots.

In NM roots, a limitation of glycolysis efficiency at Cu exposure higher than 25 µM was suggested by the over-expression of phosphoglucomutase only at low and intermediate exposure (1-25 µM Cu), together with the limitation of G3PDH accumulation, which reached a plateau at high Cu exposure (30-50 µM Cu). On the opposite, in M roots, up-regulation of an alpha-galactosidase together with the over-expression of a sucrose:sucrose 1-fructosyltransferase and a 6-phosphofructokinase pyrophosphate-dependent at intermediate Cu exposure, suggested that several carbohydrate-related enzymes cooperated together to maintain the supply of glycolysis and Krebs cycle under Cu stress. Additionally, the linear increase of G3PDH accumulation across this range of Cu exposure may promote accumulation of NADH and pyruvate at high Cu exposure.

Cu-induced impacts on mitochondria activity in both M and NM roots were shown by the decrease of ATP synthase subunit alpha and the induction of formate dehydrogenase, which respectively underpinned decrease in ATP production and increase in cellular respiration. However, higher alteration of H^+ transport and Krebs cycle in NM roots were suggested by the strong down-regulation of aconitate hydratases, succinate dehydrogenase [Ubi] flavoprotein, NADH dehydrogenase [Ubi] Fe/S protein and V-type proton ATPase subunit alpha. Together with the increase of MDH and IDH only in M, these results supported that ability to maintain correct mitochondria functioning in M cells may confer a higher Cu-tolerance in this population.

Higher Cu-induced impacts on protein metabolism in NM were suggested by the induction of several protein chaperones, CPN60-1, CPN60-2 and protein disulfide isomerase, while in M roots, over-expression of a HSP70 at intermediate and high Cu exposures may play an important role in Cu-tolerance in protecting protein metabolism. Additionally, induction of two proteasome subunits and a phytepsin, together with the over-expression of a peptidase at almost all Cu exposure, supported a better proteolysis process in M roots, which may cope with deleterious effect of Cu stress on protein metabolism in avoiding accumulation.

Increasing accumulation of SAM was suggested by the induction of SAMS by Cu stress in both populations. Due to its role in trans-methylation, trans-sulfuration and polyamine synthesis, SAM may play a central role in plants stress response and may stimulate NA and GSH production, but also ethylene synthesis.

However, down-regulation of methionine synthase only in NM roots, leading to higher accumulation in M roots at high Cu, may reflect a better ability of M cells to maintain methionine biosynthesis under Cu excess. Cysteine synthase was specifically induced in NM roots, which can reflect a higher need for cysteine to process chelation mechanisms including binding of free Cu.

Over-expression of ascorbate peroxidase and glutathione-S-transferase also probably contributed to enhance antioxidative and detoxification mechanisms in M roots, while increase in aldehyde dehydrogenase accumulation only in M roots may allow a better degradation of potentially toxic aldehydes.

To summarize, higher Cu-tolerance of M population was related in roots with maintaining of ATP and NADH production, better protection of mitochondria activity and protein metabolism but also enhanced proteolysis and chelation.

CHAPTER V: Characterization of leaf soluble proteome

Characterization of leaf soluble proteome in Metallicolous and Non-Metallicolous populations of *Agrostis capillaris* L. exposed to Cu.

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Abstract

Metallicolous (M) and non-metallicolous (NM) populations of *Agrostis capillaris* L., a pseudo-metallophyte with phenotypic plasticity for Cu tolerance, were used to investigate Cu-tolerance in plants, using a proteomic approach. Differential soluble protein accumulation was investigated in leaves of 3-month plants cultivated on perlite with a $CuSO_4$ (1-50 µM) spiked-nutrient solution. Soluble proteins extracted by the trichloroacetic acid/acetone procedure were separated using 2-DE (linear 4-7 pH gradient). Gels were CCB-stained and image analysis performed by PDQuest, and proteins identified using LC-MS/MS. Changes in photosynthetic proteins, sulfur and glutathione metabolism, transport, biotic and xenobiotic defenses as well as the differential regulation of proteins involved in signaling and secondary metabolism are discussed in relation to Cu tolerance.

Decreasing accumulation of OEE, cytochrome b6-f complex, chlorophyll a-b binding protein, and RuBisCO indicated that plants failed to maintain the production of reducing power during light dependent reactions and the carbon assimilation during light independent reactions. Up-regulation in NM leaves of sedoheptulose-1,7-bisphosphatase, RuBisCO activase and phosphoglycerate mutase indicated that reduction of RuBisCO accumulation was mainly responsible for carbon assimilation failure. Additionally, increasing accumulation of IDH suggested a higher mitochondrial respiration in both populations under Cu excess. Increasing accumulation in cysteine/methionine synthases in both populations indicated that Cu excess induced an enhanced need in S-containing amino-acids, probably to increase chelation

mechanisms, through production of glutathione (GSH), nicotianamine (NA), polyamines and phytochelatins (PC).

In NM leaves, higher impacts on photosynthesis were supported by the sharper decrease of all photosynthesis-related enzymes, and the up-regulation of a ferredoxin-NADP reductase and a metalloprotease FTSH2. A higher need in energetic compounds was revealed by the up-regulation of several glycolytic enzymes and ATPases, together with the stimulation of pentose phosphate pathway and Calvin cycle. A higher need of protein synthesis, as indicated by the up-regulation of eukaryotic initiation factor 4A, 50S ribosomal protein L10 and GTP-binding protein TypA, was coherent with the increasing accumulation of protein chaperones, i.e. ClpC2, 60kDa chaperonin, chaperonin CPN60-2, nucleoredoxin and PDI, which indicated higher Cu-induced damages on protein metabolism in NM leaves. A mitochondrial HSP70 was induced only in Cu-stressed M leaves and may better protect protein metabolism in M plants. Higher cysteine synthase accumulation in NM leaves, together with the up-regulation of glutamine synthetase, suggested an increased GSH production. Higher oxidative stress in NM leaves was indicated by up-regulation of thioredoxin and thioredoxin peroxidase.

1. Introduction

Pseudo-metallophyte species, which are able to grow on both contaminated and uncontaminated soils, constitute a relevant tool to examine mechanisms of resistance and tolerance, as there are adapted to stressful environment and adverse soil conditions. To grow on contaminated soil, metallicolous populations may have evolved molecular mechanisms enabling their survival, so comparison of tolerant and sensitive populations may provide information on mechanisms underlying tolerance. Comparison between a tolerant (metallicolous, M) population of *A. capillaris*, originated from a French wood preservation site with Cu-contaminated soils (65 - 2600 mg Cu/kg soil, Bes *et al.*, 2010), with a non-tolerant one (non-metallicolous, NM), collected on the uncontaminated soil of a forest edge (Bes, 2008) was then thought to be a good opportunity to obtain clues about Cu-tolerance.

As differences in efficiency of homeostasis and detoxification processes may explain the higher tolerance of metallicolous plants, use of proteomic tools could give new pieces of evidence to better understand the molecular mechanisms underlying metal tolerance in plants. After investigating the molecular mechanisms involved in Cu-response in roots, this chapter aimed to examine variations of protein accumulation in leaves to understand how Cu might alter plant growth. To our knowledge, similar comparisons between metal-tolerant and sensitive populations of *A. capillaris* have been conducted only at a phenotypic or physiological level,

but no work has yet been published with a proteomic approach. However, several other proteomic studies have compared populations, genotypes and cultivars, exhibiting large difference in their tolerance to abiotic stress, including metal(loid)s. Salt tolerance has been investigated in roots and leaves of *Agrostis stolonifera* tolerant and sensitive cultivars exposed to 10 dS m^{-1} NaCl for 28 days (Xu *et al.*, 2010), while response to Cu has been studied in Cu-tolerant and sensitive strains of *Ectocarpus siliculosus* exposed to 50 µg Cu/L during 10 days (Ritter *et al.*, 2010), and in roots of Cu-tolerant and sensitive *Oryza sativa* cultivars exposed to 8 µM Cu for 3 days (Song *et al.*, 2013).

Moreover, no information is available about molecular response of *A. capillaris* leaves to Cu exposure; only one study describes the response of *A. capillaris* to arsenate and arsenite, focusing on the analysis of leaf soluble proteome in plants grown for one month in As-free conditions and then exposed to arsenite and arsenate for 8 days (Duquesnoy *et al.*, 2009). However, other studies have been conducted on plant leaves for responses to Cu exposure at a proteomic level: in four-week-old *Elsholtzia splendens* plants exposed to 100 µM Cu for 3 or 6 days (Li *et al.*, 2009), and in 10-day old seedlings of *Phaseolus vulgaris* exposed to 15 or 50 µM Cu for 7 days (Cuypers *et al.*, 2005), and on algae, such as *Scytosiphon gracilis* exposed to 100 µg $Cu.L^{-1}$ for 4 days (Contreras *et al.*, 2010), which may represent sources for data comparisons. These works indicated differential accumulation of proteins under Cu stress, which were mainly related to energy, amino acid and sulfur metabolisms, and regulation of antioxidative compounds. However, no clear mechanism has yet been identified as responsible for a higher tolerance.

Most of the previous findings cited above focused on plant grown in common conditions and then short-term exposed to Cu, or other metals, and few data exist about long term Cu exposure, notably chronic exposure from germination to plant harvest. Here, both M and NM populations of *A. capillaris* L. were chronically exposed to Cu in the 1-50 µM range for a 3-month period, and differential protein accumulation was investigated in leaf soluble proteome to identify mechanisms underlying Cu-response in *A. capillaris* and higher tolerance in the M population.

2. Materials and Methods

2.1. Plants and Cu treatments

Seeds of metallicolous (M) and non-metallicolous (NM) populations were respectively collected from *A. capillaris* L. growing at a wood preservation site contaminated by Cu (Bes and Mench 2009; Mench and Bes 2009; Bes *et al.*, 2010) and at a forest edge (RN10, Km 83,

Belin Beliet, Gironde, France) in August-September 2011. Phenotypes of M and NM populations have been previously characterized on a Cu-contaminated soil series obtained with the fading technique and on Cu-spiked perlite moistened with Hoagland nutrient solution in the 1-30 µM Cu range (Bes, 2008). Seeds were sowed and plants cultivated for three months on perlite constantly bottom moistened with Hoagland n°2 nutrient solution (Hewitt, 1966) containing 1, 5, 10, 15, 20, 25, 30, 40 and 50 µM Cu (added as $CuSO_4$, $7H_20$), weekly changed. Moistened perlite was preferred than hydroponics for maintaining root ultra-structure and Si nutrition closer to soil conditions (Lux, 2010). Seeds were germinated under natural light in plastic pots (15 x 12 x 8 cm). After 28 days, plants were transferred in a growth chamber with a 14h, 27°C day and a 10h, 22°C night regime, with 220-240µmol photons $m^{-2}.s^{-1}$ light intensity and 65-75% relative humidity. After a 3-month period of growth all plants were harvested in removing perlite from roots with milliQ water. For each experimental condition (i.e. Population x Cu concentration), 3 replicates were selected randomly out of a set of 6 (previously phenotypically characterized) for the proteomic experiment. For each replicate, several leaf aliquots (1g, FW) were constituted by mixing leaf samples, taken in the median part of stems, frozen in liquid nitrogen and stored at -80°C.

2.2. Protein extraction, quantification and separation

For all aliquots (1g FW, n = 54), frozen tissues were ground in a small mortar and pestle in liquid nitrogen. Total protein was extracted following the trichloroacetic acid/acetone procedure described by Damerval *et al.* (1986) and modified by Gion *et al.*, (2005). Soluble proteins were re-solubilized in "TCT" buffer (i.e. 7M urea, 2M thiourea, 0.4% v/v Triton X-100, 4% w/v CHAPS detergent, 10 mM DTT, and 1% v/v IPG buffer) for one hour at room temperature. Samples were then centrifuged (4 min, 2 000revolutions per min, 20°C) and stored at -80°C. Protein concentration was determined in triplicates for each extract using a modified Bradford assay (Ramagli *et al.*, 1985). Protein extracts were stored at -80°C for the subsequent 2-DE steps.

For the isoelectric focusing step (IEF), 24 cm immobilized pH gradients (IPG) strips (Immobiline DryStrip, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used with a linear pH gradient ranging from 4 to 7. A mix containing 450 µg of total soluble proteins, re-suspended into 470 µL of "TCT" solution, was used to rehydrate passively acidic strips for 1h at room temperature prior to the IEF run. The IPGphor system (Amersham Biosciences, Uppsala, Sweden) was programmed at 30 (12h), 500 (1h), 1000 (1h) and finally, at 8000 V/h to achieve a total of 64 000 V/h. Strips were equilibrated in two steps with an equilibration solution (50mM TRIS-HCl, 6 M urea, 2% SDS, 30% glycerol, bromophenol blue) and DTT

(50 mM) and stirred for 15min. Iodoacetamide (125 mM) was added and the mixture was stirred for additional 15min. SDS-PAGE was carried out on batches of six or twelve gels per stage of development in a buffer (25 mM Tris, 0.2 M glycine, 0.1% SDS) at 30 W for 30 min, then at 90 W. The gels were then stained with colloidal blue (Coomassie Blue G-250).

2.3. Image analysis and spot detection

2D-gels were scanned (GS-800 Imaging densitometer; Bio-Rad). The alignment of 30 gel images, spot detection, quantification and pairing were carried out using PDQuest Advanced (v 8.0.1). Protein spots (referred for ease thereafter as spots) were automatically detected and manually corrected if necessary. For each spot, the volume was computed with background subtraction, normalized to the total volume in the gel image and expressed in %Vn. The 30 image gels were automatically aligned according to landmark spots manually selected. Spots were matched and manually corrected if necessary (Vilain *et al.*, 2004).

2.4. Statistical analysis

In this experiment, Cu was considered as a continuous variable to include the "dose" notion in the analysis. To characterize the response of each population across the range of Cu exposures, Pearson's correlation was used between spot dataset of each population (M and NM) and Cu exposure (1-50 µM). Statistical analyses were conducted on R v2.11.1 (R Foundation for Statistical Computing; Vienna, Austria) and alpha error was fixed at 0.1 because of inter-replicates variability. A clustering analysis of spot volumes was conducted on GENESIS software (v. 1.7.6).

As replicate number was too low to perform Student's tests, differential expression between M and NM populations at each Cu exposure (1-50 µM) was estimated using ratios between mean values of each population. Protein spots from M and NM populations, cultivated at the same Cu exposure (1-50 µM), were considered to display significant differences if they fulfilled the following criteria:

(i) over-expression in M population compared to NM one:

$(M_{mean} + SE_M) / (NM_{mean} - SE_{NM}) < 0.7$ and $(M_{mean} – SE_M) / (NM_{mean} + SE_{NM}) < 1.5$

(ii) over-expression in NM population compared to M one:

$(M_{mean} + SE_M) / (NM_{mean} - SE_{NM}) > 0.7$ and $(M_{mean} – SE_M) / (NM_{mean} + SE_{NM}) > 1.5$

In which M_{mean} and NM_{mean} represent average spot volumes (n = 2 or n = 3) and SE_M and SE_{NM} are standard errors on the M_{mean} and NM_{mean} respectively. The 1.5-fold ratio for significant spot alteration have been arbitrarily chosen from comparison with other proteomic studies on Cu-

tolerance (Li *et al.*, 2009; Ritter *et al.*, 2010; Song *et al.*, 2013). Ratios were calculated using Excel (Word), graphical figures were obtained on R then modified with Power Point (Word).

2.5. Protein identification by mass spectrometry

Most spots were automatically excised using "Spotcutter" (EXQuest, Bio-Rad pieces of 0.5 mm Ø and with three pieces maximum for large spots). Few ones not present in the gel part automatically cut were manually excised. Spots were rinsed twice in ultrapure water, and shrunk in Acetonitrile (ACN) for 10 min. After ACN removal, gel pieces were dried at room temperature, rehydrated in 10 ng/µL trypsin solution (T6567, Sigma-Aldrich) in 50 mM ammonium bicarbonate, and incubated overnight at 37°C.

Hydrophilic peptides were extracted with 40 mM ammonium bicarbonate containing 10% ACN at room temperature for 10 min. Hydrophobic peptides were extracted with 47% v/v ACN and 5% v/v formic acid, and this extraction step was repeated twice. All three supernatants were pooled together, concentrated in a vacuum centrifuge, and acidified with 0.1% formic acid before nanoLC-MS/MS analysis (Gion *et al.*, 2005). Peptide mixtures were analyzed by on-line capillary nanoHPLC (LC Packings, Amsterdam, The Netherlands) coupled to a nanospray LCQ Deca XP ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA). 10 µL of each peptide extract were loaded on a 300 µm ID x 5 mm PepMap C_{18} precolumn (LC Packings, Dionex, USA) at a flow rate of 20 µL/min. After 5 min desalting, peptides were online separated on a 75 µm internal diameter x 15 cm C18 PepMapTM column (LC Packings, Amsterdam, The Netherlands) with a 5-40% linear gradient of solvent B in 48 min (solvent A was 0.1% formic acid in 5% ACN, and solvent B was 0.1% formic acid in 80% ACN). The separation flow rate was set at 200 nL/min. The mass spectrometer operated in positive ion mode at a 1.8kV needle voltage and a 34V capillary voltage. Data acquisition was performed in a data-dependent mode alternating in a single run, a MS scan survey over the range m/z 300–1700 and three MS/MS scans with Collision Induced Dissociation (CID) as activation mode. MS/MS spectra were acquired using a 2 m/z unit ion isolation window, a 35% relative collision energy, and a 0.5 min dynamic exclusion duration (Gion *et al.*, 2005).

Mascot and Sequest algorithms through Proteome Discoverer 1.4 Software (Thermo Fisher Scientific Inc.) were used for protein identification in batch mode by searching against two constructed databases. The first was constructed with ESTs from NCBI (http://www.ncbi.nlm.nih.gov/) from *Agrostis* spp., including *A. capillaris*, *A. stolonifera*, *A. stolonifera* var. *palustris* and *A. scabra,* and resulted in 123,605 sequences translated in six reading frames by TRANSEQ software (http://www.ebi.ac.uk/Tools/emboss/transeq/). The

second database contained all protein sequences from *Viridiplantae* UniProt Database (31,395 entries, release 2013_09, http://www.uniprot.org/). Two missed enzyme cleavages were allowed. Mass tolerances in MS and MS/MS were set to 2 Da and 1 Da. Oxidation of methionine was searched as variable modifications and carbamidomethylation on cysteine was searched as fixed modification. Peptide validation was performed using Percolator algorithm (Käll *et al.*, 2007) and only "high confidence" peptides were retained corresponding to a 1% False Positive Rate at peptide level. A minimum of two different peptides was considered for protein validation. EST annotations were identified by searching with a protein Viridiplantae index from Swiss-Prot (BLASTX) and TrEMBL (BLASTX) database using UniProtKB (http://www.uniprot.org).

3. Results

3.1. Spots detection on 2D-gels and statistical analyzes

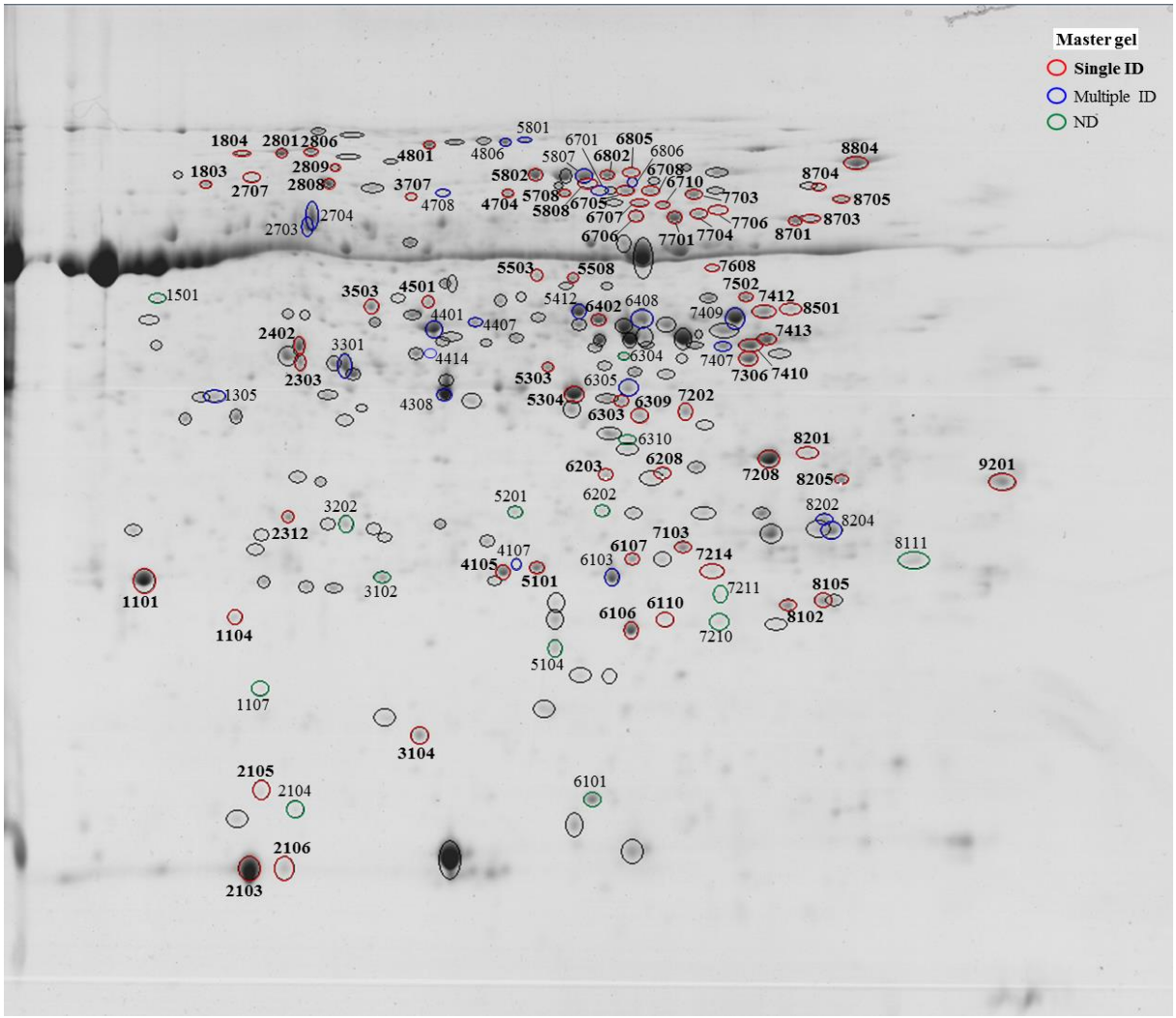


Figure 1: Reference gel (12%) showing the distribution of protein spots from *Agrostis capillaris* leaves, with locations of the 66 spots selected for identification by mass spectrometry. Spots circled in green remained unidentified, those in purple matched to 2 or 3 different identifications and those in red corresponded only to one or very similar identification (#2303, 8102 and 8105).

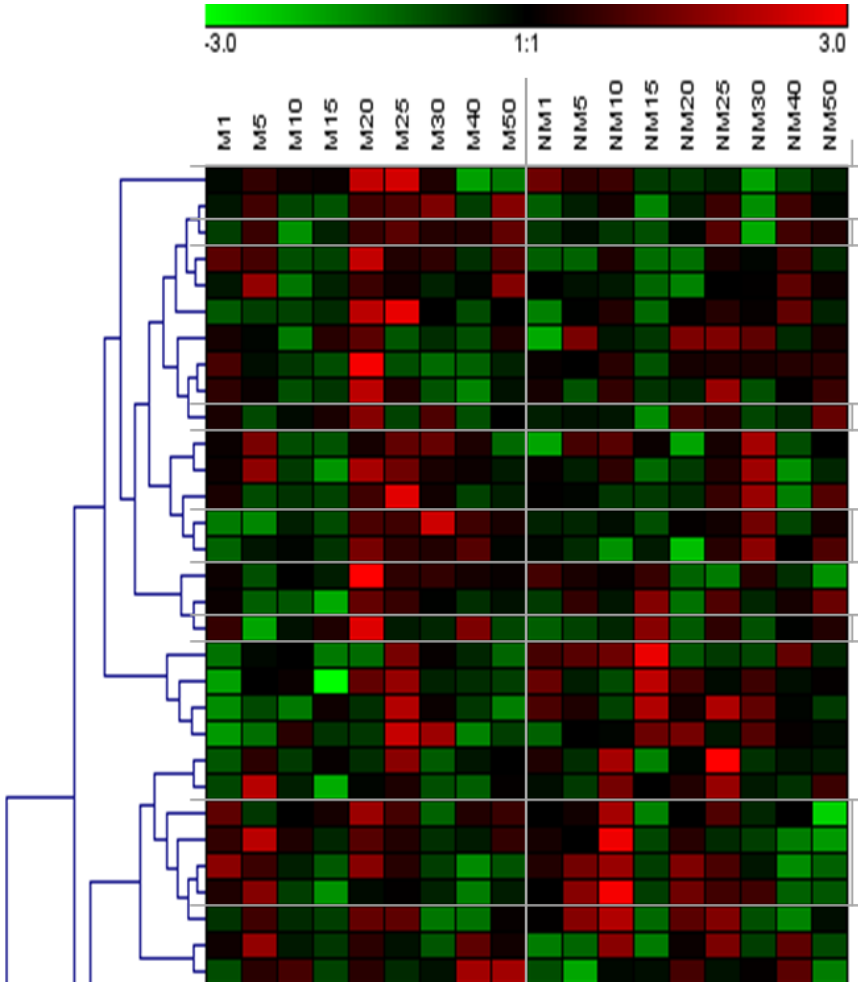
Due to the wide range of Cu exposures explored, resulting in a high amount of experimental conditions, only 214 spots were accurately delimited on 2D-gels (Fig. 1, all gel images are available in the Annex 19). To characterize differential expression of protein spots across experimental conditions, a hierarchical clustering was realized on global data (Fig. 2). To focus on the Cu effect, i.e. effect of Cu exposure on protein expression, Pearson's Correlations were computed for each population. To study the population's origin effect, i.e. differential expression between M and NM populations, ratios were calculated between M and NM mean values. Summary of statistical tests for the 214 spots are shown in Tab.1 and more data are available in Annex 20 (Variation of protein expression among Cu exposures for M and NM populations; table of mean values ± sd; summary of identification and statistical tests).

Table 1. Results of statistical tests for the 214 accurately quantified spots. Sp: spots number; rM/rNM: significance level of the Pearson's correlation for population referring to p-val = 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; R1-50: significance of comparative ratio between populations values at each exposure, -: no difference, M/NM indicated the population with higher values based on ratio >1.5.

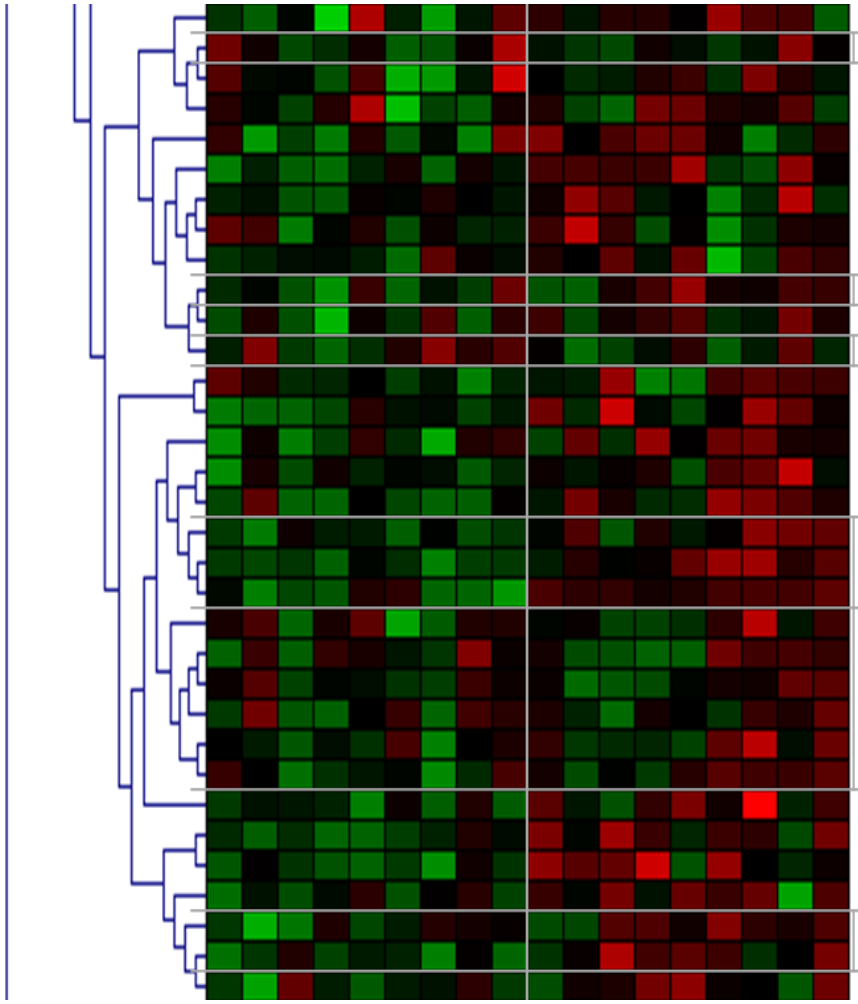
SP	rM	rNM	R1	R5	R10	R15	R20	R25	R30	R40	R50	SSP	rM	rNM	R1	R5	R10	R15	R20	R25	R30	R40	R50
1101	-	↘↘↘↘	-	-	-	-	-	-	-	-	-	5508	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
1104	-	↗↗	-	-	-	-	-	-	-	-	-	5707	-	↗	-	-	-	-	-	-	-	-	-
1105	-	-	-	-	-	-	-	-	-	-	-	5708	↗↗↗	↗↗	-	-	-	-	-	-	-	-	-
1106	-	-	-	-	-	-	-	-	-	-	-	5801	-	↗↗↗	-	-	-	-	-	-	-	-	-
1107	-	↗↗	M	M	M	M	M	-	-	M	M	5802	-	↗↗↗	-	-	-	-	-	-	-	-	-
1111	-	-	-	-	-	-	-	-	M	-	-	5806	-	-	-	-	-	NM	-	-	-	-	-
1201	-	↘	-	-	-	-	-	-	-	-	-	5807	-	↗↗↗	-	-	-	-	-	-	-	-	NM
1203	-	-	-	-	-	-	-	-	-	-	-	5808	↗↗	-	-	-	-	-	-	M	-	M	-
1205	-	-	-	-	-	-	-	-	-	-	-	6001	-	-	-	-	-	-	-	-	-	-	-
1304	-	-	-	-	-	-	-	-	-	-	-	6101	-	↗↗	-	-	-	-	-	-	-	-	-
1305	↗↗	-	-	-	-	-	-	-	-	-	-	6103	-	↘↘↘↘	-	-	-	-	-	-	-	-	-
1401	-	-	-	-	-	-	-	-	-	-	-	6106	↘↘	↘↘↘	-	-	-	-	-	-	-	-	-
1501	-	↘↘	-	-	NM	-	-	-	-	-	-	6107	-	↗↗	-	-	-	-	-	-	-	-	-
1506	↗	-	-	-	-	-	-	-	-	-	-	6108	-	-	-	-	-	-	-	-	-	-	-
1802	-	-	-	-	-	-	-	-	-	-	-	6110	↘↘↘	-	-	-	-	-	-	-	-	-	-
1803	-	-	-	-	-	-	M	-	-	-	M	6202	-	↗↗	-	-	-	-	-	-	-	-	-
1804	-	-	-	-	-	M	-	-	-	NM	-	6203	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
2101	-	-	-	-	-	-	-	-	-	-	-	6204	-	-	-	-	-	-	-	-	-	-	-
2102	-	↗	-	-	-	-	-	-	-	-	-	6207	-	-	-	-	-	-	-	-	-	-	-
2103	-	↘↘	-	-	-	-	-	-	-	-	-	6208	-	↗↗	-	-	-	-	-	-	-	-	-
2104	↘↘↘	↘↘	-	-	-	-	-	-	-	-	-	6211	-	-	-	-	-	-	-	-	-	-	-
2105	-	↘↘	-	-	-	-	-	-	-	-	-	6301	-	↗	-	-	-	-	-	-	-	-	-
2106	↘↘	-	-	-	-	-	-	-	-	-	-	6302	-	-	-	-	-	-	-	-	-	-	-
2204	-	-	-	-	-	-	-	-	-	-	-	6303	-	↗↗	-	-	-	-	-	-	-	-	-
2205	-	↗	-	-	-	-	-	-	-	-	-	6304	-	↗↗↗	-	-	-	-	-	-	-	-	-

2206	-	-	-	-	-	-	-	-	-	-	-	-	6305	-	↗↗	-	-	-	-	-	-	-	-	-	-
2211	-	-	-	-	-	-	-	-	-	-	-	-	6306	-	-	-	-	-	-	-	-	-	-	-	-	-
2301	-	-	-	-	-	-	-	-	-	-	-	-	6308	-	-	-	-	-	-	-	-	-	-	-	-	-
2303	-	↗↗↗↗	-	-	-	-	-	NM	NM	-	-	6309	↗↗↗	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
2308	↘	-	-	-	-	-	-	-	-	-	-	-	6310	-	↘↘	-	-	-	-	-	-	-	-	-	-	-
2309	-	-	-	-	-	-	-	-	-	-	-	-	6311	-	-	-	-	-	-	-	-	M	-	-	-	-
2312	-	↘↘↘	-	-	-	-	-	-	-	-	-	-	6401	-	↗	-	-	-	-	-	-	-	-	-	-	-
2402	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	6402	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
2507	-	-	-	-	NM	-	-	-	-	-	-	-	6403	-	-	NM	-	-	NM	-	-	-	-	-	-	-
2508	-	↗	-	-	-	-	-	-	-	-	-	-	6405	-	-	-	-	-	-	-	-	-	-	-	-	-
2703	↗	↗↗	-	-	-	-	-	-	-	-	-	-	6408	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
2704	↗	↗	-	-	-	M	-	-	-	-	-	-	6409	-	-	-	-	-	-	NM	-	-	-	-	-	-
2707	-	↘↘↘	-	-	-	-	-	-	-	-	-	M	6410	-	-	-	-	-	-	-	-	-	-	-	-	-
2801	↗↗	↗↗	-	-	-	-	-	-	-	-	-	-	6501	-	-	-	-	-	-	-	-	-	-	-	-	-
2806	-	↗↗↗	-	-	-	-	-	-	-	NM	-	-	6506	-	-	-	-	-	-	-	-	-	-	-	-	-
2808	-	↘	-	-	NM	-	-	-	-	-	-	-	6606	-	-	-	-	-	-	-	-	-	-	-	-	-
2809	↗	↗↗↗	-	-	-	-	-	-	-	-	-	-	6608	-	-	-	-	-	-	-	-	-	-	-	-	-
2903	-	-	-	-	-	-	-	-	-	-	-	-	6701	-	↗↗	-	-	-	-	-	-	-	-	-	-	-
3102	↘↘	↘↘↘	-	-	-	-	-	-	-	-	-	-	6702	-	-	-	-	-	-	-	-	-	-	-	-	-
3103	-	↗	-	-	-	-	-	-	-	-	-	-	6703	↘	-	-	-	-	-	-	-	-	-	-	-	-
3104	↘↘↘↘	↘↘↘↘	-	-	-	-	-	-	-	-	-	-	6705	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
3105	-	-	-	-	-	-	-	-	-	-	-	-	6706	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
3201	-	-	-	-	-	-	-	-	-	-	-	-	6707	-	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	-
3202	-	↗↗↗	-	-	M	-	-	-	-	NM	-	-	6708	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
3205	-	-	-	-	NM	-	-	-	-	-	-	-	6710	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
3301	-	↗↗	-	-	-	-	-	-	-	-	-	-	6802	-	↗↗↗	-	-	-	M	-	-	-	-	-	-	-
3303	-	-	-	-	-	-	-	-	-	-	-	-	6805	-	↗↗	-	-	-	-	-	-	-	-	-	-	-
3309	-	-	-	-	-	-	-	-	-	-	-	-	6806	-	↗↗	-	-	-	-	-	-	-	-	-	-	-
3315	-	-	-	-	-	-	-	-	-	M	-	-	6807	-	-	-	-	-	-	-	-	-	-	-	-	-
3404	↗	↗	-	-	-	-	-	-	-	-	-	-	7103	-	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	-
3406	-	-	-	-	-	-	-	-	-	-	-	-	7105	-	-	-	-	-	-	-	-	-	-	-	-	-
3503	↗↗	↗↗	-	-	-	-	-	-	-	-	-	-	7202	-	↗↗	-	-	-	-	-	-	-	-	-	-	-
3507	↗	-	-	-	-	-	-	-	-	-	-	-	7203	-	-	-	-	-	-	-	-	-	-	-	-	-
3613	-	-	-	-	-	-	-	-	-	-	-	-	7207	-	-	-	-	-	-	-	-	-	-	-	-	-
3704	-	-	-	-	-	-	-	-	-	-	-	-	7208	↘	↘↘↘↘	-	-	-	-	-	-	-	-	-	-	-
3707	↘↘	-	-	-	NM	-	-	-	-	-	-	-	7209	-	↗	-	-	-	-	-	-	-	-	-	-	-
3709	-	↗	-	-	-	-	-	-	-	-	-	-	7210	↘↘	-	-	-	-	-	-	-	-	-	-	-	-
3802	-	-	-	-	-	-	-	-	-	-	-	-	7211	↘↘	-	-	-	-	M	-	-	-	-	-	-	-
3805	-	-	-	-	-	-	-	-	-	-	-	-	7212	-	-	-	-	-	-	-	-	-	-	-	-	NM
4001	-	-	-	-	-	-	-	-	-	-	-	-	7214	↘↘↘	↘↘↘↘	-	-	-	-	-	-	-	-	-	-	-
4103	-	-	-	-	-	-	-	-	-	-	-	-	7302	↘	-	-	-	-	-	-	-	-	-	-	-	-
4104	-	-	-	-	-	-	-	-	-	M	-	-	7304	-	-	-	-	-	-	NM	-	-	-	-	-	-

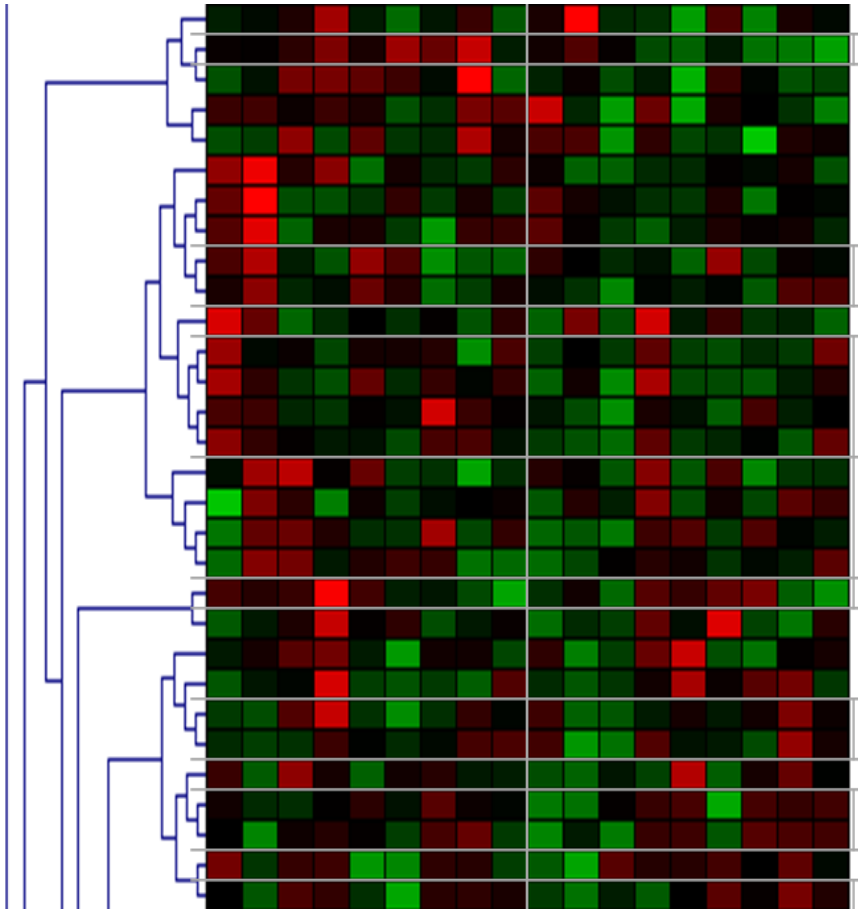
4105	↘↘	-	-	-	-	-	-	-	-	-	-	-	7306	↘	↗↗↗	-	-	-	-	-	-	-	-	-
4107	↘↘↘	↘↘↘↘	NM	-	-	-	-	-	-	-	-	-	7308	-	-	-	-	-	-	-	-	-	-	-	-
4203	↘	↘	-	-	-	-	-	-	-	-	-	-	7401	↘	-	-	-	-	-	-	-	-	-	-	-
4303	-	-	-	-	-	-	-	-	-	-	-	-	7402	-	↗	-	-	-	-	-	-	-	-	-	-
4308	↘↘↘	-	-	-	-	-	-	-	-	-	-	-	7404	-	-	-	-	-	-	-	-	-	-	-	-
4401	↗	↗↗	-	-	-	-	-	-	-	-	-	-	7407	-	↗↗	-	-	-	-	-	-	-	-	-	NM
4404	-	-	-	-	-	-	-	-	-	-	-	-	7408	-	↗	-	-	-	-	-	-	-	-	-	-
4405	-	↗	-	-	-	-	-	-	-	-	-	-	7409	-	↗↗↗	-	-	-	-	-	-	-	-	-	-
4407	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	7410	-	↗↗	-	-	-	-	-	-	-	-	-	-
4408	↘	-	-	-	-	-	-	-	-	-	-	-	7412	-	↗↗	-	-	-	-	-	-	-	-	-	-
4413	-	-	-	-	-	-	-	-	-	-	-	-	7413	↗	↗↗↗	-	-	M	-	-	-	-	-	-	-
4414	-		M	M	M	M	M	M	M	M	M	M	7414	-	↘	-	-	-	-	-	-	-	-	-	-
4501	-	↗↗	-	-	-	-	-	-	-	-	-	-	7501	-	↗	-	-	-	-	-	-	-	-	-	-
4503	-	-	-	-	-	-	-	-	-	-	-	-	7502	-	↗↗	-	-	-	-	-	-	-	-	-	-
4505	-	↗	-	-	-	-	-	-	-	-	-	-	7608	-	↗↗↗↗	-	-	-	-	-	-	-	-	-	-
4508	-	-	-	-	-	-	-	-	-	-	-	-	7701	↗	↗↗↗	-	-	-	-	-	-	-	-	-	-
4704	-	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	7703	-	↗↗	-	-	-	-	-	-	-	-	-	-
4708	↘↘	↘↘	-	-	-	-	-	-	-	-	-	-	7704	-	↗↗↗	-	-	-	-	-	-	-	-	-	-
4801	-	↗↗↗	-	-	-	-	-	-	-	-	-	-	7705	-	-	-	-	-	-	-	-	-	-	-	-
4802	-	↗	-	-	-	-	-	-	-	-	-	-	7706	-	↗↗	-	-	-	-	-	-	-	-	-	-
4805	-	-	-	-	-	-	-	-	-	-	-	-	7801	-	-	-	-	-	-	-	-	-	-	-	-
4806	-	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	7803	-	↗	-	-	-	-	-	-	-	-	-	-
5003	-	-	-	-	-	-	-	-	-	-	-	-	8102	-	↗↗↗↗	-	-	-	-	-	NM	-	-	-	-
5101	-	↗↗↗	-	-	-	-	-	-	-	-	-	NM	8105	-	↗↗↗	-	-	-	-	-	-	-	-	-	-
5103	↘	-	-	-	-	-	-	-	-	-	-	-	8106	-	↗	-	-	-	-	-	-	-	-	-	-
5104	↘↘	-	-	-	M	-	-	-	-	-	-	-	8111	↘↘	-	-	-	M	-	-	-	-	-	-	NM
5105	-	-	-	-	-	-	-	-	-	-	-	-	8201	↘↘↘	↘↘↘	-	-	-	-	-	-	-	-	-	-
5201	↗	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	8202	-	↗↗	-	-	-	-	-	-	-	-	-	-
5203	-	-	-	-	-	-	-	-	-	-	-	-	8204	-	↗↗↗	M	-	-	-	-	-	-	-	-	-
5207	-	-	-	-	-	-	-	-	-	-	-	-	8205	-	↗↗	-	-	-	-	-	-	-	-	-	NM
5210	-	-	-	-	-	-	-	-	-	-	-	-	8211	-	-	-	-	M	-	-	-	-	-	-	-
5303	-	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	8301	-	↗	-	-	-	-	-	-	-	-	-	-
5304	-	↗↗	-	-	-	M	-	-	-	-	-	-	8501	-	↗↗	-	-	-	-	-	-	-	-	-	-
5401	-	-	-	-	-	-	-	-	-	-	-	-	8701	-	↗↗↗	-	-	-	-	-	-	-	-	-	-
5404	-	↗	-	-	-	-	-	-	-	-	-	-	8702	-	↗	-	-	-	-	-	-	-	-	-	-
5412	↗↗	↗↗↗	-	-	-	-	-	-	-	-	-	-	8703	-	↗↗↗	-	-	-	-	-	-	-	-	-	-
5413	-	-	-	-	-	-	-	-	-	-	-	-	8704	↘↘	↗↗↗	-	-	-	-	NM	-	-	-	NM	
5501	-	-	-	-	-	-	-	-	-	-	-	-	8705	-	↗↗↗	-	-	-	-	-	-	-	-	NM	
5503	↗	↗↗↗	-	-	-	-	-	-	-	-	-	-	8804	-	↗↗	-	-	-	-	-	-	-	-	-	-
5507	-	-	-	-	-	-	-	-	-	-	-	-	9201	-	↗↗↗↗	-	-	-	-	-	-	-	-	-	-



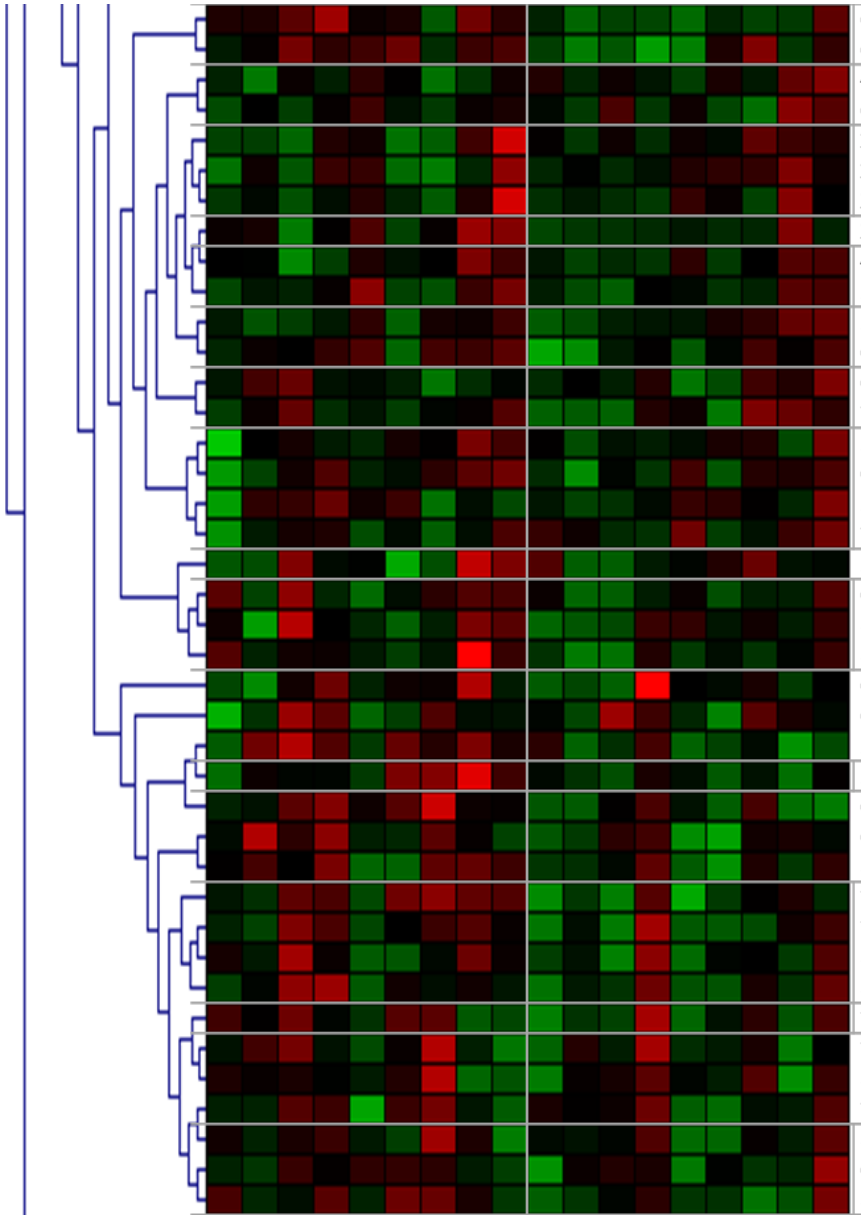
Sp	cor	ratio	ID
5103			
2101			
4501	cor NM	ns	Apyrase
2205			
4802			
6301			
2508			
2102			
4508			
6701	cor NM	ns	MID
3201			
3315			
5707			
5708	cor M/NM	ns	Phosphoglucomutase, cytoplasmic
6805	cor NM	ns	Transketolase, chloroplastic
3105			
4505			
6806	cor NM	ns	MID
7203			
5806			
6506			
7408			
1205			
2301			
2103	cor NM	ns	RuBisCO small subunit
2707	cor NM	over M	Polyphenol oxidase
2104	cor M/NM	ns	ND
2808	cor NM	over NM	Polyphenol oxidase
3704			
3303			
6405			



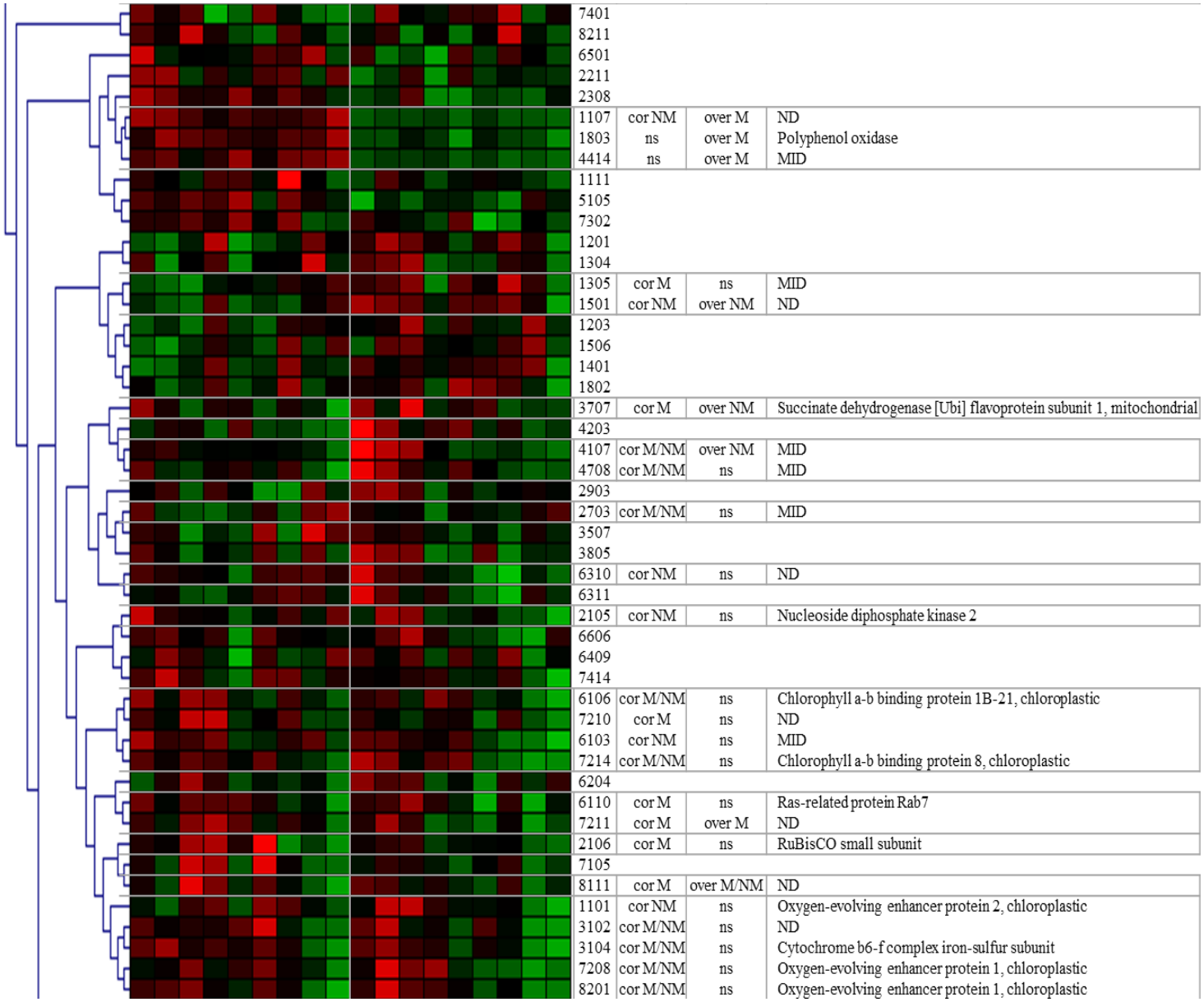
4001			
1104	cor NM	ns	50S ribosomal protein L10, chloroplastic
3309			
4103			
6306			
5401			
1105			
1106			
6108			
6304	cor NM	ns	ND
7501			
7502	cor NM	ns	RuBisCO activase A, chloroplastic
2204			
3205			
4405			
3103			
3709			
3202	cor NM	over M/NM	ND
2303	cor NM	over NM	Bark storage protein A / Glutelin type-A 1
5101	cor NM	over NM	Triosephosphate isomerase
3301	cor NM	ns	MID
4801	cor NM	ns	ATP-dependent Clp protease ATP-binding
4806	cor NM	ns	MID
5801	cor NM	ns	MID
2402	cor NM	ns	Fructose-bisphosphate aldolase
5802	cor NM	ns	Transketolase, chloroplastic
6807			
5413			
6403			
2507			
5503	cor M/NM	ns	Eukaryotic initiation factor 4A
7407	cor NM	over NM	MID
8702			



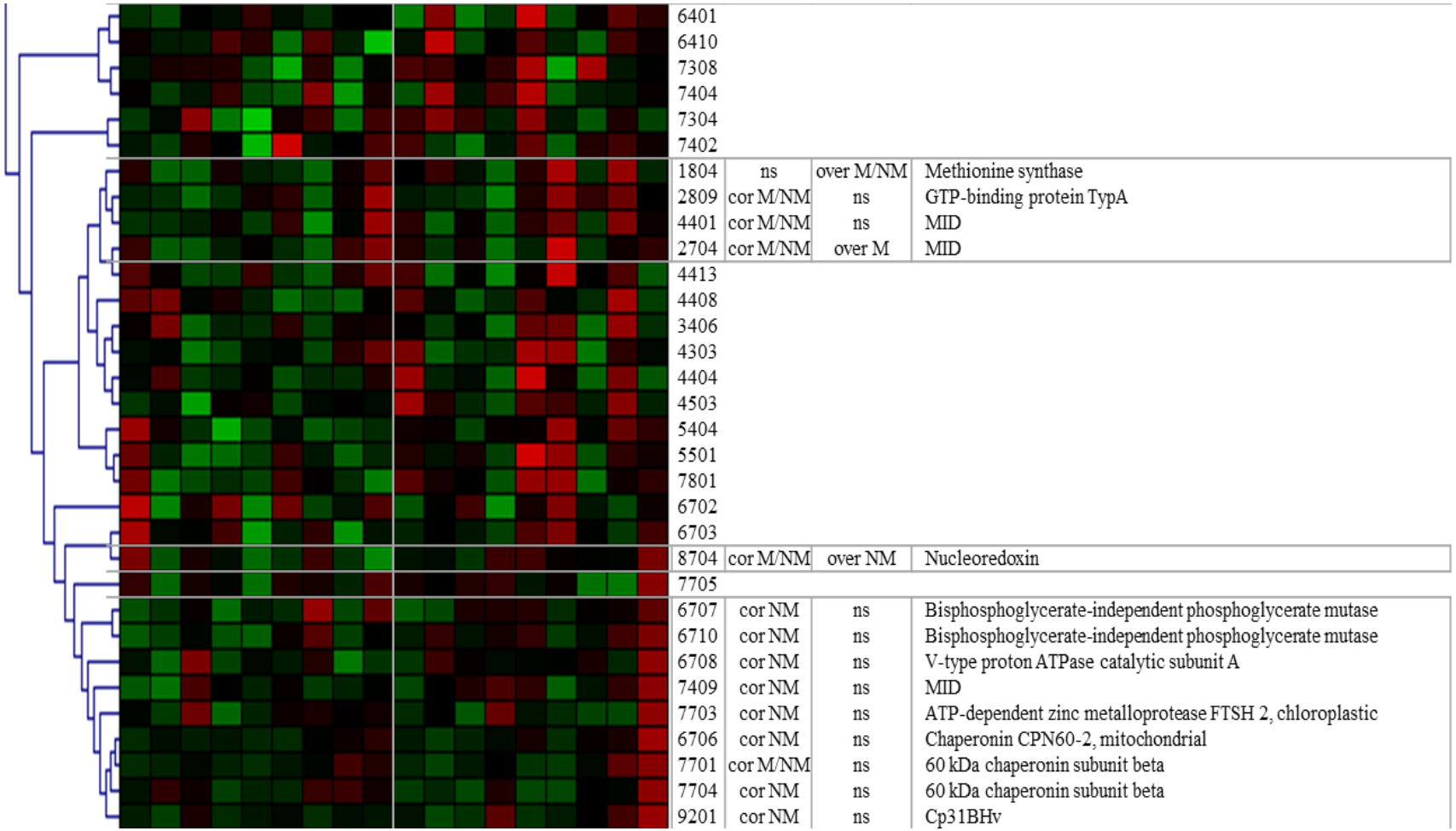
3802				
2312	cor NM	ns	Putative L-ascorbate peroxidase, chloroplastic
4104				
2206				
6302				
2309				
3613				
4805				
4105	cor M	ns	Ribulose-phosphate 3-epimerase
5303	cor NM	ns	Fructose-1,6-bisphosphatase, cytosolic
6001				
7412	cor NM	ns	Glutamine synthetase leaf isozyme, chloroplastic
8501	cor NM	ns	Glutamine synthetase, chloroplastic
7706	cor NM	ns	RuBisCO large subunit-binding protein subunit beta, chloroplastic
8703	cor NM	ns	RuBisCO large subunit-binding protein subunit alpha, chloroplastic
5104	cor M	over M	ND
6101	cor NM	ns	ND
6305	cor NM	ns	MID
8705	cor NM	over NM	Protein disulfide isomerase
4308	cor M	ns	MID
7803				
5207				
5507				
5304	cor NM	over M	Fructose-bisphosphate aldolase, chloroplastic
6202	cor NM	ns	ND
7209				
6208	cor NM	ns	Thioredoxin H-type 4
7608	cor NM	ns	Tubulin alpha
5210				
8102	cor NM	over NM	Thioredoxin peroxidase / 2-Cys peroxiredoxin BAS1



6402	cor NM	ns	Actin
6802	cor NM	over M	Transketolase, chloroplastic
4407	cor NM	ns	MID
6107	cor NM	ns	Triosephosphate isomerase
2801	cor M/NM	ns	Methionine synthase
2806	cor NM	over NM	Methionine synthase
3503	cor M/NM	ns	Isocitrate dehydrogenase [NADP], chloroplastic
3404			
4704	cor NM	ns	Phosphoglucomutase, cytoplasmic
5201	cor M/NM	ns	ND
5508	cor NM	ns	Eukaryotic initiation factor 4A
6203	cor NM	ns	Thioredoxin H-type 4
6408	cor NM	ns	MID
7103	cor NM	ns	Triosephosphate isomerase : TIM
5412	cor M/NM	ns	MID
6309	cor M/NM	ns	Cysteine synthase
5807	cor NM	over NM	MID
7202	cor NM	ns	Cysteine synthase, chloroplastic/chromoplastic
5003			
6303	cor NM	ns	Leaf Ferredoxin--NADP reductase, chloroplastic
8105	cor NM	ns	Thioredoxin peroxidase / 2-Cys peroxiredoxin BAS1
8701	cor NM	ns	60 kDa chaperonin subunit alpha
6608			
6308			
5203			
5808	cor M	over M	Heat shock 70 kDa protein 10, mitochondrial
6211			
6207			
8106			
7413	cor M/NM	over M	Phosphoribulokinase, chloroplastic
7410	cor NM	ns	Phosphoribulokinase, chloroplastic
8202	cor NM	ns	MID
8204	cor NM	over M	MID
7306	cor M/NM	ns	Sedoheptulose-1,7-bisphosphatase, chloroplastic
7212			
8301			
7207			
8205	cor NM	over NM	14-3-3-like protein A
6705	cor NM	ns	V-type proton ATPase catalytic subunit A
8804	cor NM	ns	Heat shock 70 kDa protein 7, chloroplastic



7401				
8211				
6501				
2211				
2308				
1107	cor NM	over M	ND	
1803	ns	over M	Polyphenol oxidase	
4414	ns	over M	MID	
1111				
5105				
7302				
1201				
1304				
1305	cor M	ns	MID	
1501	cor NM	over NM	ND	
1203				
1506				
1401				
1802				
3707	cor M	over NM	Succinate dehydrogenase [Ubi] flavoprotein subunit 1, mitochondrial	
4203				
4107	cor M/NM	over NM	MID	
4708	cor M/NM	ns	MID	
2903				
2703	cor M/NM	ns	MID	
3507				
3805				
6310	cor NM	ns	ND	
6311				
2105	cor NM	ns	Nucleoside diphosphate kinase 2	
6606				
6409				
7414				
6106	cor M/NM	ns	Chlorophyll a-b binding protein 1B-21, chloroplastic	
7210	cor M	ns	ND	
6103	cor NM	ns	MID	
7214	cor M/NM	ns	Chlorophyll a-b binding protein 8, chloroplastic	
6204				
6110	cor M	ns	Ras-related protein Rab7	
7211	cor M	over M	ND	
2106	cor M	ns	RuBisCO small subunit	
7105				
8111	cor M	over M/NM	ND	
1101	cor NM	ns	Oxygen-evolving enhancer protein 2, chloroplastic	
3102	cor M/NM	ns	ND	
3104	cor M/NM	ns	Cytochrome b6-f complex iron-sulfur subunit	
7208	cor M/NM	ns	Oxygen-evolving enhancer protein 1, chloroplastic	
8201	cor M/NM	ns	Oxygen-evolving enhancer protein 1, chloroplastic	



6401				
6410				
7308				
7404				
7304				
7402				
1804	ns	over M/NM	Methionine synthase
2809	cor M/NM	ns	GTP-binding protein TypA
4401	cor M/NM	ns	MID
2704	cor M/NM	over M	MID
4413			
4408			
3406			
4303			
4404			
4503			
5404			
5501			
7801			
6702			
6703			
8704	cor M/NM	over NM	Nucleoredoxin
7705			
6707	cor NM	ns	Bisphosphoglycerate-independent phosphoglycerate mutase
6710	cor NM	ns	Bisphosphoglycerate-independent phosphoglycerate mutase
6708	cor NM	ns	V-type proton ATPase catalytic subunit A
7409	cor NM	ns	MID
7703	cor NM	ns	ATP-dependent zinc metalloprotease FTSH 2, chloroplastic
6706	cor NM	ns	Chaperonin CPN60-2, mitochondrial
7701	cor M/NM	ns	60 kDa chaperonin subunit beta
7704	cor NM	ns	60 kDa chaperonin subunit beta
9201	cor NM	ns	Cp31BHv

Figure 2: Cluster of protein spots variation for the 214 accurately delimited spots (PDQuest) and identification of the 107 excised spots analyzed by LC-MS/MS. ID: most probable protein identity based on MS analysis, ND: Not Determined, MID: Multiple Identifications. Cor: Pearson's correlation; cor M, NM or M/NM: significant correlation of spot expression with Cu exposure only in M, only in NM or in both populations. Ratio: results of ratio between M and NM; over M, NM or M/NM: over-expression of spot in M, NM or both populations.

3.1.1. Cu effect

136 spots had their expression correlated with Cu exposure in at least one population (pval < 0.1, Tab. 1, Fig. 3):

26 spots were correlated with Cu exposure in both populations (Annex 21):

- 2 spot decreased in M roots but increased in NM ones,
- 14 spots increased with Cu exposure: 5 similarly in both populations, 1 more sharply in M, and 8 more sharply in NM.
- 10 spots decreased: 4 similarly in both populations, 1 more sharply in M, and 5 more sharply in NM.

19 spots were correlated with Cu exposure only in M population (Annex 22):

- 4 increased
- 15 decreased

91 spots were correlated with Cu exposure only in NM population (Annex 23):

- 80 increased
- 11 decreased

The expression of 78 spots did not exhibit any correlation with Cu exposure.

3.1.2. Population effect

40 spots were over-expressed in one population (ratio of 1.5) at least for one Cu exposure (Annex 24); 17 were over-expressed in M, 20 in NM, and 3 were first over-expressed in M at 10 or 15 µM then in NM population at 40 or 50 µM Cu (Tab. 1, Fig. 3).

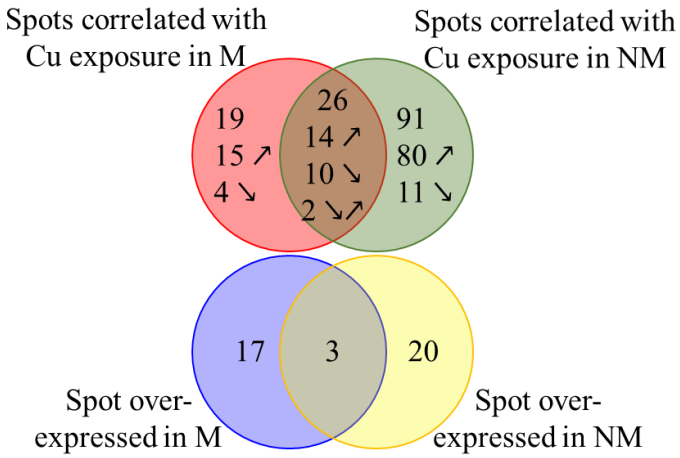


Figure 3: Venn diagram for the 136 and 40 spots which did respond to Cu treatment or population origin. Red: spots which expression was correlated with Cu exposure in M roots; Green: spots which expression was correlated with Cu exposure in NM roots; ↗: positive correlation; ↘: negative correlation; Blue: spots over-expressed in M; Yellow: spots over-expressed in NM leaves.

3.1.3. Integration of both effects

After both Cu and Population effect was examined separately, information was integrated together and synthetized in Fig. 4.

Expression of 89 spots was correlated with Cu exposure in only one population and did not differ significantly between populations:

- 14 in M (3 increased, 11 decreased)
- 75 in NM (67 increased, 8 decreased)

Expression of 22 spots was correlated with Cu exposure in both populations and did not differ significantly between populations:

- 12 increased in M and NM
- 9 decreased in M and NM
- 1 increased in M and decreased in NM

15 spots were over-expressed in one population and did not respond to Cu exposure:

- 7 over-expressed only in M
- 7 over-expressed only NM
- 1 over-expressed in M at 15 and in NM at 40 µM Cu

25 spots were over-expressed and correlated with Cu exposure in at least one population

- 3 over-expressed in M and correlated with Cu only in M (1 increased, 2 decreased)
- 5 over-expressed in M and correlated with Cu only in NM (4 increased, 1 decreased)
- 2 over-expressed in M and correlated with Cu in M and NM (2 increased)

- 1 over-expressed in NM and correlated with Cu only in M (1 decreased)
- 10 over-expressed in NM and correlated with Cu only in NM (8 increased, 2 decreased)
- 2 over-expressed in NM and correlated with Cu in M and NM (1 M/NM decreased and 1 M decreased / NM increased)

- 1 over-expressed in M at 10 µM Cu, in NM at 50 µM Cu and decreased only in M
- 1 over-expressed in M at 10 µM Cu, in NM at 40 µM Cu and increased only in NM

63 spots did not vary in response to Cu treatment or Population origin (Annex 26).

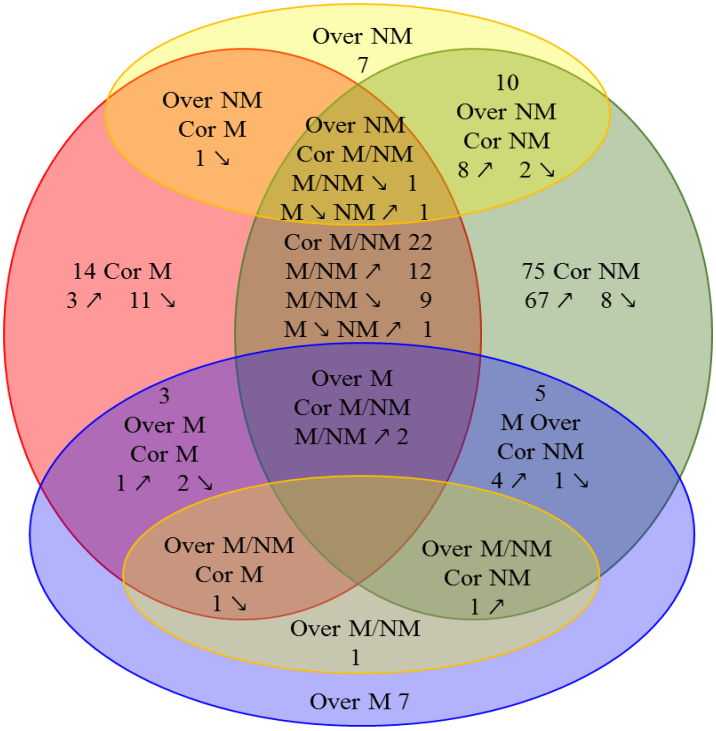


Figure 4: Adapted Venn diagram for the 151 spots wich vary among either Cu treatment or population origin. Red, cor M: spots which expression was correlated with Cu exposure in M leaves; Green, cor NM: spots which expression was correlated with Cu exposure in NM leaves; Blue, Over M: spots over-expressed in M; Yellow, Over NM: spots over-expressed in NM; cor M/NM: spots which expression was correlated with Cu exposure in M and NM leaves; Over M/NM: spots over-expressed in one population then in the other. ↗: positive correlation; ↘: negative correlation.

3.2. Protein spots excision and identification

107 out of the 214 accurately delimited spots in leaves were selected for excision (Tab. 1-2, Fig. 1) as their expression correlated with Cu exposure in at least one population ($P < 0.05$, Pearson's correlations) and/or they were over-expressed in one population at either two or more Cu exposure with a ratio > 1.5 or at one or more Cu exposure with a ratio > 2.

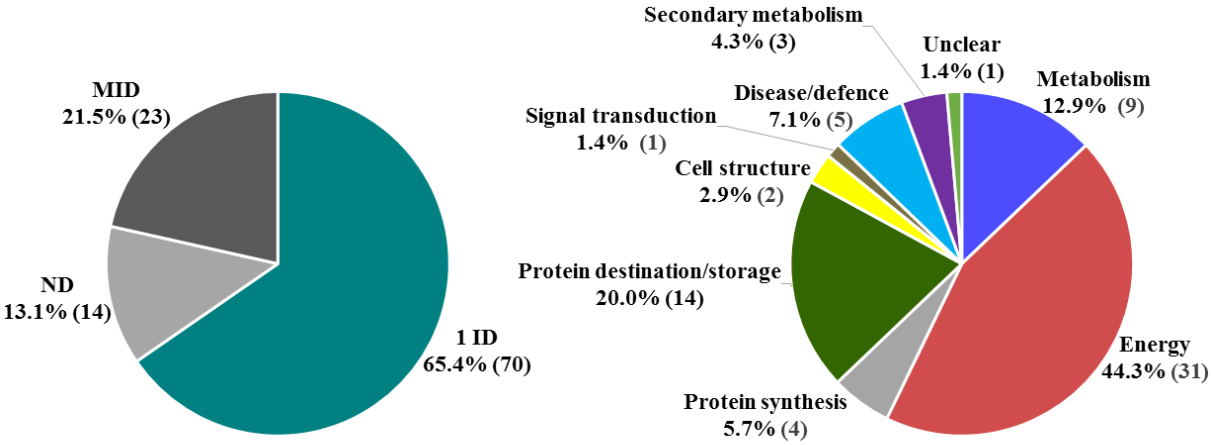
As shown in Fig. 5a, 14 (13.1%) out of the 107 excised spots characterized by LC-MS/MS remained unidentified after searching in "*Agrostis* EST" and "*Viridiplantae* proteins" databases (ND, circled in green color on the master gel picture in Fig. 1, Fig. 2 and Tab. 2). Among these 14 unidentified protein spots, three (#1501, 5201 and 6202) were matched with *Agrostis* ESTs with a function remaining uncharacterized (details available in Annex 28).

23 other spots (21.5%) matched with multiple protein identities in one or both databases (MID, circled in purple, Fig. 1, identification details available in Annex 27), while the last 70 spots (65.4%) matched with a single protein identification or two very similar identifications in case of spots 2303 and 8102/8105 (1ID, in red, Fig. 1, identification details in Annex 26).

The 70 single-match spots were assigned according to protein identifications in several functional categories (Fig. 6b) described in Bevan *et al.*, (1998), *i.e.* 12.9% Metabolism (9 spots), 44.3% Energy (31 spots), 5.7% Protein synthesis (4 spots), 20% Protein destination and storage (14 spots), 2.9% Cell structure (2 spots), 1.4% Signal transduction (1 spot), 7.1% Disease/defense (5 spots), 4.3% Secondary metabolism (3 spots) and 1.4% Unclear classification (1 spot).

Results of statistical tests for the 107 excised protein spots are presented in Table 2. Results of statistical tests of the 70 single-match spots are recorded in Tab. 3, identifications in Tab. 4, organized according to functional categories described in Fig. 6, and their functions and variations illustrated in Fig. 7.

Although all 107 excised spots were shown on heat map (Fig. 2) and in pie chart (Fig. 6a), the 23 spots with multiple identifications were not further described in results and considered for the discussion. To remember, details of protein identification for ND and MID spots are available in Annex 27 and 28.



a) 107 excised spots

b) 70 single-match spots

Figure 5: a) Results of protein spot identification for the 70 excised root spots, ND: Not Determined, MID: Multiple Identifications and 1ID: single-match Identification. b) Assignment of the 43 single-match spots in functional categories defined by Bevan *et al.* (1998).

Table 2. List of the 157 spots selected for excision, with results of protein identification and statistical tests. Sp: spots number; ID: results of protein identification (ND = non identified, MID: multiple protein identity); rM/rNM: r coefficient of Pearson's correlation for either the M or NM population, p-val = 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; Ratio 1 to ratio 50: comparative ratio between population values at each Cu exposure, -: no difference, >/>>: intensity of the difference (> indicated ratio higher than x1.5 but lower than x2, >> indicated ratio superior to x2) and M/NM indicated the population with higher values.

Sp	ID	rM	p-val		rNM	p-val		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
1101	Oxygen-evolving enhancer protein 2	-0.05	0.80	-	-0.70	0.0001	↘↘↘↘	-	-	-	-	-	-	-	-	-
1104	50S ribosomal protein L10	0.15	0.48	-	0.41	0.04	↗↗	-	-	-	-	-	-	-	-	-
1107	ND	0.06	0.772	-	0.45	0.03	↗↗	M >>	M >>	M >>	M >>	M >>	-	-	M >>	M >>
1305	MID	0.46	0.021	↗↗	-0.12	0.58	-	-	-	-	-	-	-	-	-	-
1501	ND	0.30	0.14	-	-0.47	0.02	↘↘	-	-	NM >>	-	-	-	-	-	-
1803	Polyphenol oxidase	0.11	0.59	-	-0.15	0.48	-	-	-	-	-	M >	-	-	-	M >
1804	Methionine synthase	0.33	0.11	-	0.25	0.23	-	-	-	-	M >>	-	-	-	NM >	-
2103	RuBisCO small subunit	0.03	0.89	-	-0.43	0.034	↘↘	-	-	-	-	-	-	-	-	-
2104	ND	-0.51	0.009	↘↘↘	-0.46	0.03	↘↘	-	-	-	-	-	-	-	-	-
2105	Nucleoside diphosphate kinase 2	-0.07	0.75	-	-0.42	0.04	↘↘	-	-	-	-	-	-	-	-	-
2106	RuBisCO small subunit	-0.41	0.040	↘↘	-0.28	0.18	-	-	-	-	-	-	-	-	-	-
2303	Bark storage protein A / Glutelin type-A 1	-0.04	0.84	-	0.69	0.0001	↗↗↗	-	-	-	-	-	NM >	NM >>	-	-
2312	Putative L-ascorbate peroxidase	0.13	0.53	-	-0.53	0.01	↘↘↘	-	-	-	-	-	-	-	-	-
2402	Fructose-bisphosphate aldolase	0.13	0.53	-	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
2703	MID	0.39	0.055	↗	0.40	0.05	↗↗	-	-	-	-	-	-	-	-	-
2704	MID	0.38	0.062	↗	0.34	0.09	↗	-	-	-	M >	-	-	-	-	-
2707	Polyphenol oxidase	-0.22	0.30	-	-0.59	0.002	↘↘↘	-	-	-	-	-	-	-	-	M >>
2801	Methionine synthase	0.45	0.025	↗↗	0.43	0.03	↗↗	-	-	-	-	-	-	-	-	-
2806	Methionine synthase	0.33	0.112	-	0.60	0.001	↗↗↗	-	-	-	-	-	-	-	NM >	-
2808	Polyphenol oxidase	-0.29	0.16	-	-0.36	0.08	↘	-	-	NM >>	-	-	-	-	-	-
2809	GTP-binding protein TypA	0.37	0.065	↗	0.55	0.00	↗↗↗	-	-	-	-	-	-	-	-	-
3102	ND	-0.47	0.019	↘↘	-0.54	0.01	↘↘↘	-	-	-	-	-	-	-	-	-
3104	Cytochrome b6-f complex Fe/S subunit	-0.65	0.0005	↘↘↘↘	-0.75	<0.0001	↘↘↘↘	-	-	-	-	-	-	-	-	-

3202	ND	0.00	0.98	-	0.56	0.004	↗↗↗	-	-	M >	-	-	-	-	NM >>	-
3301	MID	-0.03	0.87	-	0.44	0.026	↗↗	-	-	-	-	-	-	-	-	-
3503	Isocitrate dehydrogenase [NADP]	0.50	0.010	↗↗	0.49	0.01	↗↗	-	-	-	-	-	-	-	-	-
3707	Succinate dehydrogenase [Ubi] flavoprotein sub. 1	-0.46	0.022	↘↘	-0.27	0.19	-	-	-	NM >	-	-	-	-	-	-
4105	Ribulose-phosphate 3-epimerase	-0.40	0.045	↘↘	0.16	0.45	-	-	-	-	-	-	-	-	-	-
4107	MID	-0.56	0.004	↘↘↘	-0.75	<0.0001	↘↘↘↘	NM >	-	-	-	-	-	-	-	-
4308	MID	-0.55	0.005	↘↘↘	-0.11	0.59	-	-	-	-	-	-	-	-	-	-
4401	MID	0.36	0.077	↗	0.45	0.02	↗↗	-	-	-	-	-	-	-	-	-
4407	MID	0.14	0.49	-	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-
4414	MID	0.33	0.109	-				M >>	M >>	M >>	M >>	M >>	M >>	M >>	M >>	M >>
4501	Apyrase	0.27	0.20	-	0.45	0.02	↗↗	-	-	-	-	-	-	-	-	-
4704	Phosphoglucomutase	0.26	0.20	-	0.62	0.0009	↗↗↗↗	-	-	-	-	-	-	-	-	-
4708	MID	-0.47	0.017	↘↘	-0.49	0.01	↘↘	-	-	-	-	-	-	-	-	-
4801	ATP-dependent Clp protease ATP-binding	0.24	0.24	-	0.53	0.01	↗↗↗	-	-	-	-	-	-	-	-	-
4806	MID	0.00	0.99	-	0.73	<0.0001	↗↗↗↗	-	-	-	-	-	-	-	-	-
5101	Triosephosphate isomerase	-0.30	0.144	-	0.54	0.005	↗↗↗	-	-	-	-	-	-	-	-	NM >>
5104	ND	-0.48	0.014	↘↘	-0.03	0.88	-	-	-	M >	-	-	-	-	-	-
5201	ND	0.37	0.069	↗	0.68	0.0002	↗↗↗↗	-	-	-	-	-	-	-	-	-
5303	Fructose-1,6-bisphosphatase	-0.25	0.23	-	0.63	0.0007	↗↗↗↗	-	-	-	-	-	-	-	-	-
5304	Fructose-bisphosphate aldolase	0.03	0.88	-	0.45	0.02	↗↗	-	-	-	M >	-	-	-	-	-
5412	MID	0.50	0.011	↗↗	0.58	0.002	↗↗↗	-	-	-	-	-	-	-	-	-
5503	Eukaryotic initiation factor 4A	0.40	0.051	↗	0.57	0.00	↗↗↗	-	-	-	-	-	-	-	-	-
5508	Eukaryotic initiation factor 4A	0.33	0.10	-	0.68	0.0002	↗↗↗↗	-	-	-	-	-	-	-	-	-
5708	Phosphoglucomutase	0.51	0.010	↗↗↗	0.41	0.04	↗↗	-	-	-	-	-	-	-	-	-
5801	MID	0.13	0.55	-	0.52	0.008	↗↗↗	-	-	-	-	-	-	-	-	-
5802	Transketolase	0.03	0.87	-	0.51	0.01	↗↗↗	-	-	-	-	-	-	-	-	-
5807	MID	-0.09	0.67	-	0.52	0.01	↗↗↗	-	-	-	-	-	-	-	-	NM >
5808	Heat shock 70 kDa protein 10	0.45	0.023	↗↗	0.22	0.29	-	-	-	-	-	-	M >>	-	M >>	-

6101	ND	0.12	0.570	-	0.47	0.018	↗↗	-	-	-	-	-	-	-	-	-	-	-
6103	MID	-0.26	0.21	-	-0.68	0.0002	↘↘↘↘	-	-	-	-	-	-	-	-	-	-	-
6106	Chlorophyll a-b binding protein 1B-21	-0.43	0.034	↘↘	-0.57	0.00	↘↘↘	-	-	-	-	-	-	-	-	-	-	-
6107	Triosephosphate isomerase	0.20	0.35	-	0.41	0.04	↗↗	-	-	-	-	-	-	-	-	-	-	-
6110	Ras-related protein Rab7	-0.55	0.004	↘↘↘	-0.22	0.30	-	-	-	-	-	-	-	-	-	-	-	-
6202	ND	0.32	0.12	-	0.49	0.01	↗↗	-	-	-	-	-	-	-	-	-	-	-
6203	Thioredoxin H-type 4	0.18	0.40	-	0.68	0.0002	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6208	Thioredoxin H-type 4	0.07	0.76	-	0.48	0.01	↗↗	-	-	-	-	-	-	-	-	-	-	-
6303	Leaf Ferredoxin--NADP reductase	0.11	0.62	-	0.46	0.02	↗↗	-	-	-	-	-	-	-	-	-	-	-
6304	ND	0.28	0.173	-	0.55	0.00	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6305	MID	0.04	0.83	-	0.44	0.03	↗↗	-	-	-	-	-	-	-	-	-	-	-
6309	Cysteine synthase	0.54	0.006	↗↗↗	0.58	0.00	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6310	ND	0.08	0.69	-	-0.47	0.02	↘↘	-	-	-	-	-	-	-	-	-	-	-
6402	Actin	-0.01	0.96	-	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6408	MID	-0.27	0.19	-	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6701	MID	-0.03	0.90	-	0.44	0.029	↗↗	-	-	-	-	-	-	-	-	-	-	-
6705	V-type proton ATPase catalytic sub. A	-0.06	0.78	-	0.53	0.006	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6706	Chaperonin CPN60-2, mitochondrial	0.32	0.116	-	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6707	Phosphoglycerate mutase	0.29	0.15	-	0.65	0.0004	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6708	V-type proton ATPase catalytic sub. A	-0.16	0.46	-	0.52	0.007	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6710	Phosphoglycerate mutase	0.28	0.17	-	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-	-	-
6802	Transketolase	0.11	0.61	-	0.52	0.01	↗↗↗	-	-	-	M >	-	-	-	-	-	-	-
6805	Transketolase	0.23	0.26	-	0.47	0.018	↗↗	-	-	-	-	-	-	-	-	-	-	-
6806	MID	0.05	0.83	-	0.46	0.02	↗↗	-	-	-	-	-	-	-	-	-	-	-
7103	Triosephosphate isomerase	0.16	0.43	-	0.62	0.0010	↗↗↗↗	-	-	-	-	-	-	-	-	-	-	-
7202	Cysteine synthase	0.28	0.18	-	0.47	0.017	↗↗	-	-	-	-	-	-	-	-	-	-	-
7208	Oxygen-evolving enhancer protein 1	-0.34	0.095	↘	-0.65	0.0005	↘↘↘↘	-	-	-	-	-	-	-	-	-	-	-
7210	ND	-0.40	0.048	↘↘	-0.33	0.10	-	-	-	-	-	-	-	-	-	-	-	-

7211	ND	-0.43	0.033	↘↘	-0.34	0.10	-	-	-	-	M >	-	-	-	-	-
7214	Chlorophyll a-b binding protein 8	-0.53	0.006	↘↘↘	-0.75	<0.0001	↘↘↘↘	-	-	-	-	-	-	-	-	-
7306	Sedoheptulose-1,7-bisphosphatase	-0.38	0.062	↘	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
7407	MID	-0.05	0.81	-	0.42	0.04	↗↗	-	-	-	-	-	-	-	-	NM >
7409	MID	0.16	0.44	-	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
7410	Phosphoribulokinase	0.13	0.52	-	0.46	0.021	↗↗	-	-	-	-	-	-	-	-	-
7412	Glutamine synthetase leaf isozyme	-0.16	0.44	-	0.50	0.011	↗↗	-	-	-	-	-	-	-	-	-
7413	Phosphoribulokinase	0.35	0.087	↗	0.51	0.010	↗↗↗	-	-	M >	-	-	-	-	-	-
7502	RuBisCO activase A	0.20	0.347	-	0.41	0.05	↗↗	-	-	-	-	-	-	-	-	-
7608	Tubulin alpha	0.16	0.43	-	0.67	0.0002	↗↗↗↗	-	-	-	-	-	-	-	-	-
7701	60 kDa chaperonin subunit beta	0.34	0.098	↗	0.58	0.002	↗↗↗	-	-	-	-	-	-	-	-	-
7703	ATP-dependent zinc metalloprotease FTSH 2	0.14	0.52	-	0.48	0.014	↗↗	-	-	-	-	-	-	-	-	-
7704	60 kDa chaperonin subunit beta	0.11	0.62	-	0.55	0.005	↗↗↗	-	-	-	-	-	-	-	-	-
7706	RuBisCO large subunit-binding protein sub. beta	0.04	0.86	-	0.44	0.03	↗↗	-	-	-	-	-	-	-	-	-
8102	Thioredoxin peroxidase/2-Cys peroxiredoxin BAS1	0.13	0.55	-	0.67	0.0003	↗↗↗↗	-	-	-	-	-	NM >	-	-	-
8105	Thioredoxin peroxidase/2-Cys peroxiredoxin BAS1	0.29	0.16	-	0.53	0.01	↗↗↗	-	-	-	-	-	-	-	-	-
8111	ND	-0.45	0.025	↘↘	-0.17	0.42	-	-	-	M >	-	-	-	-	-	NM >>
8201	Oxygen-evolving enhancer protein 1	-0.54	0.005	↘↘↘	-0.60	0.00	↘↘↘	-	-	-	-	-	-	-	-	-
8202	MID	0.02	0.91	-	0.49	0.014	↗↗	-	-	-	-	-	-	-	-	-
8204	MID	-0.09	0.68	-	0.52	0.008	↗↗↗	M >	-	-	-	-	-	-	-	-
8205	14-3-3-like protein A	-0.19	0.36	-	0.44	0.030	↗↗	-	-	-	-	-	-	-	-	NM >
8501	Glutamine synthetase	-0.09	0.67	-	0.41	0.044	↗↗	-	-	-	-	-	-	-	-	-
8701	60 kDa chaperonin subunit alpha	0.22	0.30	-	0.57	0.00	↗↗↗	-	-	-	-	-	-	-	-	-
8703	RuBisCO large subunit-binding protein sub. alpha	-0.14	0.51	-	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
8704	Nucleoredoxin	-0.44	0.026	↘↘	0.60	0.001	↗↗↗	-	-	-	-	NM >	-	-	-	NM >>
8705	Protein disulfide isomerase	-0.31	0.13	-	0.57	0.003	↗↗↗	-	-	-	-	-	-	-	-	NM >
8804	Heat shock 70 kDa protein 7	-0.07	0.74	-	0.48	0.015	↗↗	-	-	-	-	-	-	-	-	-
9201	Cp31BHv	0.06	0.78	-	0.64	0.0006	↗↗↗↗	-	-	-	-	-	-	-	-	-

Table 3. Results of statistical tests for the 43 excised spots matching with a single protein identification. Sp: spots number; ID: results of protein identification after LC/MS/MS (ND = not determined); rM/rNM: r from Pearson's correlation for either M or NM population, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations values at each Cu exposure, from1 to 50 µM Cu, =: no difference, >/>>: intensity of the difference (> indicated ratio higher than x1.5 but lower than x2, >> indicated ratio superior to x2) and M/NM indicated the population with higher values.

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
	Functional category 1: Metabolism															
6309	Cysteine synthase	0.54	0.006	↗↗↗	0.58	0.002	↗↗↗	-	-	-	-	-	-	-	-	-
7202	Cysteine synthase, chloroplastic/chromoplastic	0.28	0.18	-	0.47	0.017	↗↗	-	-	-	-	-	-	-	-	-
1804	Methionine synthase	0.33	0.11	-	0.25	0.23	-	-	-	-	M >>	-	-	-	NM >	-
2801	Methionine synthase	0.45	0.025	↗↗	0.43	0.034	↗↗	-	-	-	-	-	-	-	-	-
2806	Methionine synthase	0.33	0.11	-	0.60	0.001	↗↗↗	-	-	-	-	-	-	-	NM >	-
7412	Glutamine synthetase leaf isozyme, chloroplastic	-0.16	0.44	-	0.50	0.011	↗↗	-	-	-	-	-	-	-	-	-
8501	Glutamine synthetase, chloroplastic	-0.09	0.67	-	0.41	0.044	↗↗	-	-	-	-	-	-	-	-	-
2105	Nucleoside diphosphate kinase 2	-0.07	0.75	-	-0.42	0.037	↘↘	-	-	-	-	-	-	-	-	-
4501	Apyrase	0.27	0.20	-	0.45	0.024	↗↗	-	-	-	-	-	-	-	-	-
	Functional category 2: Energy															
4704	Phosphoglucomutase, cytoplasmic	0.26	0.20	-	0.62	0.0009	↗↗↗↗	-	-	-	-	-	-	-	-	-
5708	Phosphoglucomutase, cytoplasmic	0.51	0.010	↗↗↗	0.41	0.045	↗↗	-	-	-	-	-	-	-	-	-
5303	Fructose-1,6-bisphosphatase, cytosolic	-0.25	0.23	-	0.63	0.0007	↗↗↗↗	-	-	-	-	-	-	-	-	-
2402	Fructose-bisphosphate aldolase	0.13	0.53	-	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
5304	Fructose-bisphosphate aldolase, chloroplastic	0.03	0.88	-	0.45	0.024	↗↗	-	-	-	M >	-	-	-	-	-
5101	Triosephosphate isomerase	-0.30	0.14	-	0.54	0.005	↗↗↗	-	-	-	-	-	-	-	-	NM >>
6107	Triosephosphate isomerase	0.20	0.35	-	0.41	0.042	↗↗	-	-	-	-	-	-	-	-	-
7103	Triosephosphate isomerase	0.16	0.43	-	0.62	0.0010	↗↗↗↗	-	-	-	-	-	-	-	-	-
6707	bisphosphoglycerate-independent phosphoglycerate mutase	0.29	0.15	-	0.65	0.0004	↗↗↗↗	-	-	-	-	-	-	-	-	-
6710	bisphosphoglycerate-independent phosphoglycerate mutase	0.28	0.17	-	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
3503	Isocitrate dehydrogenase [NADP], chloroplastic	0.50	0.010	↗↗	0.49	0.012	↗↗	-	-	-	-	-	-	-	-	-
3707	Succinate dehydrogenase [Ubi] flavoprotein subunit 1, mito.	-0.46	0.022	↘↘	-0.27	0.19	-	-	-	NM >	-	-	-	-	-	-

6705	V-type proton ATPase catalytic subunit A (Fragment)	-0.06	0.78	-	0.53	0.006	↗↗↗	-	-	-	-	-	-	-	-	-				
6708	V-type proton ATPase catalytic subunit A (Fragment)	-0.16	0.46	-	0.52	0.007	↗↗↗	-	-	-	-	-	-	-	-	-				
7208	Oxygen-evolving enhancer protein 1, chloroplastic	-0.34	0.095	↘	-0.65	0.0005	↘↘↘↘	-	-	-	-	-	-	-	-	-				
8201	Oxygen-evolving enhancer protein 1, chloroplastic	-0.54	0.005	↘↘↘	-0.60	0.001	↘↘↘	-	-	-	-	-	-	-	-	-				
1101	Oxygen-evolving enhancer protein 2, chloroplastic	-0.05	0.800	-	-0.70	0.0001	↘↘↘↘	-	-	-	-	-	-	-	-	-				
3104	Cytochrome b6-f complex iron-sulfur subunit	-0.65	0.0005	↘↘↘↘	-0.75	<0.0001	↘↘↘↘	-	-	-	-	-	-	-	-	-				
6106	Chlorophyll a-b binding protein 1B-21, chloroplastic	-0.43	0.034	↘↘	-0.57	0.003	↘↘↘	-	-	-	-	-	-	-	-	-				
7214	Chlorophyll a-b binding protein 8, chloroplastic	-0.53	0.006	↘↘↘	-0.75	<0.0001	↘↘↘↘	-	-	-	-	-	-	-	-	-				
6303	Leaf Ferredoxin--NADP reductase, chloroplastic	0.11	0.62	-	0.46	0.019	↗↗	-	-	-	-	-	-	-	-	-				
4105	Ribulose-phosphate 3-epimerase	-0.40	0.045	↘↘	0.16	0.45	-	-	-	-	-	-	-	-	-	-				
5802	Transketolase, chloroplastic	0.03	0.87	-	0.51	0.009	↗↗↗	-	-	-	-	-	-	-	-	-				
6802	Transketolase, chloroplastic	0.11	0.61	-	0.52	0.007	↗↗↗	-	-	-	M >	-	-	-	-	-				
6805	Transketolase, chloroplastic	0.23	0.26	-	0.47	0.018	↗↗	-	-	-	-	-	-	-	-	-				
7306	Sedoheptulose-1,7-bisphosphatase, chloroplastic	-0.38	0.062	↘	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-				
7410	Phosphoribulokinase, chloroplastic	0.13	0.52	-	0.46	0.021	↗↗	-	-	-	-	-	-	-	-	-				
7413	Phosphoribulokinase, chloroplastic	0.35	0.087	↗	0.51	0.010	↗↗↗	-	-	M >	-	-	-	-	-	-				
2103	RuBisCO small subunit	0.03	0.89	-	-0.43	0.034	↘↘	-	-	-	-	-	-	-	-	-				
2106	RuBisCO small subunit	-0.41	0.040	↘↘	-0.28	0.18	-	-	-	-	-	-	-	-	-	-				
7502	RuBisCo activase A, chloroplastic	0.20	0.35	-	0.41	0.047	↗↗	-	-	-	-	-	-	-	-	-				
	Functional category 5: Protein synthesis																			
5503	Eukaryotic initiation factor 4A	0.40	0.051	↗	0.57	0.003	↗↗↗	-	-	-	-	-	-	-	-	-				
5508	Eukaryotic initiation factor 4A	0.33	0.10	-	0.68	0.0002	↗↗↗↗	-	-	-	-	-	-	-	-	-				
1104	50S ribosomal protein L10, chloroplastic	0.15	0.48	-	0.41	0.040	↗↗	-	-	-	-	-	-	-	-	-				
2809	GTP-binding protein TypA	0.37	0.065	↗	0.55	0.005	↗↗↗	-	-	-	-	-	-	-	-	-				
	Functional category 6: Protein destination and storage																			
4801	Chaperone protein ClpC2, chloroplastic	0.24	0.24	-	0.53	0.006	↗↗↗	-	-	-	-	-	-	-	-	-				
8701	60 kDa chaperonin subunit alpha, chloroplastic	0.22	0.30	-	0.57	0.003	↗↗↗	-	-	-	-	-	-	-	-	-				
8703	60 kDa chaperonin subunit alpha	-0.14	0.51	-	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-				

7701	60 kDa chaperonin subunit beta, chloroplastic	0.34	0.098	↗	0.58	0.002	↗↗↗	-	-	-	-	-	-	-	-	-
7704	60 kDa chaperonin subunit beta	0.11	0.62	-	0.55	0.005	↗↗↗	-	-	-	-	-	-	-	-	-
7706	60 kDa chaperonin subunit beta	0.04	0.86	-	0.44	0.029	↗↗	-	-	-	-	-	-	-	-	-
8804	Heat shock 70 kDa protein 7, chloroplastic	-0.07	0.74	-	0.48	0.015	↗↗	-	-	-	-	-	-	-	-	-
5808	Heat shock 70 kDa protein 10, mitochondrial	0.45	0.023	↗↗	0.22	0.29	-	-	-	-	-	-	M >>	-	M >>	-
6706	Chaperonin CPN60-2, mitochondrial	0.32	0.12	-	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-
8704	Nucleoredoxin	-0.44	0.026	↘↘	0.60	0.001	↗↗↗	-	-	-	-	NM >	-	-	-	NM >>
8705	Protein disulfide isomerase	-0.31	0.13	-	0.57	0.003	↗↗↗	-	-	-	-	-	-	-	-	NM >
7703	ATP-dependent zinc metalloprotease FTSH 2, chloroplastic	0.14	0.52	-	0.48	0.014	↗↗	-	-	-	-	-	-	-	-	-
6110	Ras-related protein Rab7	-0.55	0.004	↘↘↘	-0.22	0.296	-	-	-	-	-	-	-	-	-	-
2303	Bark storage protein A / Glutelin type-A 1	-0.04	0.843	-	0.69	0.0001	↗↗↗↗	-	-	-	-	-	NM >	NM >>	-	-
	Functional category 9: Cell structure																		
6402	Actin	-0.01	0.96	-	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-
7608	Tubulin alpha	0.16	0.43	-	0.67	0.0002	↗↗↗↗	-	-	-	-	-	-	-	-	-
	Functional category 10: Signal transduction																		
8205	14-3-3-like protein A	-0.19	0.36	-	0.44	0.030	↗↗	-	-	-	-	-	-	-	-	NM >
	Functional category 11: Disease/defense																		
2312	Putative L-ascorbate peroxidase, chloroplastic	0.13	0.53	-	-0.53	0.006	↘↘↘	-	-	-	-	-	-	-	-	-
6203	Thioredoxin H-type 4	0.18	0.40	-	0.68	0.0002	↗↗↗↗	-	-	-	-	-	-	-	-	-
6208	Thioredoxin H-type 4	0.07	0.76	-	0.48	0.015	↗↗	-	-	-	-	-	-	-	-	-
8102	Thioredoxin peroxidase / 2-Cys peroxiredoxin BAS1	0.13	0.55	-	0.67	0.0003	↗↗↗↗	-	-	-	-	-	NM >	-	-	-
8105	Thioredoxin peroxidase / 2-Cys peroxiredoxin BAS1	0.29	0.16	-	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
	Functional category 20: Secondary metabolism																		
1803	Polyphenol oxidase	0.11	0.59	-	-0.15	0.48	-	-	-	-	-	M >	-	-	-	M >
2707	Polyphenol oxidase	-0.22	0.30	-	-0.59	0.002	↘↘↘	-	-	-	-	-	-	-	-	M >>
2808	Polyphenol oxidase	-0.29	0.16	-	-0.36	0.078	↘	-	-	NM >>	-	-	-	-	-	-
	Functional category 12: Unclear classification																		
9201	Cp31BHv	0.06	0.78	-	0.64	0.0006	↗↗↗	-	-	-	-	-	-	-	-	-

Table 4. Identification details for the 70 spots analyzed by LC-MS/MS which matched with a single protein identity; only the best match between both databases is shown. Sp: spot number; Db: consulted database, V: *Viridiplantae* of Uniprot and A: *Agrostis* spp. EST database; ID: Protein identity; Uniprot: Uniprot Accession; gb Access: Genbank Accession; eval: e-value of NCBI blastx; Cov: % of sequence coverage between experimental and database; (nb): number of peptides matched between both sequences; peptids: list of matched peptides. Details of identification and peptide lis were consigned in Annex 26.

Sp	Db	ID	Uniprot	cov (nb)	Genbank / e-value
		Functional category 1: Metabolism			
6309	A	Cysteine synthase EC = 2.5.1.47	I1HC84	62.07 (6)	GR282134_5 / 2e-64
7202	A	Cysteine synthase, chloroplastic/chromoplastic	M8AZ01	60.69 (5)	GR282134_5 / 2e-63
1804	A	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase : Methionine synthase EC = 2.1.1.14	M7ZHT1	25.78 (6)	DV856495_2 / 1e-121
2801	V	Methionine synthase	Q42662	18.98 (10)	
2806	V	Methionine synthase	P93263	10.85 (5)	
7412	V	Glutamine synthetase leaf isozyme, chloroplastic EC = 6.3.1.2	P13564	11.52 (3)	
8501	V	Glutamine synthetase, chloroplastic	P25462	9.46 (3)	
2105	V	Nucleoside diphosphate kinase 2, chloroplastic EC = 2.7.4.6	P47923	11.3 (3)	
4501	A	Apyrase EC = 3.6.1.5	B9U140	6.69 (2)	DV858912_5 / 5e-24
		Functional category 2: Energy			
4704	V	Phosphoglucomutase, cytoplasmic 2 EC = 5.4.2.2	P93805	23.33 (10)	
5708	V	Phosphoglucomutase, cytoplasmic	Q9SNX2	22.38 (10)	
5303	A	Fructose-1,6-bisphosphatase, cytosolic EC = 3.1.3.11	D8L9K9	38.96 (8)	DV862215_3 / 5e-85
2402	A	Fructose-bisphosphate aldolase EC = 4.1.2.13	I1GXE4	29.69 (6)	DV858099_2 / 1e-104
5304	V	Fructose-bisphosphate aldolase, chloroplastic	Q40677	22.68 (8)	
5101	A	Triosephosphate isomerase EC = 5.3.1.1	E0X6V4	73.51 (11)	GR278906_4 / 8e-103
6107	A	Triosephosphate isomerase	E0X6V4	67.03 (8)	GR278906_4 / 9e-103
7103	V	Triosephosphate isomerase, chloroplastic	P46225	44.97 (12)	
6707	V	2,3-bisphosphoglycerate-independent phosphoglycerate mutase EC = 5.4.2.12	P30792	13.77 (6)	
6710	V	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	P30792	15.56 (8)	
3503	V	Isocitrate dehydrogenase [NADP], chloroplastic (Fragment)	Q40345	20.32 (8)	
3707	V	Succinate dehydrogenase [Ubi] flavoprotein subunit 1, mitochondrial	O82663	7.89 (4)	
6705	V	V-type proton ATPase catalytic subunit A (Fragment)	Q40002	27.07 (13)	
6708	V	V-type proton ATPase catalytic subunit A (Fragment)	Q40002	33.79 (15)	

7208	A	Oxygen-evolving enhancer protein 1, chloroplastic	M8AE10	61.9 (19)	DV859364_2 / 3e-169
8201	A	Oxygen-evolving enhancer protein 1, chloroplastic	M8AE10	53.65 (11)	DV859364_2 / 3e-169
1101	A	Oxygen-evolving enhancer protein 2, chloroplastic	M7YV65	41.31 (9)	DV853316_3 / 4e-123
3104	A	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic EC = 1.10.9.1	Q7X9A6	40.58 (8)	DV853200_2 / 7e-141
6106	A	Chlorophyll a-b binding protein 1B-21, chloroplastic	Q9SDM1	9.06 (3)	DY543567_5 / 2e-118
7214	A	Chlorophyll a-b binding protein 8, chloroplastic	M8A6M9	24.74 (5)	DV856057_1 / 1e-123
6303	A	Ferredoxin--NADP reductase, leaf isozyme, chloroplastic EC = 1.18.1.2	M8B795	34.23 (12)	DV855685_1 / 2e-137
4105	A	Ribulose-phosphate 3-epimerase EC = 5.1.3.1	I1H9A1	25.55 (5)	DV856160_1 / 3e-142
5802	V	Transketolase, chloroplastic EC = 2.2.1.1	Q7SIC9	10.07 (7)	
6802	V	Transketolase, chloroplastic	Q7SIC9	9.78 (7)	
6805	A	Transketolase, chloroplastic	M8APV9	28.09 (4)	DV863383_1 / 2e-56
7306	V	Sedoheptulose-1,7-bisphosphatase, chloroplastic EC = 3.1.3.37	P46285	30.28 (9)	
7410	V	Phosphoribulokinase, chloroplastic EC = 2.7.1.19	P26302	36.63 (10)	
7413	V	Phosphoribulokinase, chloroplastic	P26302	31.93 (9)	
2103	A	Ribulose-1,5-bisphosphate carboxylase small subunit EC = 4.1.1.39	Q9SDY8	52.69 (9)	GR279297_6 / 1e-74
2106	A	Ribulose-1,5-bisphosphate carboxylase small subunit	Q9SDY8	48.5 (7)	GR279297_6 / 1e-74
7502	A	Ribulose bisphosphate carboxylase/oxygenase activase A, chloroplastic	Q40073	44.44 (10)	DV855440_2 / 0
		Functional category 5: Protein synthesis			
1104	A	50S ribosomal protein L10, chloroplastic	M8BNG8	12.77 (2)	DY543708_6 / 5e-42
5503	V	Eukaryotic initiation factor 4A EC = 3.6.4.13	P41378	35.02 (12)	
5508	V	Eukaryotic initiation factor 4A	P41378	43.48 (18)	
2809	A	GTP-binding protein TypA	G3K3T1	20.22 (3)	DV864812_1 / 2e-78
		Functional category 6: Protein destination and storage			
4801	V	Chaperone protein ClpC2, chloroplastic	Q2QVG9	32.75 (24)	
8701	V	60 kDa chaperonin subunit alpha, chloroplastic (Fragment): CPN-60 alpha	P08823	42.54 (18)	
8703	V	60 kDa chaperonin subunit alpha, chloroplastic (Fragment): CPN-60 alpha	P08823	28.55 (13)	
7701	V	60 kDa chaperonin subunit beta, chloroplastic (Fragment): CPN-60 beta	Q43831	50.7 (23)	
7704	V	60 kDa chaperonin subunit beta, chloroplastic (Fragment): CPN-60 beta	Q43831	49.1 (22)	
7706	V	60 kDa chaperonin subunit beta, chloroplastic (Fragment): CPN-60 beta	Q43831	38.28 (15)	
8804	V	Heat shock 70 kDa protein 7, chloroplastic	Q9LTX9	9.47 (8)	
5808	V	Heat shock 70 kDa protein 10, mitochondrial	Q9LDZ0	8.94 (5)	

6706	V	Chaperonin CPN60-2, mitochondrial: HSP60-2	Q05046	19.3 (10)	
8704	A	Nucleoredoxin EC = 1.8.1.8	N1R275	21.24 (5)	DV853833_1 / 2e-96
8705	A	Protein disulfide isomerase EC = 5.3.4.1	Q9FEG4	54.42 (11)	EV519572_1 / 4e-135
7703	V	ATP-dependent zinc metalloprotease FTSH 2, chloroplastic EC = 3.4.24.-	Q655S1	36.09 (16)	
6110	V	Ras-related protein Rab7	P31022	15.53 (3)	
2303	A	Bark storage protein A	M8CRB0	17.89 (4)	DV857196_1 / 8e-131
		Glutelin type-A 1	M7Z0L4	7.64 (2)	DV856120_3 / 2e-105
		Functional category 9: Cell structure			
6402	V	Actin	Q05214	59.68 (17)	
7608	A	Tubulin alpha-1 chain	O22347	50.3 (11)	DV858436_1 / 4e-150
		Functional category 10: Signal transduction			
8205	V	14-3-3-like protein A	P29305	49.62 (12)	
		Functional category 11: Disease/defense			
2312	A	Putative L-ascorbate peroxidase, chloroplastic EC = 1.11.1.11	M8BMC6	21.32 (6)	DV855736_2 / 6e-101
6203	A	Thioredoxin H-type 4	M8CV70	23.5 (5)	DV865481_2 / 3e-85
6208	A	Thioredoxin H-type 4	M8CV70	30.77 (7)	DV865481_2 / 3e-85
8102	A	Thioredoxin peroxidase EC = 1.11.1.15	O81480	25.78 (5)	DV856996_5 / 5e-129
	V	2-Cys peroxiredoxin BAS1, chloroplastic (Fragment) EC = 1.11.1.15	P80602	35.24 (5)	
8105	A	Thioredoxin peroxidase	O81480	48.42 (7)	DV865047_4 / 1e-101
	V	2-Cys peroxiredoxin BAS1, chloroplastic (Fragment)	P80602	30 (6)	
		Functional category 20: Secondary metabolism			
1803	A	Polyphenol oxidase EC = 1.10.3.1	Q6PLR1	32.89 (4)	GR279139_4 / 3e-22
2707	A	Polyphenol oxidase	Q6PLR1	32.89 (4)	GR279139_4 / 3e-22
2808	A	Polyphenol oxidase	Q6PLR0	39.54 (8)	DV854107_3 / 4e-34
		Functional category 12: Unclear classification			
9201	A	Cp31BHv	O81988	30.03 (8)	DV853271_2 / 4e-118

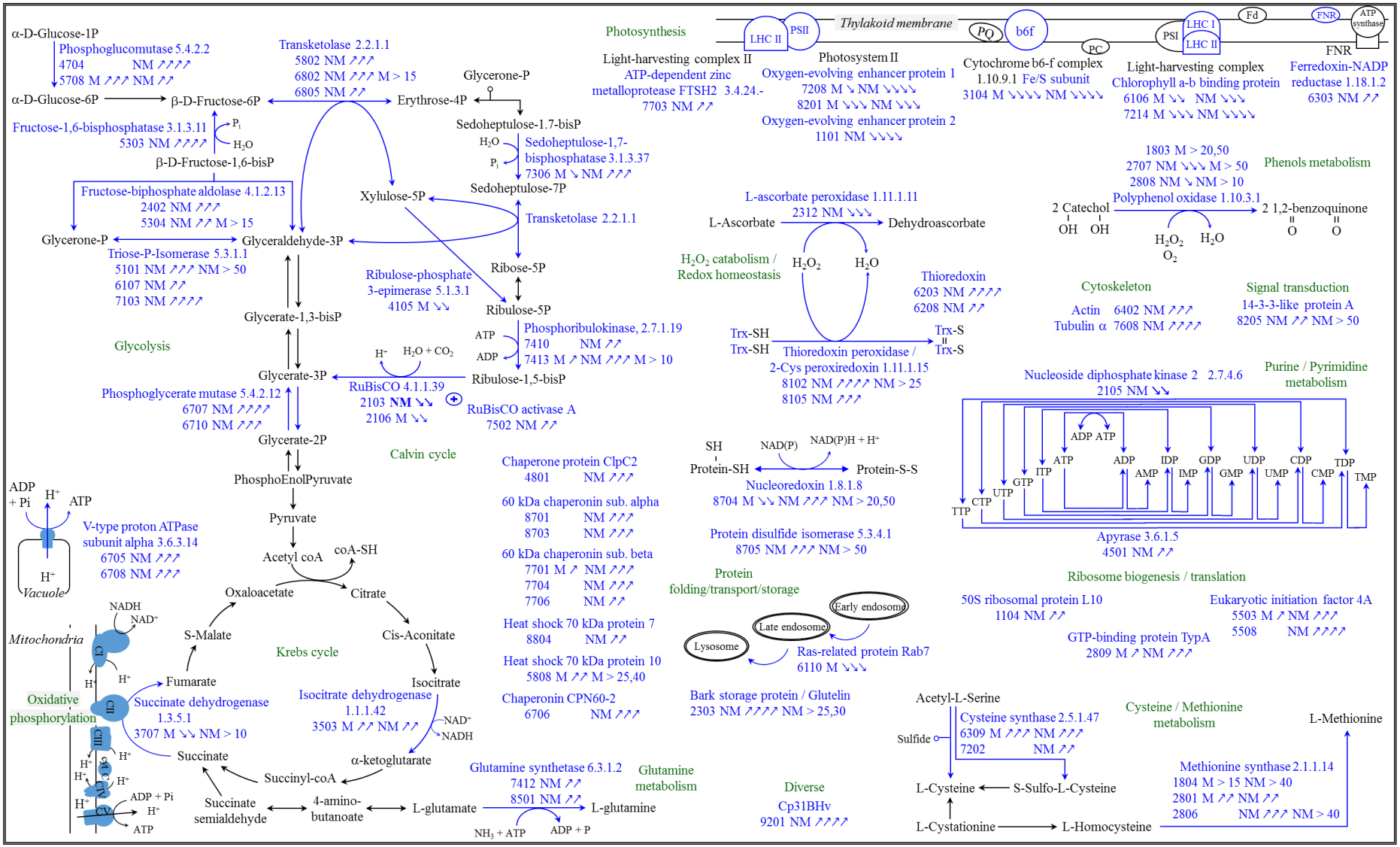


Figure 6: Functions of the 70 identified proteins (in blue) in plant metabolic processes. Enzymes are represented by their name and EC. Spot numbers and identifications referred to Tab. 3 and 4. Variation of root spots refers to Tab. 3. M / NM: Metallicolous / Non-Metallicolous population of *A. capillaris*. ↗ / ↘: positive / negative correlation (Pearson); p-val: 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; M/NM > 1-50: population with higher expression at 1-50 µM Cu (ratio > x1.5).

3.3. Pattern of protein accumulation

Description of protein spot expression and identification was made according to the functional categories presented in Fig. 5b and referred to Tab. 3-4 and Fig. 6, so no further reference to these tables are cited in the text. To simplify the reading, 'M leaves' and 'NM leaves' were abbreviated by M and NM, and 'protein spot expression' by 'expression', if no additional indication is provided. To shorten the text, formula such as 'protein spot matched as XX' or 'protein spot identified as XX' were not used and protein identities were cited directly (Tab. 4). Additionally, 'positively/negatively correlated with Cu exposure' were replaced by 'increased/decreased' or 'down-/up-regulated'.

3.3.1. Functional category 1: Metabolism

Enzymes belonging to cysteine/methionine metabolism were identified, two cysteine synthase spots (#6309 and 7202) were up-regulated in NM leaves ($r = 0.58$ and 0.47, p-values = 0.002 and 0.017 respectively), but only #6309 was also significantly up-regulated in M ($r = 0.54$, p-val = 0.006) and none differed between populations according to ratios. Three methionine synthase spots (#1804, 2801 and 2806) were differentially expressed among experimental conditions, i.e. #1804 was over-expressed in M at 15 µM Cu (ratio > 2) and in NM at 40 µM ($1.5 < \text{ratio} < 2$), #2801 was up-regulated by Cu exposure in both M ($r = 0.45$, p-val = 0.025) and NM leaves ($r = 0.43$, p-val = 0.034), while #2806 was up-regulated only in NM ($r = 0.60$, p-val = 0.001) and over-expressed in NM at 40 µM ($1.5 < \text{ratio} < 2$).

Expression of two glutamine synthetase spots (#7412 and 4501) increased only in NM ($r = 0.50$ and 0.41, p-values = 0.011 and 0.044 respectively) and did not differed between population according to ratios.

Two enzymes involved in purine/pyrimidine metabolism did respond to Cu exposure only in NM, while a nucleoside diphosphate kinase 2 (#2105) decreased ($r = -0.42$, p-val = 0.037), an apyrase (#4501) increased with Cu exposure ($r = 0.45$, p-val = 0.025).

3.3.2. Functional category 2: Energy

Expression of the ten spots of glycolysis-related enzymes increased in NM leaves, i.e. phosphoglucomutase (#4704 and 4708, $r = 0.62$ and 0.41, p-values = 0.0009 and 0.045), fructose-1,6-bisphosphatase (#5303, $r = 0.63$, p-val = 0.0007), fructose-bisphosphate aldolase (#2402 and 5304, $r = 0.61$ and 0.4, p-values = 0.001 and 0.024), triosephosphate isomerase (#5101, 6107 and 7103, $r = 0.54$, 0.41 and 0.62, p-values = 0.005, 0.042 and 0.001), and phosphoglycerate mutase (#6707 and 6710, $r = 0.65$ and 0.53, p-values = 0.0004 and 0.007),

while only one phosphoglucomutase (#5708) was also up-regulated in M leaves (r = 0.51, p-val = 0.01). Ratios indicated over-expression of one fructose-bisphosphate aldolase (#5304) in M at 15 µM Cu (1.5 < ratio < 2) and one triosephosphate isomerase (#5101) in NM at 50 µM Cu (ratio >2).

Expressions of two Krebs-related enzymes did respond to Cu exposure, while an Isocitrate dehydrogenase (#3503) was up-regulated in both M (r = 0.50, p-val = 0.01) and NM (r = 0.49, p-val = 0.012), a succinate dehydrogenase [Ubiquinone] flavoprotein subunit 1 (#3707) was down-regulated only in M (r = -0.46, p-val = 0.022) and over-expressed in NM at 10 µM Cu (1.5 > ratio > 2).

Expression of two V-type proton ATPase catalytic subunit A (#6705 and 6708) increased only in NM leaves (r = 0.53 and 0.52, p-values = 0.006 and 0.007) but did not differ significantly between populations according to ratios.

Several photosynthesis-related spots did respond to Cu exposure but did not differ significantly between populations according to ratios. Expression of three oxygen-evolving enhancer spots (#7208 and 8201 as protein 1 and #1101 as protein 2) decreased in NM (r = -0.65, -0.60 and -0.70, p-values = 0.0005, 0.001 and 0.0001 respectively) but only two (#7208 and 8201) decreased also in M (r = -0.34 and -0.54, p-values = 0.095 and 0.005). A cytochrome b6-f complex iron-sulfur subunit (#3104), and two chlorophyll a-b binding proteins (#6106 and 7214) were down-regulated in both M (r = -0.65, -0.43 and -0.53, p-values = 0.0005, 0.034 and 0.006 respectively) and NM leaves (r = -0.75, -0.57 and -0.75, p-values < 0.0001, = 0.003 and < 0.0001 respectively), while a ferredoxin-NADP reductase (#6303) was up-regulated only in NM (r = 0.46, p-val = 0.019).

Expression of the three transketolase spots (#5802, 6802 and 6805), involved in pentose phosphate pathway, increased only in NM leaves (r = 0.51, 0.52 and 0.47, p-values = 0.009, 0.007 and 0.018 respectively) and only one, #6802, was over-expressed in M at 10 µM Cu. A ribulose-phosphate 3-epimerase (#4105) was down-regulated only in M (r = -0.40, p-val = 0.045) but did not differ between populations according to ratios.

Expression of enzymes involved in Calvin cycle did respond to Cu in one or both populations. Two RuBisCO small subunit spots (#2103 and 2106), decreased respectively in NM (r = -0.43, p-val = 0.034) and M (r = -0.41, p-val = 0.04); however, considering levels of expression (between 3.9 and 16.2% for #2103 and between 0.5 and 0.11% for #2106), the decrease in NM was the dominant effect. A RuBisCO activase A (#7502) was up-regulated

only in NM (r = 0.41, p-val = 0.047) and none of these spots differed between populations according to ratios.

A sedoheptulose-1,7-bisphosphatase (#7306) and two phosphoribulokinase spots (#7410 and 7413) were up-regulated in NM leaves (r = 0.53, 0.46 and 0.51, p-values = 0.007, 0.021 and 0.01 respectively); #7306 was down-regulated in M (r = -0.38, p-val = 0.062), while #7413 was up-regulated in M (r = 0.35, p-val = 0.087) and over-expressed in M at 10 µM.

3.3.3. Functional category 5: Protein synthesis

One 50S ribosomal protein L10 (#1104) and an eukaryotic initiation factor 4A (#5508) spots were up-regulated only in NM (r = 0.41 and 0.68, p-values = 0.04 and 0.0002), while another eukaryotic initiation factor 4A (#5503) and a GTP-binding protein TypA (#2809) spots were up-regulated in both M (r = 0.40 and 0.37, p-values = 0.051 and 0.065) and NM (r = 0.57 and 0.55, p-values = 0.003 and 0.005).

3.3.4. Functional category 6: Protein destination and storage

Seven chloroplastic protein chaperones were up-regulated markedly in NM, i.e. chaperone protein ClpC2 (#4801, r = 0.53, p-val = 0.006), 60 kDa chaperonin subunit alpha (#8701 and 8703, r = 0.57 and 0.61, p-values = 0.003 and 0.00) and beta (#7701, 7704 and 7706, r = 0.58, 0.55 and 0.44, p-values = 0.002, 0.005 and 0.029 respectively) and a heat shock 70 kDa protein 7 (#8804, r = 0.48, p-val = 0.015), while only one 60 kDa chaperonin subunit beta (#7701) was also up-regulated in M leaves (r = 0.34, p-val = 0.098). These seven spots did not differ significantly between populations according to ratios.

Two other mitochondrial protein chaperones, i.e. a heat shock 70 kDa protein 10 (#5808) and a chaperonin CPN60-2 (#6706) were respectively up-regulated in M (r = 0.45, p-val = 0.023) and NM leaves (r = 0.55, p-val = 0.004), and #6706 was also over-expressed in M at 25 and 40 µM Cu (ratio > 2).

Expression of a protein disulfide isomerase (#8705), increased with Cu exposure only in NM (r = 0.57, p-val = 0.003), leading to significant over-expression in NM at 50 µM Cu (1.5 < ratio < 2). Similarly, a chloroplastic ATP-dependent zinc metalloprotease FTSH 2 (#7703) was up-regulated only in NM (r = 0.48, p-val = 0.014) but no significant difference occurred between populations according to ratios. In contrast, expression of a nucleoredoxin (#8704) decreased in M (r = -0.44, p-val = 0.026) but increased in NM (r = 0.60, p-val = 0.001) leading to significant over-expression in NM at 20 (1.5 < ratio < 2) and 50 µM Cu (ratio > 2). A Ras-related protein Rab7 (#6110) was down-regulated only in M (r = -0.55, p-val = 0.004) but did not differ significantly between populations according to ratios

Spot 2303 matched with two close protein identities, bark storage protein A and glutelin type A1, indicating that this spot was probably a storage protein, which differed partially from already characterized sequences. Expression of #2303 increased only in NM ($r = 0.69$, p-val = 0.0001) leading to over-expression in NM at 25 ($1.5 < ratio < 2$) and 30 µM Cu ($ratio > 2$).

3.3.5. Functional category 9: Cell structure

Expression of both cytoskeleton-related protein spots, i.e. actin (#6402) and tubulin alpha (#7608), increased sharply only in NM ($r = 0.55$ and 0.67, p-values = 0.004 and 0.0002) but did not differ significantly between populations according to ratios.

3.3.6. Functional category 10: Signal transduction

Expression of a 14-3-3-like protein A (#8205), increased only in NM ($r = 0.44$, p-val = 0.03), leading to significant over-expression in NM at 50 µM Cu ($1.5 < ratio < 2$).

3.3.7. Functional category 11: Disease/defense

All proteins involved in redox homeostasis did respond to Cu exposure only in NM; while a chloroplastic L-ascorbate peroxidase (#2312) was down-regulated ($r = -0.53$, p-val = 0.006), two thioredoxin H-type 4 spots (#6203 and 6208 $r = 0.68$ and 0.48, p-values = 0.0002 and 0.015) were up-regulated. Two other spots (#8102 and 8105) matched with two close protein identities, thioredoxin peroxidase and 2-Cys peroxiredoxin BAS1, indicating a peroxidase function. These two peroxidases were also up-regulated only in NM leaves ($r = 0.67$ and 0.53, p-values = 0.0003 and 0.007) but only #8102 was over-expressed in NM at 25 µM ($1.5 < ratio < 2$).

3.3.8. Functional category 20: Secondary metabolism

Three polyphenol oxidase spots were differentially expressed between populations, i.e. #1803 in M at 20 and 50 µM Cu ($1.5 < ratio < 2$), #2707 in M at 50 µM Cu ($ratio > 2$), and #2808 in NM at 10 µM Cu ($ratio > 2$). While #1803 did not respond to Cu exposure, expression of #2707 and 2808 decreased only in NM ($r = -0.59$ and -0.36, p-values = 0.002 and 0.078).

3.3.9. Functional category 12: Unclear classification

A Cp31BHv spot (#9201) was up-regulated by Cu exposure only in NM ($r = 0.64$, p-val = 0.0006) but did not differ significantly between populations according to ratio.

4. Discussion

4.1. General comments

In leaves of M and NM *A. capillaris* populations exposed to increasing Cu concentrations in nutrient solution (1-50 µM), 214 spots were accurately quantified in all experimental conditions. Higher spot amounts, i.e. 381 and 420 reproducible spots, have been respectively recorded in leaves of 1 month-old plants of *A. capillaris* exposed to arsenic for 8 days (Duquesnoy *et al.*, 2009) and of *A. stolonifera* cultivars exposed to salt-stress for 28 days (Xu *et al.*, 2010). In Duquesnoy *et al.* (2009) several new spots have been recorded under As stress conditions, while in our study, most spots did respond to either Cu treatment or population origin but any new spot was not detected in excess Cu conditions. Experimental design may explain such differences as plants were first grown for one month on As-free vermiculite then exposed to As stress, whereas in our experiment plants were permanently exposed to Cu, from germination to harvest. In Duquesnoy *et al.* (2009), short-term mechanisms of acclimation (resistance) are presumed to occur, while in our experiment, long term resistance ones were assumed to take place.

The marked differences in Cu-tolerance between M and NM populations of *A. capillaris* reported at the phenotypic level were accompanied by major changes in the protein profiles of leaves, as 151 out of 214 spots did respond to either Population- and/or Cu (Table 1). However, protein spots expression in leaves was more influenced by Cu exposure than by populations origin, as only 40 spots were differentially expressed between populations (Ratios) whereas 136 were correlated with Cu exposure (Pearson's correlations). Main differences found between M and NM leaves did stand in the response to Cu exposure: although only two of the 26 spots varying in both populations showed really opposite pattern with a decrease in M leaves and an increase in NM ones and 15 varied more intensively in one population. Additionally, 110 spots were either up- or down-regulated by Cu in only one population (19 in M and 91 in NM leaves). Among spots differentially expressed in leaves between populations, similar spot numbers were over-expressed only in M (17) and NM (20) leaves and only 3 spots were over-expressed in M at low Cu exposure (10-15 µM), and in NM at high Cu exposure (40-50 µM). In overall, spots up-regulated (119) by Cu exposure were more abundant than down-regulated spots (25). Only 29 spots did respond to both Cu and population origin, deserving more attention.

Proportionally, less spots varied in response to Cu in 28-days old *Elsholtzia splendens* plants exposed to 100 µM Cu for 6 days (Li *et al.*, 2009): i.e. 65% of the 214 quantified spots were either up- or down-regulated, while only 6 out of around 1 000 spots detected in *E.*

splendens were up-or down regulated under Cu stress. Such difference in protein pattern has been not observed in an experiment comparing tolerant and sensitive strains of *Ectocarpus siliculosus*, exposed to Cu (50 µg Cu/L during 10 days, Ritter *et al.*, 2010),

Some spots, which remained unidentified, could deserve additional analysis as they might potentially be involved in the higher Cu-tolerance of the metallicolous population. In particular, spot 1107 was over-expressed in M at almost all Cu concentrations tested but up-regulated by Cu exposure only in NM leaves and spots 5104, 7210, 7211 and 8111 were down-regulated by Cu exposure only in M leaves.

4.2. Involvement of proteins in metabolic pathways

Nearly half of the identified protein spots (31 out of 70 spots) were involved in energy metabolism, participating in glycolysis, pentose phosphate pathway or light dependent and independent phases of photosynthesis (referred therefore as photosynthesis and Calvin cycle, respectively), (Tab. 3, 4 Fig.7).

4.2.1. Energy metabolism

Reduced accumulation of proteins involved in light dependent reactions of photosynthesis supported Cu-induced impacts in both populations. Impacts on photosystem II were shown by the decrease of oxygen-evolving enhancer proteins (#1101, 7208 and 8201), which stabilizes the manganese cluster of the oxygen-evolving complex, the primary site of water oxidation. Impacts on cytochrome b6-f complex and of light-harvesting complexes were indicated by the reduction of cytochrome b6-f complex iron-sulfur subunit (#3104) and chlorophyll a-b binding proteins (#6106 and 7214).

Stronger decreases occurred in NM, suggesting higher disturbance of photosynthetic apparatus in this population. In parallel, accumulation of a ferredoxin-NADP reductase (FNR #6303), which plays a major role in regulating electron flow during photosynthesis, increased in NM but did not vary in M, indicating disruption of normal electron flow in NM, together with the decrease of photosynthesis-related proteins. Increased accumulation of FNR may protect, at least partially, the chloroplast from oxidative stress.

Among all enzymes involved in glycolysis, pentose phosphate pathway or Calvin cycle, only RuBisCO small subunit spots were down-regulated in both M (#2106) and NM leaves (#2103) in the 1-50 µM range of Cu-exposure. However, regarding to relative spot expression, i.e. between 5.5 and 12.5% for #2103 and between 0.17 and 0.5% for #2106, the down-regulation of #2103 in NM leaves had more impact on total RuBisCO content and indicated a

sharper decrease in NM leaves. As sedoheptulose-1,7-bisphosphatase (#7306), RuBisCO activase (#7502) and phosphoribulokinase (7410 and 7413) spots were sharply induced in NM leaves, Cu-induced impacts on carbon fixation, which contributed to growth reduction, resulted mainly from the altered RuBisCO accumulation and were more intense in the NM population.

Accumulation of RuBisCO large and small subunits is severely reduced by Cu, Cd and Hg excess, less sharply by Co and Li but not altered by Zn or Sr in leaf segments of *O. sativa* floated in contaminated solutions, indicating that Cu directly targets carbon assimilation (250 µM for 72h; Hajduch *et al.*, 2001), while it is not affected by excess Zn.

In *A. stolonifera* exposed to salinity stress for 28 days, enzymes involved in light dependent reactions of photosynthesis, i.e. cytochrome f, OEE, PSI subunit N, light-harvesting complex I and cytochrome b6–f complex Fe/S subunit, are up-regulated while those involved in light independent reactions, i.e. RuBisCO large subunits, RuBisCO activase, phosphoglycerate kinase and chloroplastic aldolase, are down-regulated (Xu *et al.*, 2010). Similarly, in *A. capillaris* exposed to arsenic stress, RuBisCO small and large subunits are down-regulated, while oxygen-evolving enhancer protein are up-regulated (Duquesnoy *et al.*, 2009).

Under salt or As excess, plants are able to maintain the production of ATP and NADH but are disturbed in carbon assimilation. On the opposite, here under Cu excess, plants failed to maintain both the production of reducing power and the carbon assimilation, as most proteins involved in light dependent reactions of photosynthesis, but also RuBisCO decreased in both populations. Additionally, NM plants exhibited a disruption of electron flow, as reflected by the increase of ferredoxin-NADP reductase.Glycolysis flow was also markedly stimulated in NM leaves, regarding to up-regulation of phosphoglucomutase (#4704), fructose-bisphosphate aldolase (FBP aldolase, #2402 and 5304), triosephosphate isomerase (TIM, #5101, 6107 and 7103), and phosphoglycerate mutase (#6707 and 6710), while only one phosphoglucomutase (#5708) increased significantly in M.

Increasing production of β-D-fructose-6P and glyceraldehyde-3P, suggested by the induction of fructose-1,6-bisphosphatase (#5303), fructose-bisphosphate aldolases and triose-phosphate-isomerases, may provide additional supply for transketolases (#5802, 6802 and 6805), involved in non-oxidative reactions of pentose phosphate pathway, which accumulation also drastically increased in NM leaves.

Increasing accumulation of above-mentioned energy-related enzymes, together with the sharp increase of two V-type proton ATPase catalytic subunit A spots (#6705 and 6708) only

in NM leaves indicated a higher need in energetic compounds to support chelation, repairing and detoxification processes, induced by Cu excess.

Stimulation of pentose phosphate pathway and Calvin cycle, through increasing accumulation of FBP aldolases, TIM, transketolases, sedoheptulose-1,7-bisphosphatase, RuBisCO activase (#7502) and phosphoribulokinase may contribute to counterpart the decline of carbon fixation related to the decrease in RuBisCO accumulation.

Isocitrate dehydrogenase (#3503), which catalyzes in the Krebs cycle the oxidative decarboxylation of isocitrate into α-ketoglutarate and CO_2 using NAD^+/NADH, was up-regulated in both populations, indicating an increase of mitochondrial respiration under Cu stress.

4.2.2. Amino acid metabolism

A cysteine (CS, #6309) and a methionine (#2801) synthase spots were up-regulated by Cu exposure in both populations but additional spots (#7202, 1804 and 2806) were induced or over-expressed (at high Cu exposure) only in NM leaves. This indicated that although a higher need in cysteine and methionine existed in both populations under Cu stress, NM exhibited a greater stimulation of cysteine/methionine biosynthesis. Methionine synthase catalyzes the transfer of a methyl group from 5-methyltetrahydrofolate to L-homocysteine resulting in the formation of methionine, while cysteine synthase catalyzes the transfer of a hydrogen disulfide to an O_3-acetyl-L-serine resulting in the formation of L-cysteine. Increasing amount of these two main S-containing amino-acids may promote production of derived metabolites, such as polyamines and GSH. Stimulation of GSH production in NM, was also suggested by the increasing accumulation of two glutamine synthetase spots (#7412 and 8501) only in NM leaves.

Induction of cysteine synthase by Cu excess has been reported in a sensitive strain of *Ectocarpus siliculosus*, but not in the tolerant strain (50 µg Cu/L during 10 days; Ritter *et al.*, 2010) but induction by Al stress has been also recorded in leaves of *O. sativa* (75 µM for 3 days; Yang *et al.*, 2013). Increasing accumulation of cysteine/methionine synthases in both populations but of glycolysis enzymes only in NM may indicate that chelation in leaves of tolerant plants was sufficient to cope with deleterious Cu effects, without disturbing normal flow of glycolysis.

4.2.3. Protein synthesis, folding, destination and storage

Increasing accumulation of proteins involved in protein synthesis, i.e. GTP-binding protein TypA (#2809), eukaryotic initiation factor 4A (#5503 and 5508) and 50S ribosomal

protein L10 (#1104) was significant only in NM (p-val < 0.05) and pointed out a higher need in protein synthesis processes for this population, to maintain cell functioning under Cu excess.

As Cu is known to impact protein metabolism, it was not surprising to find a stronger accumulation in protein chaperones in NM population, which could prevent and reverse incorrect protein interactions, folding and aggregations. All chloroplastic chaperones were significantly up-regulated only in NM leaves (p-val < 0.05), i.e. chaperone protein ClpC2 (#4801), 60kDa chaperonin subunit alpha (#8701 and 8703) and beta (#7701, 7704 and 7706) and heat shock 70 kDa protein 7 (#8804), which pointed out stronger Cu-induced impacts on chloroplasts compared to M population.

Increase in chloroplastic ATP-dependent zinc metalloprotease FTSH 2 also confirmed higher impacts on photosynthesis, as it is involved in thylakoid formation and in the removal of damaged component of the photosystem II. Additionally, up-regulation of a mitochondrial chaperonin CPN60-2 (#6706), a nucleoredoxin (#8705) and a protein disulphide-isomerase (PDI, #8705) only in NM indicated higher accumulation of misfolded proteins and pointed out an increased need for protection of protein metabolism.

On the opposite, over-expression at 25 and 40 µM Cu of a mitochondrial heat shock 70 kDa protein 10 (HSP70, #5808), which was induced only in M, may better protect protein metabolism compared to NM population. Together with the increased accumulation of proteins involved in ribosome biogenesis / translation, increasing accumulation of protein chaperones suggested a higher turnover of protein in NM compared to M, involving stimulation of protein synthesis and folding processes. A stimulated protein turn over in NM may also explain the observed stimulation of glutamine synthetase, by an increased requirement in N assimilation (DalCorso *et al.*, 2013). Induction of a HSP70 has been reported in a tolerant strain of *E. siliculosus* under chronic Cu stress, but it does not vary in a sensitive strain (50 µg Cu/L during 10 days; Ritter *et al.*, 2010), confirming that these proteins may participate in enhancing Cu tolerance in plant cells.

4.2.4. Disease/defense

Due to its redox-active properties, Cu catalyzes the formation of hydroxyl radicals via Haber-Weiss and Fenton-like reactions, generating reactive oxygen species (ROS), which cause oxidative stress in cells (Noctor and Foyer, 1998). As expected, Cu exposure induced up-regulation of ROS detoxifying enzymes, such as thioredoxin peroxidases (#8102 and 8105) and thioredoxin (#6203 and 6208), which increased only in NM, suggesting that oxidative stress was higher in NM leaves. As Cu may be bound by S residues, Cu chelation has been proposed

to compete with H_2O_2 detoxification. Increasing accumulation of thioredoxin and thioredoxin peroxidase may both enhance Cu chelation and H_2O_2 detoxification. Down-regulation of a chloroplastic L-ascorbate peroxidase (#2312) may favor the accumulation of L-ascorbic acid which may chelate free Cu in cells.

Globally, polyphenol oxidases (PPO, #1803, 2707 and 2808) decreased in NM leaves only leading to over-expression in M at 50 µM. Polyphenol oxidase is a tetramer containing four Cu atoms per molecule, and binding sites for two aromatic compounds and oxygen. Higher accumulation of PPO in M leaves may contribute to enhance both H_2O_2 detoxification and production of phenols, which can chelate Cu.

4.2.5. Other functional categories

Enzymes belonging to purine/pyrimidine metabolism were affected by Cu excess only in NM, with down-regulation of a nucleoside diphosphate kinase 2 (#2105) and up-regulation of an apyrase (#4501), indicating a higher sensitivity to Cu exposure. Decrease of nucleoside diphosphate kinase may support a slowing down of cellular processes as Cu rose, while it was maintained in M plants.

Both actin (#6402) and tubulin alpha (#7608) were up-regulated by Cu exposure only in NM leaves, while negative impacts of Cu on cytoskeleton is reported in most studies. Increase in cytoskeleton components may contribute to maintain correct cell functions under Cu stress, by its implication in cell division, organelle movement, cohesion or jonction among cells and cell structure.

Cp31BHv (#9201) increased sharply with Cu exposure in NM leaves but did not respond to Cu in M leaves. Its function in biological processes has not yet been described. The only information available about its molecular function concerns a nucleotide binding capacity.

Enhanced accumulation of V-type H^+-ATPase in NM was coherent with the up-regulation of a 14-3-3-like protein A (#8205), as 14-3-3 proteins are known for being positive regulators of plasma membrane H^+-ATPase that governs the electrochemical gradient across the plasma membrane and is essential to control ion transport and cytosolic pH. The 14-3-3 proteins are involved in regulating signal transduction pathways, hormone signaling, transcription factors, metabolism, apoptosis, adhesion, cellular proliferation, differentiation, and survival, and ion homeostasis (Mhawech, 2005; Fuglsang *et al.*, 2006). They also interact with several proteins involved in ethylene biosynthesis, e.g. ACC synthase, ETO-like protein, and SAMS. (Chang *et al.*, 2009)

5. Conclusion

In both M and NM populations, Cu excess altered accumulation of various component of the photosynthesis process, i.e. photosystem II, cytochrome b6-f complex and light-harvesting complexes, as shown by the down-regulation of oxygen-evolving enhancer proteins, cytochrome b6-f complex iron-sulfur subunit, chlorophyll a-b binding protein. Additionally, Cu impacted carbon assimilation in decreasing RuBisCO accumulation, which indicated that plants failed to maintain both the production of reducing power during light dependent reactions and the carbon assimilation during light independent reactions. In particular, up-regulation in NM of several other enzymes involved in dark reactions, i.e. sedoheptulose-1,7-bisphosphatase, RuBisCO activase and phosphoglycerate mutase, indicated that reduction of RuBisCO was mainly responsible for carbon assimilation failure. Increase of isocitrate dehydrogenase indicated also an increase in mitochondrial respiration in both populations under Cu excess.

Increasing accumulation in cysteine/methionine synthases in both populations indicated that Cu excess induced an enhanced need in S-containing amino-acids, probably to increase chelation mechanisms, through production of glutathione (GSH), nicotianamine, polyamines or phytochelatins. Higher cysteine synthase accumulation in NM leaves, together with the up-regulation of glutamine synthetase, probably indicated an increased GSH production.

Higher impacts on NM photosynthesis were pointed out by the sharper decrease of all photosynthesis-related enzymes (i.e. oxygen-evolving enhancer protein 1 and 2, cytochrome b6-f complex Fe-S subunit, chlorophyll a-b binding protein and RuBisCO), but also by the increase of a ferredoxin-NADP reductase, which indicated an alteration of electron flow during the photosynthesis process, and of a metalloprotease FTSH2, which is involved in the removal of damaged components of the photosystem II.

Moreover, up-regulation of several enzymes involved in glycolysis, i.e. phosphoglucomutase, fructose-bisphosphate aldolase, triosephosphate isomerase, phosphoglycerate mutase, only in NM leaves indicated that normal glycolysis flow was altered under Cu stress. Together with the up-regulation of ATPases, it revealed a higher need in energetic compounds to perform chelation or detoxification, and maintain cell growth. In particular, stimulation of pentose phosphate pathway and Calvin cycle, through increasing accumulation of fructose-bisphosphate aldolases, triosephosphate isomerase, transketolases, sedoheptulose-1,7-bisphosphatase, RuBisCO activase and phosphoribulokinase, may contribute to counteract the decline of carbon fixation related to the decreasing RuBisCO accumulation.

Accumulation of damaged or misfolded proteins under Cu stress was shown in NM leaves by the increasing accumulation of protein chaperones, i.e. ClpC2, 60kDa chaperonin, chaperonin CPN60-2, nucleoredoxin and protein disulfide isomerase. It was then logical to find a stimulation of protein synthesis processes, as indicated by the up-regulation of eukaryotic initiation factor 4A, 50S ribosomal protein L10 and GTP-binding protein TypA, to allow the replacement of degraded or damaged proteins. Interestingly, a mitochondrial HSP70 was specifically induced by Cu in M leaves, leading to a higher accumulation at high Cu exposure. In providing a better protection of protein metabolism, this HSP may contribute to the higher tolerance of the M population. Higher oxidative stress in NM leaves was also indicated by up-regulation of thioredoxin and thioredoxin peroxidase. Down-regulation of a chloroplastic L-ascorbate peroxidase may also favor the accumulation of L-ascorbic acid to chelate free Cu in NM cells.

CHAPTER VI: Establishment of qPCR procedure

Feasibility of a transcriptomic characterization of Cu-tolerance in metallicolous and non-metallicolous populations of *Agrostis capillaris* L.

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Abstract

This work aimed at characterizing both M and NM populations by a multi-scale approach, from phenotype to proteomic levels. However, in this experiment, the feasibility of transcriptomic (qPCR) approach on *A. capillaris* was investigated, to increase the knowledge about Cu-tolerance in M and NM populations, using a new approach. Combining data on transcript and protein accumulation may improve the knowledge on proteins underlying plant responses to Cu excess, and notably those involved in the higher Cu tolerance reported for the M population at a phenotypic level.

RNA extraction and cDNA synthesis by TR-PCR were achieved using commercial kits for all experimental conditions, i.e. root and leaf tissues of 50-days-old M and NM plants exposed to 1, 5, 25 and 40 µM Cu. Primer design was successfully performed for all 20 tested genes, i.e. 8 housekeeping genes: *EF1*, *RuBisCO*, *Ubi*, *ABC*, *APRT*, *Cyc*, *L2* and *YLS 8*, and 12 genes of interest, i.e. *Act 101*, *Act 3*, *GAPDH*, *Glx I*, *MetE*, *SAMS*, *Cu/Zn-SOD*, *TIM*, *Tub alpha*, *HMA5*, *NAS* and *RAN*. No efficient primer couple was found for RAN, implying further tests for this particular gene. In contrast for all other genes, a stable and specific couple of primers was identified and provided efficient amplification after PCR.

1. Introduction

A preliminary experiment has been carried out to evaluate the use of transcriptomic analysis for unraveling molecular mechanisms underlying differential Cu-tolerance between M and NM *A. capillaris* populations. Such experiment was a prerequisite as *A. capillaris* is a non-model unsequenced plant species, poorly known at genetic and transcriptomic levels.

Combining data on transcript and protein accumulations, released by transcriptomic and proteomic techniques, would expand the knowledge on proteins involved in plant responses to excess Cu and particularly on protein underlying the higher Cu tolerance of M bentgrass plants at the phenotypic level.

The first step consisted in selecting genes of interest, and prospecting if enough sequences were available for such transcriptomic analyses. Based on preliminary experiment (Chapt. II), the transcripts of 8 genes were retained for possible involvement in differential Cu tolerance between M and NM bentgrass plants, i.e. *Actin*, *G3PDH* (or *GAPDH*), *Glyoxalase I* (GlxI), *Cu/Zn-SOD*, *SAMS*, *TIM*, and *Tubulin α*. Three additional genes, i.e. *HMA5*, *NAS* and *RAN*, were chosen for their functions in Cu tolerance based on the literature.

Transcript levels for above-mentioned genes did respond to several abiotic stresses. For example, *G3PDH* and *TIM* transcript levels increase in rice (*Oryza sativa)* cell cultures under NaCl and cold stress (+2% NaCl culture solution/10°C; Umeda *et al.*, 1994). Over-expression of Glyoxalase I in tobacco (*Nicotiana tabacum*) seedlings does increase tolerance to salt stress (800 mM NaCl; Veena *et al.*, 1999) and its accumulation increases in cotyledons of *Brassica juncea* exposed to Zn (200 mM ZnCl; Veena *et al.*, 1999).

A second step was to select housekeeping genes from literature and then to assess the feasibility to use them as reference genes. Selection of housekeeping genes, with steady accumulation across experimental conditions (i.e. Cu exposures and plant populations), is a prerequisite of qPCR analyses. Availability of at least 2-3 housekeeping genes is necessary for avoiding errors (Thellin *et al.*, 1999; Vandesompele *et al.*, 2002). *Actin* has been often used as control gene (Wang, 2003; Xu *et al.*, 2007 and 2008; Han *et al.*, 2008), but its expression did respond to Cu exposure in several plant species (Remans *et al.*, 2008), which led to its selection as candidate genes. Expression of the *18S rRNA* has been chosen as reference to study differential transcript accumulation in *Chlamydomonas reinhardtii* under increasing Cu exposure, i.e. 10, 50, 100, 150 and 200µM Cu for 48h (Luis *et al.*, 2006). According to literature, expressions of other genes than actin are more stable under Cu exposure, i.e. *Fbox proteins*, or proteins from SAND family, *YLS8* and *Ubiquitin* in *Arabidopsis thaliana* seedlings exposed to

0.5 or 2 µM Cu for 1hour (Remans *et al.*, 2008) or *APRT*, *EF1*, *L2* and *Cyc* in potatoes exposed to cold and salt stress (Nicot *et al.*, 2005). Out of the 18 housekeeping genes determined in soybean seedlings exposed to 130 stressful growth conditions (Libault *et al.*, 2008) four were selected as potential candidates: *CDPK-related protein kinase, Fbox proteins, metalloproteases* and *ATP Binding Cassette (ABC) transporters*.

To our knowledge, no transcriptomic study has been reported for *A. capillaris.* Therefore this study aimed at: (1) developing a total RNA extraction protocol for *A. capillaris*; and (2) achieving analysis of transcripts matching with proteins selected from the preliminary proteomic study, in testing primers, reference genes, and performing qPCR analysis. For *Agrostis* spp., three methods are published to extract total RNAs. A Promega kit has been used for *A. scabra* to identify genes involved in heat stress (control: 20°C, stressed: 40°C, Tian *et al.*, 2009). Two methods have been used for*A. capillaris*: one based on trizol and chloroform to study phylogenics in *Agrostis* genus (Rotter *et al.*, 2007) and the RNeasy Plant Mini Kit of Qiagen for an EST analysis (Dinler and Budak, 2008).

To evaluate the efficiency of the transcriptomic procedure, accumulation of transcripts was studied for a set of candidate genes (*i.e. Act3*, *Act 101*, *GAPDH*, *Glx I*, *MetE*, *SAM*, *Cu/Zn-SOD*, *Tub alpha*, and *TIM*) in roots and leaves of *A. capillaris* exposed to increasing Cu exposure (1, 5, 25 and 40 µM). Underlined hypotheses were: i) May the transcript accumulation depend on replicates, plant populations, Cu exposures and tissues? and ii) May a relation exist between transcript and protein accumulation?

2. Material and Methods

2.1. Plant culture and sampling

New plant batches, from the same seed lots, were cultivated in the same conditions than those previously described (Chapt. III, IV and V) for a 50-day growth period and with four Cu exposures (1, 5, 25 and 40 µM Cu). Three plastic pots (15 x 12 x 8 cm) were sown with around 20-30 seeds, and plants were progressively thinned to conserve between 5 and 10 individuals germinated the same day and similarly developed. However, at high exposures (25 and 40 µM Cu), phytotoxic impacts of Cu on plant growth led to a reduced number of replicates, not sufficient for statistical analyses. At day 50, apical parts of roots and youngest leaves of at least 5 individuals were frozen in liquid nitrogen then stored in plastic tubes at -80°C for further transcriptomic analyses. For each pot, all tissue samples collected were pooled together in the same tube to form one replicate and were used for RNA extraction.

Tab. 1: Number of individuals used for transcriptomic analyses in Metallicolous (M) and Non-Metallicolous (NM) populations of *A. capillaris* exposed to 1, 5, 25 et 40 µM Cu. Rep: replicate, nd: no available data.

Population	M				NM			
Cu exposure	1 µM	5 µM	25 µM	40 µM	1 µM	5 µM	25 µM	40 µM
Rep #1	8	7	6	6	8	7	7	5
Rep #2	6	7	nd	7	6	7	nd	nd
Rep #3	8	8	6	6	8	8	5	nd

2.2. Sequences

As *A. capillaris* genome is still unsequenced, the number of available EST sequences is restricted but 21,656 sequences were found in NCBI EST database (http://www.ncbi.nlm.nih.gov/nucest/) for *A. capillaris*, *A. stolonifera*, *A. stolonifera* var. *palustris* and *A. scabra*. An EST database was created during proteomic analyses (Chapt. II) and *Agrostis* EST accessions were available for most of the relevant genes.

For some other genes, sequences were first searched in model species such as *A. thaliana* or *Oryza sativa* using NCBI database then homolog sequences were further found in *Agrostis* EST using nBLAST function (Nicot, 2005; Tian *et al.*, 2009). Functions of the retained homolog *Agrostis* EST sequences were then confirmed using BLASTx to avoid errors. Blast performing, nucleotide and functional blast, nblast and xblast were carried out using blast tools of NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Extraction, DNase and purification

RNA extraction procedure was performed using Qiagen RNeasy Plant Mini Kit (Xu *et al.*, 2007; Dinler and Budak, 2008; Remans *et al.*, 2008). Frozen tissues of root and shoot aliquots were ground in liquid nitrogen using a small mortar and pestle then 80 mg and 60 mg of powder were respectively used for root and leaf extraction, using RLT lysis buffer. Mix was loaded on QIAshredder spin column to separate cell fragments from RNA by centrifugation. Supernatant was washed and filtered in a RNeasy spin column adsorbing total RNA, eluded with 30 µL of RNase free water, then collected in an Eppendorf tube (1.5mL) and stored at -80°C for further use.

300ng of total RNA (+7 µL milliQ H_2O and 2 µL bromophenol blue) together with 0.8 µL of 1Kb maker (0.5 $\mu g.\mu L^{-1}$, Gene Ruler, 1Kb Plus DNA Ladder, Thermoscientifics) were visualized on TBE gel (1.2% agar, 1 µL Gel red for 30 mL of TBE 0.5x) revealed by UV using Gene Genius BioImaging System. Image acquisition was made using Gene Snap and Gene tools from Syngene (Veena *et al.*, 1999).

A DNase assay was performed using Promega RQ1 RNase-Free DNase; RNAs were placed in DNase mix (4 µL DNase 10x Reaction Buffer + 0.3 µL RNasin Plus RNase Inhibitor + 5 µL RNase-Free DNase + 0.7 µL autoclaved milliQ H_2O) for 30 min at 37°C. DNase was followed by a purification using Qiagen RNeasy Plant Mini Kit (RNeasy spin column) and purified RNA was eluded with RNase free water and stored at -80°C after dosage.

2.4. RT-PCR, cDNA synthesis

Synthesis of cDNA was conducted using Biorad IScript cDNA Synthesis kit (1 µL Iscript RT + 4 µL Iscript buffer 5x + qsp 1 µg RNA + qsp 20 µL milliQ H_2O), and RT-PCR cycle performed on Gene Amp PCR System 9700 thermocycler (Applied Biosystems) was fixed as followed: 5 min at 25°C, 30 min at 42°C and 5 min at 85°C. Dilutions (100 µL, 1/10X) were made to perform tests on primer specificity and efficiency, and stored at -20°C.

2.5. Primer design pre-selection using PCR

Primer3 software was used to design specific primers of each analyzed genes (12 interest genes and 8 housekeeping genes), with parameters fixed as followed: 50% of G and C bases, minimal size of 20 nucleotides, hybridization temperature around 60°C (Libault *et al.*, 2008) and an amplicon size comprised between 100 and 150 nucleotides. To avoid multi-hybridizations and check for primer specificity, research of homology between primers and EST sequences was processed using nblast on all EST sequences available for *Agrostis* spp. and multiple homologies were investigated using Clustal W (Veena *et al.*, 1999). Non-specific primers were eliminated for potential candidate group, leading to the selection of two primers pairs for each gene.

MilliQ H_2O was added to primers, synthesized by Eurogentec Company, to obtain 100 µM concentrations for each. Working solution (5 µM) was made to test primer efficiency on cDNA diluted solutions (1/10). Biolabs 'Taq DNA polymerase with standard Taq Buffer' was used to perform PCR (2 µL buffer + 0.8 µL of each primer + 0.8 µL DNTP + 0.1 µL Taq polymerase + 2.5 µL cDNA solution (or 2.5 µL H_2O for control) + 13 µL milliQ H_2O, with buffer made of 10 mM Tris-HCl, 50 mM KCl and 1.5 mM $MgCl_2$, pH 8.3 at 25°C). PCR cycle parameters were fixed as followed, 5 min at 94°C, 30 cycles of {20sec at 94°C ,20sec at 60°C, 20sec at 72°C}, 10 min at 72°C. Amplicon quantities and size were visualized on TAE gel (2.5% agar, TAE 0.5x, 1 µL Gel red for 30 mL of TAE), revealed by UV, to estimate the efficiency of PCR and primers. Stability of pre-selected primer couples was tested on a cDNA elution range (1/10, 1/100, 1/1000 and 1/2000), using qPCR, as described below.

2.6. qPCR

qPCR was performed using Biorad IQ SYBR green kit (Libault *et al.*, 2008). 2 µL of reaction mix containing 10 µL d'Iscript + 0.6 µL of each primer + 6.8 µL autoclaved milliQ H_2O (qsp. 18 µL) were added to 2 µL of each dilution of tested genes on plates from 'Hard-Shell® Thin-Wall 96-Well Skirted PCR' (96 spots, Biorad) or to 2 µL H_2O for negative controls. Fluorescence was measured using Biorad thermocycler (MJ Research, PTC 200) and qPCR cycle parameters were : 5 min at 95°C, 40 cycles {15s at 95°C, 45s at 60°C} ; fluorescence was measured at every 0.1°C between 60°C and 95°C, to establish melting curves. Results were analyzed using Opticon Monitor 3 software.

2.7. Selection of housekeeping genes

Once primers were established and tested by PCR, selection of more stable genes under Cu exposure was performed using RefFinder software (Zsori *et al.*, 2013), which gave a hierarchical list of best housekeeping genes established from various interfaces: GeNorm, Best keeper and NormFinder. Classification is based on Ct difference (ΔCt), i.e. intersection of fluorescence curves and fixed threshold, which must be minimal for a good control gene.

3. Results

3.1. Establishment of qPCR procedure

3.1.1. Gene sequences

Agrostis capillaris EST sequences were available for *Act 101*, *Act3, SAMS, Cu/Zn SOD, TIM* and *Tub alpha*. The available EST sequences of *GAPDH*, *Glx I, SAMS*, *TIM*, *Cu/Zn SOD* and *MetE* from *A. stolonifera* were blasted for homology in *A. capillaris* and resulted in specific sequence selection. The *HMA5*, *RAN* and *NAS* EST sequences were first found in *Oryza sativa* then homolog sequences were identified in *A. capillaris* using nblast, and their functions were confirmed using xblast. Eight housekeeping genes were retained for further evaluation under Cu stress. *Agrostis capillaris* EST sequence was available for *RuBisCO*. Ubiquitin sequence was not annotated in *A. capillaris* but primers were available in Li (2005). For *EF1*, *L2, YLS8, ABC, APRT* and *Cyc*, EST sequences were first found in *O. sativa* and then homolog sequences were screened in *A. capillaris* using nblast, and their functions were confirmed using xblast.

3.1.2. RNA extraction and purification

Out of the three RNA extraction procedures previously used on *Agrostis* spp. and presented in introduction, i.e. Promega kit (Tian *et al.*, 2009), trizol and chloroform extraction

(Rotter *et al.*, 2007) and Qiagen RNeasy Plant Mini Kit (Dinler and Budak, 2008), the Qiagen Kit was chosen for testing because it is simple and easy in routine use.

After testing both lysis buffer and a quantity range to establish optimal extraction parameters, 80 mg and 60 mg of powder were respectively retained for extraction in roots and leaves, and RLT lysis buffer was chosen. Efficiency of DNase/purification was also clearly visible in TBE gels, and was retained to complete extraction procedure (Fig. 1).

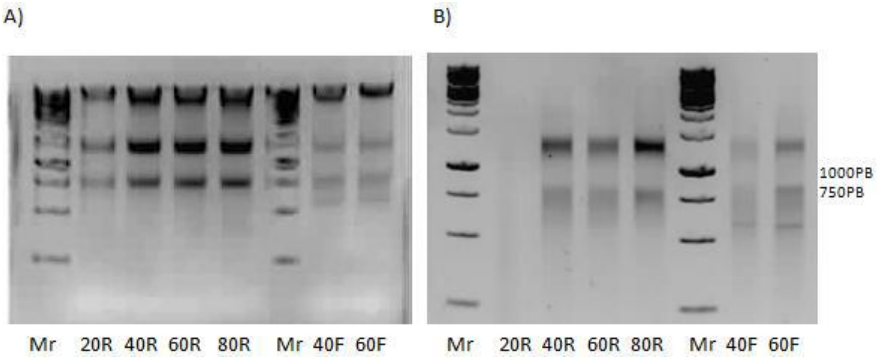

Figure 1: TBE gel (TBE 0.5X, 1.2% agar, Gel red, 300ng total RNA) illustrating quantity range and DNase efficiency: a) before DNase, b) after DNase, Mr: MW Marker, R: Roots, F: leaves, 20, 40, 60, 80: mg of crushed powder used for the extraction.

3.1.3. Primer and housekeeping gene selection

Amplification of both pairs of primers designed for each gene, visualized on TAE gels, is presented in Fig. 2.

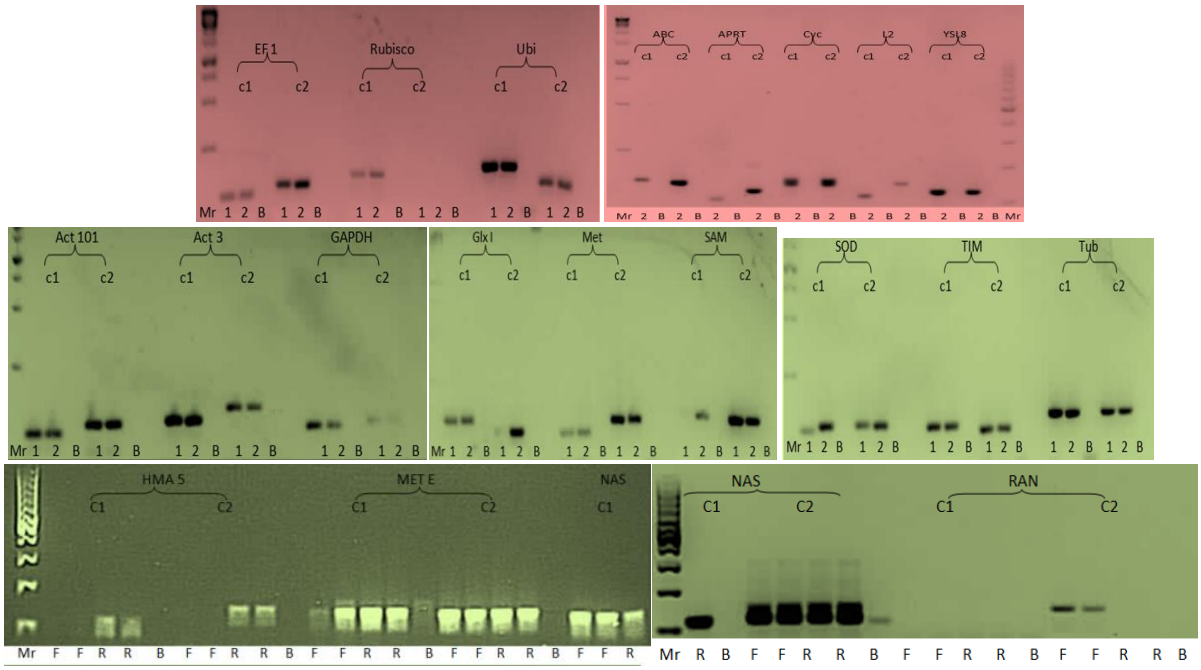

Figure 2: Visualization of amplicon amplification, using PCR, on TAE gels (TAE 1X, 2.5% agar). Mr: MW Marker, R: Roots, F: leaves, B: negative control (H_2O), 1/2: technical replicated of PCR,c1/c2: primer pair 1 and 2, red gels: housekeeping genes (EF1, *RuBisCO*, *Ubi*, *ABC*, *APRT*, *Cyc*, *L2* and *YLS8*),

green gels: interest genes (*Act 101*, *Act3*, *GAPDH*, *Glx I*, *MetE*, *SAM*, *Cu/Zn SOD*, *TIM*, *Tubα*, *HMA5*, *Met E*, *NAS* and *RAN*).

Concerning interest genes (in green, Fig. 2), amplification was not obtained for both *HMA5* primer pairs in leaves, while it was successful in roots. With pair 1 of primers, *RAN* amplification was unsuccessful in both roots and leaves, while with pair 2 amplification occurred only in leaves, with a slow rate.

Low accumulation occurred with primer pair 2 of *GAPDH* and pair 1 of *MetE*. For reference genes (in red, Fig. 2), amplification was lacking only for pair 2 (c2) of *RuBisCO*. Intensity of amplification depended on the primer pair used, the one showing the highest amplification was conserved for further stability test, i.e. pair 1 for *RuBisCO*, *Ubi*, *L2*, *YLS8*, *Act3*, *GAPDH*, *Cu/Zn SOD*, *TIM*, *Tub alpha*, and pair 2 for *EF1*, *ABC*, *APRT*, *Cyc*, *Act 101*, *Glx I*, *MetE*, *SAMS*, *HMA5*, *NAS* and *RAN*.

Table 2: List of primer pairs retained for qPCR after primer selection procedure, and corresponding sequences. c1/c2: primer pair 1 or 2, see Fig. 1, red lines: sequences of housekeeping gene primers (*EF1*, *RuBisCO*, *Ubi*, *ABC*, *APRT*, *Cyc*, *L2* and *YLS8*), green lines: sequences of interest gene primers (*Act 101*, *Act 3*, *GAPDH*, *Glx I*, *MetE*, *SAM*, *Cu/Zn SOD*, *TIM*, *Tubα*, *HMA5*, *NAS* and *RAN*).

Gene	Left primer (5'-3')	Right primer (3'-5')
EF1 (c1)	GACGCGGGTATTGTGAAGAT	TTTGTCTCATGTCACGCACA
RuBisCO (c1)	TATCACATCGAGCCTGTTGC	AGAGCACGTAGGGCTTTGAA
Ubi (c2)	TTCACCTCCCAGGTCATCAT	CTTCTTCTCTGGGGGAAACC
ABC (c2)	ACGAGGCGAGCACTTCTAAA	CTCCTGGGCAAACTCGTAAG
APRT (c2)	GGGACGATTGTTGCTGCTAT	CCCAGGGAACTTATTGCTGA
Cyc (c1)	GATCTGATCTCCTGCGGTTC	CAGAATCCAAACAGGGGAAA
L2 (c1)	CAACCCTGACAACGGAACTT	GTTCTTCCTCCACCAGCAAC
YLS 8 (c2)	GCCAGCATGTAACCCTTGAT	TAGACAGCAGGTCCCGTTTC
Act 101 (c2)	AGCTCGCATATGTGGCTCTT	TCTCTGCCCCAATGGTAATC
Act3 (c1)	ACCCTCCAATCCAGACACTG	CTCGACTATGTTCCCCGGTA
GAPDH (c1)	CTCAAGGGCATTTTGGGTTA	CGAAGTTGTCGTTCAAAGCA
Glx I (c2)	TGCAATCCCTTCTTGAGGAC	AAGTTATCCTTCGCCCGTCT
MetE (c2)	ATGGATTTGGTGGCTTTGAG	CAGGACGCATTCAGGAAAAT
SAM (c2)	CAAGGCCTCTGCTTAAGTGC	GCCACACCAAAATACCAACC
Cu/Zn SOD (c2)	TGAGGATGACTTGGGGAAAG	ACAGAAGTGAAGGCCGAAAA
TIM (c1)	TGGTGCAGCTACTGTGGTTC	TAATAACCCGCGACAAAAGG
Tubα (c1)	CAGGCTTGTGTCTCAGGTCA	GAGATCACTGGGGCATAGGA
HMA5 (c2)	ATGGGGTAAACGACTCACCA	GAGAGATCGATTGCGGTGAT
NAS (c2)	CGCACCAGAAGATGAAGGAG	GATCGGGCCAATATTAATCG
RAN	none	None

Pre-selection of pair 1 for *RuBisCO*, *L2*, *Act3*, *GAPDH*, *TIM* and *Tub alpha* or pair 2 for *ABC*, *APRT*, *Act 101*, *Glx I*, *MetE*, *SAMS*, *HMA5* and *NAS*, was confirmed by stability tests and primers were retained for further qPCR analyses. However, pairs pre-selected for *EF1*, *Ubi*, *Cyc*, *YLS 8* and *Cu/Zn SOD*, did not pass through stability tests, so the stability of the second available pair was tested according to the same procedure, and led to final selection of primer pair 1 for *EF1* and pair 2 for *Ubi*, *Cyc*, *YLS 8* and *Cu/Zn SOD* (Tab. 2). Pair 2 of *RAN* identified in leaves did not pass stability tests, therefore any analysis could be conducted for this gene.

Stability of the eight pre-selected housekeeping genes was tested using qPCR and only two, i.e. *APRT-Cyc* in roots and *Ubi-Cyc* in leaves, were conserved as housekeeping genes in our experimental conditions, i.e. populations M and NM exposed to 1, 5, 25 and 40 µM.

3.2. Transcripts accumulation

Unfortunately, due to inexperience and incidents, several mistakes were made during qPCR procedure and replicates lost, leading to unusable dataset for statistical analysis. At high Cu exposure (25-40 µM Cu), plant growth were insufficient in some replicates (one for M25 and NM25 and two for NM40, one replicate for NM at 40 µM, Fig. 3), preventing any statistical analyses at 25 µM and 40 µM Cu, as 3 replicates was already a very low number for statistical tests. Low Cu exposures (1 and 5 µM Cu) may be compared but cDNA syntheses were performed using different reaction mixes (see section 2.4), preventing any comparison among replicates between experimental conditions.

The number of replicates was also reduced due to failure of extraction or qPCR procedures. In fact, only one replicate was available for leaves (except for exposure at 40 µM Cu, for which no replicate was successfully analyzed), and for three interest genes, *i.e. HMA5*, *MetE* and *NAS*; consequently, data were neither shown nor discussed.

In roots, three replicates were available for M at 1, 5 and 40 µM Cu but only NM5 was complete (Fig. 5), so some careful comments were only made for 1 and 5 µM Cu exposures. For instance, in M roots, higher accumulation was obtained at 1 and 5 µM Cu for *TIM*, *Act101*, *Act 3* and *GlxI* at 5 µM Cu. This deserves further analyses to make any conclusions. Accumulation of *SOD* increased in both populations when Cu increased.

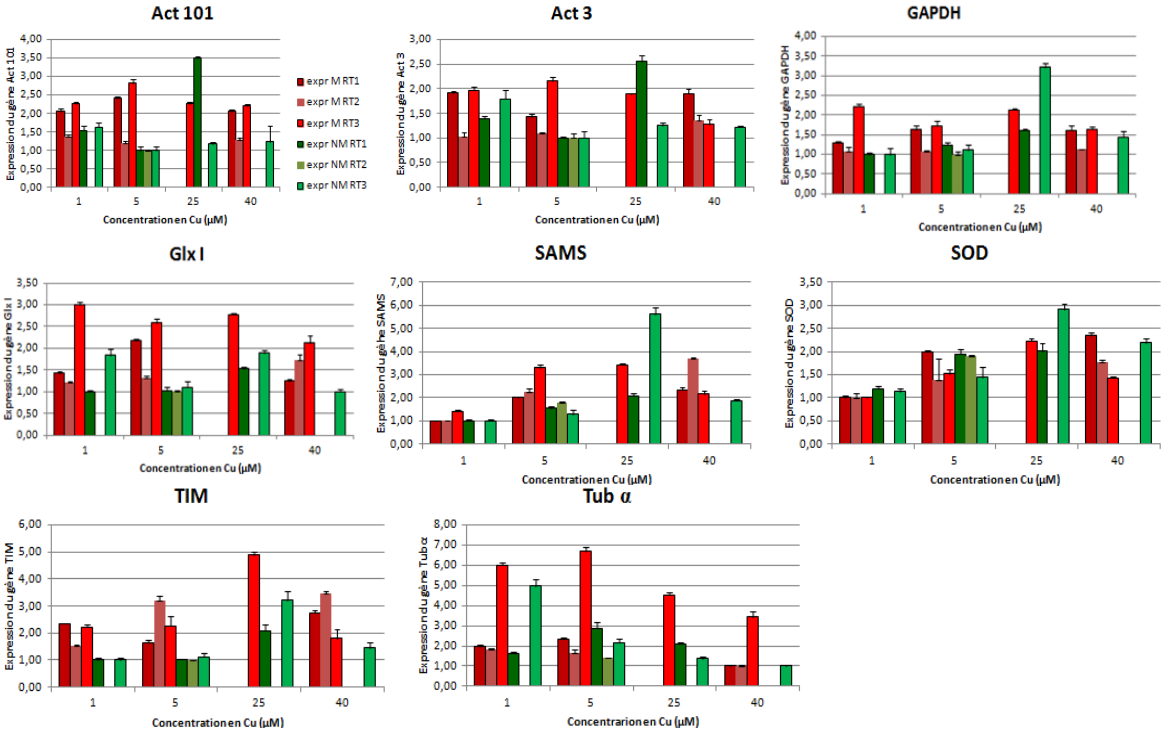

Figure 3: Transcript accumulation for Act 101, Act 3, GAPDH, GlxI, SAMS, SOD, TIM and Tub alpha, in roots of M (red) and NM (green) plants of *Agrostis capillaris* exposed to 1, 5, 25 and 40 µM Cu. Results normalized using APRT and Cyc as housekeeping genes. Error bares indicated variability for three technical replicates.

4. Discussion

4.1. Range of Cu exposure

Selected Cu exposure range assessed at the transcriptomic level may not be relevant. In fact interval between Cu exposures was likely excessive, leading to phenotypes too different to be efficiently compared. Indeed, at high Cu exposure (25-40 µM), NM plants exhibited intense phytotoxic symptoms. Interval of 2 to 5 µM Cu would be more useful to assess transcriptomic changes induced by Cu stress.

A higher number of replicates is a key factor to consider for further experiments, to avoid potential reduction of replicate number, due to sample lost during storage or experimental failure. To proceed statistical analyses, six replicates would be relevant to increase results reliability.

Gene expression depends on the growth period (Alaoui-Sossé *et al.*, 2004). Therefore comparing short and long term exposure may increase the knowledge on transcriptomic changes induced by Cu stress. Only 11 genes of interest were considered in this preliminary experiment, it would be interesting to increase the number of targeted genes but also of housekeeping genes investigated.

4.2. Gene variation

Accumulation of *SOD* transcripts was apparently up-regulated by Cu exposure. Induction of Fe- and Mn-SODs transcript in response to Cu excess has previously been found in *C. reinhardtii*. However, this induced accumulation of transcripts was not related to an enhanced SOD activity, which might be explained by the replacement of the proper cofactor by Cu, leading to enzyme inactivation (Luis *et al.*, 2006). In this case, Fe- and Mn-SODs may act as Cu-chelators.

4.3. Application on *Agrostis capillaris*

Because of experimental failures, no statistical analysis could be applied to the dataset. Consequently, a discussion on the relationships between transcript and protein accumulation was not possible. However, this preliminary experiment indicated that such approach needs further attention as it is applicable for *A. capillaris*. In fact, primers were successfully designed and tested, amplification of amplicons was also successful for all tested genes except *RAN*.

5. Conclusion

RNA extraction, DNase and cDNA synthesis procedure was achieved for all experimental conditions, i.e. root and leaf tissues of M and NM plants exposed to 1, 5, 25 and 40 µM Cu. Primer design was successfully performed for all 20 tested genes, i.e. 8 housekeeping genes: *EF1*, *RuBisCO*, *Ubi*, *ABC*, *APRT*, *Cyc*, *L2* and *YLS 8*, and 12 genes of interest: *Act 101*, *Act 3*, *GAPDH*, *Glx I*, *MetE*, *SAMS*, *Cu/Zn-SOD*, *TIM*, *Tub alpha*, *HMA5*, *NAS* and *RAN*. However, no efficient primer pair was found for RAN, requiring further tests for this particular gene. For all other genes, a stable and specific primer pair was identified and provided amplification.

CHAPTER VII: General discussion

There is a lack of knowledge on mechanisms underlying Cu-tolerance and low shoot:root ratio in grassy species such as *A. capillaris,* even though several histological and physiological processes have been suggested, e.g. root uptake limitation and efflux, differential accumulation between roots and aerial parts, and enzymatic and non-enzymatic systems to quench ROS damages. In comparing metallicolous and non-metallicolous populations grown on an increasing range of Cu exposure (1-50 µM), this study aimed at elucidating the mechanisms underlying Cu response and tolerance in plants.

Using the proteomic approach, the experiment focused on the differential accumulation of soluble proteins under increasing stress, which would help to explain and understand the impacts observed and measured at the phenotypic level. The first part of this discussion consisted more in a 'summary' of root and leaf proteomic results section, written to facilitate comparison between roots and leaf profiles and between population responses to Cu.

1. Comparison of proteomic profiles between roots and leaves

Roots and leaves exhibited different profiles, with a higher number of accurately quantified spots in roots (419 spots) than in leaves (214 spots). However, more spots did respond proportionally to Cu in leaves (136 spots, 63.6%), than in roots (199 spots, 47.5%), while more spots were over-expressed in one population (ratio > 1.5 at one Cu exposure minimum) in roots (95 spots, 22.7%) than in leaves (40 spots, 18.7%).

More spots were excised from roots (157 spots, 37.5%) than from leaves (107 spots, 50%) 2D-gels (Fig. 1), for being differentially expressed among Cu treatment (p-val <0.05) or/and between populations. After searching in both '*Agrostis*-EST' and '*Viridiplantae* proteins' databases, more spots remained unidentified (ND) in roots (48 spots, 30.6%) than in leaves (14 spots, 13.1%). However, a similar number of spots matched with multiple protein identities (MID) in roots (24 spots, 15.3%) and leaves (23 spots, 21.5%), resulting in a higher proportion of MID spots in leaves. As a non-model species, low information was available for searching and proteins may differ significantly from other species, or been specific to *Agrostis capillaris*, thus limiting protein identification, especially in roots. Similarly, in *A. stolonifera*, a non-negligible portion of protein spots remained unidentified in both roots (16 out of 40) and leaves (32 out of 148) after searching in green plant NCBI database (Xu and Huang, 2010a).

Although more spots resulted in a single protein identity in roots, proportionally, a higher amount of leaf spots were identified, i.e. 85 out of 157 (54.1%) in roots and 70 out of 107 (65.4%) in leaves.

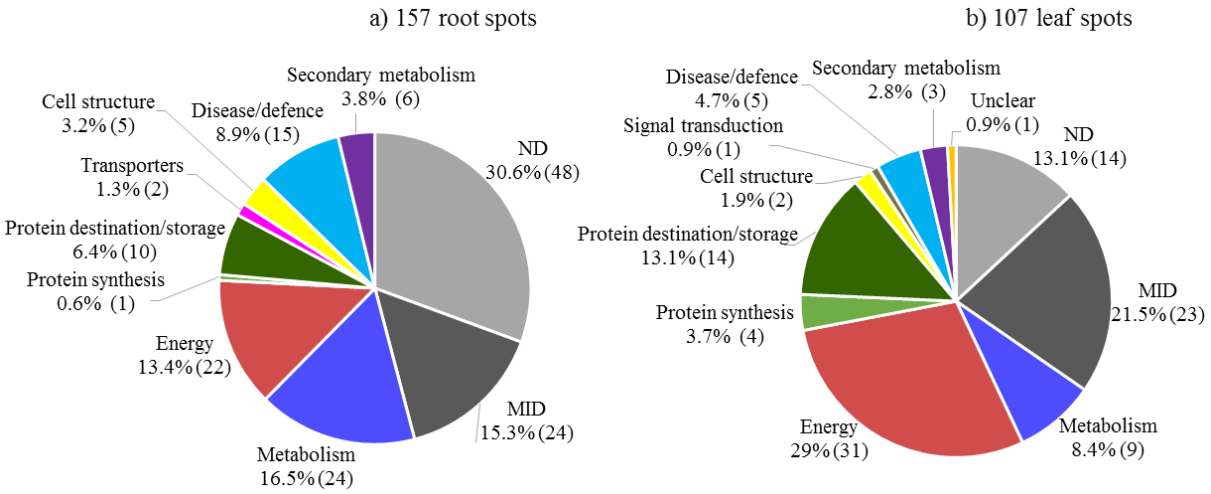

Figure 1: Assignment of protein spots from a) roots and b) leaves in functional categories defined by Bevan *et al.* (1998), with the addition of two categories, ND: Not Determined and MID: Multiple Identifications

The single-match spots were assigned to functional categories (Fig. 1) as described in Bevan *et al.*, (1998) and resulted in 87 different protein identities, indicating that numerous spots matched with the same protein identity. 32 protein identities were found in at least two different spots, of which 15 proteins were found in both roots and leaves (Annex 29). Such 'multiple spots for a single protein' were also reported in several other proteomic studies on stress response in plants (Xu *et al.*, 2010; Irazusta *et al.*, 2012; Zhao *et al.*, 2012; Song *et al.*, 2013; Weng *et al.*, 2013) and may be due to expression of isoforms derived from different genes of multigene families, differing in amino acid sequence, chemical and physical properties.

Observation of different patterns of expression among spots matched with the same protein identification, e.g. glutamine synthetase, S-adenosylmethionine synthase, ketol-acid reductoisomerase, or ATP synthase subunit alpha, suggesting that different isoforms of these enzymes may respond differently to Cu exposure in each population.

While most functional categories were identified in roots and leaves, i.e. Metabolism, Energy, Protein synthesis, Protein destination and storage, Cell structure, Disease/defense, Secondary metabolism, the proportion of each category differed between tissues. Few additional categories were found only in one tissue, i.e. Transporters in roots, Signal transduction and Unclear classification in leaves (Fig. 1).

Table 1: Proteins identified from root and leaf spots. Sp: spots number; T: tissue, R: roots and L: leaves; ID: protein identity; rM/rNM: significance of Pearson's correlation, referring to p-val = 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; R1 to R50: comparative ratio between population values at each Cu exposure, -: no difference, >/>>: intensity of the difference, M/NM indicated the population with higher values, > ratio higher than x1.5 but lower than x2, >> ratio superior to x2.

ID	Sp	T	rM	rNM	R1	R5	R10	R15	R20	R25	R30	R40	R50
Functional category 1: Metabolism													
Glutamine synthetase EC = 6.3.1.2	5404	R	-	↗↗↗	-	-	-	-	-	-	-	-	-
	7518	R	↘↘	-	-	-	-	-	-	-	-	-	-
	7412	L	-	↗↗	-	-	-	-	-	-	-	-	-
	8501	L	-	↗↗	-	-	-	-	-	-	-	-	-
Cysteine synthase EC = 2.5.1.47	5309	R	-	↗↗↗	-	-	-	-	-	-	-	-	-
	6303	R	-	↗↗↗	-	-	-	-	-	-	-	-	-
	6309	L	↗↗↗	↗↗↗	-	-	-	-	-	-	-	-	-
	7202	L	-	↗↗	-	-	-	-	-	-	-	-	-
Methionine synthase EC = 2.1.1.14	2802	R	↘	↘↘↘↘	-	-	-	-	-	-	-	M>	-
	1804	L	-	-	-	-	-	M>>	-	-	-	NM>	-
	2801	L	↗↗	↗↗	-	-	-	-	-	-	-	-	-
	2806	L	-	↗↗↗	-	-	-	-	-	-	-	NM>	-
Functional category 2: Energy													
Phosphoglucomutase, cytoplasmic EC = 5.4.2.2	4705	R	-	↘↘	NM>>	NM>>	-	NM>>	-	NM>	-	-	-
	4704	L	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
	5708	L	↗↗↗	↗↗	-	-	-	-	-	-	-	-	-
Fructose-bisphosphate aldolase EC = 4.1.2.13	2425	R	↘↘	-	-	-	-	-	-	-	-	-	-
	2402	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
	5304	L	-	↗↗	-	-	-	M>	-	-	-	-	-
Triosephosphate isomerase EC = 5.3.1.1	6209	R	↘↘	-	-	-	M>	-	-	-	-	-	-
	5101	L	-	↗↗↗	-	-	-	-	-	-	-	-	NM>>
	6107	L	-	↗↗	-	-	-	-	-	-	-	-	-
	7103	L	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
Isocitrate dehydrogenase [NADP] EC = 1.1.1.42	2525	R	↗↗	↗	-	-	-	-	-	-	-	-	-
	3503	L	↗↗	↗↗	-	-	-	-	-	-	-	-	-
Succinate dehydrogenase [Ubi] flavoprotein sub. 1	3718	R	↘	↘↘↘	-	-	-	-	-	-	-	-	-
	4702	R	-	↘↘↘↘	-	-	-	-	-	-	-	-	-
	3707	L	↘↘	-	-	-	NM>	-	-	-	-	-	-
V-type proton ATPase catalytic subunit A	6706	R	-	↘↘↘	-	-	-	-	-	-	-	-	-
	6705	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
	6708	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
Functional category 6: Protein destination and storage													
Heat shock 70 kDa protein 10, mitochondrial	4716	R	-	-	-	-	M>>	-	-	M>	M>	M>>	-
	5808	L	↗↗	-	-	-	-	-	-	M>>	-	M>>	-
Chaperonin CPN60-2, mitochondrial	6629	R	-	↗↗↗	-	-	-	-	-	-	-	-	-
	6706	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
Protein disulfide isomerase EC = 5.3.4.1	1504	R	-	↗↗	-	-	-	-	-	-	-	-	-
	8705	L	-	↗↗↗	-	-	-	-	-	-	-	-	NM>
Functional category 9: Cell structure													
Actin	5514	R	-	↘↘	-	-	-	-	-	-	-	-	-
	6402	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
Alpha tubulin	7605	R	-	↘↘↘	-	-	-	-	-	-	-	-	-
	7608	L	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
Functional category 11: Disease/defense													
L-ascorbate peroxidase 1 EC = 1.11.1.11	1211	R	↘↘↘↘	↘↘↘↘	-	-	-	-	-	-	-	-	-
	1220	R	↘↘↘	↘↘↘↘	-	-	-	-	-	-	-	-	-
Probable L-ascorbate peroxidase 6	2312	R	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
L-ascorbate peroxidase 2	6203	R	-	-	M>>	-	-	-	-	-	-	-	M>>
	6212	R	-	↘↘	-	-	-	-	-	-	-	-	-
	6213	R	-	↘↘↘↘	-	-	-	-	-	-	-	M>	-
	7205	R	-	↘↘	-	-	M>	-	-	-	M>>	-	-
Putative L-ascorbate peroxidase, chloroplastic	2312	L	-	↘↘↘	-	-	-	-	-	-	-	-	-

Table 2: Proteins identified from multiple root or leaf spots. Sp: spots number; T: tissue, R: roots and L: leaves; ID: protein identity; rM/rNM: significance of Pearson's correlation, referring to p-val = 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; R1 to R50: comparative ratio between population values at each Cu exposure, -: no difference, >/>>: intensity of the difference, M/NM indicated the population with higher values, > ratio higher than x1.5 but lower than x2, >> ratio superior to x2.

ID	Sp	T	rM	rNM	R1	R5	R10	R15	R20	R25	R30	R40	R50
Functional category 1: Metabolism													
Alanine aminotransferase 2	2618	R	-	↗↗	-	-	-	-	-	-	-	-	-
	2623	R	-	-	-	-	-	-	-	-	-	-	M>
S-adenosylmethionine synthase	3526	R	↗↗	-	-	-	-	-	-	-	-	-	-
	4541	R	↗	↗↗	-	-	-	-	-	-	-	-	-
	5506	R	↗↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-
Methylthioribose-1-phosphate	5425	R	-	↗↗↗	-	-	M>>	-	-	-	-	NM>>	-
isomerase	5426	R	↗	↗↗↗↗	-	-	M>	-	-	-	NM>>	-	NM>
Ketol-acid reductoisomerase	2725	R	↗	-	-	-	NM>>	-	-	-	-	-	-
	3701	R	↗	↗↗	-	-	-	-	-	-	-	-	-
	3709	R	↗↗↗↗	-	-	-	-	-	-	-	-	M>>	-
	3712	R	↗↗	-	-	-	-	-	-	-	-	-	-
Phenylalanine / Phenylalanine/tyrosine	2724	R	↘↘	↘↘	-	-	-	-	-	-	-	-	-
ammonia-lyase	3707	R	-	↘↘	-	-	M>	-	-	-	-	-	-
Functional category 2: Energy													
bisphosphoglycerate-independent	6707	L	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
phosphoglycerate mutase EC=5.4.2.12	6710	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
Aconitate hydratase	2801	R	-	↘↘↘	-	-	-	-	-	-	-	-	-
	2805	R	-	↘↘↘	-	-	-	-	-	-	-	-	M>
	2810	R	↘	↘↘	-	-	-	-	-	-	-	-	-
	2818	R	-	↘↘	-	M>	-	-	-	-	-	-	-
	3802	R	-	↘↘↘	-	-	-	-	-	-	-	-	-
NADH dehydrogenase [Ubi] Fe-S	3815	R	-	↘↘↘↘	-	-	-	-	-	-	-	-	-
protein 1	4801	R	-	↘↘↘	-	-	-	-	-	-	-	-	-
ATP synthase subunit alpha	4601	R	↘↘	-	-	-	-	-	-	-	-	-	-
	6617	R	-	↘↘↘	-	-	-	-	-	-	-	-	-
Oxygen-evolving enhancer protein 1,	7208	L	↘	↘↘↘↘	-	-	-	-	-	-	-	-	-
chloroplastic	8201	L	↘↘↘	↘↘↘	-	-	-	-	-	-	-	-	-
Transketolase, chloroplastic	5802	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
EC = 2.2.1.1	6802	L	-	↗↗↗	-	-	-	M>	-	-	-	-	-
	6805	L	-	↗↗	-	-	-	-	-	-	-	-	-
RuBisCO small subunit EC = 4.1.1.39	2103	L	-	↘↘	-	-	-	-	-	-	-	-	-
	2106	L	↘↘	-	-	-	-	-	-	-	-	-	-
Phosphoribulokinase, chloroplastic	7410	L	-	↗↗	-	-	-	-	-	-	-	-	-
EC = 2.7.1.19	7413	L	↗	↗↗↗	-	-	M>	-	-	-	-	-	-
Formate dehydrogenase	513	R	↗↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-
	1503	R	↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-
	1507	R	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
Functional category 5: Protein synthesis													
Eukaryotic initiation factor 4A	5503	L	↗	↗↗↗	-	-	-	-	-	-	-	-	-
	5508	L	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
Functional category 6: Protein destination and storage													
60 kDa chaperonin subunit alpha	8701	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
	8703	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
60 kDa chaperonin subunit beta	7701	L	↗	↗↗↗	-	-	-	-	-	-	-	-	-
	7704	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
	7706	L	-	↗↗	-	-	-	-	-	-	-	-	-
Mitochondrial-processing peptidase	1618	R	-	-	M>>	M>>	M>>	M>>	M>>	M>>	-	M>	M>
subunit alpha	1626	R	↘↘↘	-	-	-	-	-	M>	-	-	-	-

Table 2 (suite)

ID	Sp	T	rM	rNM	R1	R5	R10	R15	R20	R25	R30	R40	R50
Functional category 9: Cell structure													
Beta-tubulin	7616	R	↘↘	↘↘↘	-	-	-	-	-	-	-	-	-
	7617	R	↘↘↘	↘↘↘	-	-	-	-	-	-	-	-	-
	7626	R	↘↘	↘	-	-	-	-	-	-	-	-	-
Functional category 11: Disease/defense													
Thioredoxin peroxidase / 2-Cys peroxiredoxin BAS1 EC = 1.11.1.15	8102	L	-	↗↗↗↗	-	-	-	-	-	NM>	-	-	-
	8105	L	-	↗↗↗	-	-	-	-	-	-	-	-	-
Thioredoxin H-type 4	6203	L	-	↗↗↗↗	-	-	-	-	-	-	-	-	-
	6208	L	-	↗↗	-	-	-	-	-	-	-	-	-
Glutathione S-transferase	217	R	↘	-	M>	M>>	M>>	M>>	-	M>	M>	M>	-
	6205	R	↘↘	-	-	-	-	-	-	-	-	-	-
Superoxide dismutase [Mn]	2210	R	↗	↗↗↗	-	-	-	-	-	-	-	-	-
	3202	R	↗↗	↗↗↗↗	-	-	-	-	-	-	-	-	-
Functional category 20: Secondary metabolism													
Polyphenol oxidase EC = 1.10.3.1	1803	L	-	-	-	-	-	-	M>	-	-	-	M>
	2707	L	-	↘↘↘	-	-	-	-	-	-	-	-	M>>
	2808	L	-	↘	-	-	NM>>	-	-	-	-	-	-

1.1. Energy metabolism

While in roots the 'Energy' category regrouped only a quarter of the 85 single-match spots (24.7%), in leaves almost half (45%) of the 70 spots belonged to these categories (Fig. 1). All energy processes, i.e. glucose/fructose metabolism, glycolysis, pentose phosphate pathway, Krebs Cycle, photosynthesis, respiration and electron transfer were altered (induced or repressed) by Cu exposure in either roots or leaves, but different patterns were observed among populations and tissues (Tab. 2, Fig. 2).

Obviously, Cu excess altered photosynthesis and carbon fixation only in leaves, in down-regulating oxygen-evolving enhancer proteins, cytochrome b6-f complex iron-sulfur subunit, chlorophyll a-b binding protein and RuBisCO, more intensively in NM leaves. In NM, up-regulation of sedoheptulose-1,7-bisphosphatase, RuBisCO activase and phosphoglycerate mutase indicated that reduction of RuBisCO was responsible for failure in carbon assimilation and enhanced accumulation of a metalloprotease FTSH2 pointed out stronger Cu-induced damages on photosystem II for this population. The increase of a ferredoxin-NADP reductase in NM leaves indicated an alteration of electron flow during the photosynthesis process, and may provide a higher production of NADH under increasing Cu excess.

A higher need for energetic compounds and reducing power was suggested by the up-regulation of V-type ATPases and of glycolysis-involved enzymes, i.e. phosphoglucomutase, FBP aldolase, TIM, phosphoglycerate mutase, only in NM leaves. In particular, stimulation of pentose phosphate pathway and Calvin cycle, through increasing accumulation of FBP

aldolases, TIM, transketolases, sedoheptulose-1,7-bisphosphatase, RuBisCO activase and phosphoribulokinase, may contribute to counteract the decline of carbon fixation related to the sharp decrease of RuBisCO accumulation in NM.

In roots, Cu induced the up-regulation of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) in both populations, although more sharply in M roots, which may promote the production of pyruvate and NADH. Impacts on mitochondria activity occurred in both M and NM roots, as shown by the decrease of ATP synthase subunit alpha and the increase of formate dehydrogenase, which respectively underpinned reduced ATP production and higher cellular respiration.

Higher impacts on mitochondria integrity in NM roots were related to the down-regulation of enzymes involved in the Krebs cycle / Oxidative phosphorylation, i.e. aconitate hydratase, succinate dehydrogenase and NADH dehydrogenase. Additionally, a limitation of glycolysis efficiency at Cu exposure higher than 25 µM was suggested by the over-expression of phosphoglucomutase only at low and intermediate exposure (1-25 µM Cu), and the limitation of G3PDH accumulation, which reached a plateau at high Cu exposure (30-50 µM Cu).

In M roots, up-regulation of an alpha-galactosidase and over-expression of a sucrose:sucrose 1-fructosyltransferase and a 6-phosphofructokinase pyrophosphate-dependent at intermediate Cu exposure suggested that several carbohydrate-related enzymes cooperated together to maintain the supply of glycolysis and Krebs cycle under Cu stress. Additionally, the linear increase of G3PDH accumulation across this range of Cu exposure may promote accumulation of NADH and pyruvate at high Cu exposure. This suggested that Cu tolerance in *A. capillaris* may involve the maintenance of glycolysis activity. No or smaller alterations of H^+ transport and Krebs cycle in M roots, together with the increase of MDH and IDH supported that ability to maintain energy production in M cells may confer a higher Cu-tolerance in this population.

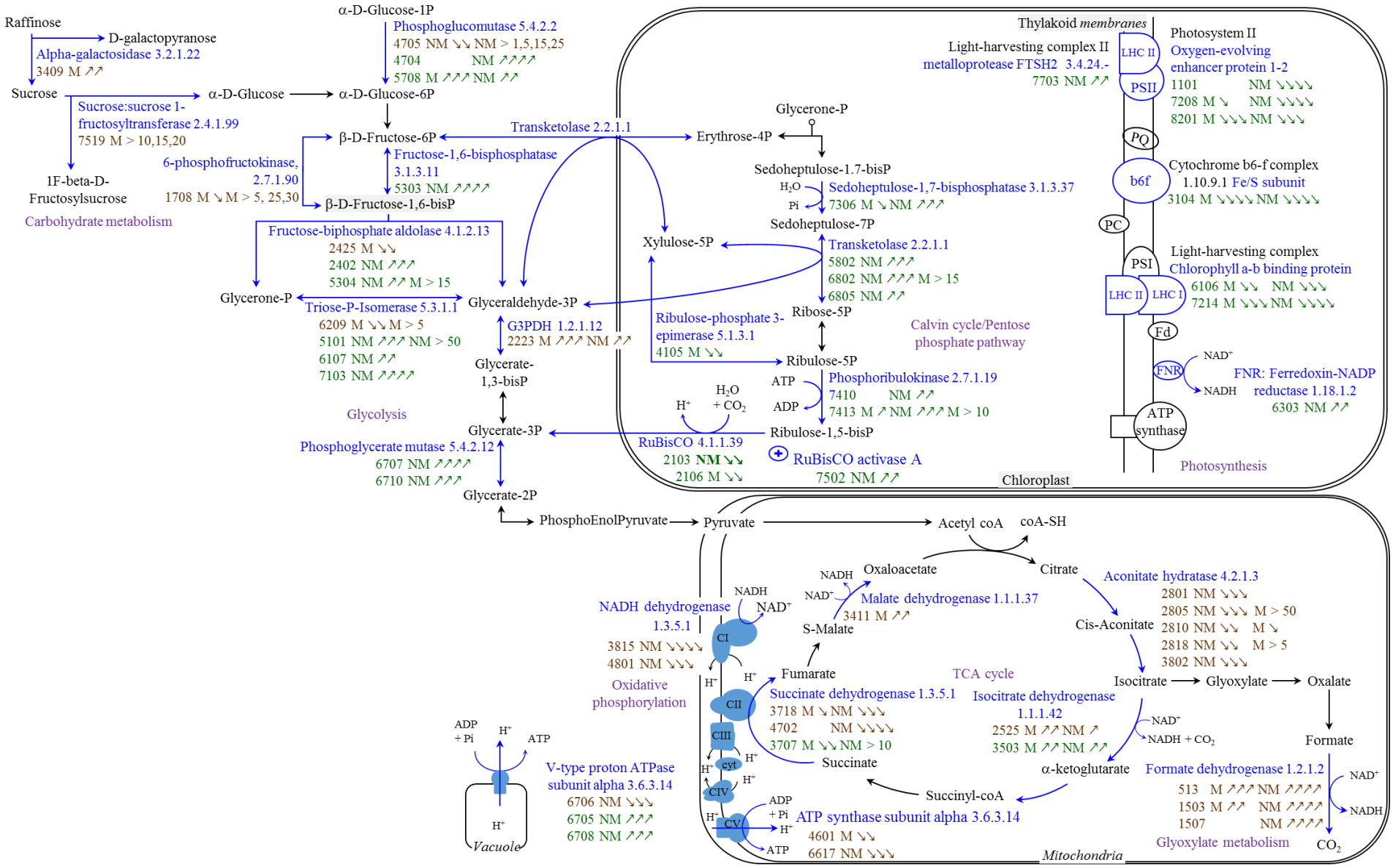

Figure 2: Expression profiles of protein spots involved in energy metabolism in roots (brown) and leaves (green). Enzymes are represented by their name and EC. ↗ / ↘: positive / negative correlation (Pearson); p-val: 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; M/NM > 1-50: population with higher expression at 1-50 µM Cu (ratio > x1.5).

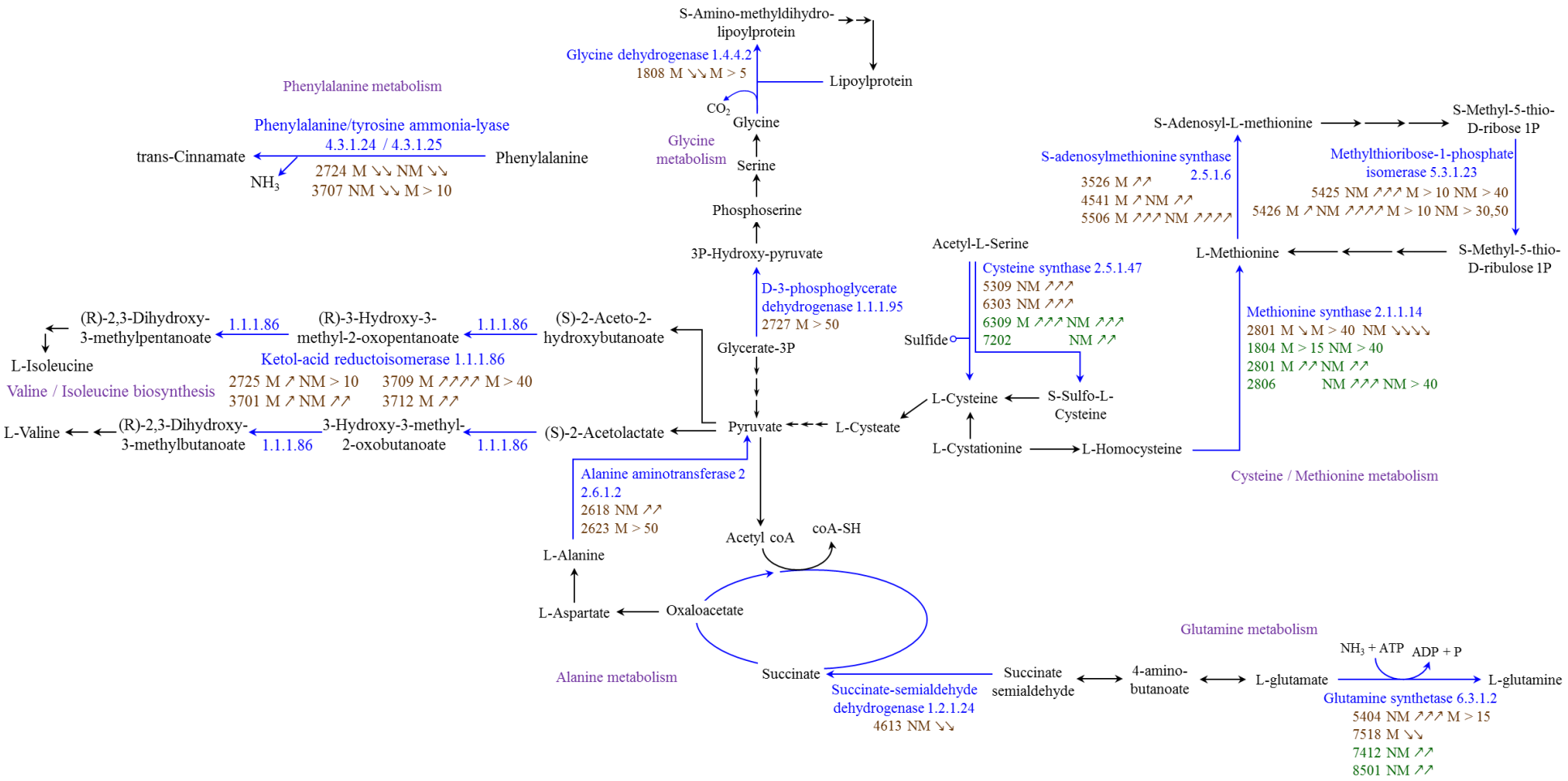

Figure 3: Expression profiles of protein spots involved in amino acid metabolism in roots (brown) and leaves (green). Enzymes are represented by their name and EC. ↗ / ↘: positive / negative correlation (Pearson); p-val: 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; M/NM > 1-50: population with higher expression at 1-50 µM Cu (ratio > x1.5).

1.2. Primary metabolism

1.2.1. Amino acids

Several molecular changes occurring in response to Cu exposure were related to the modification of amino acid metabolism and synthesis of other metabolites derived from amino acids. The 'Metabolism' category was one of the three main functional categories influenced by Cu in both roots and leaves, although it was more represented in roots (31% vs 13%). Among the eleven proteins involved in amino acid metabolism, only three were identified from both roots and leaves and exhibited different patterns under increasing Cu. Cysteine synthase (CS) and glutamine synthetase (GS) were found from two root and leaf spots, while methioninesynthase (MS) was identified from one root and two leaf spots.

The increase of CS but decrease of MS in NM roots indicated that cysteine production was preferentially stimulated compared to methionine one. As cysteine is an amino-acid central in metal chelation, enhanced accumulation probably reflected an increasing need to process chelation mechanisms including binding of free Cu. In roots, three S-adenosylmethionine synthase spots (SAMS) were up-regulated in one or both populations, suggesting an enhanced accumulation of S-adenosylmethionine (SAM). Due to SAM role in trans-methylation, trans-sulfuration and polyamine synthesis, SAM may play a central role in plants stress response and may stimulate nicotianamine (NA) and glutathione (GSH) production, but also ethylene synthesis. However, down-regulation of methionine synthase only in NM roots, leading to higher accumulation in M roots at high Cu, may reflect a better ability of M cells to maintain methionine biosynthesis under Cu excess.

Two CS and two MS spots were up-regulated in NM leaves, while only one of each increased also in M. Enhanced accumulation of CS and MS indicated that Cu excess induced an enhanced need in S-containing amino-acids in both populations but more intense in NM. Up-regulation of CS and GS in NM roots and leaves, probably indicated a higher production of GSH and derived products such as phytochelatins (PC) under Cu excess. Additionally, enhanced accumulation of GS may indicate a higher nitrogen assimilation in NM plants.

In roots, several proteins involved in Glycine (glycine dehydrogenase, D-3-phosphoglycerate dehydrogenase), Alanine (alanine aminotransferase), Valine/Leucine (ketol-acid reductoisomerase), and Phenylalanine (phenylalanine / phenylalanine-tyrosine ammonia-lyase) metabolism were differentially regulated by Cu or population origin. Globally, accumulation of ketol-acid reductoisomerases increased under Cu treatment in one or both populations, more intensively in M roots, indicating that Cu excess induced valine and

isoleucine biosynthesis from pyruvate. Accumulation of two phenylalanine ammonia-lyases (PAL) decreased under Cu excess, first in both populations and second only in NM. Decreasing PAL accumulation can lead to reduced production of lignin or to alteration of lignin composition. Together with the respective down- and up-regulation of caffeoyl-CoA O-methyltransferase and cinnamyl alcohol dehydrogenase only in NM, the decrease of a second PAL only in NM roots may indicate a stronger alteration of lignin biosynthesis in this population.

1.2.2. Nucleotide metabolism

Accumulation of spots belonging to Purine / Pyrimidine metabolism were altered in roots of both populations but only in NM leaves and different proteins were identified from each tissue. While an adenosine kinase and a nucleoside diphosphate kinase 2 were down-regulated, an adenine phosphoribosyltransferase 1 and an apyrase were up-regulated. Alteration of purine metabolism by Cu excess was recorded in roots of both populations, but only in NM leaves, indicating that a better maintenance of purine metabolism may contribute to the better fitness of M plants.

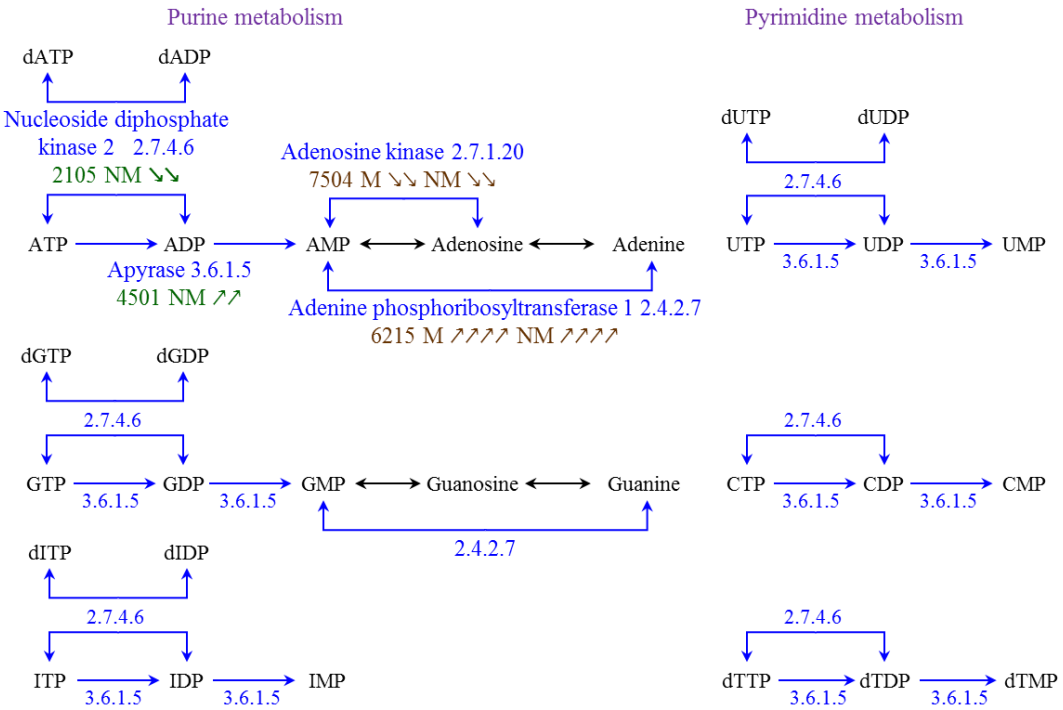

Figure 4: Expression profiles of protein spots involved in nucleotide metabolism in roots (brown) and leaves (green). Enzymes are represented by their name and EC. ↗ / ↘: positive / negative correlation (Pearson); p-val: 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; M/NM > 1-50: population with higher expression at 1-50 µM Cu (ratio > x1.5).

1.3. Protein synthesis, transport, folding and proteolysis

Among the 21 proteins involved in protein synthesis, transport, folding and proteolysis, only two, i.e. mitochondrial chaperonin CPN60-2 and protein disulfide isomerase (PDI), were identified in roots and leaves of the NM population but none was identified in roots and leaves of both populations.

Only one nucleoredoxin was differentially expressed in leaves of both populations, i.e. down- regulated in M but up-regulated in NM. Most proteins involved in 'protein folding/refolding' were differentially expressed only in NM. A heat shock 70 kDa protein 10 (HSP70 P1) was over-expressed at intermediate and high Cu exposure (10, 25, 30 and 40 µM Cu) in M roots and up-regulated in M leaves, leading to over-expression at 25 and 40 µM Cu. Higher accumulation of this protein at high Cu exposure seems to contribute to enhance Cu-tolerance in both M roots and leaves by protecting mitochondrial protein metabolism under Cu excess.

Cu excess induced a strong up-regulation of several protein chaperones in roots and leaves of the NM population. A PDI and a chaperonin CPN60-2 were up-regulated in both roots and leaves, while a mitochondrial chaperonin CPN60-1 increased only in roots, indicating a Cu-induced accumulation of misfolded proteins and enhanced need to protect protein metabolism. Up-regulation of several chloroplastic protein chaperones, i.e. a chaperone protein ClpC2, several 60kDa chaperonins subunit alpha (2 spots) and beta (3 spots) and a heat shock 70 kDa protein 7, supported that Cu strongly impacted protein metabolism in chloroplasts of NM plants. In preventing and reversing incorrect protein interactions, folding and aggregations, these proteins may protect cells against the accumulation of damaged or misfolded proteins related to Cu excess.

All proteins involved in protein synthesis or proteolysis were found from only one tissue and differentially regulated in only one population. Among the four proteins related to translation, one 40S ribosomal protein was down-regulated in M roots, while a 50S ribosomal protein, two eukaryotic initiation factors, and a GTP-binding protein TypA were up-regulated only in NM leaves.

Among the six proteins involved in proteolysis, one was up-regulated in NM leaves and related to higher photic damages in photosystem II (see section 1.1. Energy), while the five other were differentially regulated only in M roots. Two proteasome subunits, i.e. proteasome subunit beta type and 26S proteasome non-ATPase regulatory subunit 14 and a phytepsin were induced by Cu exposure, while a mitochondrial-processing peptidase subunit alpha was over-

expressed at all Cu exposure except 30 µM, and a second one over-expressed at 20 µM and down-regulated by Cu exposure. A cysteine proteinase inhibitor 12 (also called cystatin) was over-expressed at 50 µM. Over-expression or up-regulation of these enzymes supported the existence of a better proteolysis process in M roots, which may counteract the toxic effect of Cu on protein metabolism in avoiding accumulation of damaged proteins.

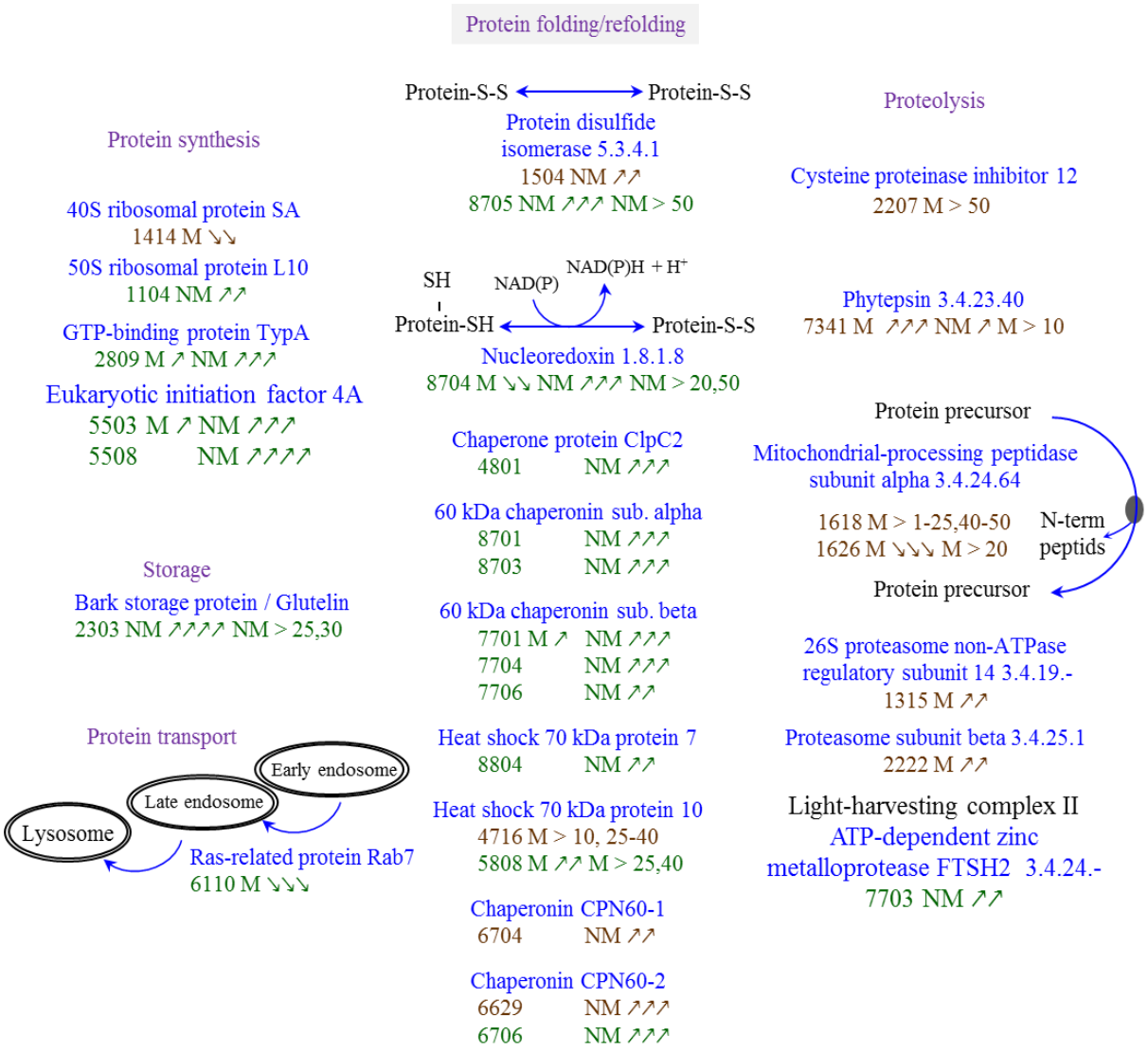

Figure 5: Expression variation of protein spots related to protein metabolism and catabolism in roots (brown) and leaves (green). Enzymes are represented by their name and EC. ↗ / ↘: positive / negative correlation (Pearson); p-val: 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; M/NM > 1-50: population with higher expression at 1-50 µM Cu (ratio > x1.5).

1.4. Stress response / Detoxification

Free Cu in cells may increase accumulation of H_2O_2, through Fenton reactions, which levels are controlled by cells by adapting redox homeostasis. In roots of both populations, two ascorbate peroxidases (APx1) were down-regulated while a superoxide dismutase (SOD) was up-regulated, suggesting an increasing accumulation of $O_2^{\circ-}$ and H_2O_2. The down-regulation of APx could indicate an accumulation of H_2O_2 and/or a decrease in AsA, as APX became rapidly

unstable in case of AsA deprivation and inactivated by high levels of H_2O_2. Stronger accumulation of $O_2^{\cdot -}$ and H_2O_2 was suggested in NM roots by the up-regulation of an additional SOD and down-regulation of three more APx. Down-regulation of an alcohol dehydrogenase only in NM roots but up-regulation of aldehyde dehydrogenase only in M, respectively underpinned a better detoxification of alcohols and aldehydes in M roots. In M roots, over-expression of three APx2 at low (1 and 15 µM) or high Cu exposure (30 and 50 µM Cu), indicated the involvement of these antioxidative enzymes in the Cu-tolerance of the M population, probably by improving H_2O_2 detoxification.

Higher accumulation of one glutathione-S-transferase (GST) in M roots compared to NM ones at almost all Cu exposures (1-10 and 25-40 µM) may provide a better protection of roots against accumulation of toxic compounds by increasing conjugation of various hydrophobic or electrophilic compounds, including free Cu.

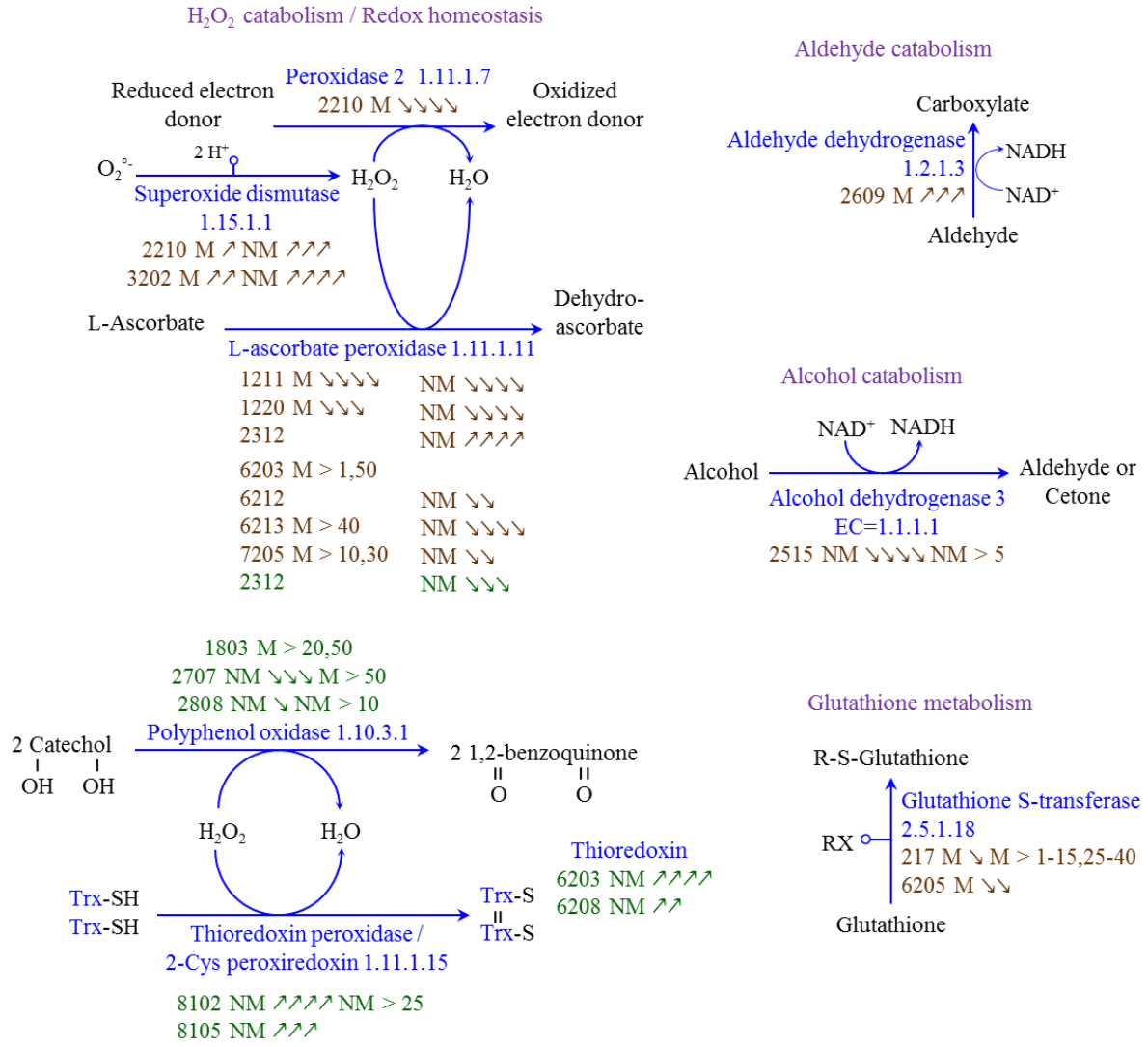

Figure 6: Expression variation of protein spots related to stress response and detoxification in roots (brown) and leaves (green). Enzymes are represented by their name and EC. ↗ / ↘: positive / negative correlation (Pearson); p-val: 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; M/NM > 1-50: population with higher expression at 1-50 µM Cu (ratio > x1.5)

An enhanced accumulation of ROS in NM leaves was suggested by the up-regulation of a metalloprotease FTSH2 involved in removal of damaged reaction center D1 proteins from photosystem II, which is known to be damaged by the presence and accumulation of ROS molecules or cationic radicals generated through photochemical reactions. Existence of a higher oxidative stress in NM leaves was also suggested by the up-regulation of thioredoxin and thioredoxin peroxidase. Down-regulation of a chloroplastic L-ascorbate peroxidase may also favor the accumulation of L-ascorbic acid to chelate free Cu in NM cells.

1.5. Other pathways

Proteins belonging to cytoskeleton exhibited different patterns in roots and leaves. Tubulins beta decreased in roots of M and NM but were not identified in leaves. Tubulin alpha and actin spots were down-regulated in roots but up-regulated in leaves of the NM population.

2. Integration of phenotypic, physiological and proteomic results

Differences between both populations tested have been demonstrated at increasing levels in associating ecological and proteomic approaches. All results pointed out Cu-induced impacts on both populations but also demonstrated the higher tolerance of the M population, originated from the Cu-contaminated soil and the stronger impacts in NM plants. Overall, proteomic results supported the Cu induced impacts reported at the phenotypic level, and confirmed higher impacts in NM population compared to M one.

2.1. Common Cu-induced impacts in both populations

Excess Cu impacted plant growth, altered root architecture (coralloid roots), and induced chlorotic symptoms in both populations. Roots accumulated most part of the Cu uptake, with higher concentrations in tissues compared to leaves (1.6 to 15.6 higher for M and 1.4 to 25.8 for NM from 1 to 50 µM Cu). Uptake patterns also differed: in roots, Cu concentrations, but also inter-replicates variability, increased sharply, while in shoots, Cu concentrations exhibited an increase between 1 and 15 µM Cu, then a plateau between 15 and 30 µM followed by another increase after 30 µM Cu. This confirmed the excluder phenotype already reported for this population for Cu excess and suggested that major mechanisms of chelation and sequestration were developed in roots. Cu concentrations increased drastically in roots of both populations, with a 45- and 70-fold increase occurring between 1 and 50 µM Cu in M and NM respectively. However, the variability between population replicates increased greatly with increasing Cu exposure indicating variability in the plant capacity to accumulate Cu in roots. On the opposite,

variation of foliar Cu concentrations would be more limited, with a 4.6- and 3.7-fold increase in M and NM respectively and a small inter-replicate variability.

Cu induced alteration in ionome accumulations, with differences observed among both tissues and population origin. Main differences between populations were observed for Ca, Fe, K, Al, Na, and Zn while B, Mg, Mn, and P behaved quite similarly. An increasing P uptake and translocation was measured in roots and shoots of both populations, was related to the increased accumulation of enzymes needing ATP and NADP under Cu stress. In parallel, Mg and Mn increased also in roots and shoots of both populations, more sharply in shoots, probably to support changes in cell metabolism and photosynthesis in leaves.

2.1.1. Impacts on roots

Reduction of root growth was related in both populations with alteration of electron transport, reduction of ATP production but increase of CO_2 production in mitochondria, as shown by the decreasing accumulation of ATP synthase subunit alpha and the up-regulation of two formate dehydrogenases. Up-regulation of a glyceraldehyde-3-phosphate dehydrogenase (G3PDH) in roots of both populations indicated that this protein was involved in plant response to Cu and suggested that Cu altered glycolysis flow to support a higher need in energetic compounds. An adenine phosphoribosyltransferase 1 was sharply induced, while an adenosine kinase was repressed by increasing Cu exposure, indicating that purine metabolism was altered by Cu excess in both populations.

The down-regulation of APx could indicate an accumulation of H_2O_2 and/or a decrease in AsA, as APX became rapidly unstable in case of AsA deprivation and inactivated by high levels of H_2O_2. However, considering that APx possess a heme B containing Fe, the decreased accumulation of APx may also be related to a possible Fe deficiency in roots. An increasing accumulation of ROS in roots was also pointed out by the up-regulation of superoxide dismutase (SOD). Impairment of cell integrity was suggested by the down-regulation of several tubulins beta in both populations, while down-regulation of phenylalanine ammonia-lyase could lead to reduced production of lignin or alteration of lignin composition.

Among the four enzymes involved in Cysteine/Methionine metabolism, only SAMS was up-regulated in roots of both species, suggesting SAM involvement in *A. capillaris* response to Cu excess. The precise consequences of an increasing SAM amount remained unclear, as it is involved in three key metabolic pathways: trans-methylation, trans-sulfuration and polyamine synthesis. However, down-regulation of tricetin 3',4',5'-O-trimethyltransferase and flavone 3'-O-methyltransferase did not suggest a higher need for methyl groups.

In roots, although no significant correlation existed between Fe concentrations and Cu exposure, the existence of a Fe deficiency cannot be excluded, as an increased need in Fe may result from Cu stress. If the increased uptake of Fe did not occur simultaneously with Cu, a Fe deficiency may occur in cells. In fact, the Fe concentration measured on a mix of all tissues did not indicate the real availability for physiological processes within cells. Moreover, as one of the less mobile element in plants, Fe deficiency may occur only in some parts of the root system and been masked by the global measurement. Additionally, the logarithmic model fitted on Fe concentrations in M roots indicated a decrease as Cu exposure rose.

At the proteomic level, strong decrease of Fe-containing enzymes in one of both populations supported the hypothesis of an effective but not measurable Fe deficiency in M and NM roots. While two APx1 decreased in both populations, several enzymes decreased only in NM, i.e. aconitases (5 spots), NADH dehydrogenase [Ubi] iron-sulfur protein 1 (2 spots) and APx2 (3 spots), or only in M roots, i.e. peroxidase and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase. Additionally, two methylthioribose-1-phosphate isomerases, known to be induced by Fe deficiency in roots, were up-regulated in NM roots.

2.1.2. Impacts on leaves

The progressive yellow coloration of plant leaves observed at high Cu exposure suggested degradation of the photosynthetic apparatus and Fe deficiency. Despite the small variation of foliar Cu concentrations, very intense changes in protein accumulation were revealed by the proteomic analysis, indicating that an accurate toxicity was triggered by a small variation of Cu content in leaves. This toxicity was probably due to the intense impact of Cu on photosynthesis processes. In leaves of both populations, Cu excess altered multiple components involved in light dependent reactions, i.e. photosystem II, cytochrome b6-f complex and light-harvesting complexes, as shown by the down-regulation of oxygen-evolving enhancer proteins, cytochrome b6-f complex iron-sulfur subunit and chlorophyll a-b binding protein. On the opposite, failure of carbon assimilation was mainly attributed to reduced RuBisCO accumulation, as shown by the up-regulation in NM of several other enzymes involved in dark reactions, i.e. sedoheptulose-1,7-bisphosphatase, RuBisCO activase and phosphoglycerate mutase. These results indicated that plants of both populations failed to maintain both the production of reducing power and the carbon assimilation during photosynthesis processes.

A decrease of shoot Fe concentrations confirmed the existence of Fe deficiency in leaves of both populations, which was suggested at the phenotypic level, by the leaf discoloration. Variability of color depending on leaf age, with young yellow and old green leaves, suggested that Fe deficiency was variable among leaf age and more intense in young leaves, which was

coherent with the fact that Fe is one of the less mobile element in plant tissues and is poorly remobilized from old tissues. The decreasing Fe concentrations in shoots of both populations, was probably responsible for the sharp decrease of the cytochrome b6-f complex Fe/S subunit. These results indicated that Cu excess induced Fe deficiency in leaves and confirmed that Fe/Cu antagonism contributed to photosynthesis disruption under Cu stress.

A cysteine and a methionine synthase were up-regulated in leaves of both populations, indicating that Cu excess induced an enhanced need in S-containing amino-acids. A nucleoredoxin was identified from M and NM leaves but exhibited opposite patterns, while its accumulation increased in M, decreased in NM, resulting in an over-expression in NM at 25 and 40 µM.

2.2. Differences between populations

Phenotypic characterization indicated a lower Cu-tolerance for the NM population, with stronger reduction of growth and more intense coralloid and chlorotic symptoms, which may be explained at the proteomic level by the alteration of protein accumulation.

In NM roots, the more marked growth reduction could be related to a higher disruption of H^+ transport/ATP production and Krebs cycle in mitochondria, as suggested by the strong down-regulation of aconitate hydratase, succinate and NADH dehydrogenases and V-type proton ATPase subunit alpha only in NM. Additionally, a limitation of glycolysis efficiency at Cu exposure higher than 25 µM was suggested by the over-expression of phosphoglucomutase only at low and intermediate exposures (1-25 µM Cu), and the limitation of G3PDH accumulation, which reached a plateau at high Cu exposures (30-50 µM Cu). Accumulation of a MS decreased, while accumulation of two CS increased, indicating that thiol groups were mainly used for cysteine biosynthesis. Together with the up-regulation of a GS it suggested a higher production of GSH and derivatives such as MTs and PC, which are involved in Cu homeostasis and tolerance. Additionally, enhanced accumulation of GS may indicate a higher Nitrogen assimilation in NM plants. Two mitochondrial chaperonins (CPN60-1 and CPN60-2) and a protein disulfide isomerase (PDI) were sharply up-regulated in NM roots, indicating more Cu-induced impacts on mitochondria and protein metabolism, and probable accumulation of misfolded proteins. Up-regulation of a second SOD and down-regulation of an alcohol dehydrogenase respectively suggested a higher accumulation of ROS and toxic alcohols under Cu excess.

Decreased accumulation of K+ voltage-gated channel probably explained the decrease of K concentration in NM roots exposed over 20 µM Cu, while the maintenance of such

transporter supported the linear increase in M root K concentration on this range of Cu exposure. As one of the three primary macronutrients, K^+ has various functions in plants so over-expression of a K^+ voltage-gated channel (#1414) in M roots at 40 µM Cu probably conferred an advantage for this population, permitting a higher K^+ uptake at high Cu excess, or a lower K^+ leakage induced by Cu.

Phenotypic characterization indicated higher chlorosis symptoms and stronger growth reduction, which may be attributed at the proteomic level to the more intense down-regulation of all identified photosynthesis-related enzymes, i.e. OEE, cytochrome b6-f complex Fe-S subunit, chlorophyll a-b binding protein and RuBisCO. In particular, the sharp decrease of RuBisCO accumulation indicated a strong failure of carbon assimilation. Additionally, the increase of a ferredoxin-NADH reductase indicated an alteration of electron flow during the photosynthesis process, but may provide a higher production of NADH under increasing Cu excess. As previously mentioned, the Fe deficiency induced by the decrease in foliar Fe concentrations was probably responsible for the sharp decrease of cytochrome b6-f complex Fe/S subunit, and OEE proteins. Following this hypothesis, the stronger decrease of Fe in NM leaves may also explain the stronger down-regulation of these proteins. Additionally, the decrease of chloroplastic APx only in NM leaves may suggest either a decrease of AsA content or a stronger Fe deficiency in chloroplast compared to the M population.

The enhanced accumulation of a metalloprotease FTSH2 in NM, which is involved in the removal of damaged D1, pointed out stronger Cu-induced damages on photosystem II for this population. As this photosystem II reaction center D1 protein is known to be damaged by the presence and accumulation of ROS molecules or cationic radicals generated through photochemical reactions (Yamamoto 2001), a higher production of such compounds was suggested in NM chloroplasts. This was confirmed by the up-regulation of several thioredoxin and thioredoxin peroxidase only in this population. Higher impacts on chloroplasts were also suggested by the increase of several chloroplastic protein chaperones, i.e. a chaperone protein ClpC2, several 60kDa chaperonins subunit alpha (2 spots) and beta (3 spots) and a heat shock 70 kDa protein 7, and by the up-regulation of chloroplastic CS, MS and GS, which indicated a higher need in S-containing amino-acids and production of GSH to process chelation and detoxification mechanisms.

A higher need for energetic compounds and reducing power in NM leaves was shown by the up-regulation of V-type ATPases and of several enzymes involved in glycolysis, i.e. phosphoglucomutase, FBP aldolase, TIM, phosphoglycerate mutase, which confirmed the higher need for P supply suggested by the stronger P translocation in NM plants. Stimulation

of pentose phosphate pathway and Calvin cycle, through increasing accumulation of FBP aldolases, TIM, transketolases, sedoheptulose-1,7-bisphosphatase, RuBisCO activase and phosphoribulokinase, may contribute to counteract the decline of carbon fixation related to the sharp decrease of RuBisCO accumulation. Enhanced accumulation of V-type H^+-ATPase was coherent with the up-regulation of a 14-3-3-like protein A, as 14-3-3 proteins are known for being positive regulators of plasma membrane H^+-ATPase that governs the electrochemical gradient across the plasma membrane and is essential to control ion transport and cytosolic pH. Down-regulation of a nucleoside diphosphate kinase 2 and up-regulation of an apyrase only in NM leaves also indicated a higher alteration of Purine/Pyrimidine metabolism under Cu excess.

As Cu altered protein metabolism and induced the accumulation of numerous proteins, it was consistent to find a stimulation of protein synthesis processes in leaves, as indicated by the up-regulation of eukaryotic initiation factor 4A, 50S ribosomal protein L10 and GTP-binding protein TypA. Together with the increased accumulation of protein chaperones, it suggested a higher stimulation of protein synthesis and folding processes. A stimulated protein turn over in NM leaves may also explain the enhanced accumulation of GS, by an increased requirement in N assimilation. A mitochondrial CPN60-2 chaperonin and a PDI were sharply up-regulated in NM leaves, indicating more Cu-induced impacts on mitochondria and protein metabolism, and probable accumulation of misfolded proteins. None of the identified proteins involved in proteolysis was differentially expressed in NM roots, which could indicate that plants failed to improve the proteolysis processes under Cu excess, which was also consistent with the probable accumulation of misfolded and damaged proteins. Actin and tubulin alpha spots were up-regulated only in NM leaves, indicating changes on cytoskeleton, probably to support cell division and maintain cell integrity.

A higher oxidative stress in NM leaves was suggested by the up-regulation of thioredoxin and thioredoxin peroxidases. Down-regulation of a chloroplastic L-ascorbate peroxidase may also favor the accumulation of L-ascorbic acid to chelate free Cu in NM cells. Globally, polyphenol oxidases (PPO) were down-regulated only in NM leaves, leading to over-expression in M at 50 µM Cu. PPO is a tetramer containing four Cu atoms per molecule, and binding sites for two aromatic compounds and oxygen. Higher accumulation of PPO in M leaves may contribute to enhance the storage of Cu through protein incorporation, favor H_2O_2 detoxification and production of phenols, which can chelate Cu.

In M roots, up-regulation of an alpha-galactosidase and over-expression of a sucrose:sucrose 1-fructosyltransferase and a 6-phosphofructokinase pyrophosphate-dependent at intermediate Cu exposure suggested that several carbohydrate-related enzymes cooperated

together to maintain the supply of glycolysis and Krebs cycle under Cu stress. Additionally, the linear increase of G3PDH accumulation across this range of Cu exposure may promote accumulation of NADH and pyruvate at high Cu exposure. This suggested that Cu tolerance in *A. capillaris* may involve the maintenance of glycolysis activity. Up-regulation of IDH and MDH in M roots may provide an increasing amount of NADH but also of citric and malic acid, which can chelate Cu and protect mitochondria from free Cu^{2+}. Together with the small alterations of H^+ transport and Krebs cycle, it suggested a better mitochondria functioning under Cu stress.

A heat shock 70 kDa protein 10 (HSP70 P1) appeared to be involved in the higher tolerance of the M population as it was more accumulated in both roots and leaves of this population. In roots, this HSP70 was over-expressed at intermediate and high Cu exposure (10, 25, 30 and 40 µM Cu) and in leaves, it was up-regulated by Cu exposure, resulting in over-expression at 25 and 40 µM Cu. Enhanced accumulation of this HSP may contribute to enhance tolerance by protecting protein metabolism under Cu excess. Several proteins involved in proteolysis did respond to Cu and were over-expressed in M roots. Two proteasome subunits, i.e. proteasome subunit beta type and 26S proteasome non-ATPase regulatory subunit 14 and a phytepsin were induced by Cu exposure. A mitochondrial-processing peptidase subunit alpha was over-expressed at all Cu concentrations except at 30 µM, and a second one over-expressed at 20 µM and down-regulated by higher Cu exposure. A cysteine proteinase inhibitor 12 (also called cystatin) was over-expressed at 50 µM. Over-expression or up-regulation of these enzymes supported the existence of a better proteolysis process in M roots, which may counteract the toxic effect of Cu on protein metabolism by avoiding accumulation of damaged proteins.

Several cytoplasmic APx2 were down-regulated only in NM roots leading to over-expression in M at high Cu exposure, which indicated the involvement of these antioxidative enzymes in the Cu-tolerance of the M population, probably by improving H_2O_2 detoxification. The sharp down-regulation of a peroxidase may also provide an increased accumulation of reduced electron donor to quench ROS and protect M cells against oxidative damages. The M population did not exhibit down-regulation of mitochondrial Fe-containing proteins, but showed a decreased accumulation of cytoplasmic L-ascorbate peroxidases and peroxidase 2. Cells may avoid Fe deficiency in mitochondria, by limiting production of Fe-proteins in cytoplasm and favoring the supply for mitochondria. One glutathione-S-transferase (GST) was over-expressed in M at almost all Cu exposures (1-10 and 25-40 µM). As GSTs catalyze the conjugation of GSH with a large variety of substrates, including Cu, higher accumulation in M may promote root protection against accumulation of various hydrophobic or electrophilic

compounds, including free Cu, by increasing conjugation. Additionally, the up-regulation of aldehyde dehydrogenase only in M roots may also provide a better degradation of potentially toxic aldehydes in mitochondria.

3. What about the processes involved in the higher Cu tolerance of M plants?

This thesis increased our knowledge on plant response to increasing Cu exposure in both M and NM populations of *A. capillaris* in the 1-50 µM Cu range. However, the second main aim was to elucidate the mechanisms enabling higher Cu-tolerance in M plants. Indeed, phenotypic, physiological and/or proteomic results obtained during this multidisciplinary-approach permitted to refute and validate several hypotheses about the mechanisms underlying higher Cu-tolerance in M.

The possibility of a reduced Cu-uptake/accumulation in M roots was refuted at low and high Cu exposures by the determination of root Cu concentrations, which did not differ between populations. However, at intermediate Cu exposures (25-30 µM Cu), reduced accumulation in M roots was suggested by the higher Cu concentrations in NM roots compared to M ones. Decreasing Cu uptake may be achieved through rhizosphere mechanisms, which were not studied in this work, through an increase of biomass production and/or alteration of transporter accumulation and activity. In our case, a dilution effect was strongly supported by the higher biomass of M plants, but similar mineral masses in both populations. No proteomic evidence supported a decrease of transporter accumulation in roots, but the possibility cannot be excluded as the extraction procedure was designed for soluble proteins and not membrane ones. Similar experiments on differential accumulation of membrane proteome may help to elucidate the variation of transporter accumulation under Cu excess.

The equivalent or lower Cu concentrations but the higher fitness and growth of M roots strongly suggested a better efficiency to store Cu in tissues or to cope with its deleterious effects on cell integrity. The better ability to cope with deleterious effects of Cu excess in M roots was confirmed by the proteomic experiment.

The hypothesis of a lower Cu translocation from roots to shoots in M plants, preventing Cu toxicity in leaves, was excluded by the measurement of foliar Cu concentrations, which were higher in M at 5, 15, 20 and 40 µM Cu and similar in both populations at other Cu exposures.

The higher Cu mineral mass of M shoots at Cu exposure equal or higher than 10 µM Cu, resulted from a higher Cu concentration and relatively stable production of dry matter. Although lower Cu concentrations were measured in NM leaves at moderate and high Cu exposures,

proteomic results indicated higher impacts on chloroplasts, as shown by the stronger disruption of photosynthesis processes and the sharp up-regulation of chaperones and antioxidative enzymes. This supported the existence of a better efficiency to cope with the deleterious effects of Cu excess in M leaves, and even more suggested a high need for Cu in this population

Modifications of ion uptake and translocation to shoots appeared to contribute to enhance Cu tolerance in M plants, as major difference in Ca, Fe, K, Al, Na and Zn accumulation patterns occurred between populations.

These experiments supported an antagonism between Fe and Cu in both populations under Cu excess. Root Fe concentrations did not vary, which suggested Fe deficiency as an increasing Fe need probably resulting from the increasing Cu accumulation. This hypothesis was supported at the proteomic level by the decrease of several Fe-containing enzymes in one of both populations, i.e. aconitases, APx1 and APx2, NADH dehydrogenase [Ubi] Fe/S protein 1, peroxidase and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, and the up-regulation of Methylthioribose-1-phosphate isomerases, known to be induced by Fe deficiency in roots.

Fe concentrations decreased in shoots of both populations, more markedly in NM, suggesting a Fe deficiency in shoots, which may be responsible for the chlorosis symptoms through the alteration of light dependent photosynthesis processes. Implication of Fe deficiency in photosynthesis alteration was confirmed by the sharp decrease of Cytochrome b6-f complex Fe/S subunit in both populations. Results suggested that M plants were able to cope with the enhanced Cu foliar concentrations but unable to counterpart the deleterious effects of the Fe deficiency, while NM plants were impacted by both Cu excess and Fe deficiency. A possible explanation for the less intense chlorotic symptoms in M plants would consist in maintaining sufficient Fe supply for chloroplast metabolism, probably through a re-allocation of Fe in cells (deprivation in cytoplasm to favor chloroplasts supply for example). However, further analyses will be necessary to identify the distribution in plant tissues depending on their age and to understand the precise role of the Cu-induced Fe deficiency in the plant response to Cu excess and in the higher tolerance of the M population.

Cu exposure induced an increasing Ca uptake and translocation, resulting in increasing Ca concentrations in both roots and shoots. The increase was more marked in NM roots but similar in shoots of both populations, indicating that the lower Ca concentrations in M leaves were rather due to a limitation of Ca uptake by roots than to a limitation of Ca translocation. As Cu is known to modify stability of Ca channels, and induces increasing Ca flux into cells, a better regulation of Ca uptake by roots may participate to enhance Cu tolerance in M plants.

Na concentrations decreased in roots of both populations, suggesting that reduction of Na uptake was a common mechanism for both populations in response to Cu excess. However, higher concentrations in M between 25 and 40 µM indicated a better ability to accumulate Na in M roots at intermediate Cu excess. Additionally, the higher concentrations in NM shoots at almost all Cu exposure tested indicated lower root-to-shoot translocation in M plants even at low Cu exposure. The fact that M plants have evolved two mechanisms to reduce Na concentrations in leaves suggested that Na regulation plays an important role in M Cu tolerance.

K concentrations increased in NM roots between 1 and 25 µM but decreased at Cu exposure higher than 25 µM, while they increased linearly in M roots, indicating a limitation of K uptake in NM roots at Cu higher than 25 µM. This variation was explained at the molecular level by the reduced accumulation of a voltage-gated potassium channel and the probable K deficiency in roots was suggested by the higher K translocation from roots to shoots. As a major plant nutrient, such limitation of K uptake may contribute to growth reduction in the NM population at Cu exposure higher than 25 µM, while the higher translocation may reflect a higher need of P in leaves in response to Cu excess. So the better maintenance of K uptake in M roots may contribute to enhance Cu tolerance in providing enough K supply to maintain cellular processes.

Al concentrations decreased in roots of both populations, probably due to Cu/Al competition for root uptake, but increased in M shoots only, indicating an enhanced and higher translocation in M plants in response to Cu excess. Avoiding Al deprivation in leaves may contribute to support cell functions and be involved in the higher tolerance of the M population.

Concluding remarks – Take Home message

First, this work confirmed that existence of plant species with phenotypic plasticity regarding tolerance represents a good opportunity to study mechanism of tolerance, in comparing tolerant and sensitive genotypes/populations/cultivars. More particularly, it showed that *Agrostis capillaris*, as a metallophyte evolving populations tolerant to various metals, is a good candidate to study tolerance to metal(loid), including Cu.

Secondly, this thesis also confirmed that multi-disciplinary approaches are key strategies to better understand plant responses to stresses. Proteomic represents a useful tool to elucidate differential accumulation of proteins by metal(loid) stress. In these experiments, proteomics results supported and explained - at least partially – the Cu-induced impacts observed at the plant scale. Use of transcriptomic approach to characterize differential accumulation of transcripts under Cu stress, appeared to be applicable in these populations and deserves also further investigations to complement knowledge gained by proteomic approach.

In comparing two populations differing by their Cu tolerance, i.e. tolerant (M) vs sensitive (NM), this thesis improved knowledge about i) *A. capillaris* response to Cu excess and ii) molecular mechanisms underlying higher Cu tolerance in the M population. Differences between M and NM populations tested have been demonstrated at phenotypic, physiological and proteomic levels. Results indicated the existence of Cu-induced impacts common to both populations but indicated stronger impacts in NM plants/higher tolerance for the M population. Impacts on photosynthesis process was demonstrated at phenotypic and proteomic levels for both populations but might result from Cu excess and/or from Cu-induced Fe deficiency. Results suggested that M plants were able to cope with the enhanced foliar Cu concentrations but unable to counterpart the deleterious effects of Fe deficiency, while NM plants suffered from both Fe deficiency and foliar Cu increase. These experiments permitted to exclude the possibility of a reduced Cu translocation from roots to shoots in M plants, but the possibility of a reduced uptake and/or accumulation in M roots at intermediate Cu exposures (25-30 µM Cu) deserved further investigations. Results confirmed the hypothesis of a better Cu management in M roots and leaves and a better ability to cope with deleterious effects of Cu excess, such as ROS production, or impacts on protein metabolism. To summarize, results suggested that M plants have developed ability to deal with Cu excess in leaves enabling a better carbon assimilation, while protection of roots contribute to maintain and regulate nutrient uptake.

Futur works

Two types of futur works can be distinguished at the end of this thesis. The first concerns the study of remarkable results obtained in these experiments, while second regrouped reflexions about methods and improvement of Cu tolerance investigations using *A. capillaris*.

The involvement of Cu-induced Fe deficiency in plant response to Cu excess deserves more attention. It would be really interesting to study the Cu-response in tissues with different development stages, i.e. young, mature or old leaves or root parts. As young, intermediate and old leaves exhibited clear phenotypical differences under increasing Cu stress, measurement of nutrient concentration or protein profile may provide knowledge about the cooperation between plant parts to cope with Cu. In fact, decrease in Fe content and chlorosis symptoms were more marked in young leaves while old ones exhibited bronzing symptoms and purple coloration. The mapping of Cu and Fe distribution in plant tissues could be realized by using for example imaging mass spectrometric techniques such as Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS; Becker *et al.*, 2009).

The possibility of a reduced Cu uptake and accumulation in M roots at intermediate Cu exposures (20-40 µM Cu) deserves also more analyses, as different strategies may occur depending on the level of Cu exposure. This range of Cu exposure should be investigated more precisely in increasing the number of tested concentrations and proteomic analyses must focused on membranous transporters.

Several important questions remain about Cu tolerance in the M population. Did the tolerance occur during germination and/or growth? What are the consequence of Cu excess on plant reproduction, number of flowers, seed quality and quantity? Better results about germination under Cu exposure could be achieved by cultivating seeds in Petri dishes containing solidified culture medium; it would indicate if population differentiation occurs during germination, i.e. % of germination success, mean time of germination, phenotypic symptoms. Then, the rate of mortality could also be monitored in better controlling the number of individuals per population, for example in using a one-by-one separation among plants.

This work confirmed that multi-scale approaches (integrative biology) coupling phenotypic and physiological characterization together with "-omics" approaches, is one of the keys to gain information on molecular mechanisms underlying changes induced by Cu-stress observed at the plant scale. The proteomic approach increased the knowledge about *A. capillaris* response to Cu excess, but also exhibited some limits. The very large biological characteristics exhibited by proteins, i.e. size, mass, charge, hydrophobicity, conformation or

post-translational modifications make impossible the extraction of an entire proteome by following a single proteomic protocol. Additionally, accumulation of dominant proteins such as RuBisCO, or of cell wall compound and plant metabolites may disturb the extraction step. Separation and colorations gel-based techniques have also their own technical limits, due to inherent limit of detection, separation and quantification (DalCorso *et al.*, 2013). All these limitations indicated that cooperation of several proteomic approaches is necessary for obtaining the maximum information about differential regulation of protein accumulation under Cu stress. For further verification of the changes in proteomic profiling, analyzes by immuno-blotting may be necessary, as used by Zhao *et al.* (2011) and use of new bioinformatics tools could also improve interpretation of protein involvement in biological pathways (Antonov *et al.*, 2009)

Furthermore, as proteomic approach inform only on protein accumulation, it appears also necessary to complement results by a biochemical approach in measuring enzyme activities, as they could also be either activated or inhibited together with being down- or up-expressed. Use of transcriptomic technics may also be helpful to characterize differential accumulation of transcripts under Cu stress, and identify regulation processes between expression of transcripts and accumulation of the corresponding proteins.

Another option to investigate Cu tolerance would be the subpooling of M population to compare individuals with very high tolerance (phenotype not affected by Cu or even higher biomass) to individuals with moderate and low tolerance. We can also imagine a comparison between non-tolerant individuals from M population to individuals of NM population.

Isolation of such highly tolerant genotypes through screening on increasing Cu exposure may permit the creation of highly tolerant cultivars, available for further application in phytoremediation of Cu-contaminated soils or for Cu-tolerance investigations, in providing plant material with limited genetic variability. Increasing contrasts between compared populations may also highlight the most performant mechanisms of Cu tolerance.

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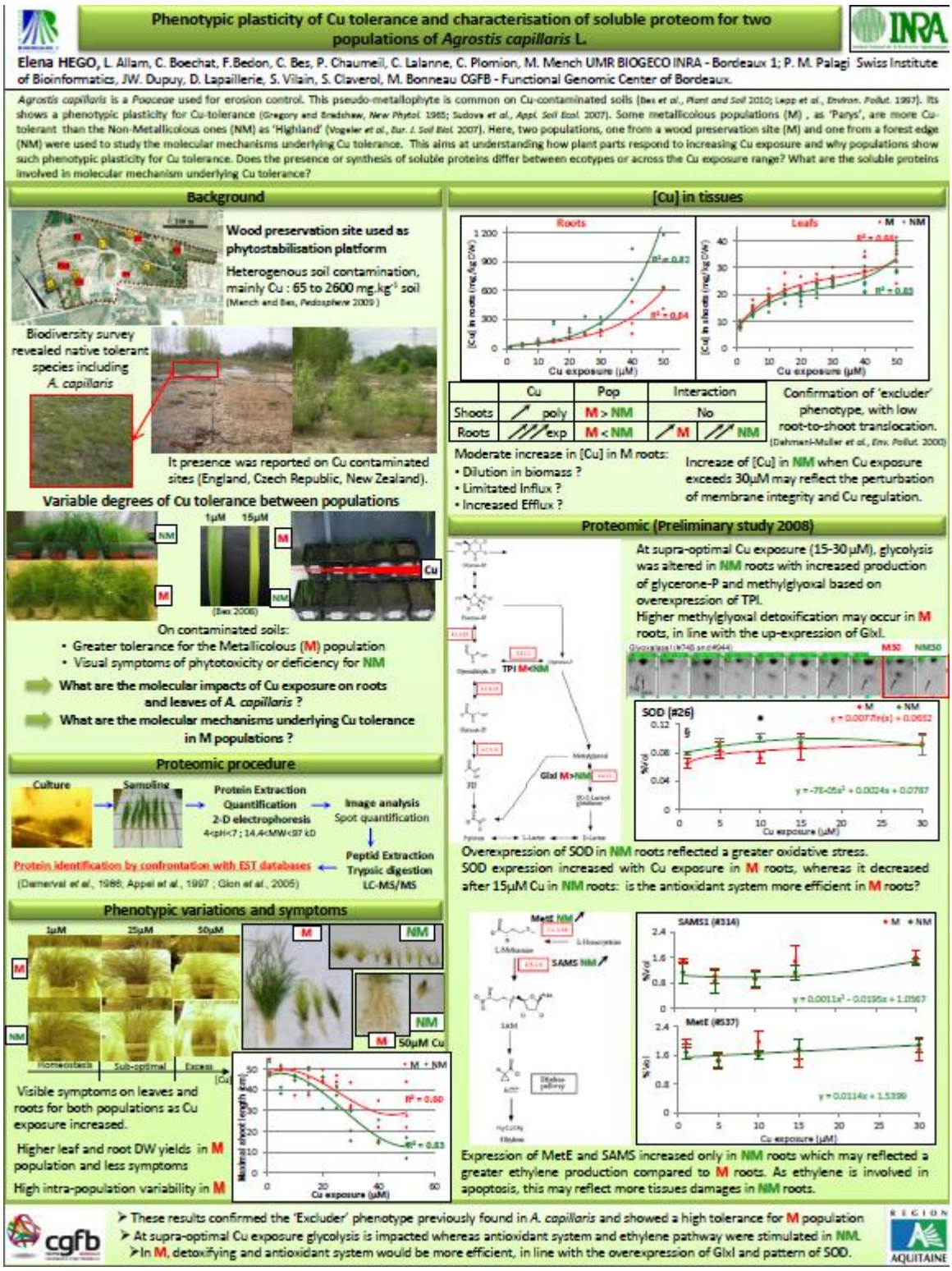
Poster

Plant Biology Congress 2012 (Fribourg, July 29 – August 3 2012)



Oral presentations

11th International Conference on the Biogeochemistry of Trace Elements (Florence, July 3-7 2011)

Florence, March 22 2011

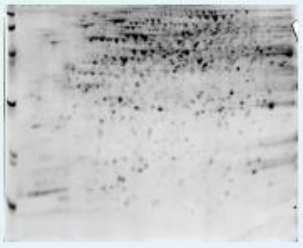
11th ICOBTE, Scientific Program of the Symposium 4: Plant and soil microbial community responses to trace element induced stress: information by 'omic' approaches.
Organizers: Gian Attilio Sacchi, Nicola Tomasi, Giacomo Pietramellara, Loretta Landi, Paolo Nannipieri, Giancarlo Renella, Tuesday July 5th 2011, 11:10 – 12:50 and 2:30 – 5:10

To: Mrs Elena Hego
UMR BIOGECO, INRA,
Talence, France

We are pleased to inform you that your paper entitled 'Proteomic characterization of metallicolous and non metallicolous population of *Agrostis capillaris* exposed to Cu' has been accepted as oral presentation for the Symposium 4 of the 11th International Conference on the Biogeochemistry of Trace Elements (11th ICOBTE).

Proteomic characterization of metallicolous and non-metallicolous populations of *Agrostis capillaris* exposed to Cu.

Elena Hego[1], C.M. Bes[1], F. Bedon[1], M. Mench[1], P.M. Palagi[3], P. Chaumeil[1], A. Barre[2], S. Claverol[2], J.W. Dupuy[2], M. Bonneu[2], C. Lalanne[1], and C. Plomion[1]



[1]UMR BIOGECO INRA 1202, University of Bordeaux 1, Talence, France. mench@bordeaux.inra.fr. [2]Pôle Protéomique de la Plateforme Génomique Fonctionnelle Bordeaux, Université Bordeaux 2, Bordeaux, France. [3]Swiss Institute of Bioinformatics, CMU, Genève 4, Switzerland.

9th International Phytotechnologies Conference (Hasselt, September 11-14 2012)

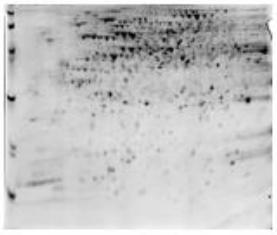
Dear Colleagues

Thank you all for your abstract submission for the IPS Conference on Phytotechnologies which will take place September 11-14, 2012 in Hasselt, Belgium. Due to the high number of abstracts, abstract selection for platform presentations was very competitive. Sessions chairs came to a conclusion regarding the selection and classification of abstracts for platform and poster presentations. For sure, it's going to be an exciting conference, covering a wide range of topics.

We would like to inform you that your abstract has been accepted for a **full platform presentation.** In the attached file you can find your selected abstract + the session in which it has been classified.

Proteomic characterization of metallicolous and non-metallicolous populations of *Agrostis capillaris* exposed to Cu.

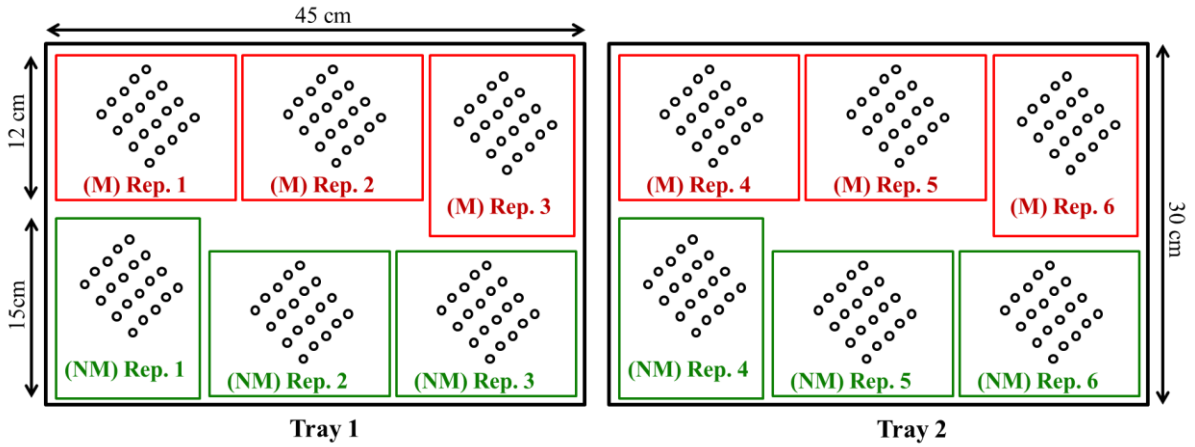
Elena Hego, L. Allam, C. Boechat, F. Bedon, C. Bes, P. Chaumeil, C. Lalanne, C. Plomion, M. Mench - UMR BIOGECO INRA - Bordeaux 1 ; JW. Dupuy, D. Lapaillerie, S. Vilain, S. Claverol, M. Bonneau - Functional Genomic Center of Bordeaux FRANCE
P. M. Palagi Swiss Institute of Bioinformatics SWITZERLAND



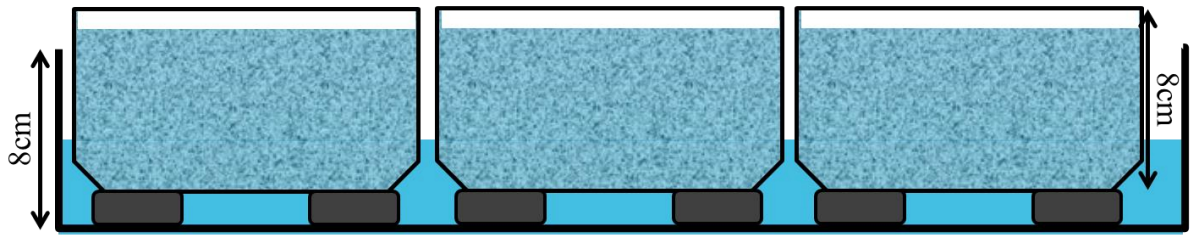
B i o G e C o

Annex 1 - Culture of *A. capillaris* populations exposed to Cu

For each Cu exposure, *i.e.* 1, 5, 10, 15, 20, 25, 30, 40 and 50 µM Cu, 6 plastic pots (15 x 12 x 8 cm), therefor reffered as replicates, were sown for each population, and 2 sets of 3 replicates were arranged in 2 different plastic trays. Pots were perced in the center and raised with 2cm plastic blocks to permit Hoagland solution (added with CuSO4, 7H2O) to imbibate the perlite by capillarity. Seeds of bot populations were collected in August 2011 and sown in September 2011 after 2 days in 4°C.



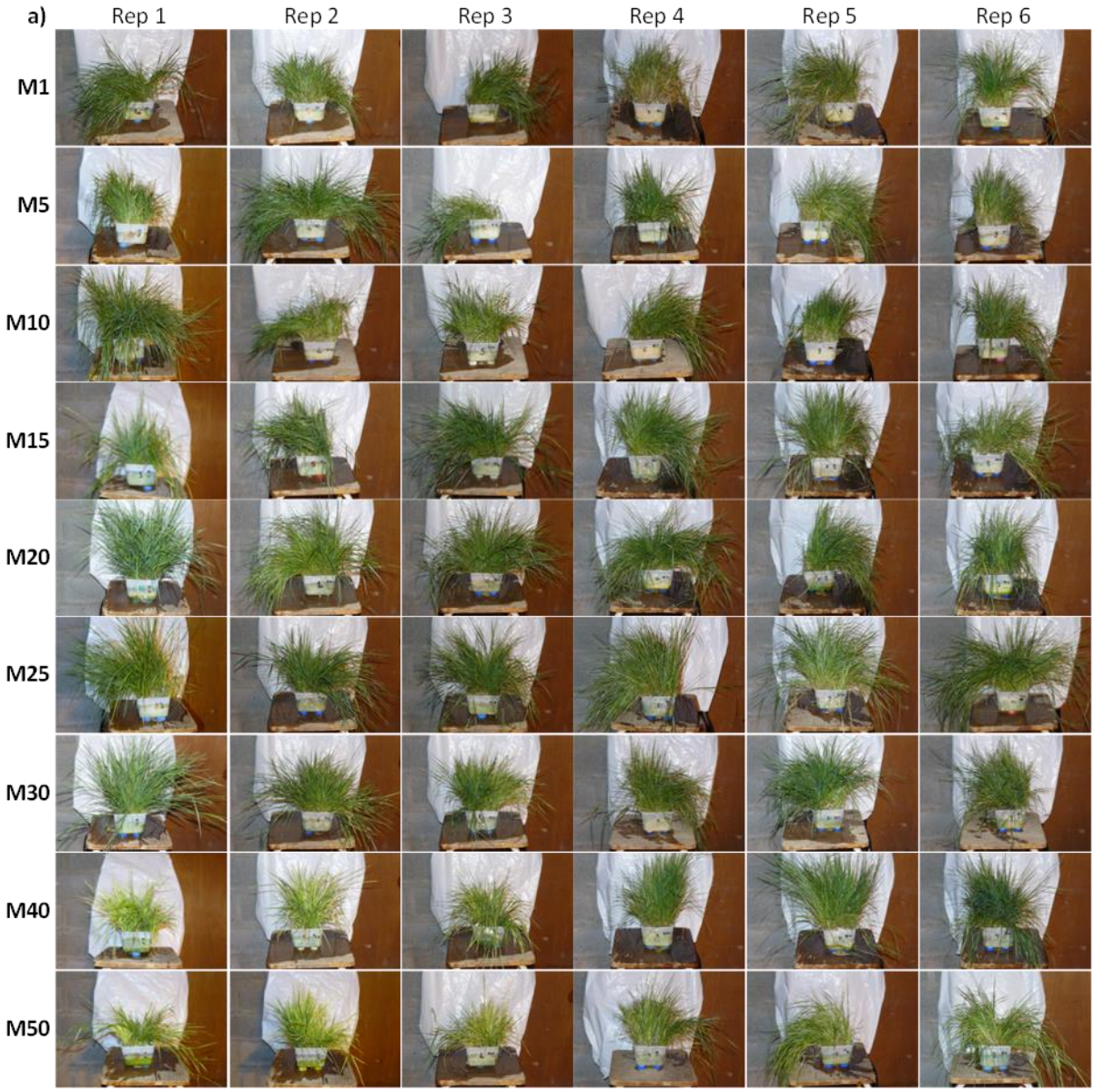
Disposition of replicates in trays.



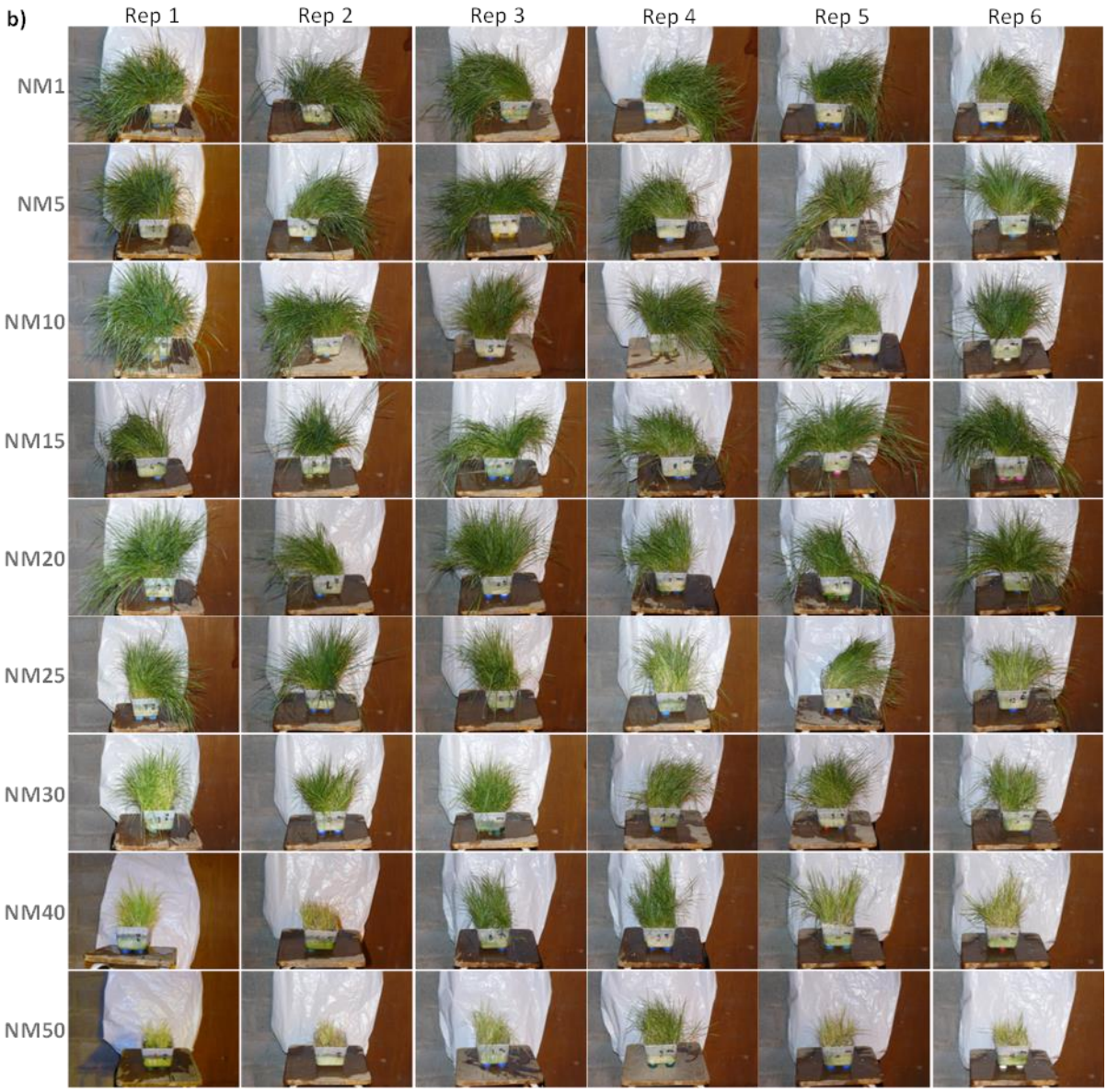
Disposition of replicates in one tray.

Annex 2 - Phenotypes of M and NM populations exposed to Cu

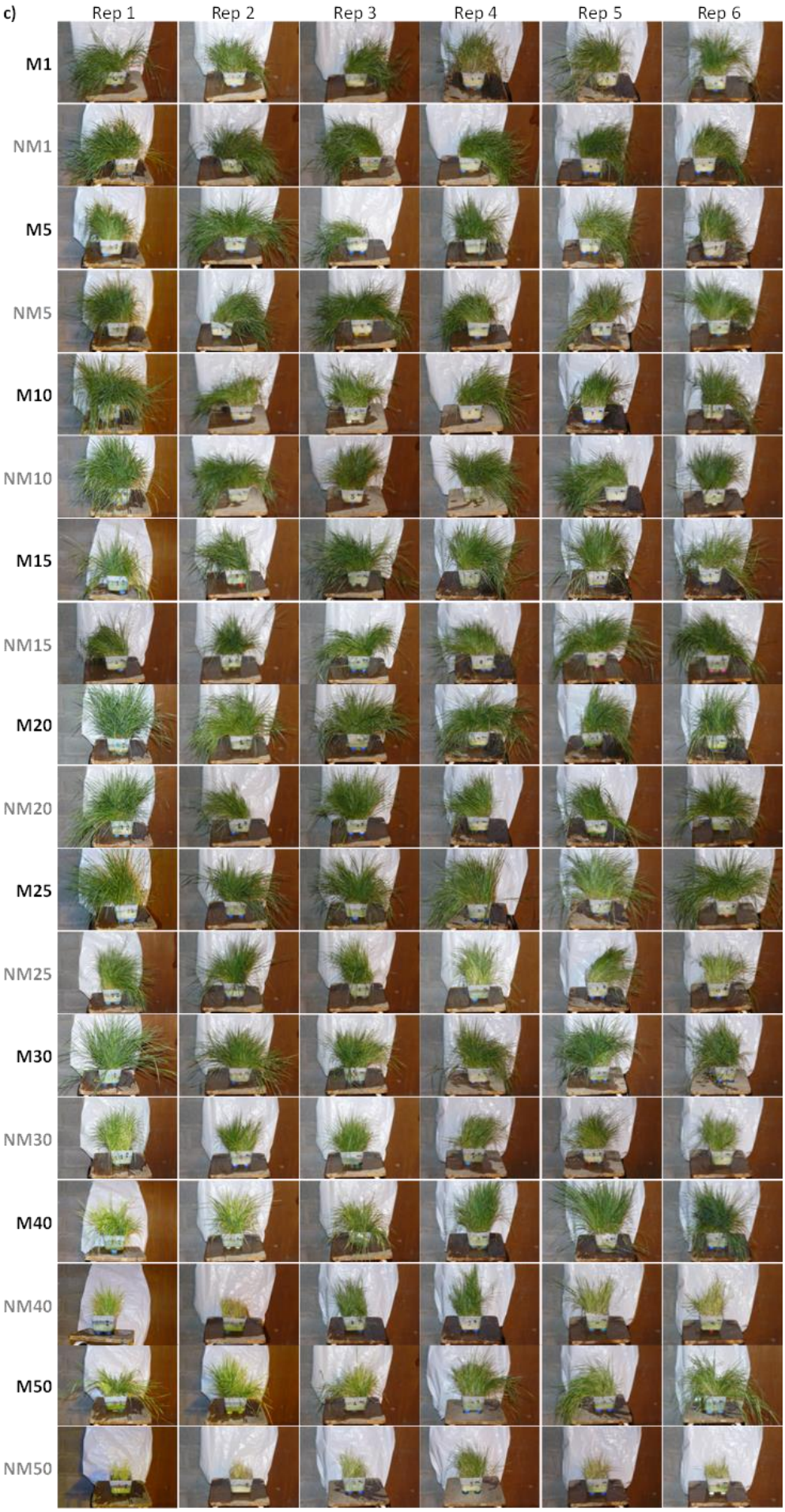
Pictures of the six replicates of *Agrostis capillaris* populations (M: Metallicolous, NM: Non-Metallicolous) exposed to nine Cu concentrations (1, 5, 10, 15, 20, 25, 30, 40 and 50 µM Cu added as CuSO4), cultivated for three months on perlite spiked with Hoagland solution.



Replicates of M population



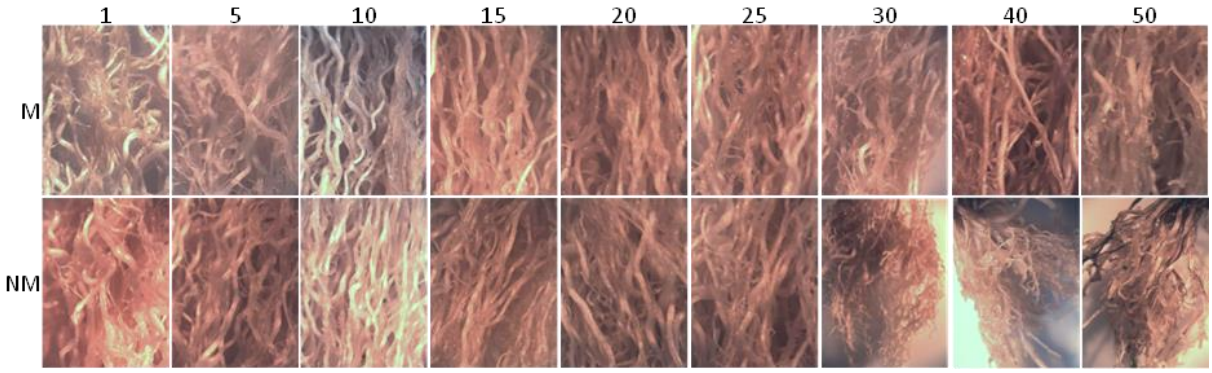
Replicates of NM population



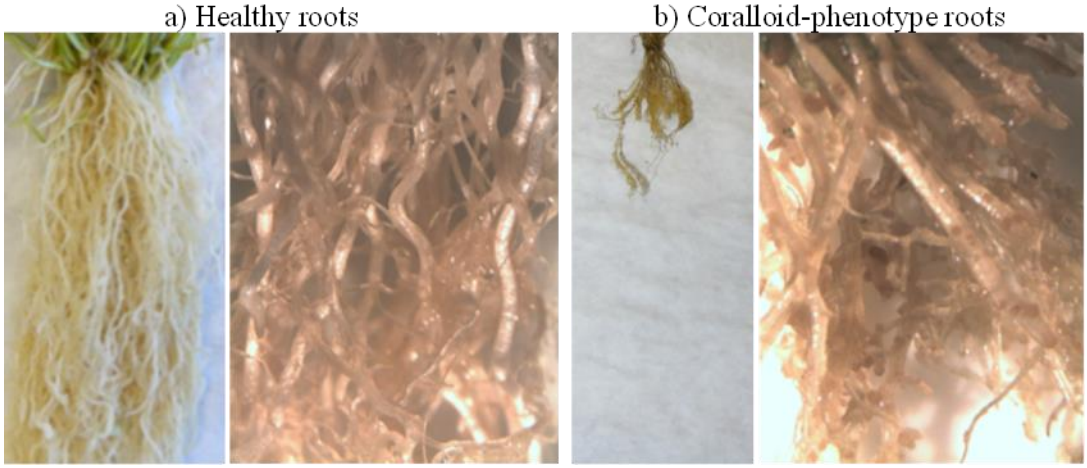
Replicates of both populations.

Annex 3 - Cu impacts on roots

Impacts of Cu exposure on roots of M and NM populations exposed to increasing Cu exposure (1-50 µM Cu). Pictures from binocular microscope.



Global and close aspect of a) healthy and b) impacted roots from M and NM populations of *Agrostis capillaris* respectively, exposed to 50 µM Cu.



Annex 4 - Mean values of growth parameters

Mean values of growth parameters ($\pm$ sd, n = 6) in a) roots and b) shoots with significant differences between M and NM population (Student's test) indicated by symbols near the highest mean for each comparison (0.001< *** < 0.001 < ** < 0.01 < * < 0.05 < # < 0.1 < ns < 1). FW: Fresh Weight; DW: Dry Weight in g; Lmean: Mean length of shoots in cm; Lmax: Maximal length of shoots in cm; [X]: Concentration of X in tissues in $mg.kg^{-1}$ DW, Cu: Copper, Al: Aluminum; B: Bore; Ca: Calcium; Fe: Iron; Mg: Magnesium; Mn: Manganese; P: Phosphorus; K: Potassium; Na: Sodium; Zn: Zinc; r: roots and s: shoots.

Growth parameters in roots

Cond.	FWr	DWr	[Cu]r	[Al]r	[B]r	[Ca]r	[Fe]r	[Mg]r	[Mn]r	[P]r	[K]r	[Na]r	[Zn]r
M1	**1.13** ns ± 0.22	**0.14** ns ± 0.03	**12.01** ± 1.6	**125.41** ± 17.21	**3.6** ± 0.8	**2204** ± 421	**85.47** ns ± 27.31	**1440** ± 0.370	**23.14** ± 4.95	**1663** ± 384	**16731** ± 6165	**1497** ns ± 593	**17.92** ± 3.16
M5	**1.32** ns ± 0.35	**0.15** # ± 0.03	**39.44** ns ± 11.36	**137.78** ns ± 43.77	**5.92** ± 1.75	**2203** ± 374	**96.01** ns ± 24.15	**1415** ± 0.154	**26.69** ns ± 13.24	**2156** ns ± 559	**20110** ± 5461	**1379** ± 267	**19.38** ± 7.05
M10	**1.21** * ± 0.26	**0.14** * ± 0.03	**60.33** ± 20.59	**105.66** ± 22.26	**6.05** ± 2.54	**2071** ± 333	**68.09** ± 15.88	**1526** ± 0.275	**26.83** ns ± 4.78	**2092** ± 421	**20550** ± 5400	**1617** ns ± 541	**18.44** ± 2.99
M15	**1.45** ** ± 0.29	**0.16** * ± 0.03	**89.24** ± 31.37	**113.92** ns ± 26.35	**5.2** ± 1.19	**2257** ± 428	**71.83** ± 8.3	**1627** ns ± 0.388	**32.56** ns ± 26.04	**1949** ± 668	**23052** 8697	**1283** ns ± 240	**17.15** ± 3.53
M20	**1.19** * ± 0.38	**0.13** * ± 0.03	**142.01** ± 37.15	**100.41** ± 16.64	**4.1** ± 1.54	**2652** ± 672	**69.22** ± 20.14	**1552** ± 0.405	**26** ± 13.83	**2090** ± 454	**31183** ns ± 6970	**1082** ns ± 128	**20.35** ± 11.2
M25	**1.55** ** ± 0.12	**0.17** * ± 0.02	**157.17** ± 43.34	**92.26** ± 15.67	**6.11** ± 2.44	**2600** ± 622	**70.24** ± 25.48	**1640** ± 0.295	**25.5** ± 15.74	**2069** ± 374	**28920** ns ± 3607	**1192** * ± 216	**20.34** ± 6.64
M30	**1.52** ** ± 0.35	**0.17** ** ± 0.04	**167.94** ± 44.15	**89.08** ± 17.24	**5.8** ± 2.73	**2263** ± 309	**61.63** ± 12.5	**1614** ± 0.295	**24.1** ± 13.56	**2075** ± 716	**26145** ns ± 8931	**1260** ** ± 176	**18.42** ± 5.84
M40	**1.61** ** ± 0.44	**0.17** * ± 0.04	**365.46** ± 165.12	**112.37** ns ± 68.24	**7.94** ± 4.75	**2483** ± 354	**70.81** ± 11.39	**1868** ns ± 0.522	**57.12** ± 52.1	**2727** ± 1059	**33173** ns ± 13366	**1242** # ± 172	**20.32** ± 6.73
M50	**1.59** ** ± 0.6	**0.17** ** ± 0.07	**542.7** ± 249.15	**79.66** ± 19.66	**9.81** ± 5.59	**2896** ± 791	**76.08** ± 27.91	**2191** ± 0.753	**70.41** ± 63.22	**3559** ± 1409	**35331** * ± 10749	**1155** ± 205	**31.63** ± 7.64
NM1	**0.97** ± 0.03	**0.12** ± 0.01	**12.05** ns ± 2.73	**120.69** ± 20.06	**9.12** ns ± 6.32	**2707** ns ± 941	**82.9** ± 20.82	**1529** ns ± 0.458	**26.64** ns ± 5.83	**2262** ns ± 862	**20304** ns ± 5538	**1395** ± 277	**21.01** ns ± 9.66
NM5	**1** ± 0.18	**0.12** ± 0.02	**30.46** ± 8.86	**122.64** ± 40.64	**6.82** ns ± 2.57	**2539** ns ± 507	**89.59** ± 18.72	**1511** ns ± 0.380	**25.02** ± 9.76	**1974** ± 553	**20491** ns ± 8999	**1499** ns ± 508	**19.59** ns ± 6.73
NM10	**0.78** ± 0.12	**0.09** ± 0.02	**77.93** ns ± 26.49	**107.65** ns ± 28.96	**8.8** ns ± 6.99	**2559** ns ± 696	**84.87** ns ± 28.35	**1601** ns ± 0.375	**24.65** ± 5.16	**2242** ns ± 488	**21361** ns ± 6420	**1268** ± 405	**24.25** ns ± 10.2
NM15	**0.82** ± 0.17	**0.1** ± 0.04	**157.71** ns ± 80.85	**109.37** ± 35.65	**5.73** ns ± 2.5	**2418** ns ± 352	**81.22** ns ± 24.4	**1450** ± 0.282	**24.79** ± 7.53	**2350** ns ± 828	**24302** ns ± 8945	**1111** ± 382	**21.79** ns ± 4.55
NM20	**0.73** ± 0.2	**0.08** ± 0.02	**171.93** ns ± 34.24	**108.22** ns ± 24.26	**9.92** ns ± 12.84	**2808** ns ±510	**76.13** ns ± 16.47	**1595** ns ± 0.386	**26.01** ns ± 8.63	**2371** ns ± 523	**24996** ± 3030	**977** ± 283	**24.38** ns ± 10.25
NM25	**0.92** ± 0.33	**0.1** ± 0.04	**270.79** * ± 70.73	**107.5** ns ± 20.28	**8.22** ns ± 3.88	**3021** ns ± 451	**85.53** ns ± 25.56	**1770** ns ± 0.227	**28.52** ns ± 8.56	**2752** # ± 616	**26237** ± 3284	**831** ± 71	**24.49** ns ± 8.06
NM30	**0.61** ± 0.15	**0.08** ± 0.01	**311.99** * ± 98.16	**98.8** ns ± 4.36	**6.16** ns ± 3.38	**2696** ns ± 709	**103.59** ns ± 86.82	**1687** ns ± 0.517	**36.13** ns ± 19.24	**2837** ns ± 1248	**22465** ± 6030	**927** ± 135	**19.97** ns ± 5.48
NM40	**0.53** ± 0.37	**0.08** ± 0.06	**612.32** ns ± 275.36	**96.11** ± 29.67	**10.08** ns ± 3.65	**4076** ns ± 2 472	**84.89** ns ± 27.1	**1846** ± 0.732	**110.11** ns ± 74.08	**4101** ns ± 2283	**22031** ± 6997	**1006** ± 206	**33.01** ns ± 14.34
NM50	**0.23** ± 0.17	**0.03** ± 0.02	**839.13** ns ± 295.72	**100.24** # ± 15.59	**19.78** # ± 8.94	**7318** * ± 2848	**107.36** # ± 25.37	**2717** ns ± 0.725	**255.37** ns ± 247.2	**4157** ns ± 837	**20513** ± 4093	**1283** ns ± 203	**52.95** # ± 19.57

Growth parameters in shoots

Cond.	FWs	DWs	Lmean	Lmax	[Cu]s	[Al]s	[B]s	[Ca]s	[Fe]s	[Mg]s	[Mn]s	[P]s	[K]s	[Na]s	[Zn]s
M1	**1.4** ± 0.15	**0.42** ns ± 0.05	**30.5** ± 1.9	**48.67** ± 3.5	**7.65** ± 0.85	**22.67** ± 4.13	**14.2** ± 2.84	**3522** ± 440	**63.72** ± 22.21	**2434** ± 211	**45.98** ± 3.62	**2185** ± 225	**22356** ± 2515	**392.66** ± 149.94	**8.07** ± 1.54
M5	**1.98** ns ± 0.94	**0.52** ns ± 0.13	**33.83** ± 3.5	**50.75** ns ± 6.8	**15.25** # ± 1.67	**18.58** ns ± 2.52	**17.77** ± 10.45	**4266** ± 958	**61.62** ± 5.95	**2868** ± 599	**67.64** ns ± 20.32	**2807** ns ± 624	**28320** ns ± 5381	**351.6** ± 47.99	**10.06** ns ± 2.45
M10	**2.2** # ± 0.79	**0.52** * ± 0.13	**34** ± 4.2	**46.58** ns ± 7.4	**18.67** ns ± 1.9	**21.58** ± 5.7	**19.19** ± 7.07	**3966** ± 525	**53.87** ± 12.11	**2783** ± 440	**56.01** ± 17.46	**2823** ± 641	**27277** ns ± 4859	**322.31** ± 65.18	**9.08** ns ± 2.63
M15	**2.23** ns ± 1.04	**0.6** * ± 0.19	**33.42** * ± 3.1	**59.25** # ± 10.6	**20.67** ns ± 2.37	**20.49** ± 6.21	**27.56** ns ± 8.2	**4722** ± 773	**52.25** ± 15.04	**2976** ns ± 607	**65.23** ± 21.72	**2712** ± 878	**26986** ns ± 7648	**356.38** ± 101.4	**9** ± 2.2
M20	**1.95** # ± 0.74	**0.45** ** ± 0.09	**31.5** # ± 2.9	**52.92** * ± 5.9	**22.95** * ± 2.72	**19.56** ± 3.84	**23.3** ± 3.86	**4938** ± 460	**48.56** ± 12.72	**3185** ± 255	**60.04** ± 9.26	**3086** ± 584	**29411** ns ± 3091	**378.18** ± 104.85	**7.48** ± 0.89
M25	**1.98** * ± 0.31	**0.46** ** ± 0.08	**31.83** * ± 3	**51.67** # ± 9.5	**26.37** * ± 2.38	**18.38** ± 2.83	**28.71** ± 4.5	**5929** ± 746	**56.58** ns ± 8.53	**3622** ± 258	**65.87** ± 9.94	**3392** ± 746	**30778** ns ± 4694	**455.77** ± 60.8	**8.8** ± 1.98
M30	**2.01** *** ± 0.43	**0.48** *** ± 0.11	**29.33** ** ± 3.1	**46.25** ** ± 7.8	**24.58** ± 1.88	**19.17** ± 4.58	**28.83** ± 11.86	**5211** ± 925	**50.19** ± 10.09	**3393** ± 484	**67.42** ± 18.11	**3205** ± 708	**27727** ± 4105	**361.73** ± 53.29	**8.38** ± 1.86
M40	**2.43** * ± 1.07	**0.48** ** ± 0.15	**27.17** *** ± 2.8	**37.83** # ± 6	**29.88** * ± 3.3	**24.37** ± 7.11	**42.24** ± 11.71	**6768** ± 776	**43.97** ns ± 3.07	**4269** ± 845	**128.12** ± 48.63	**5262** ± 2269	**37231** ns ± 9737	**428.58** ± 76.34	**13.51** ± 5.05
M50	**2.34** ** ± 0.82	**0.49** ** ± 0.19	**26.33** *** ± 4.5	**42.33** *** ± 8.3	**35.13** ns ± 2.77	**31.59** ns ± 3.55	**44.65** ± 16.32	**6941** ± 545	**45.16** ns ± 5.94	**4782** ± 679	**131.6** ± 58.56	**5340** ± 1585	**39893** ± 9131	**490.02** ± 98.52	**14.69** ± 4.2
NM1	**1.49** ns ± 0.33	**0.42** ± 0.03	**35.42** ** ± 2.2	**52.42** ns ± 5.6	**8.71** ns ± 1.47	**24.76** ns ± 11.78	**21.22** ns ± 12.8	**4295** * ± 567	**74.27** ns ± 15.21	**2600** ns ± 602	**51.46** ns ± 6.06	**2601** ns ± 511	**24538** ns ± 5163	**555.72** # ± 124	**11.75** ns ± 4.29
NM5	**1.44** ± 0.3	**0.4** ± 0.04	**34.08** ns ± 4.1	**47.58** ± 5.7	**13.31** ± 1.68	**16.96** ± 4.06	**21.94** ns ± 7.43	**4654** ns ± 378	**72.07** ns ± 11.78	**2896** ns ± 448	**55.44** ± 8.09	**2609** ± 446	**24591** ± 3902	**586.27** ** ± 127.1	**9.01** ± 1.34
NM10	**1.29** ± 0.39	**0.3** ± 0.05	**30** ± 3.8	**43.75** ± 6.2	**16.96** ± 1.7	**21.69** ns ± 7.22	**26** ns ± 6.74	**5098** ** ± 433	**63.36** ns ± 13.56	**3072** ns ± 408	**58.43** ns ± 8.9	**2918** ns ± 488	**26265** ± 4102	**520.3** ** ± 92.89	**8.7** ± 1.17
NM15	**1.33** ± 0.26	**0.33** ± 0.08	**28.83** ± 3.1	**47.5** ± 6	**19.85** ± 1.82	**36.47** ns ± 34.88	**22.12** ± 3.31	**5062** ns ± 419	**73.69** ns ± 39.07	**2779** ± 305	**66.51** ns ± 11.39	**3181** ns ± 645	**25840** ± 3719	**469.91** # ± 30.58	**12.15** ns ± 6.67
NM20	**1.18** ± 0.37	**0.26** ± 0.05	**42.83** ± 4.3	**42.83** ± 4.3	**19.02** ± 2.18	**24.32** ns ± 6.13	**27.87** ns ± 6.28	**5598** # ± 657	**51.83** ns ± 11.09	**3276** ns ± 495	**65.99** ns ± 9.25	**3332** ns ± 610	**28432** ± 4498	**498.07** ns ± 137.72	**9.85** # ± 2.19
NM25	**1.23** ± 0.46	**0.27** ± 0.08	**26.67** ± 2.5	**41.17** ± 4.1	**22.12** ± 2.54	**38.85** ns ± 37.96	**29.67** ns ± 10.03	**6338** ns ± 815	**50.09** ± 6.18	**3941** ns ± 454	**88.16** # ± 22.01	**4064** ns ± 916	**30085** ± 3912	**582.14** * ± 78.39	**11.9** ns ± 3.69
NM30	**0.89** ± 0.28	**0.17** ± 0.03	**21.17** ± 2.5	**30.92** ± 3.6	**25.14** ns ± 1.49	**27.71** * ± 5.12	**36.19** ns ± 14.52	**7243** ** ± 1072	**56.28** ns ± 9.23	**4232** * ± 551	**108.13**# ± 37.55	**4740** # ± 1317	**32882** ns ± 6105	**634.7** ** ± 123.51	**12.48** ** ± 2.18
NM40	**0.58** ± 0.33	**0.16** ± 0.09	**17.5** ± 3.3	**29.92** ± 6.6	**23.92** ± 3.29	**18.6** ns ± 8.77	**62.58** ns ± 36.52	**9414** # ± 2599	**35.25** ± 10.07	**5325** ns ± 1581	**174.66**ns ± 94.53	**5570** ns ± 2605	**34420** ± 11587	**912.04** * ± 322.16	**15.01** ns ± 6.61
NM50	**0.18** ± 0.12	**0.06** ± 0.03	**10.83** ± 3	**20** ± 5.4	**32.58** ± 5.02	**21.31** ± 12.46	**88.43** * ± 32.52	**15040**** ± 3713	**43.99** ± 29.79	**7860** ** ± 1305	**242.24**# ± 91.77	**7770** * ± 1168	**46043** ns ± 8647	**1781.44*** ± 902.21	**23.6** * ± 5.91

Annex 5 - Student's tests on growth parameters

P-values of Student's tests applied at each Cu exposure to estimate the differences between M and NM populations exposed to 1-50 µM Cu and referring to Annex 4; alpha = 10%.

Cu (µM)	1	5	10	15	20	25	30	40	50
FWr (g)	0.18	0.11	**0.012**	**0.003**	**0.049**	**0.007**	**0.001**	**0.002**	**0.003**
FWs (g)	0.60	0.26	**0.052**	0.11	**0.072**	**0.014**	**0.0009**	**0.011**	**0.002**
DWr (g)	0.20	**0.091**	**0.017**	**0.013**	**0.017**	**0.017**	**0.005**	**0.022**	**0.006**
DWs (g)	0.92	0.13	**0.014**	**0.021**	**0.004**	**0.005**	**0.0009**	**0.003**	**0.004**
Lmean (cm)	**0.004**	0.92	0.15	**0.042**	**0.050**	**0.015**	**0.001**	**0.0006**	**0.0002**
Lmax (cm)	0.24	0.44	0.53	**0.064**	**0.012**	**0.058**	**0.005**	**0.074**	**0.0008**
[Cu]r (mg/kg)	0.98	0.20	0.27	0.12	0.22	**0.015**	**0.020**	0.12	0.12
[Cu]s (mg/kg)	0.20	**0.097**	0.16	0.55	**0.0314**	**0.0213**	0.62	**0.0170**	0.35
[Al]r (mg/kg)	0.70	0.58	0.91	0.82	0.57	0.22	0.27	0.64	**0.098**
[Al]s (mg/kg)	0.72	0.47	0.98	0.36	0.18	0.28	**0.0197**	0.28	0.13
[B]r (mg/kg)	0.11	0.53	0.44	0.68	0.36	0.33	0.85	0.44	**0.066**
[B]s (mg/kg)	0.28	0.49	0.15	0.21	0.20	0.85	0.40	0.28	**0.0296**
[Ca]r (mg/kg)	0.31	0.26	0.20	0.53	0.69	0.25	0.25	0.21	**0.016**
[Ca]s (mg/kg)	**0.0383**	0.43	**0.0042**	0.41	**0.0993**	0.43	**0.0096**	**0.0728**	**0.0043**
[Fe]r (mg/kg)	0.87	0.65	0.28	0.45	0.57	0.37	0.33	0.32	**0.094**
[Fe]s (mg/kg)	0.40	0.12	0.27	0.29	0.67	0.20	0.34	0.11	0.93
[Mg]r (mg/kg)	0.74	0.62	0.73	0.43	0.87	0.45	0.79	0.96	0.29
[Mg]s (mg/kg)	0.58	0.94	0.31	0.54	0.73	0.21	**0.0287**	0.23	**0.0019**
[Mn]r (mg/kg)	0.33	0.82	0.50	0.55	1.00	0.72	0.28	0.22	0.16
[Mn]s (mg/kg)	0.12	0.26	0.79	0.91	0.33	**0.0781**	**0.0642**	0.36	**0.0508**
[P]r (mg/kg)	0.20	0.62	0.62	0.42	0.39	**0.066**	0.27	0.26	0.44
[P]s (mg/kg)	0.14	0.58	0.80	0.36	0.53	0.23	**0.0522**	0.85	**0.0217**
[K]r (mg/kg)	0.36	0.94	0.83	0.83	0.11	0.25	0.47	0.14	**0.026**
[K]s (mg/kg)	0.42	0.24	0.73	0.77	0.70	0.81	0.15	0.69	0.30
[Na]r (mg/kg)	0.74	0.65	0.28	0.42	0.47	**0.012**	**0.008**	**0.079**	0.34
[Na]s (mg/kg)	**0.0915**	**0.0074**	**0.0036**	**0.0542**	0.15	**0.0183**	**0.0029**	**0.0191**	**0.0237**
[Zn]r (mg/kg)	0.52	0.96	0.27	0.104	0.57	0.40	0.67	0.12	**0.061**
[Zn]s (mg/kg)	0.12	0.43	0.78	0.35	**0.0620**	0.14	**0.0097**	0.69	**0.0225**

Annex 6 - Correlations and models for growth parameters

Pearson's correlations between growth parameter and Cu exposure and models fitting set of data. Significance symbols refer to 0.001< *** < 0.001 < ** < 0.01 < * < 0.05 < # < 0.1 < ns < 1. CorP: r coefficient and significance of Pearson's Correlation. Regression: (R^2) Type of model and significance of each variable tested (Cu √Cu Cu^2 Cu^3. or LnCu). Model types = Lin: Linear; Log: Logarithm; SqR: Square root; Squ: Square; P: Polynomial model degree 2; P2: Polynomial model degree 3 and for these two last, significances are indicated in a decreasing order (Cu^3/Cu^2/Cu and Cu^2/Cu).

	CorP. (M)	Regression (M)	Model equation (M)	CorP. (NM)	Regression (NM)	Model equation (M)
FWr	0.36 **	(0.14) SqR. **	$FWr_M = 0.08 \sqrt{Cu} + 1.05$	-0.66 ***	(0.46) Squ. ***	$FWr_{NM} = - 0.0003 Cu^2 + 0.92$
FWs	0.23 #	-	-	-0.75 ***	(0.35) Log. ***	$FWs_{NM} = - 0.27 \ln(Cu) + 1.78$
DWr	0.22 ns	-	-	-0.56 ***	-	-
DWs	-0.02 ns	-	-	-0.86 ***	(0.67) Squ. ***	$DWs_{NM} = - 0.0001 Cu^2 + 0.36$
L_{mean}	-0.52 ***	(0.37) P2 ***/#/*	$L_{meanM} = 0.0004Cu^3 - 0.03Cu^2 + 0.58Cu + 30.66$	-0.91 ***	(0.84) Lin. ***	$L_{meanNM} = - 0.48 Cu + 36.35$
L_{max}	-0.36 **	(0.26) P2 **/#/*	$L_{maxM} = 0.001Cu^3 - 0.08Cu^2 + 1.62Cu + 44.91$	-0.84 ***	(0.71) Lin. ***	$L_{maxNM} = - 0.62Cu + 53.04$
[Cu]r	0.81 ***	-	-	0.85 ***	-	-
[Cu]s	0.92 ***	(0.9) P2 ***/**/***	$[Cu]_M = 0.0005Cu^3 - 0.05Cu^2 + 1.52Cu + 7.18$	0.89 ***	(0.84) P2 ***/#/***	$[Cu]_{NM} = 0.0005Cu^3 - 0.04Cu^2 + 1.31Cu + 7.52$
[Al]r	-0.36 **	-	-	-0.27 *	(0.07) Log. *	$[Al]_{NM} = - 6.55 \ln Cu + 125.25$
[Al]s	0.40 **	(0.36) P2 ***/***	$[Al]_M = 0.01 Cu2 - 0.45 Cu + 22.92$	-0.02 ns	-	-
[B]r	0.42 **	-	-	0.34 *	-	-
[B]s	0.7 ***	-	-	0.69 ***	-	-
[Ca]r	0.34 *	(0.12) Lin. *	$[Ca]_M = 12.70 Cu + 2126.64$	0.58 ***	-	-
[Ca]s	0.81 ***	(0.66) Lin. ***	$[Ca]_M = 70.66 Cu + 3601.41$	0.82 ***	(0.66) Lin. ***	$[Ca]_{NM} = 191.86 Cu + 2792.93$
[Fe]r	-0.2 ns	(0.08) Log. *	$[Fe]_M = -5.47 \ln(Cu) + 88.86$	0.15 ns	-	-
[Fe]s	-0.41 **	-	-	-0.49 ***	-	-
[Mg]r	0.44 ***	(0.22) Squ. ***	$[Mg]_M = 0.28 Cu^2 + 1456.15$	0.5 ***	(0.32) P */***	$[Mg]_{NM} = 0.76Cu^2 - 18.34Cu + 1606.74$
[Mg]s	0.78 ***	(0.61) Lin. ***	$[Mg]_M = 45.07 Cu + 2386.28$	0.83 ***	(0.68) Lin. ***	$[Mg]_{NM} = 96.27 Cu + 1901.24$
[Mn]r	0.38 **	-	-	0.52 ***	-	-
[Mn]s	0.62 ***	-	-	0.74 ***	-	-
[P]r	0.49 ***	(0.3) P */***	$[P]_M = 0.98 Cu2 - 19.68 Cu + 1999.54$	0.54 ***	-	-
[P]s	0.64 ***	(0.41) Lin. ***	$[P]_M = 63.72 Cu + 2036$	0.78 ***	(0.61) Lin. ***	$[P]_{NM} = 101.16 Cu + 1884.03$
[K]r	0.55 ***	(0.3) Lin. ***	$[K]_M = 365.83 Cu + 18165.75$	0.03 ns	(0.07) P. #/ns	$[K]_{NM} = - 7.62Cu^2 + 391.50Cu + 19392.67$
[K]s	0.58 ***	(0.34) Squ. ***	$[K]_M = 5.87 Cu^2 + 25842.23$	0.67 ***	(0.5) Squ. ***	$[K]_{NM} = 7.97Cu^2 + 24696.72$
[Na]r	-0.29 *	(0.09) Lin. *	$[Na]_M = - 94.61 Cu + 1551.34$	-0.26 #	(0.29) P ***/*	$[Na]_{NM} = 0.78Cu^2 - 45.45Cu + 1580.18$
[Na]s	0.36 **	-	-	0.6 ***	-	-
[Zn]r	0.39 **	(0.15) Lin. **	$[Zn]_M = 0.20 Cu + 16.17$	0.54 ***	(0.44) P2 #/**/***	$[Zn]_{NM} = 0.001Cu^3 - 0.05Cu^2 + 0.82Cu + 19.03$
[Zn]s	0.47 ***	(0.33) P2 **/***	$[Zn]_M = 0.005 Cu^2 - 0.16 Cu + 9.46$	0.58 ***	(0.43) Squ. ***	$[Zn]_{NM} = 0.005 Cu^2 + 9.2$

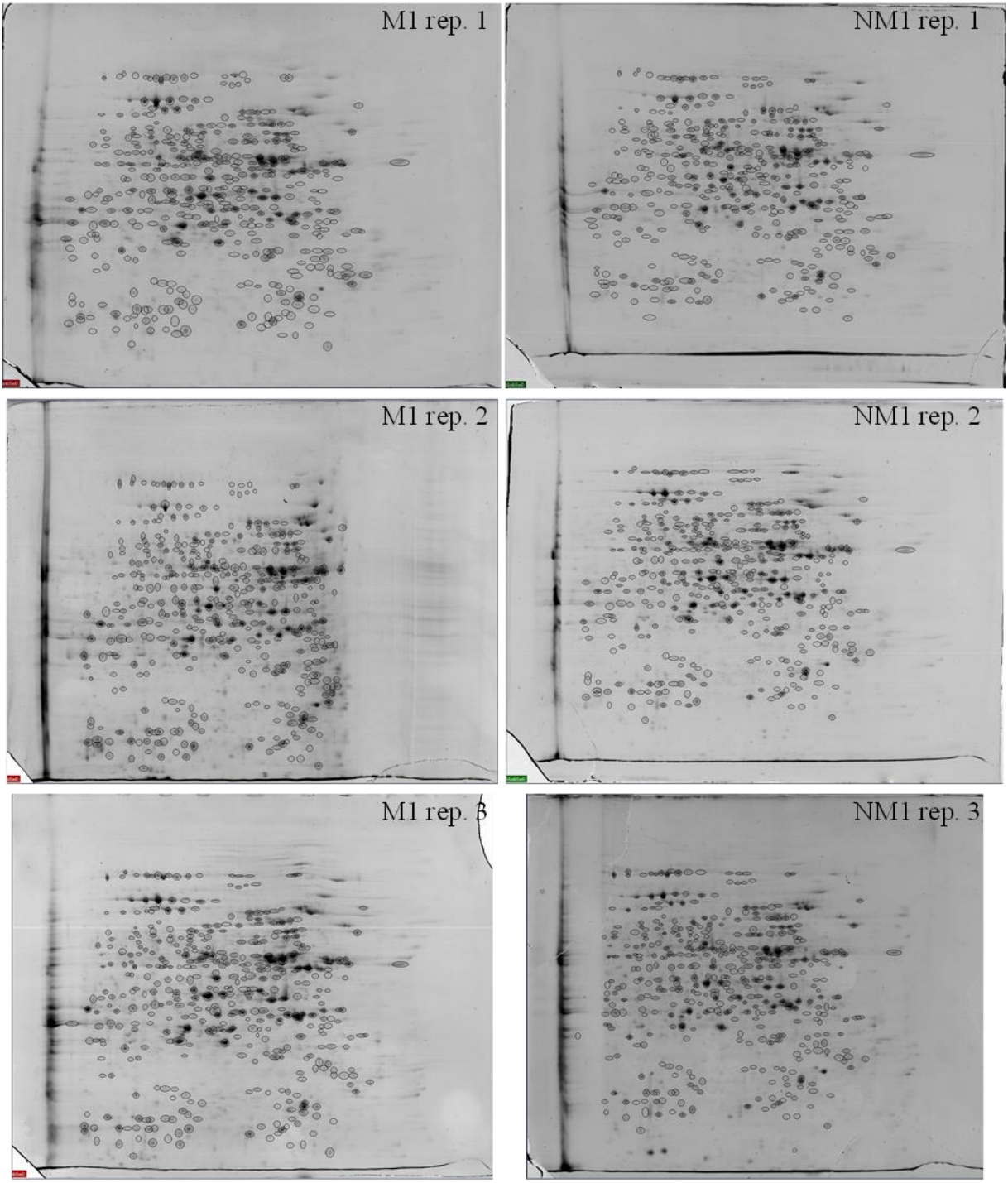
Annex 7 - Shoot / Root ratios

Shoot / Root ratios of growth parameters mean values (n = 6, list in legend of Tab. 2, with the exception of Length, available only for shoots) of both population (M, NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40 and 50 µM). Mean Shoot / Root ratios among all Cu exposure are indicated for each parameters and population at the end of the line.

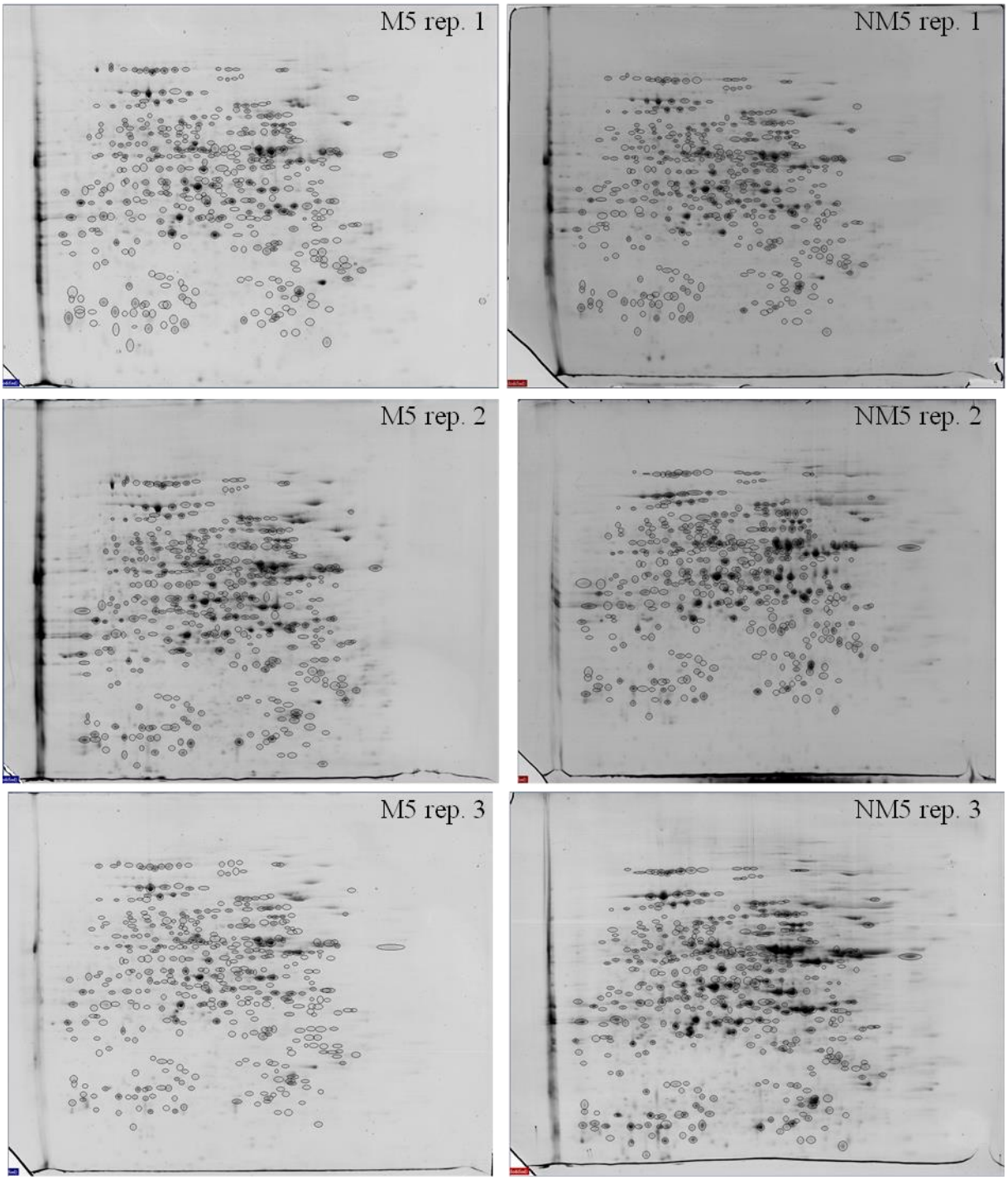
Cu exposure (µM)	1	5	10	15	20	25	30	40	50	Mean S/R
FW S/R (M)	1.25	1.51	1.82	1.54	1.64	1.28	1.32	1.51	1.47	**1.48**
FW S/R (NM)	1.53	1.44	1.66	1.63	1.61	1.33	1.46	1.09	0.76	**1.39**
DW S/R (M)	3.01	3.33	3.74	3.73	3.44	2.77	2.84	2.85	2.94	**3.18**
DW S/R (NM)	3.51	3.35	3.44	3.32	3.19	2.62	2.25	2	1.76	**2.83**
[Cu] S/R (M)	0.64	0.39	0.31	0.23	0.16	0.17	0.15	0.08	0.06	**0.24**
[Cu] S/R (NM)	0.72	0.44	0.22	0.13	0.11	0.08	0.08	0.04	0.04	**0.21**
[Al] S/R (M)	0.18	0.13	0.20	0.18	0.19	0.20	0.22	0.22	0.40	**0.21**
[Al] S/R (NM)	0.21	0.14	0.20	0.33	0.22	0.36	0.28	0.19	0.21	**0.24**
[B] S/R (M)	3.94	3.00	3.17	5.29	5.69	4.70	4.97	5.32	4.55	**4.52**
[B] S/R (NM)	2.33	3.22	2.96	3.86	2.81	3.61	5.87	6.21	4.47	**3.93**
[Ca] S/R (M)	1.60	1.94	1.91	2.09	1.86	2.28	2.30	2.73	2.40	**2.12**
[Ca] S/R (NM)	1.59	1.83	1.99	2.09	1.99	2.10	2.69	2.31	2.06	**2.07**
[Fe] S/R (M)	0.75	0.64	0.79	0.73	0.70	0.81	0.81	0.62	0.59	**0.72**
[Fe] S/R (NM)	0.90	0.80	0.75	0.91	0.68	0.59	0.54	0.42	0.41	**0.67**
[Mg] S/R (M)	1.69	2.03	1.82	1.83	2.05	2.21	2.10	2.29	2.18	**2.02**
[Mg] S/R (NM)	1.70	1.92	1.92	1.92	2.05	2.23	2.51	2.88	2.89	**2.22**
[Mn] S/R (M)	1.99	2.53	2.09	2.00	2.31	2.58	2.80	2.24	1.87	**2.27**
[Mn] S/R (NM)	1.93	2.22	2.37	2.68	2.54	3.09	2.99	1.59	0.95	**2.26**
[P] S/R (M)	1.31	1.30	1.35	1.39	1.48	1.64	1.54	1.93	1.50	**1.49**
[P] S/R (NM)	1.15	1.32	1.30	1.35	1.41	1.48	1.67	1.36	1.87	**1.43**
[K] S/R (M)	1.34	1.41	1.33	1.17	0.94	1.06	1.06	1.12	1.13	**1.17**
[K] S/R (NM)	1.21	1.20	1.23	1.06	1.14	1.15	1.46	1.56	2.24	**1.36**
[Na] S/R (M)	0.26	0.26	0.20	0.28	0.35	0.38	0.29	0.35	0.42	**0.31**
[Na] S/R (NM)	0.40	0.39	0.41	0.42	0.51	0.70	0.68	0.91	1.39	**0.65**
[Zn] S/R (M)	0.45	0.52	0.49	0.52	0.37	0.43	0.45	0.66	0.46	**0.49**
[Zn] S/R (NM)	0.56	0.46	0.36	0.56	0.40	0.49	0.63	0.45	0.45	**0.48**

Annex 8 - 2D-gels from roots soluble proteome

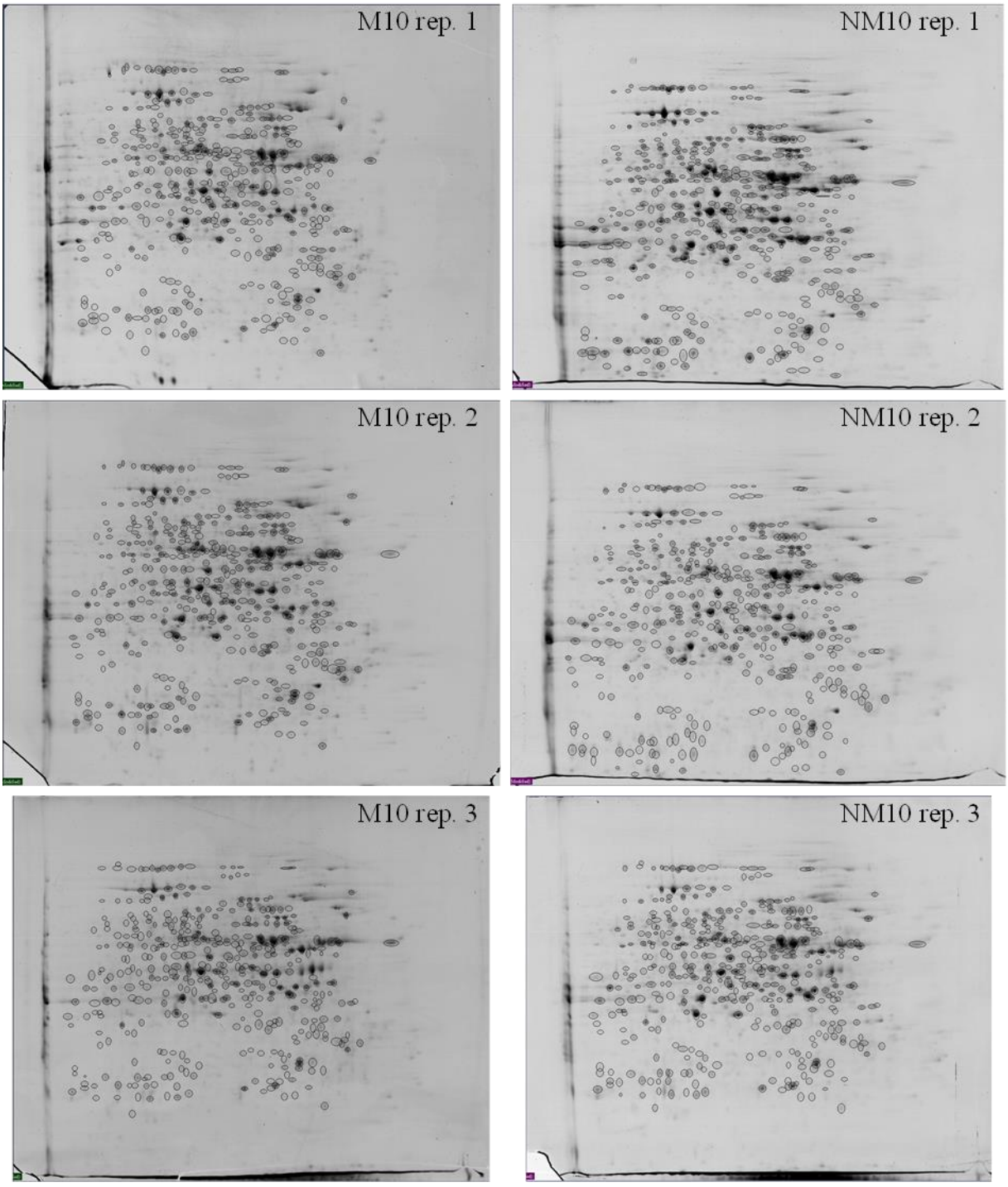
Distribution of soluble protein spots from *Agrostis capillaris* roots, for M and NM populations exposed to nine Cu concentrations (1, 5, 10, 15, 20, 25, 30, 40 and 50 µM). pI from 4 to 7.



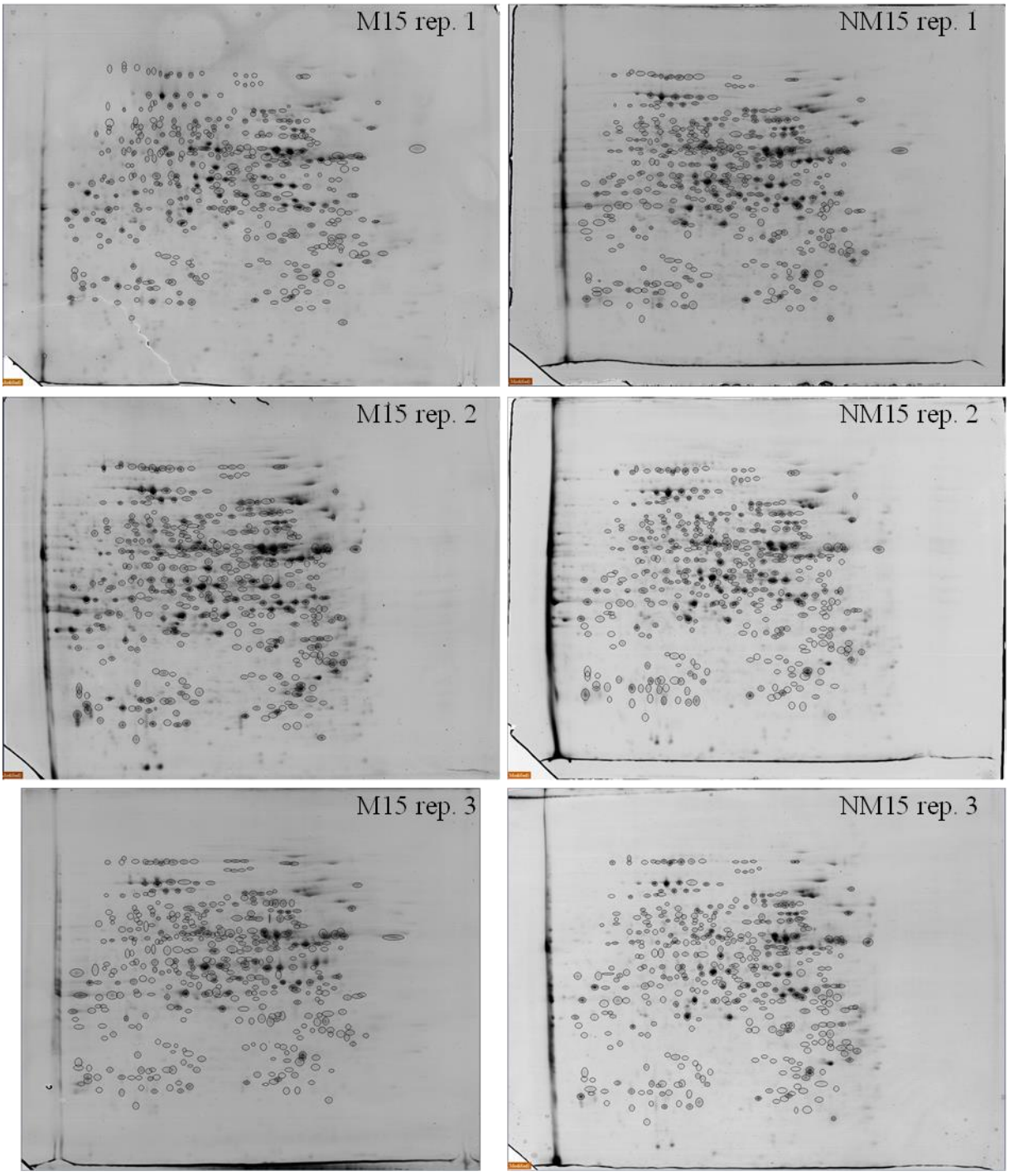
Root replicates at 1µM



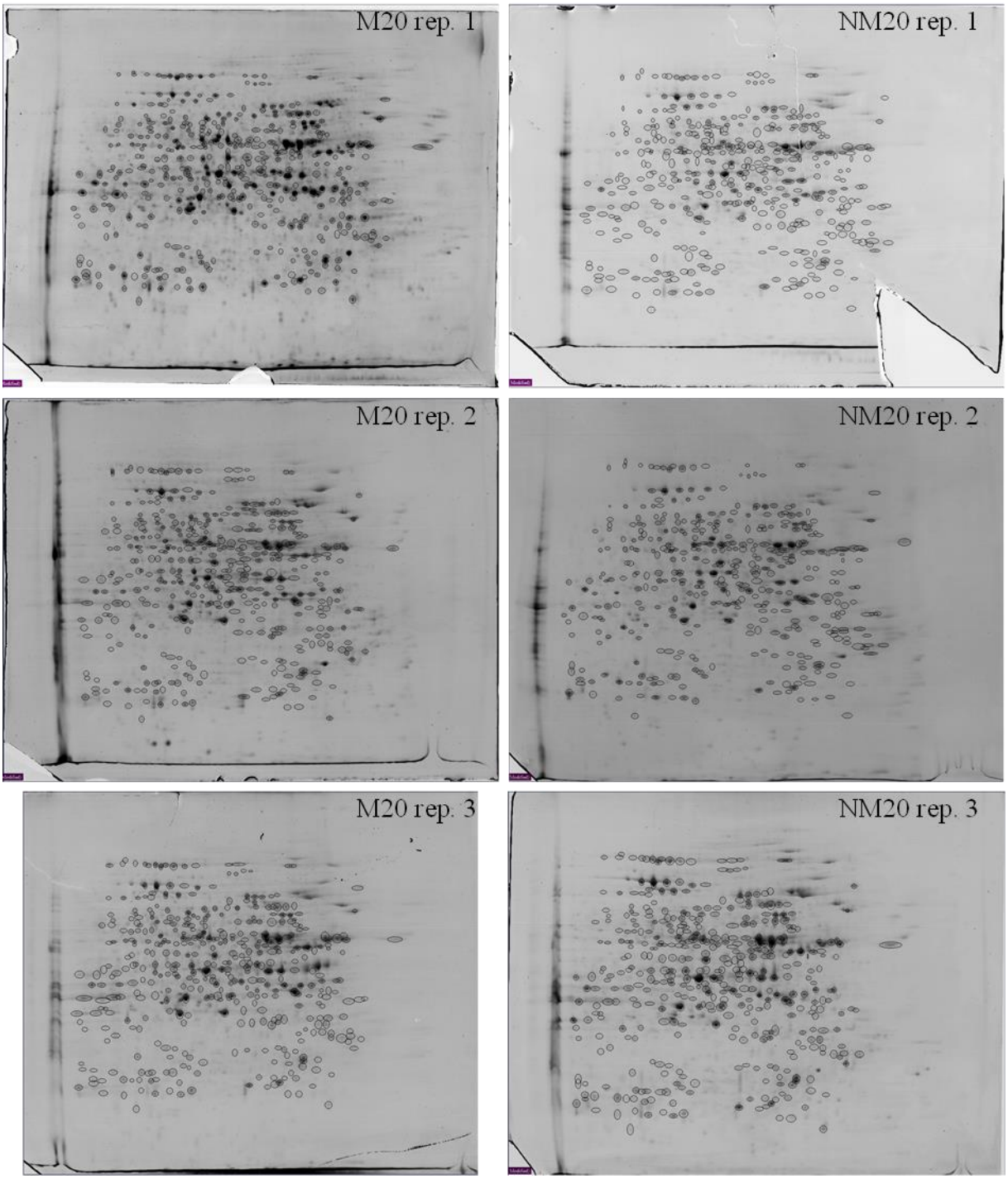
Root replicates at 5µM



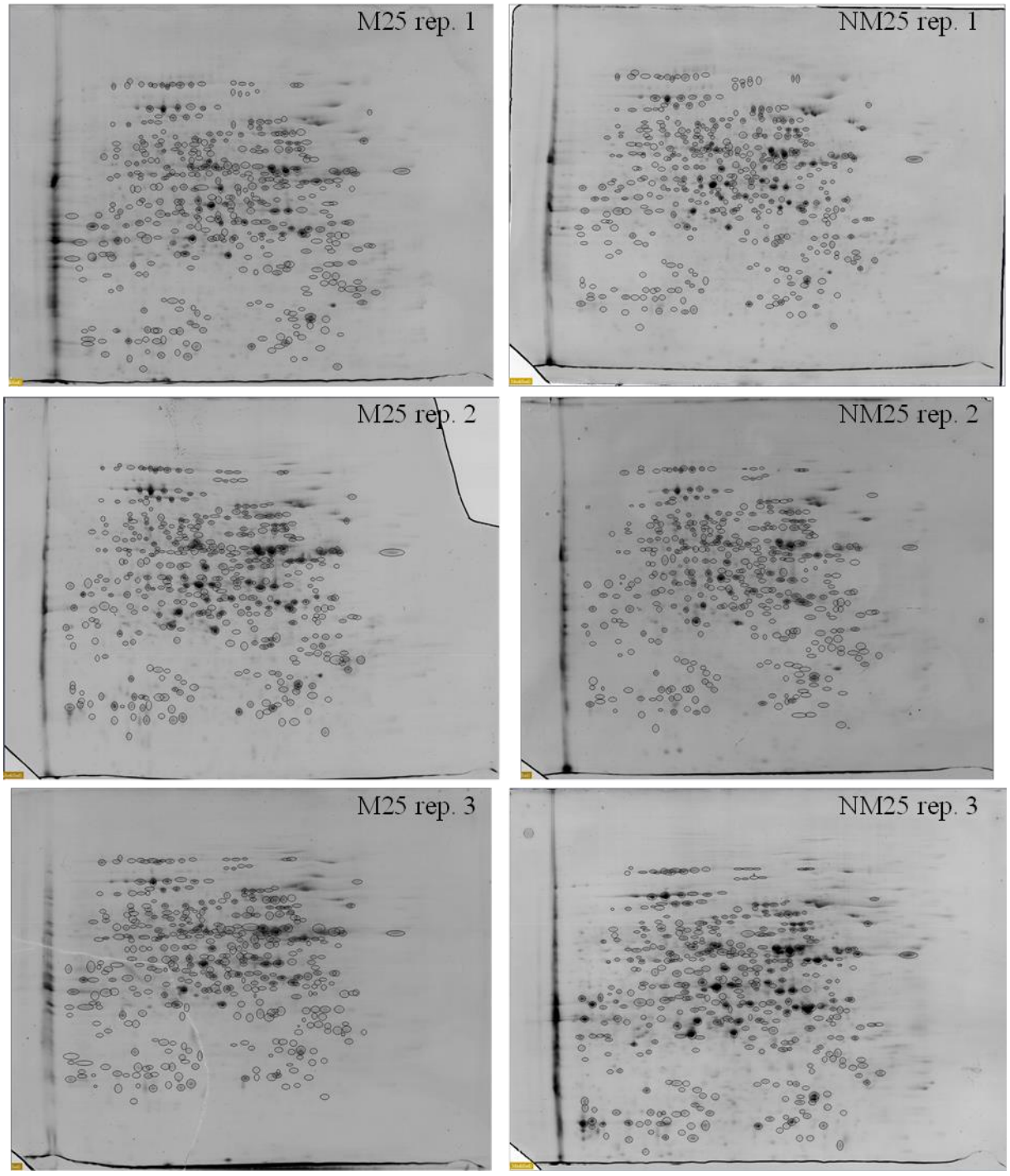
Root replicates at 10µM



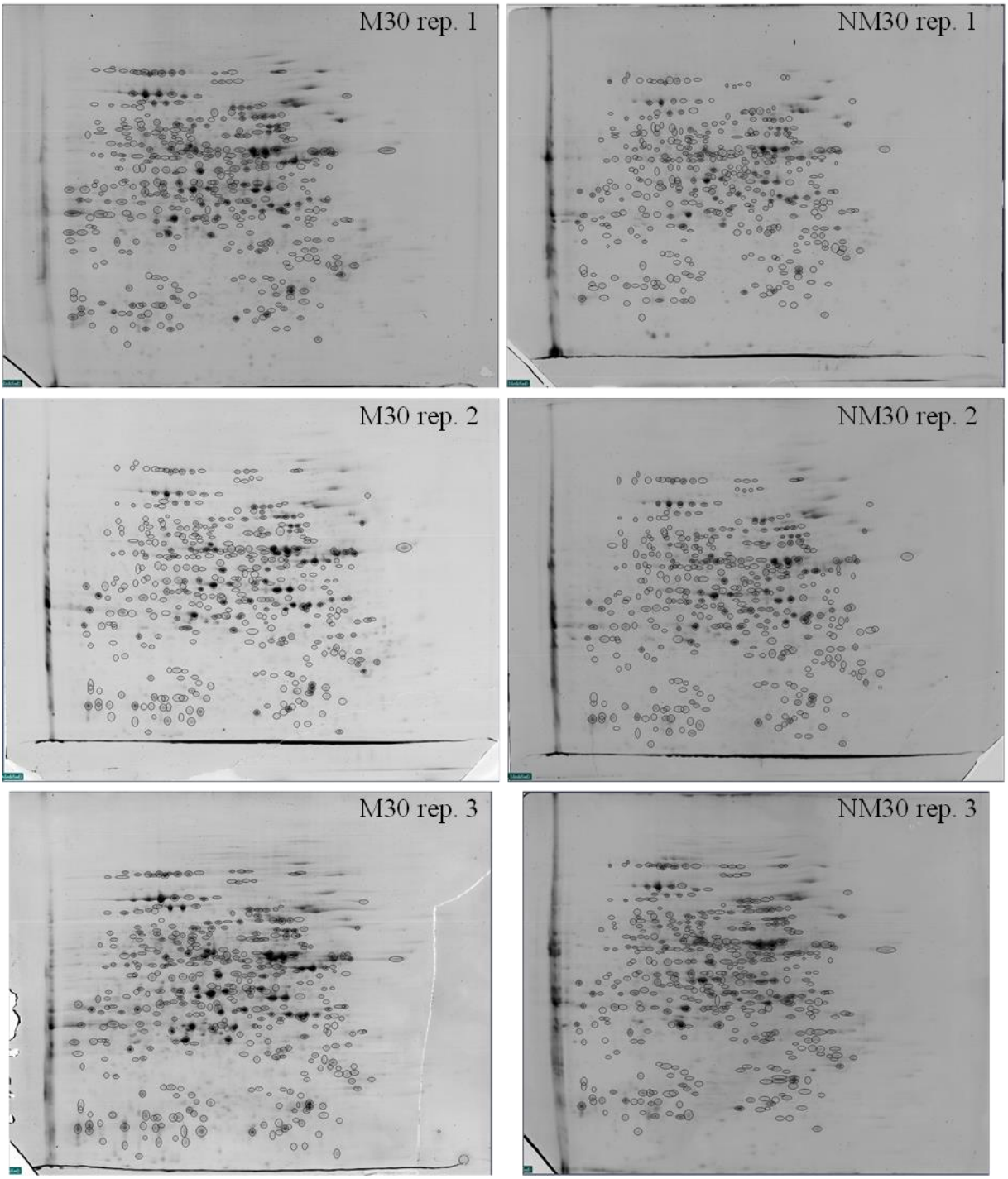
Root replicates at 15µM



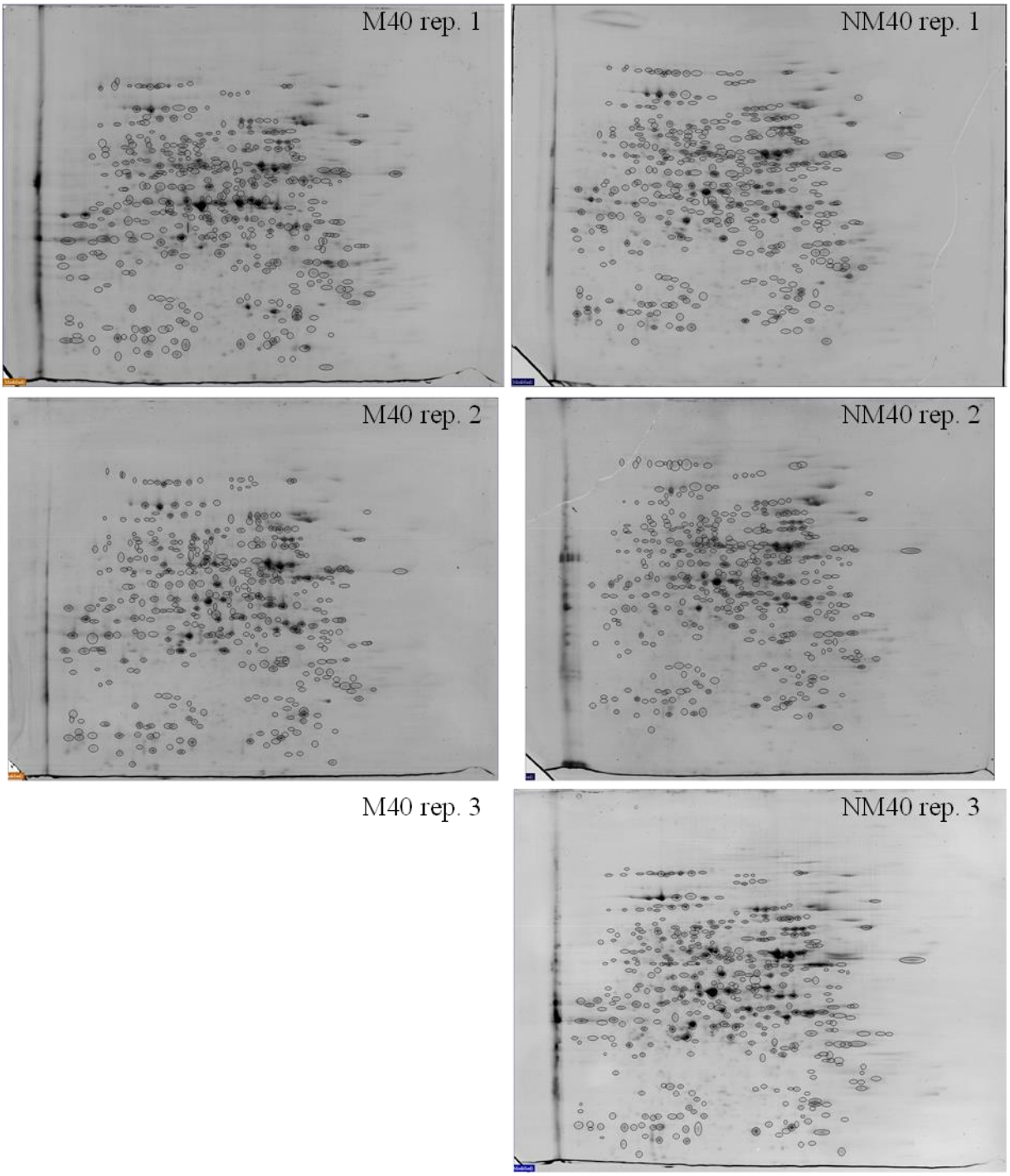
Root replicates at 20µM



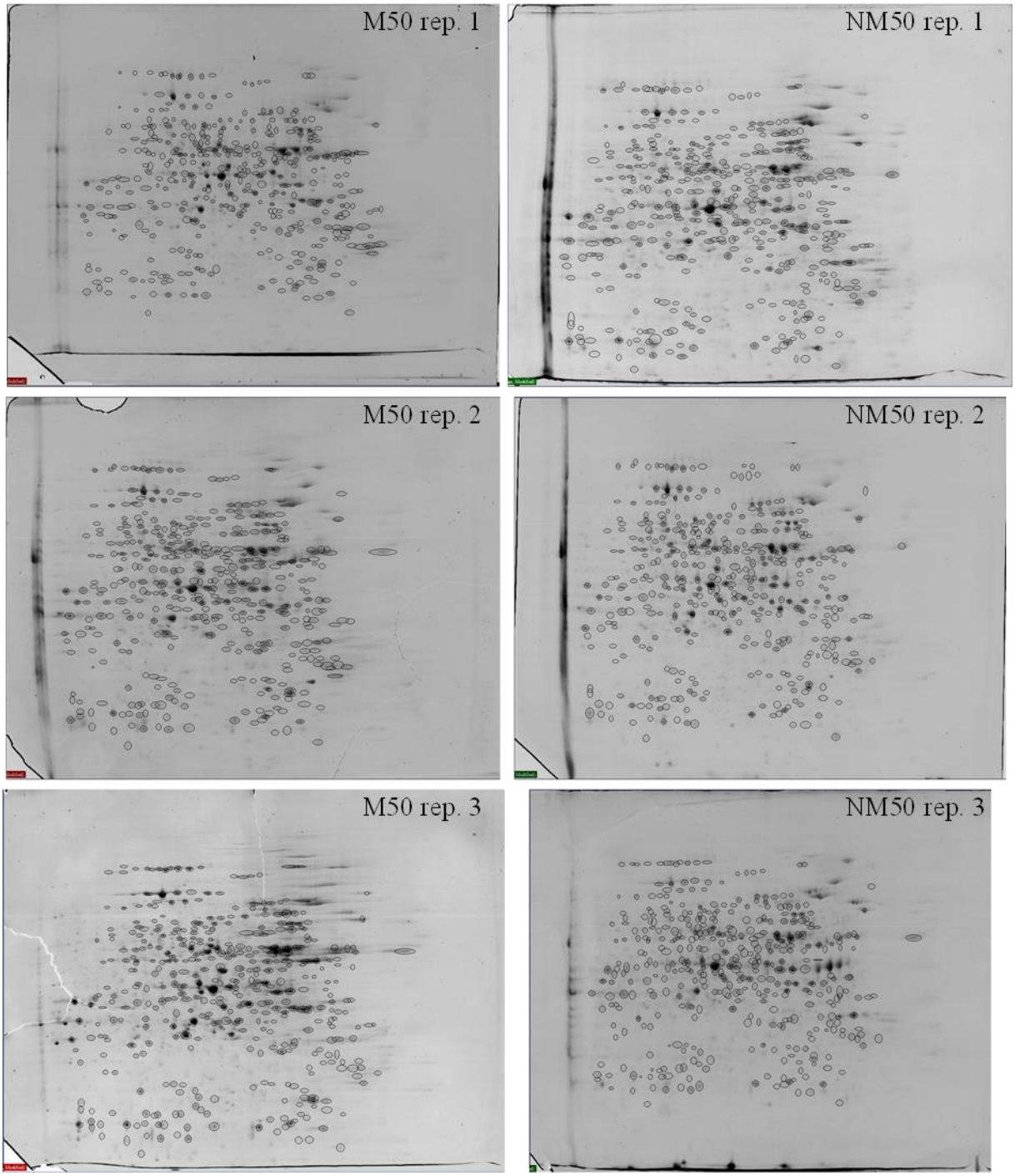
Root replicates at 25M



Root replicates at 30µM



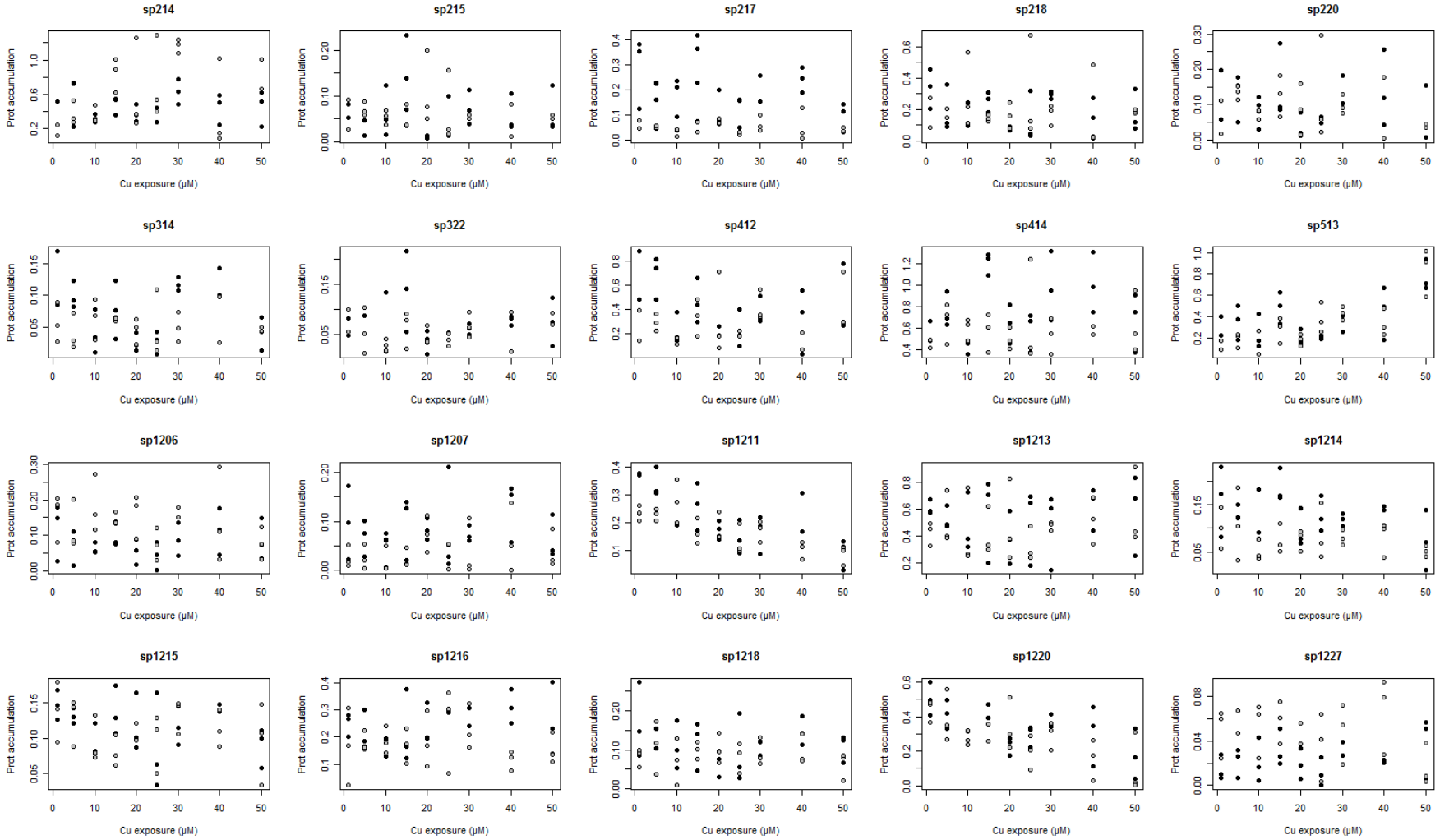
Root replicates at 40µM



Root replicates at 50µM

Annex 9 - Description of the 419 root spots

Spots 214 to 1227



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations.

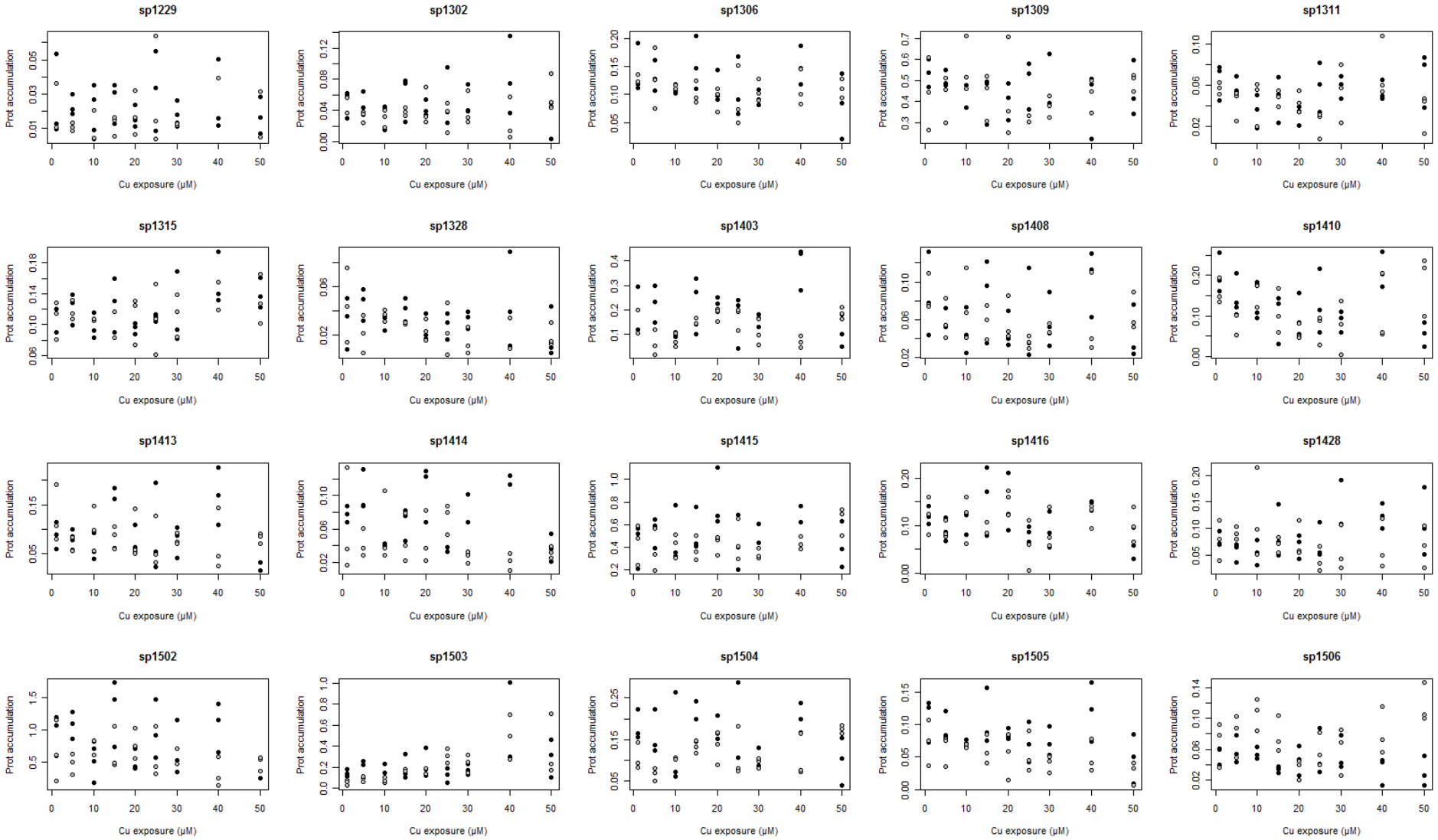
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
214	0.382 ± 0.191	0.176 ± 0.09	0.560 ± 0.289	0.373 ± 0.132	0.338 ± 0.055	0.364 ± 0.098	0.480 ± 0.106	0.842 ± 0.197	0.379 ± 0.1	0.630 ± 0.548	0.357 ± 0.118	0.741 ± 0.477	0.628 ± 0.147	1.164 ± 0.079	0.446 ± 0.176	0.416 ± 0.52	0.452 ± 0.203	0.833 ± 0.242
215	0.068 ± 0.021	0.059 ± 0.045	0.049 ± 0.038	0.071 ± 0.016	0.062 ± 0.056	0.054 ± 0.016	0.147 ± 0.082	0.051 ± 0.027	0.032 ± 0.039	0.109 ± 0.079	0.042 ± 0.049	0.066 ± 0.078	0.074 ± 0.037	0.056 ± 0.004	0.058 ± 0.041	0.046 ± 0.05	0.064 ± 0.051	0.055 ± 0.005
217	0.286 ± 0.142	0.062 ± 0.022	0.204 ± 0.038	0.049 ± 0.007	0.178 ± 0.077	0.030 ± 0.014	0.336 ± 0.098	0.058 ± 0.023	0.118 ± 0.069	0.072 ± 0.011	0.122 ± 0.062	0.027 ± 0.005	0.220 ± 0.06	0.063 ± 0.031	0.241 ± 0.049	0.054 ± 0.065	0.095 ± 0.057	0.039 ± 0.009
218	0.336 ± 0.127	0.179 ± 0.132	0.188 ± 0.148	0.186 ± 0.03	0.195 ± 0.084	0.298 ± 0.237	0.254 ± 0.065	0.142 ± 0.021	0.134 ± 0.098	0.161 ± 0.082	0.132 ± 0.161	0.292 ± 0.33	0.291 ± 0.025	0.171 ± 0.068	0.147 ± 0.129	0.179 ± 0.266	0.176 ± 0.134	0.187 ± 0.011
220	0.127 ± 0.099	0.064 ± 0.067	0.127 ± 0.068	0.134 ± 0.019	0.083 ± 0.047	0.074 ± 0.014	0.151 ± 0.106	0.126 ± 0.058	0.036 ± 0.036	0.086 ± 0.072	0.057 ± 0.013	0.125 ± 0.149	0.138 ± 0.04	0.099 ± 0.027	0.138 ± 0.108	0.091 ± 0.122	0.056 ± 0.085	0.040 ± 0.006
314	0.127 ± 0.059	0.057 ± 0.031	0.099 ± 0.021	0.041 ± 0.028	0.041 ± 0.034	0.064 ± 0.032	0.077 ± 0.046	0.063 ± 0.004	0.026 ± 0.015	0.046 ± 0.02	0.027 ± 0.017	0.050 ± 0.051	0.117 ± 0.011	0.051 ± 0.023	0.121 ± 0.029	0.063 ± 0.05	0.041 ± 0.026	0.047 ± 0.005
322	0.065 ± 0.023	0.078 ± 0.031	0.076 ± 0.02	0.056 ± 0.046	0.075 ± 0.084	0.029 ± 0.012	0.138 ± 0.081	0.064 ± 0.038	0.036 ± 0.024	0.046 ± 0.02	0.046 ± 0.01	0.039 ± 0.013	0.062 ± 0.01	0.067 ± 0.025	0.079 ± 0.009	0.055 ± 0.056	0.075 ± 0.048	0.081 ± 0.016
412	0.685 ± 0.283	0.268 ± 0.179	0.680 ± 0.175	0.294 ± 0.069	0.231 ± 0.128	0.151 ± 0.03	0.468 ± 0.182	0.339 ± 0.153	0.180 ± 0.09	0.325 ± 0.34	0.231 ± 0.153	0.199 ± 0.023	0.382 ± 0.115	0.424 ± 0.125	0.325 ± 0.267	0.137 ± 0.099	0.444 ± 0.293	0.507 ± 0.291
414	0.606 ± 0.106	0.453 ± 0.053	0.757 ± 0.162	0.667 ± 0.193	0.500 ± 0.163	0.601 ± 0.101	1.211 ± 0.103	0.571 ± 0.176	0.643 ± 0.18	0.501 ± 0.099	0.589 ± 0.183	0.681 ± 0.492	0.982 ± 0.325	0.535 ± 0.165	1.016 ± 0.281	0.582 ± 0.052	0.679 ± 0.275	0.635 ± 0.282
513	0.314 ± 0.125	0.132 ± 0.063	0.353 ± 0.161	0.185 ± 0.071	0.240 ± 0.164	0.195 ± 0.127	0.488 ± 0.146	0.277 ± 0.12	0.201 ± 0.074	0.181 ± 0.057	0.207 ± 0.017	0.380 ± 0.138	0.363 ± 0.095	0.429 ± 0.065	0.445 ± 0.247	0.337 ± 0.126	0.773 ± 0.141	0.837 ± 0.224
1206	0.117 ± 0.08	0.157 ± 0.068	0.067 ± 0.049	0.121 ± 0.071	0.062 ± 0.015	0.182 ± 0.082	0.098 ± 0.034	0.145 ± 0.019	0.054 ± 0.036	0.160 ± 0.062	0.043 ± 0.039	0.075 ± 0.046	0.088 ± 0.048	0.160 ± 0.017	0.113 ± 0.067	0.146 ± 0.135	0.085 ± 0.059	0.076 ± 0.045
1207	0.098 ± 0.075	0.025 ± 0.022	0.068 ± 0.038	0.026 ± 0.025	0.066 ± 0.008	0.019 ± 0.026	0.095 ± 0.065	0.024 ± 0.02	0.083 ± 0.022	0.074 ± 0.037	0.084 ± 0.112	0.035 ± 0.029	0.074 ± 0.016	0.039 ± 0.058	0.127 ± 0.06	0.063 ± 0.07	0.062 ± 0.045	0.039 ± 0.039
1211	0.326 ± 0.082	0.234 ± 0.027	0.340 ± 0.052	0.229 ± 0.021	0.191 ± 0.001	0.276 ± 0.077	0.260 ± 0.087	0.167 ± 0.045	0.175 ± 0.033	0.179 ± 0.05	0.144 ± 0.059	0.133 ± 0.055	0.163 ± 0.069	0.171 ± 0.037	0.215 ± 0.079	0.103 ± 0.032	0.092 ± 0.054	0.085 ± 0.035
1213	0.610 ± 0.054	0.423 ± 0.089	0.528 ± 0.081	0.506 ± 0.2	0.473 ± 0.217	0.426 ± 0.285	0.562 ± 0.317	0.416 ± 0.176	0.383 ± 0.199	0.480 ± 0.303	0.504 ± 0.284	0.328 ± 0.124	0.475 ± 0.284	0.473 ± 0.03	0.616 ± 0.158	0.517 ± 0.173	0.585 ± 0.301	0.578 ± 0.291
1214	0.163 ± 0.075	0.101 ± 0.043	0.132 ± 0.016	0.108 ± 0.078	0.117 ± 0.057	0.051 ± 0.021	0.188 ± 0.035	0.075 ± 0.031	0.097 ± 0.04	0.076 ± 0.023	0.128 ± 0.038	0.088 ± 0.06	0.118 ± 0.014	0.079 ± 0.016	0.131 ± 0.021	0.081 ± 0.037	0.074 ± 0.064	0.052 ± 0.011
1215	0.146 ± 0.021	0.137 ± 0.042	0.131 ± 0.011	0.127 ± 0.034	0.094 ± 0.024	0.095 ± 0.032	0.136 ± 0.034	0.080 ± 0.022	0.117 ± 0.041	0.113 ± 0.013	0.087 ± 0.068	0.097 ± 0.041	0.117 ± 0.027	0.133 ± 0.024	0.124 ± 0.032	0.112 ± 0.026	0.089 ± 0.027	0.096 ± 0.058
1216	0.249 ± 0.042	0.166 ± 0.142	0.217 ± 0.072	0.181 ± 0.037	0.172 ± 0.038	0.187 ± 0.05	0.221 ± 0.136	0.170 ± 0.065	0.240 ± 0.075	0.185 ± 0.103	0.298 ± 0.006	0.243 ± 0.158	0.252 ± 0.052	0.232 ± 0.084	0.311 ± 0.062	0.115 ± 0.036	0.257 ± 0.134	0.154 ± 0.056
1218	0.168 ± 0.095	0.083 ± 0.024	0.125 ± 0.026	0.109 ± 0.067	0.108 ± 0.061	0.071 ± 0.06	0.117 ± 0.062	0.099 ± 0.022	0.068 ± 0.034	0.102 ± 0.039	0.092 ± 0.089	0.082 ± 0.039	0.108 ± 0.021	0.092 ± 0.036	0.146 ± 0.037	0.096 ± 0.039	0.108 ± 0.035	0.062 ± 0.036
1220	0.501 ± 0.094	0.440 ± 0.064	0.414 ± 0.083	0.391 ± 0.15	0.298 ± 0.031	0.273 ± 0.042	0.373 ± 0.108	0.289 ± 0.056	0.234 ± 0.053	0.345 ± 0.151	0.294 ± 0.062	0.195 ± 0.099	0.365 ± 0.04	0.296 ± 0.081	0.304 ± 0.175	0.155 ± 0.116	0.180 ± 0.144	0.110 ± 0.172
1227	0.015 ± 0.011	0.050 ± 0.022	0.021 ± 0.013	0.060 ± 0.012	0.021 ± 0.02	0.053 ± 0.025	0.032 ± 0.017	0.058 ± 0.019	0.019 ± 0.014	0.043 ± 0.01	0.012 ± 0.013	0.036 ± 0.031	0.033 ± 0.009	0.049 ± 0.027	0.022 ± 0.002	0.066 ± 0.034	0.038 ± 0.028	0.017 ± 0.019

Mean values (± sd, n=2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
214		0.07	0.72	-	0.36	0.075	↗	=	=	=	=	=	=	=	=	=
215		-0.06	0.78	-	-0.08	0.71	-	=	=	=	=	=	=	=	=	=
217	Glutathione S-transferase : GST EC=2.5.1.18	-0.34	0.082	↘	-0.06	0.77	-	M >	M >>	M >>	M >>	=	M >	M >	M >	=
218		-0.24	0.22	-	-0.04	0.83	-	=	=	=	=	=	=	=	=	=
220		-0.13	0.54	-	-0.15	0.48	-	=	=	=	=	=	=	=	=	=
314		-0.15	0.47	-	-0.02	0.91	-	=	=	=	=	=	=	M >	=	=
322		-0.06	0.78	-	0.15	0.48	-	=	=	=	=	=	=	=	=	=
412		-0.25	0.22	-	0.23	0.28	-	=	=	=	=	=	=	=	=	=
414		0.18	0.36	-	0.07	0.74	-	=	=	=	M >	=	=	=	=	=
513	Formate dehydrogenase, mitochondrial EC=1.2.1.2	0.52	0.006	↗↗↗	0.77	<0.001	↗↗↗↗	=	=	=	=	=	=	=	=	=
1206		0.04	0.85	-	-0.25	0.20	-	=	=	=	=	=	=	=	=	=
1207		0.03	0.90	-	0.23	0.24	-	=	=	=	=	=	=	=	=	=
1211	L-ascorbate peroxidase 1 EC=1.11.1.11	-0.66	<0.001	↘↘↘↘	-0.76	<0.001	↘↘↘↘	=	=	=	=	=	=	=	=	=
1213		0.06	0.76	-	0.19	0.35	-	=	=	=	=	=	=	=	=	=
1214	ND	-0.38	0.047	↘↘	-0.25	0.21	-	=	=	=	M >	=	=	=	=	=
1215		-0.30	0.13	-	-0.15	0.47	-	=	=	=	=	=	=	=	=	=
1216		0.31	0.12	-	-0.09	0.65	-	=	=	=	=	=	=	=	M >	=
1218		-0.12	0.56	-	-0.14	0.49	-	=	=	=	=	=	=	=	=	=
1220	L-ascorbate peroxidase 1, cytosolic EC=1.11.1.11	-0.55	0.003	↘↘↘	-0.66	0.77	↘↘↘↘	=	=	=	=	=	=	=	=	=
1227		0.29	0.16	-	-0.29	0.14	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 1229 to 1506



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

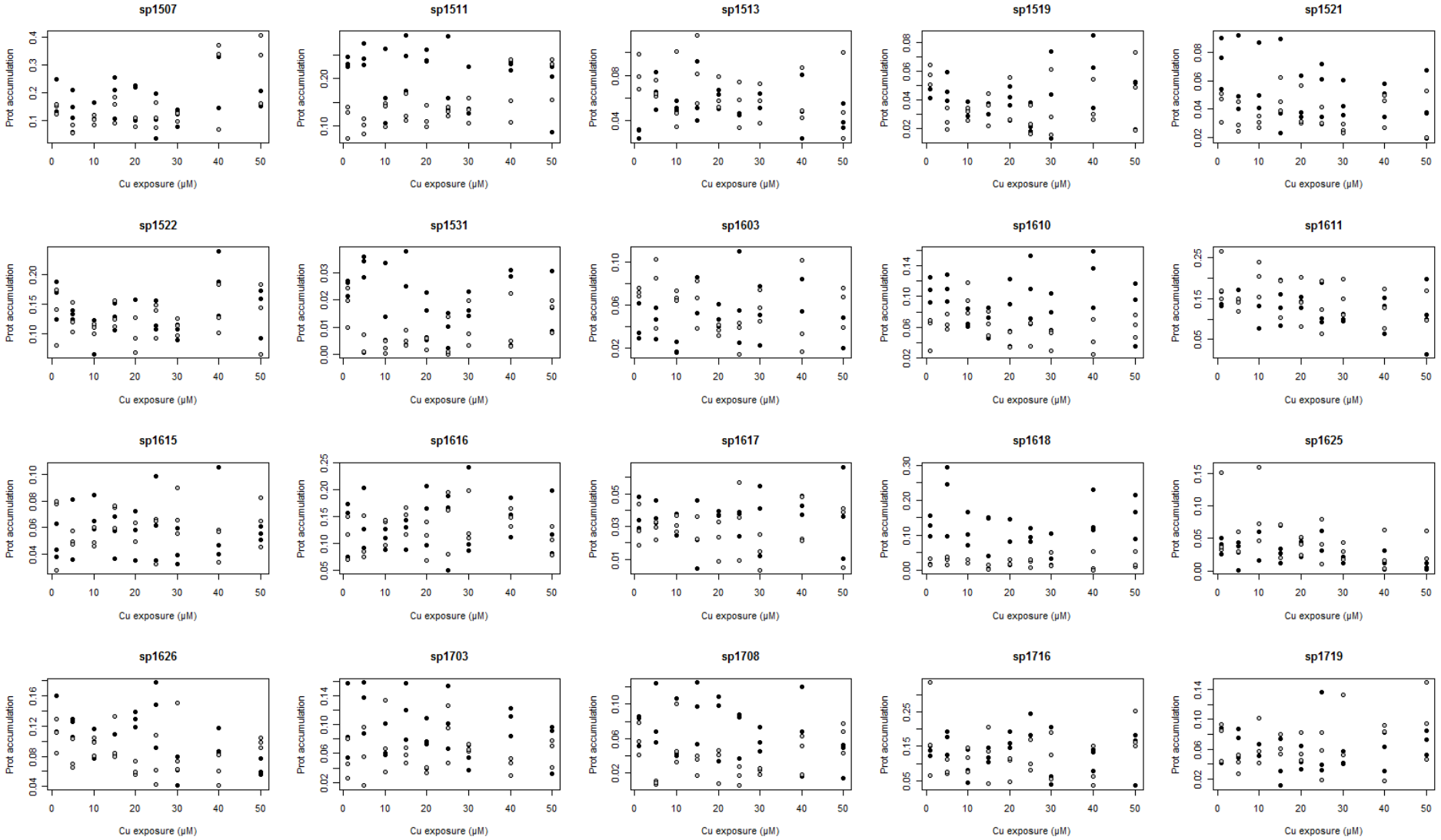
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
1229	0.025 ± 0.025	0.023 ± 0.018	0.023 ± 0.006	0.011 ± 0.002	0.024 ± 0.013	0.009 ± 0.01	0.026 ± 0.012	0.012 ± 0.006	0.016 ± 0.007	0.018 ± 0.013	0.032 ± 0.023	0.027 ± 0.032	0.018 ± 0.008	0.013 ± 0.001	0.026 ± 0.021	0.039	0.017 ± 0.011	0.018 ± 0.019
1302	0.050 ± 0.018	0.047 ± 0.014	0.048 ± 0.015	0.033 ± 0.007	0.034 ± 0.017	0.030 ± 0.011	0.060 ± 0.029	0.038 ± 0.005	0.043 ± 0.01	0.043 ± 0.024	0.052 ± 0.038	0.034 ± 0.02	0.051 ± 0.019	0.041 ± 0.022	0.082 ± 0.05	0.026 ± 0.028	0.033 ± 0.025	0.061 ± 0.023
1306	0.141 ± 0.045	0.127 ± 0.007	0.133 ± 0.028	0.129 ± 0.055	0.105 ± 0.004	0.115 ± 0.004	0.154 ± 0.048	0.102 ± 0.021	0.111 ± 0.029	0.093 ± 0.022	0.108 ± 0.053	0.092 ± 0.053	0.094 ± 0.015	0.107 ± 0.02	0.151 ± 0.035	0.110 ± 0.032	0.081 ± 0.059	0.111 ± 0.017
1309	0.537 ± 0.066	0.439 ± 0.172	0.505 ± 0.041	0.422 ± 0.11	0.407 ± 0.063	0.563 ± 0.132	0.423 ± 0.116	0.430 ± 0.11	0.406 ± 0.088	0.438 ± 0.239	0.492 ± 0.114	0.323 ± 0.016	0.481 ± 0.129	0.377 ± 0.05	0.404 ± 0.158	0.430 ± 0.077	0.450 ± 0.133	0.494 ± 0.04
1311	0.065 ± 0.018	0.057 ± 0.006	0.059 ± 0.008	0.042 ± 0.015	0.035 ± 0.016	0.045 ± 0.022	0.048 ± 0.023	0.047 ± 0.008	0.031 ± 0.009	0.044 ± 0.011	0.059 ± 0.024	0.023 ± 0.013	0.059 ± 0.011	0.053 ± 0.029	0.054 ± 0.01	0.074 ± 0.03	0.068 ± 0.027	0.035 ± 0.019
1315	0.108 ± 0.016	0.108 ± 0.024	0.122 ± 0.02	0.118 ± 0.013	0.097 ± 0.017	0.106 ± 0.001	0.127 ± 0.035	0.106 ± 0.019	0.095 ± 0.007	0.110 ± 0.031	0.109 ± 0.005	0.107 ± 0.045	0.115 ± 0.047	0.113 ± 0.027	0.155 ± 0.034	0.131 ± 0.021	0.140 ± 0.019	0.131 ± 0.032
1328	0.032 ± 0.022	0.045 ± 0.031	0.047 ± 0.013	0.021 ± 0.016	0.031 ± 0.007	0.036 ± 0.005	0.041 ± 0.01	0.030 ± 0.001	0.025 ± 0.011	0.024 ± 0.009	0.028 ± 0.011	0.024 ± 0.021	0.033 ± 0.008	0.015 ± 0.011	0.047 ± 0.04	0.022 ± 0.018	0.020 ± 0.021	0.019 ± 0.01
1403	0.205 ± 0.089	0.152 ± 0.069	0.227 ± 0.074	0.064 ± 0.052	0.100 ± 0.009	0.075 ± 0.028	0.234 ± 0.118	0.151 ± 0.015	0.223 ± 0.03	0.184 ± 0.026	0.166 ± 0.109	0.169 ± 0.045	0.160 ± 0.025	0.106 ± 0.052	0.384 ± 0.088	0.069 ± 0.024	0.110 ± 0.066	0.188 ± 0.025
1408	0.084 ± 0.045	0.086 ± 0.02	0.060 ± 0.011	0.059 ± 0.021	0.047 ± 0.024	0.074 ± 0.037	0.084 ± 0.044	0.058 ± 0.018	0.047 ± 0.019	0.058 ± 0.023	0.057 ± 0.05	0.036 ± 0.007	0.058 ± 0.029	0.050 ± 0.006	0.102 ± 0.035	0.060 ± 0.044	0.043 ± 0.028	0.066 ± 0.02
1410	0.201 ± 0.049	0.159 ± 0.032	0.153 ± 0.046	0.086 ± 0.028	0.128 ± 0.048	0.157 ± 0.031	0.101 ± 0.062	0.109 ± 0.055	0.098 ± 0.053	0.059 ± 0.019	0.129 ± 0.08	0.071 ± 0.037	0.088 ± 0.026	0.073 ± 0.066	0.211 ± 0.044	0.106 ± 0.085	0.056 ± 0.029	0.184 ± 0.073
1413	0.087 ± 0.027	0.126 ± 0.058	0.088 ± 0.011	0.065 ± 0.015	0.062 ± 0.028	0.100 ± 0.045	0.136 ± 0.065	0.085 ± 0.023	0.076 ± 0.028	0.083 ± 0.051	0.091 ± 0.092	0.069 ± 0.051	0.076 ± 0.032	0.079 ± 0.013	0.168 ± 0.059	0.071 ± 0.064	0.045 ± 0.037	0.082 ± 0.01
1414	0.078 ± 0.01	0.062 ± 0.063	0.103 ± 0.025	0.042 ± 0.017	0.037 ± 0.007	0.057 ± 0.043	0.068 ± 0.019	0.047 ± 0.029	0.107 ± 0.034	0.047 ± 0.031	0.042 ± 0.011	0.074 ± 0.018	0.067 ± 0.036	0.027 ± 0.007	0.120 ± 0.006	0.021 ± 0.01	0.037 ± 0.017	0.032 ± 0.007
1415	0.431 ± 0.196	0.439 ± 0.182	0.543 ± 0.135	0.367 ± 0.186	0.483 ± 0.251	0.420 ± 0.105	0.534 ± 0.196	0.385 ± 0.107	0.805 ± 0.265	0.427 ± 0.086	0.428 ± 0.243	0.456 ± 0.181	0.496 ± 0.095	0.339 ± 0.048	0.630 ± 0.134	0.436 ± 0.058	0.417 ± 0.204	0.643 ± 0.12
1416	0.121 ± 0.019	0.122 ± 0.04	0.090 ± 0.025	0.090 ± 0.017	0.109 ± 0.024	0.117 ± 0.05	0.158 ± 0.073	0.091 ± 0.013	0.142 ± 0.063	0.152 ± 0.027	0.084 ± 0.017	0.059 ± 0.052	0.089 ± 0.038	0.091 ± 0.042	0.144 ± 0.011	0.123 ± 0.026	0.061 ± 0.033	0.101 ± 0.037
1428	0.079 ± 0.015	0.078 ± 0.038	0.057 ± 0.018	0.090 ± 0.012	0.054 ± 0.024	0.121 ± 0.085	0.090 ± 0.05	0.070 ± 0.015	0.068 ± 0.023	0.076 ± 0.034	0.072 ± 0.034	0.040 ± 0.023	0.114 ± 0.074	0.058 ± 0.042	0.123 ± 0.024	0.066 ± 0.046	0.110 ± 0.064	0.066 ± 0.04
1502	0.952 ± 0.31	0.658 ± 0.469	1.078 ± 0.206	0.476 ± 0.16	0.470 ± 0.265	0.754 ± 0.122	1.309 ± 0.514	0.672 ± 0.336	0.517 ± 0.164	0.778 ± 0.232	0.989 ± 0.453	0.603 ± 0.391	0.678 ± 0.415	0.554 ± 0.132	1.066 ± 0.372	0.326 ± 0.232	0.456 ± 0.168	0.493 ± 0.109
1503	0.144 ± 0.035	0.059 ± 0.029	0.197 ± 0.085	0.090 ± 0.027	0.148 ± 0.092	0.076 ± 0.027	0.191 ± 0.117	0.174 ± 0.005	0.229 ± 0.136	0.174 ± 0.032	0.125 ± 0.068	0.308 ± 0.066	0.182 ± 0.052	0.238 ± 0.084	0.531 ± 0.41	0.491 ± 0.21	0.293 ± 0.181	0.374 ± 0.295
1504	0.181 ± 0.037	0.107 ± 0.033	0.160 ± 0.054	0.067 ± 0.015	0.132 ± 0.114	0.104 ± 0.002	0.196 ± 0.049	0.132 ± 0.016	0.175 ± 0.03	0.131 ± 0.04	0.158 ± 0.112	0.112 ± 0.061	0.100 ± 0.026	0.094 ± 0.013	0.201 ± 0.036	0.104 ± 0.053	0.099 ± 0.058	0.174 ± 0.009
1505	0.110 ± 0.034	0.073 ± 0.035	0.095 ± 0.023	0.063 ± 0.024	0.070 ± 0.006	0.068 ± 0.004	0.107 ± 0.044	0.060 ± 0.023	0.084 ± 0.009	0.053 ± 0.036	0.072 ± 0.031	0.054 ± 0.032	0.073 ± 0.022	0.040 ± 0.012	0.120 ± 0.046	0.050 ± 0.025	0.048 ± 0.038	0.026 ± 0.018
1506	0.053 ± 0.012	0.069 ± 0.029	0.058 ± 0.018	0.080 ± 0.027	0.055 ± 0.008	0.106 ± 0.021	0.034 ± 0.004	0.077 ± 0.023	0.046 ± 0.019	0.035 ± 0.013	0.053 ± 0.03	0.058 ± 0.021	0.053 ± 0.023	0.059 ± 0.031	0.034 ± 0.018	0.081 ± 0.031	0.030 ± 0.019	0.117 ± 0.025

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
1229		-0.10	0.63	-	0.20	0.37	-	=	=	=	=	=	=	=	=	=
1302		0.08	0.69	-	0.20	0.34	-	=	=	=	=	=	=	=	=	=
1306		-0.26	0.18	-	-0.19	0.35	-	=	=	=	=	=	=	=	=	=
1309		-0.18	0.38	-	-0.04	0.86	-	=	=	=	=	=	=	=	=	=
1311		0.17	0.39	-	0.01	0.98	-	=	=	=	=	=	=	=	=	=
1315	26S proteasome non-ATPase regulatory subunit 14 EC=3.4.19.-	0.41	0.032	↗↗	0.30	0.13	-	=	=	=	=	=	=	=	=	=
1328		-0.15	0.45	-	-0.38	0.054	↘	=	=	=	=	=	=	=	=	=
1403	ND	0.05	0.79	-	0.22	0.27	-	=	=	=	=	=	=	=	M >>	=
1408		-0.05	0.82	-	-0.20	0.31	-	=	=	=	=	=	=	=	=	=
1410		-0.29	0.14	-	0.06	0.75	-	=	=	=	=	=	=	=	=	=
1413		0.03	0.89	-	-0.22	0.27	-	=	=	=	=	=	=	=	=	=
1414	Probable voltage-gated potassium channel subunit beta	-0.11	0.58	-	-0.32	0.099	↘	=	=	=	=	=	=	=	M >>	=
1415		0.00	0.98	-	0.37	0.060	↗	=	=	=	=	=	=	=	=	=
1416		-0.21	0.29	-	-0.05	0.79	-	=	=	=	=	=	=	=	=	=
1428	glyceraldehyde-3-phosphate dehydrogenase / UDP-arabinopyranose mutase	0.45	0.019	↗↗	-0.27	0.17	-	=	=	=	=	=	=	=	=	=
1502		-0.22	0.28	-	-0.28	0.15	-	=	=	=	=	=	=	=	=	=
1503	Formate dehydrogenase, mitochondrial EC=1.2.1.2	0.40	0.036	↗↗	0.73	<0.001	↗↗↗↗	=	=	=	=	=	=	=	=	=
1504	Protein disulfide isomerase-like 2-1 EC=5.3.4.1	-0.21	0.29	-	0.42	0.029	↗↗	=	=	=	=	=	=	=	=	=
1505	ND	-0.26	0.19	-	-0.51	0.007	↘↘↘	=	=	=	=	=	=	=	=	=
1506	ND	-0.39	0.045	↘↘	0.20	0.32	-	=	=	=	=	=	=	=	=	NM >

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 1507 to 1719



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

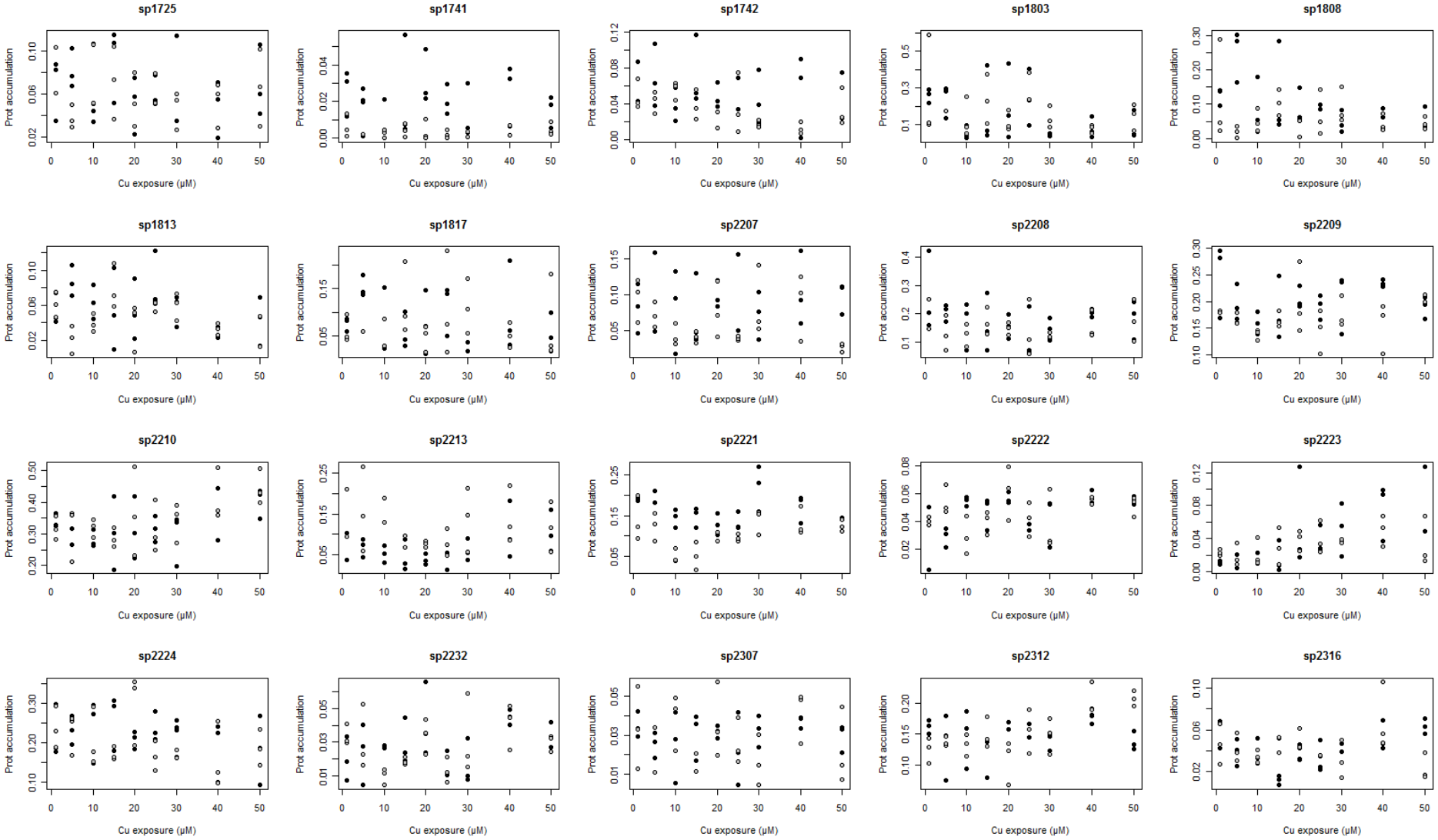
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
1507	0.169 ± 0.069	0.146 ± 0.018	0.157 ± 0.051	0.068 ± 0.016	0.147 ± 0.034	0.103 ± 0.017	0.192 ± 0.077	0.145 ± 0.049	0.183 ± 0.071	0.089 ± 0.019	0.112 ± 0.08	0.117 ± 0.045	0.117 ± 0.034	0.116 ± 0.016	0.270 ± 0.107	0.260 ± 0.168	0.171 ± 0.032	0.301 ± 0.126
1511	0.234 ± 0.01	0.114 ± 0.036	0.248 ± 0.024	0.100 ± 0.016	0.176 ± 0.079	0.129 ± 0.027	0.237 ± 0.059	0.134 ± 0.03	0.245 ± 0.013	0.117 ± 0.024	0.194 ± 0.083	0.130 ± 0.009	0.162 ± 0.054	0.133 ± 0.027	0.228 ± 0.01	0.167 ± 0.067	0.172 ± 0.075	0.209 ± 0.047
1513	0.029 ± 0.004	0.082 ± 0.015	0.066 ± 0.016	0.067 ± 0.008	0.053 ± 0.005	0.061 ± 0.035	0.061 ± 0.027	0.084 ± 0.03	0.060 ± 0.008	0.063 ± 0.014	0.046 ± 0.001	0.056 ± 0.02	0.051 ± 0.013	0.056 ± 0.017	0.051 ± 0.028	0.059 ± 0.024	0.043 ± 0.011	0.057 ± 0.039
1519	0.043 ± 0.004	0.058 ± 0.007	0.048 ± 0.01	0.026 ± 0.008	0.034 ± 0.005	0.031 ± 0.005	0.035 ± 0.004	0.034 ± 0.011	0.043 ± 0.006	0.036 ± 0.017	0.026 ± 0.011	0.025 ± 0.01	0.044 ± 0.03	0.035 ± 0.023	0.061 ± 0.025	0.037 ± 0.015	0.042 ± 0.019	0.047 ± 0.027
1521	0.073 ± 0.018	0.043 ± 0.01	0.060 ± 0.028	0.033 ± 0.011	0.059 ± 0.024	0.031 ± 0.004	0.050 ± 0.035	0.049 ± 0.012	0.045 ± 0.016	0.039 ± 0.015	0.056 ± 0.019	0.034 ± 0.007	0.046 ± 0.013	0.026 ± 0.003	0.048 ± 0.012	0.041 ± 0.012	0.047 ± 0.017	0.031 ± 0.019
1522	0.161 ± 0.033	0.132 ± 0.047	0.133 ± 0.008	0.125 ± 0.025	0.101 ± 0.03	0.110 ± 0.008	0.129 ± 0.023	0.131 ± 0.023	0.114 ± 0.038	0.096 ± 0.029	0.126 ± 0.027	0.128 ± 0.03	0.105 ± 0.013	0.112 ± 0.014	0.186 ± 0.054	0.138 ± 0.042	0.142 ± 0.043	0.131 ± 0.06
1531	0.025 ± 0.003	0.018 ± 0.007	0.033 ± 0.004	0.003 ± 0.004	0.018 ± 0.014	0.003 ± 0.002	0.022 ± 0.017	0.006 ± 0.003	0.015 ± 0.009	0.005 ± 0.003	0.009 ± 0.007	0.005 ± 0.008	0.018 ± 0.005	0.010 ± 0.009	0.021 ± 0.015	0.010 ± 0.011	0.019 ± 0.011	0.015 ± 0.006
1603	0.042 ± 0.017	0.072 ± 0.004	0.044 ± 0.014	0.075 ± 0.033	0.019 ± 0.005	0.068 ± 0.005	0.059 ± 0.024	0.062 ± 0.022	0.049 ± 0.011	0.037 ± 0.005	0.064 ± 0.043	0.032 ± 0.015	0.050 ± 0.027	0.059 ± 0.015	0.069 ± 0.021	0.051 ± 0.045	0.034 ± 0.02	0.061 ± 0.019
1610	0.108 ± 0.016	0.055 ± 0.022	0.111 ± 0.017	0.066 ± 0.01	0.070 ± 0.012	0.097 ± 0.02	0.068 ± 0.02	0.065 ± 0.016	0.089 ± 0.034	0.041 ± 0.011	0.111 ± 0.041	0.055 ± 0.017	0.080 ± 0.024	0.046 ± 0.014	0.127 ± 0.038	0.045 ± 0.023	0.083 ± 0.042	0.062 ± 0.014
1611	0.145 ± 0.019	0.195 ± 0.062	0.146 ± 0.027	0.136 ± 0.016	0.121 ± 0.04	0.199 ± 0.043	0.124 ± 0.039	0.164 ± 0.052	0.141 ± 0.014	0.141 ± 0.061	0.127 ± 0.053	0.126 ± 0.065	0.101 ± 0.009	0.153 ± 0.044	0.116 ± 0.046	0.126 ± 0.049	0.108 ± 0.092	0.122 ± 0.041
1615	0.048 ± 0.013	0.062 ± 0.029	0.055 ± 0.023	0.051 ± 0.005	0.069 ± 0.013	0.051 ± 0.008	0.054 ± 0.016	0.070 ± 0.01	0.055 ± 0.019	0.059 ± 0.008	0.065 ± 0.032	0.055 ± 0.019	0.044 ± 0.014	0.070 ± 0.018	0.064 ± 0.037	0.049 ± 0.014	0.055 ± 0.005	0.064 ± 0.019
1616	0.135 ± 0.052	0.113 ± 0.04	0.141 ± 0.057	0.104 ± 0.042	0.109 ± 0.019	0.127 ± 0.026	0.120 ± 0.028	0.145 ± 0.026	0.156 ± 0.055	0.108 ± 0.036	0.135 ± 0.074	0.145 ± 0.059	0.143 ± 0.087	0.142 ± 0.049	0.150 ± 0.036	0.148 ± 0.017	0.132 ± 0.06	0.106 ± 0.026
1617	0.037 ± 0.01	0.030 ± 0.013	0.038 ± 0.007	0.028 ± 0.006	0.033 ± 0.008	0.031 ± 0.005	0.024 ± 0.021	0.032 ± 0.008	0.038 ± 0.001	0.022 ± 0.012	0.033 ± 0.008	0.034 ± 0.024	0.036 ± 0.022	0.014 ± 0.011	0.043 ± 0.006	0.031 ± 0.015	0.038 ± 0.028	0.028 ± 0.02
1618	0.127 ± 0.03	0.022 ± 0.01	0.212 ± 0.103	0.028 ± 0.012	0.113 ± 0.049	0.023 ± 0.005	0.114 ± 0.062	0.007 ± 0.006	0.103 ± 0.037	0.021 ± 0.009	0.099 ± 0.021	0.021 ± 0.012	0.063 ± 0.037	0.026 ± 0.022	0.156 ± 0.065	0.020 ± 0.03	0.157 ± 0.063	0.026 ± 0.024
1625	0.039 ± 0.013	0.074 ± 0.068	0.028 ± 0.023	0.039 ± 0.018	0.041 ± 0.022	0.093 ± 0.06	0.024 ± 0.011	0.054 ± 0.029	0.039 ± 0.015	0.039 ± 0.014	0.041 ± 0.017	0.044 ± 0.035	0.018 ± 0.005	0.031 ± 0.013	0.016 ± 0.015	0.027 ± 0.031	0.006 ± 0.005	0.033 ± 0.025
1626	0.134 ± 0.024	0.108 ± 0.023	0.120 ± 0.013	0.079 ± 0.02	0.098 ± 0.02	0.095 ± 0.012	0.108 ± 0.025	0.099 ± 0.03	0.129 ± 0.01	0.063 ± 0.01	0.139 ± 0.044	0.071 ± 0.034	0.061 ± 0.019	0.096 ± 0.047	0.095 ± 0.019	0.061 ± 0.02	0.064 ± 0.011	0.098 ± 0.006
1703	0.098 ± 0.053	0.051 ± 0.028	0.128 ± 0.036	0.056 ± 0.04	0.074 ± 0.024	0.078 ± 0.051	0.119 ± 0.039	0.058 ± 0.011	0.086 ± 0.02	0.038 ± 0.004	0.107 ± 0.044	0.089 ± 0.04	0.055 ± 0.017	0.067 ± 0.005	0.106 ± 0.02	0.044 ± 0.012	0.073 ± 0.036	0.063 ± 0.02
1708	0.074 ± 0.02	0.059 ± 0.019	0.083 ± 0.037	0.008 ± 0.002	0.063 ± 0.038	0.059 ± 0.037	0.092 ± 0.036	0.031 ± 0.012	0.080 ± 0.041	0.032 ± 0.021	0.069 ± 0.029	0.016 ± 0.011	0.058 ± 0.014	0.022 ± 0.004	0.068 ± 0.052	0.044 ± 0.023	0.038 ± 0.021	0.063 ± 0.017
1716	0.137 ± 0.014	0.185 ± 0.137	0.166 ± 0.036	0.087 ± 0.023	0.090 ± 0.049	0.113 ± 0.035	0.123 ± 0.021	0.128 ± 0.081	0.166 ± 0.024	0.090 ± 0.038	0.169 ± 0.082	0.118 ± 0.047	0.103 ± 0.091	0.123 ± 0.067	0.117 ± 0.034	0.083 ± 0.06	0.128 ± 0.08	0.188 ± 0.055
1719	0.073 ± 0.027	0.074 ± 0.027	0.071 ± 0.02	0.040 ± 0.013	0.073 ± 0.026	0.067 ± 0.031	0.039 ± 0.032	0.065 ± 0.013	0.046 ± 0.016	0.061 ± 0.02	0.069 ± 0.059	0.053 ± 0.032	0.047 ± 0.009	0.106 ± 0.047	0.059 ± 0.026	0.064 ± 0.041	0.070 ± 0.017	0.097 ± 0.052

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
1507	Formate dehydrogenase, mitochondrial EC=1.2.1.2	0.16	0.41	-	0.61	0.0008	↗↗↗	=	=	=	=	=	=	=	=	=
1511	Protein disulfide isomerase-like 2-1 / FBP aldolase cytoplasmic	-0.29	0.15	-	0.65	0.0003	↗↗↗	M >	M >	=	=	M >	=	=	=	=
1513		-0.07	0.73	-	-0.28	0.15	-	NM >	=	=	=	=	=	=	=	=
1519		0.14	0.50	-	0.03	0.88	-	=	=	=	=	=	=	=	=	=
1521		-0.33	0.094	↘	-0.17	0.40	-	=	=	=	=	=	=	=	=	=
1522		0.16	0.42	-	0.08	0.69	-	=	=	=	=	=	=	=	=	=
1531	ND	-0.24	0.22	-	0.21	0.30	-	=	M >>	=	=	=	=	=	=	=
1603	ND	0.19	0.37	-	-0.26	0.19	-	=	=	NM >>	=	=	=	=	=	=
1610		0.03	0.90	-	-0.27	0.18	-	=	=	=	=	=	=	=	=	=
1611	mitochondrial processing peptidase α-chain precursor / 6-phosphogluconate dehydrogenase, decarboxylating 2	-0.30	0.13	-	-0.39	0.043	↘↘	=	=	=	=	=	=	=	=	=
1615		0.04	0.85	-	0.06	0.76	-	=	=	=	=	=	=	=	=	=
1616		0.08	0.69	-	0.12	0.56	-	=	=	=	=	=	=	=	=	=
1617		0.12	0.54	-	-0.08	0.71	-	=	=	=	=	=	=	=	=	=
1618	Mitochondrial-processing peptidase subunit α EC=3.4.24.64	-0.04	0.82	-	0.04	0.84	-	M >>	M >>	M >>	M >>	M >>	M >>	=	M >	M >
1625	ND	-0.52	0.005	↘↘↘	-0.37	0.054	↘	=	=	=	=	=	=	=	=	=
1626	mitochondrial processing peptidase α-chain precursor	-0.54	0.004	↘↘↘	-0.16	0.43	-	=	=	=	=	M >	=	=	=	=
1703		-0.25	0.21	-	0.01	0.96	-	=	=	=	=	M >	=	=	M >	=
1708	6-phosphofructokinase, pyrophosphate dependent EC=2.7.1.90	-0.32	0.098	↘	0.14	0.47	-	=	M >>	=	=	=	M >	M >	=	=
1716		-0.09	0.65	-	0.06	0.77	-	=	=	=	=	=	=	=	=	=
1719		-0.07	0.74	-	0.30	0.13	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 1725 to 2316



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

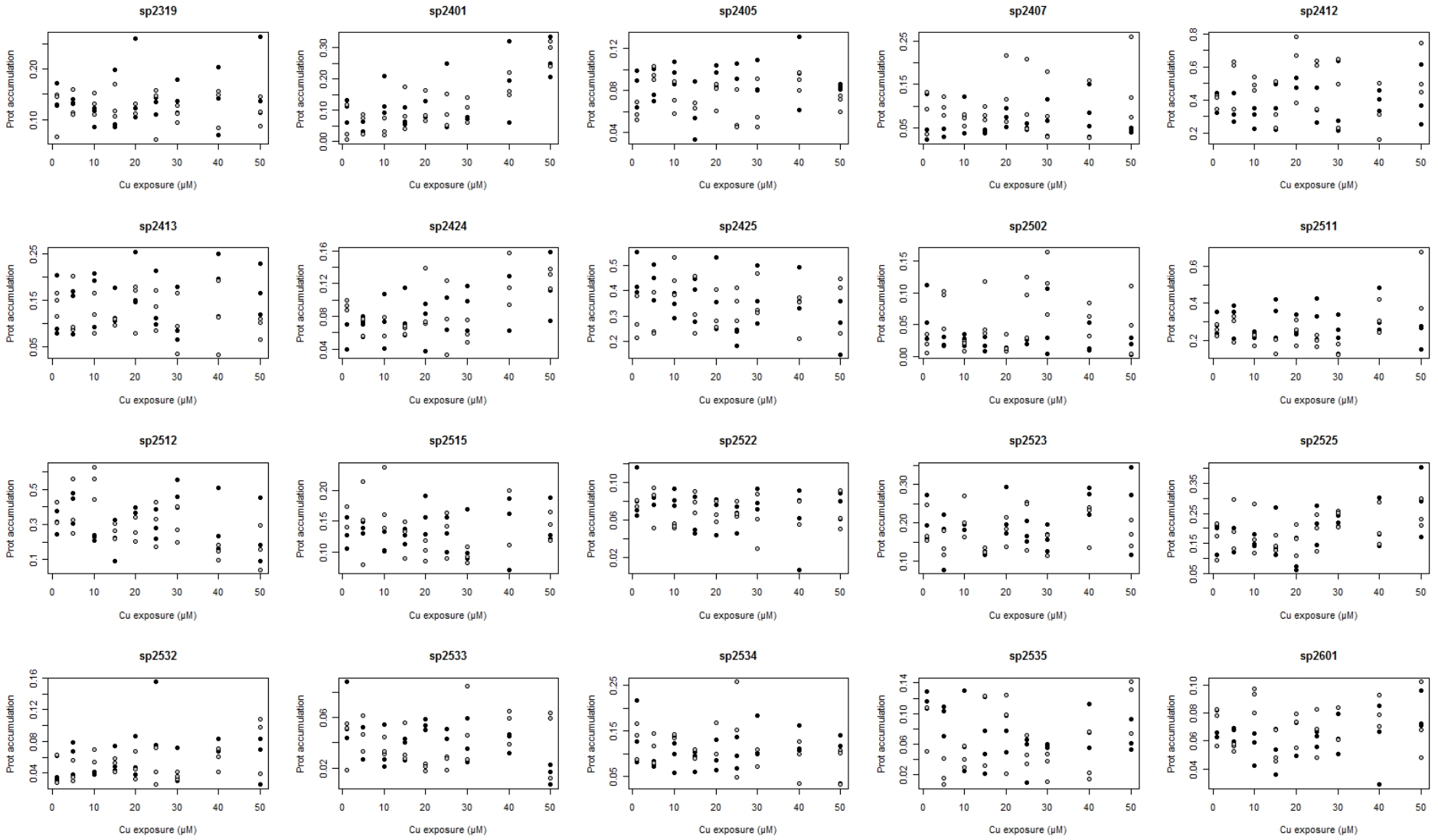
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
1725	0.068 ± 0.029	0.075 ± 0.024	0.082 ± 0.018	0.038 ± 0.01	0.062 ± 0.039	0.069 ± 0.031	0.091 ± 0.034	0.071 ± 0.034	0.052 ± 0.026	0.053 ± 0.025	0.062 ± 0.014	0.060 ± 0.016	0.058 ± 0.048	0.047 ± 0.018	0.048 ± 0.026	0.052 ± 0.021	0.069 ± 0.033	0.066 ± 0.035
1741	0.026 ± 0.012	0.006 ± 0.006	0.023 ± 0.004	0.002 ± 0.001	0.009 ± 0.01	0.003 ± 0.002	0.023 ± 0.029	0.004 ± 0.004	0.032 ± 0.015	0.004 ± 0.006	0.021 ± 0.008	0.002 ± 0.002	0.013 ± 0.015	0.003 ± 0.002	0.025 ± 0.017	0.003 ± 0.003	0.015 ± 0.009	0.005 ± 0.003
1742	0.057 ± 0.026	0.048 ± 0.017	0.069 ± 0.035	0.043 ± 0.012	0.038 ± 0.019	0.056 ± 0.01	0.072 ± 0.039	0.038 ± 0.017	0.048 ± 0.014	0.024 ± 0.01	0.059 ± 0.022	0.037 ± 0.034	0.045 ± 0.03	0.017 ± 0.004	0.054 ± 0.046	0.012 ± 0.007	0.049 ± 0.036	0.034 ± 0.021
1803	0.258 ± 0.038	0.267 ± 0.279	0.237 ± 0.09	0.173	0.054 ± 0.036	0.130 ± 0.106	0.178 ± 0.214	0.236 ± 0.135	0.203 ± 0.206	0.117 ± 0.055	0.244 ± 0.154	0.311 ± 0.101	0.042 ± 0.009	0.136 ± 0.059	0.081 ± 0.058	0.078 ± 0.021	0.089 ± 0.077	0.145 ± 0.072
1808	0.124 ± 0.025	0.120 ± 0.148	0.250 ± 0.074	0.020 ± 0.017	0.085 ± 0.084	0.051 ± 0.033	0.126 ± 0.136	0.105 ± 0.038	0.091 ± 0.051	0.036 ± 0.027	0.094 ± 0.007	0.068 ± 0.066	0.048 ± 0.032	0.090 ± 0.052	0.062 ± 0.028	0.044 ± 0.025	0.063 ± 0.043	0.044 ± 0.018
1813	0.053 ± 0.018	0.061 ± 0.014	0.087 ± 0.018	0.022 ± 0.016	0.064 ± 0.02	0.040 ± 0.01	0.054 ± 0.046	0.079 ± 0.026	0.054 ± 0.034	0.038 ± 0.027	0.085 ± 0.033	0.059 ± 0.006	0.056 ± 0.018	0.060 ± 0.015	0.031 ± 0.007	0.033 ± 0.007	0.043 ± 0.028	0.036 ± 0.02
1817	0.075 ± 0.014	0.062 ± 0.029	0.154 ± 0.022	0.059	0.067 ± 0.074	0.047 ± 0.034	0.058 ± 0.038	0.120 ± 0.077	0.076 ± 0.067	0.046 ± 0.028	0.112 ± 0.054	0.107 ± 0.111	0.036 ± 0.02	0.111 ± 0.058	0.101 ± 0.096	0.051 ± 0.027	0.053 ± 0.041	0.076 ± 0.092
2207	0.082 ± 0.034	0.095 ± 0.031	0.099 ± 0.056	0.072 ± 0.017	0.081 ± 0.059	0.043 ± 0.016	0.072 ± 0.051	0.041 ± 0.008	0.099 ± 0.019	0.077 ± 0.039	0.082 ± 0.065	0.039 ± 0.003	0.073 ± 0.033	0.086 ± 0.049	0.105 ± 0.052	0.087 ± 0.047	0.098 ± 0.022	0.026 ± 0.006
2208	0.262 ± 0.14	0.182 ± 0.059	0.207 ± 0.03	0.130 ± 0.062	0.169 ± 0.086	0.128 ± 0.04	0.160 ± 0.103	0.173 ± 0.048	0.157 ± 0.042	0.148 ± 0.023	0.122 ± 0.09	0.141 ± 0.1	0.146 ± 0.04	0.123 ± 0.009	0.203 ± 0.014	0.157 ± 0.049	0.184 ± 0.068	0.176 ± 0.075
2209	0.249 ± 0.07	0.181 ± 0.001	0.196 ± 0.034	0.166 ± 0.011	0.159 ± 0.021	0.138 ± 0.009	0.182 ± 0.06	0.165 ± 0.015	0.205 ± 0.021	0.199 ± 0.068	0.191 ± 0.024	0.146 ± 0.041	0.205 ± 0.057	0.177 ± 0.029	0.234 ± 0.007	0.156 ± 0.047	0.190 ± 0.021	0.207 ± 0.007
2210	0.341 ± 0.022	0.318 ± 0.037	0.315 ± 0.046	0.312 ± 0.087	0.282 ± 0.027	0.320 ± 0.029	0.304 ± 0.115	0.287 ± 0.03	0.316 ± 0.098	0.366 ± 0.141	0.316 ± 0.041	0.315 ± 0.082	0.294 ± 0.082	0.342 ± 0.063	0.366 ± 0.083	0.414 ± 0.083	0.403 ± 0.048	0.445 ± 0.055
2213	0.078 ± 0.036	0.133 ± 0.068	0.068 ± 0.023	0.157 ± 0.105	0.051 ± 0.021	0.159 ± 0.041	0.044 ± 0.039	0.086 ± 0.017	0.037 ± 0.013	0.075 ± 0.008	0.039 ± 0.022	0.078 ± 0.034	0.060 ± 0.027	0.139 ± 0.08	0.105 ± 0.071	0.141 ± 0.07	0.105 ± 0.051	0.117 ± 0.062
2221	0.189 ± 0.003	0.139 ± 0.055	0.174 ± 0.041	0.124 ± 0.035	0.145 ± 0.023	0.049 ± 0.017	0.148 ± 0.025	0.050 ± 0.034	0.128 ± 0.027	0.101 ± 0.012	0.134 ± 0.023	0.095 ± 0.009	0.221 ± 0.057	0.138 ± 0.031	0.171 ± 0.035	0.133 ± 0.035	0.137 ± 0.013	0.124 ± 0.014
2222	0.033 ± 0.024	0.040 ± 0.003	0.029 ± 0.007	0.055 ± 0.011	0.055 ± 0.003	0.030 ± 0.014	0.047 ± 0.012	0.040 ± 0.008	0.057 ± 0.004	0.062 ± 0.019	0.034 ± 0.004	0.042 ± 0.012	0.043 ± 0.018	0.038 ± 0.022	0.057 ± 0.006	0.056 ± 0.003	0.055 ± 0.003	0.052 ± 0.007
2223	0.011 ± 0.002	0.023 ± 0.004	0.013 ± 0.008	0.020 ± 0.014	0.015 ± 0.007	0.022 ± 0.017	0.016 ± 0.019	0.030 ± 0.022	0.057 ± 0.061	0.039 ± 0.012	0.036 ± 0.017	0.040 ± 0.02	0.052 ± 0.032	0.036 ± 0.002	0.077 ± 0.035	0.051 ± 0.018	0.065 ± 0.056	0.034 ± 0.03
2224	0.219 ± 0.067	0.236 ± 0.053	0.231 ± 0.037	0.227 ± 0.052	0.238 ± 0.079	0.206 ± 0.073	0.259 ± 0.07	0.171 ± 0.018	0.208 ± 0.022	0.294 ± 0.089	0.238 ± 0.037	0.164 ± 0.038	0.242 ± 0.012	0.169 ± 0.012	0.188 ± 0.077	0.158 ± 0.084	0.182 ± 0.088	0.186 ± 0.046
2232	0.020 ± 0.013	0.034 ± 0.006	0.024 ± 0.018	0.030 ± 0.019	0.027 ± 0.001	0.010 ± 0.005	0.029 ± 0.014	0.019 ± 0.002	0.042 ± 0.022	0.034 ± 0.011	0.015 ± 0.008	0.013 ± 0.008	0.017 ± 0.013	0.032 ± 0.024	0.045 ± 0.005	0.041 ± 0.013	0.037 ± 0.009	0.030 ± 0.005
2307	0.035 ± 0.007	0.034 ± 0.021	0.025 ± 0.007	0.026 ± 0.013	0.025 ± 0.018	0.038 ± 0.015	0.031 ± 0.012	0.017 ± 0.005	0.032 ± 0.003	0.036 ± 0.019	0.022 ± 0.019	0.026 ± 0.012	0.032 ± 0.008	0.016 ± 0.013	0.037 ± 0.003	0.041 ± 0.013	0.029 ± 0.007	0.022 ± 0.02
2312	0.162 ± 0.01	0.125 ± 0.021	0.135 ± 0.053	0.138 ± 0.007	0.147 ± 0.047	0.133 ± 0.018	0.132 ± 0.049	0.150 ± 0.025	0.154 ± 0.018	0.109 ± 0.035	0.152 ± 0.013	0.155 ± 0.036	0.140 ± 0.014	0.148 ± 0.029	0.175 ± 0.008	0.204 ± 0.025	0.138 ± 0.015	0.207 ± 0.012
2316	0.052 ± 0.014	0.046 ± 0.019	0.039 ± 0.013	0.042 ± 0.013	0.038 ± 0.013	0.035 ± 0.006	0.012 ± 0.005	0.048 ± 0.008	0.037 ± 0.008	0.049 ± 0.011	0.032 ± 0.016	0.035	0.042 ± 0.005	0.031 ± 0.018	0.053 ± 0.015	0.070 ± 0.031	0.063 ± 0.008	0.023 ± 0.013

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
1725		-0.18	0.37	-	-0.06	0.77	-	=	=	=	=	=	=	=	=	=
1741	ND	-0.09	0.65	-	0.01	0.97	-	=	M >>	=	=	M >	M >>	=	=	=
1742	ND	-0.11	0.59	-	-0.46	0.015	↘↘	=	=	=	=	=	=	=	=	=
1803		-0.36	0.063	↘	-0.28	0.19	-	=	=	=	=	=	=	NM >	=	=
1808	Glycine dehydrogenase [decarboxylating], mitochondrial EC=1.4.4.2	-0.48	0.012	↘↘	-0.15	0.46	-	=	M >>	=	=	=	=	=	=	=
1813		-0.33	0.091	↘	-0.13	0.51	-	=	M >	=	=	=	=	=	=	=
1817		-0.19	0.34	-	0.06	0.79	-	=	-	=	=	=	=	=	=	=
2207	Cysteine proteinase inhibitor 12 : Cystatin	0.10	0.62	-	-0.20	0.32	-	=	=	=	=	=	=	=	=	M >>
2208		-0.17	0.39	-	0.05	0.79	-	=	=	=	=	=	=	=	=	=
2209		0.00	0.99	-	0.20	0.32	-	=	=	=	=	=	=	=	=	=
2210	Superoxide dismutase [Mn] EC=1.15.1.1	0.34	0.080	↗	0.53	0.005	↗↗↗	=	=	=	=	=	=	=	=	=
2213		0.33	0.094	↗	-0.07	0.73	-	=	=	NM >	=	=	=	=	=	=
2221		-0.10	0.61	-	0.23	0.26	-	=	=	M >	M >	=	=	=	=	=
2222	Proteasome subunit beta type EC=3.4.25.1	0.41	0.033	↗↗	0.22	0.27	-	=	=	=	=	=	=	=	=	=
2223	Glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic EC=1.2.1.12	0.59	0.001	↗↗↗	0.40	0.037	↗↗	=	=	=	=	=	=	=	=	=
2224		-0.27	0.17	-	-0.32	0.11	-	=	=	=	=	=	=	=	=	=
2232		0.32	0.11	-	0.19	0.33	-	=	=	M >	=	=	=	=	=	=
2307		0.09	0.65	-	-0.09	0.67	-	=	=	=	=	=	=	=	=	=
2312	Probable L-ascorbate peroxidase 6, chloroplastic EC=1.11.1.11	0.05	0.80	-	0.69	<0.001	↗↗↗↗	=	=	=	=	=	=	=	=	=
2316	ND	0.38	0.052	↗	-0.07	0.72	-	=	=	=	NM >>	=	=	=	=	M >

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 2319 to 2601



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

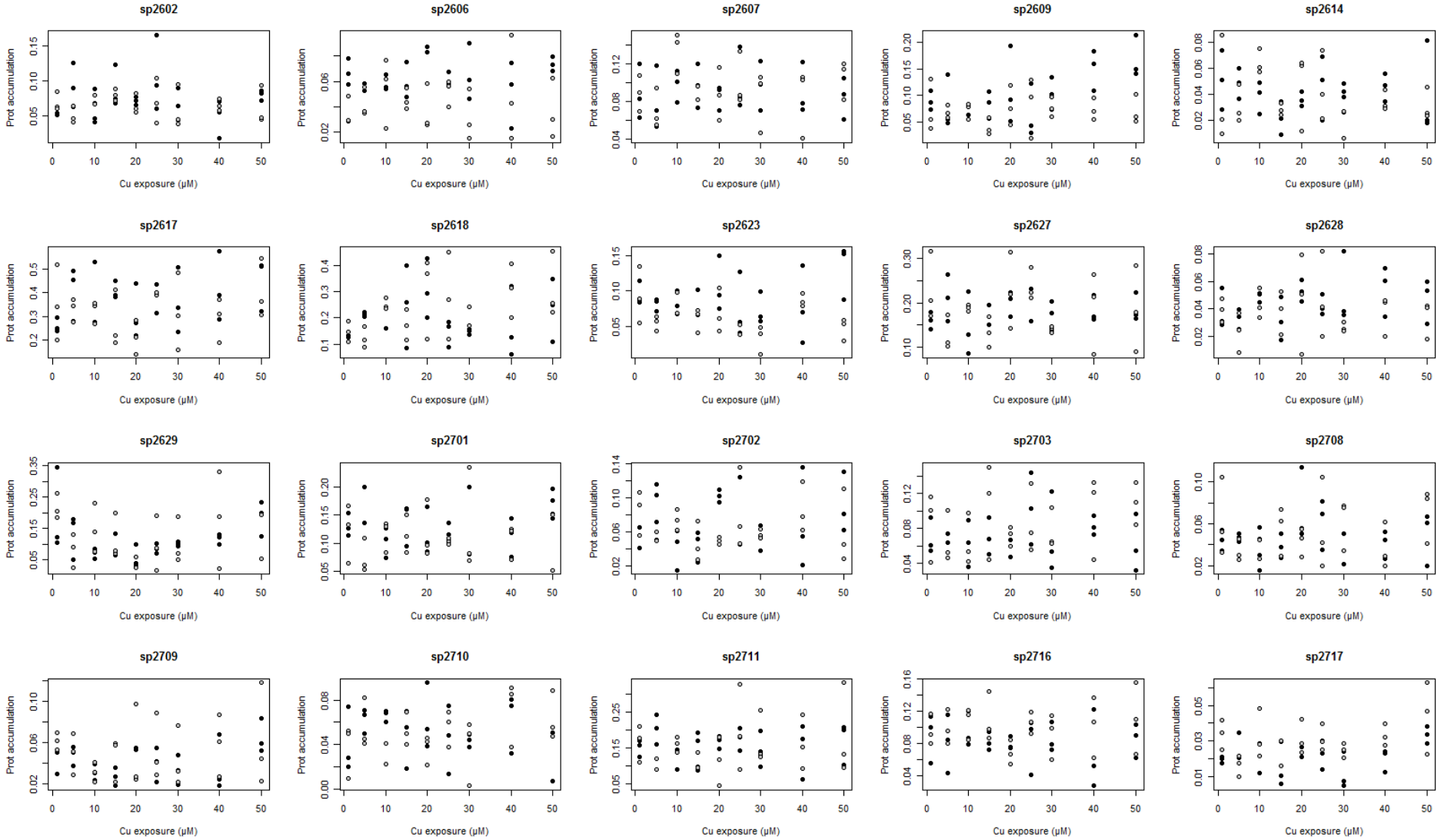
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
2319	0.143 ± 0.025	0.121 ± 0.047	0.135 ± 0.004	0.128 ± 0.028	0.109 ± 0.02	0.131 ± 0.022	0.124 ± 0.063	0.132 ± 0.034	0.163 ± 0.085	0.118 ± 0.011	0.130 ± 0.017	0.122 ± 0.053	0.143 ± 0.034	0.111 ± 0.017	0.138 ± 0.066	0.130 ± 0.04	0.171 ± 0.081	0.116 ± 0.029
2401	0.101 ± 0.036	0.050 ± 0.06	0.041 ± 0.019	0.062 ± 0.034	0.139 ± 0.063	0.042 ± 0.028	0.077 ± 0.029	0.100 ± 0.069	0.096 ± 0.03	0.105 ± 0.051	0.117 ± 0.116	0.097 ± 0.051	0.077 ± 0.003	0.104 ± 0.04	0.193 ± 0.131	0.178 ± 0.039	0.264 ± 0.065	0.287 ± 0.042
2405	0.085 ± 0.018	0.060 ± 0.009	0.082 ± 0.016	0.096 ± 0.007	0.097 ± 0.011	0.073 ± 0.016	0.059 ± 0.028	0.065 ± 0.003	0.095 ± 0.011	0.077 ± 0.014	0.081 ± 0.03	0.058 ± 0.02	0.090 ± 0.017	0.064 ± 0.024	0.097 ± 0.035	0.089 ± 0.009	0.084 ± 0.003	0.069 ± 0.008
2407	0.065 ± 0.055	0.086 ± 0.049	0.041 ± 0.01	0.099 ± 0.022	0.077 ± 0.042	0.069 ± 0.013	0.041 ± 0.005	0.082 ± 0.016	0.074 ± 0.022	0.132 ± 0.078	0.051 ± 0.007	0.112 ± 0.086	0.071 ± 0.043	0.095 ± 0.076	0.096 ± 0.049	0.071 ± 0.076	0.043 ± 0.005	0.151 ± 0.097
2412	0.402 ± 0.067	0.394 ± 0.043	0.341 ± 0.088	0.529 ± 0.158	0.297 ± 0.066	0.495 ± 0.04	0.357 ± 0.138	0.351 ± 0.143	0.463 ± 0.077	0.611 ± 0.205	0.360 ± 0.106	0.530 ± 0.159	0.374 ± 0.228	0.457 ± 0.209	0.397 ± 0.061	0.326 ± 0.171	0.412 ± 0.185	0.564 ± 0.159
2413	0.124 ± 0.069	0.144 ± 0.026	0.136 ± 0.051	0.127 ± 0.065	0.164 ± 0.062	0.121 ± 0.044	0.130 ± 0.041	0.105 ± 0.009	0.183 ± 0.062	0.143 ± 0.056	0.141 ± 0.063	0.131 ± 0.043	0.109 ± 0.06	0.098 ± 0.065	0.187 ± 0.067	0.114 ± 0.08	0.171 ± 0.056	0.092 ± 0.023
2424	0.066 ± 0.024	0.094 ± 0.006	0.074 ± 0.005	0.062 ± 0.012	0.074 ± 0.033	0.064 ± 0.013	0.081 ± 0.03	0.064 ± 0.005	0.072 ± 0.031	0.095 ± 0.039	0.077 ± 0.022	0.078 ± 0.045	0.093 ± 0.028	0.061 ± 0.014	0.096 ± 0.033	0.122 ± 0.032	0.115 ± 0.042	0.127 ± 0.012
2425	0.454 ± 0.086	0.288 ± 0.086	0.439 ± 0.071	0.289 ± 0.092	0.345 ± 0.049	0.452 ± 0.075	0.377 ± 0.089	0.332 ± 0.114	0.379 ± 0.141	0.314 ± 0.079	0.224 ± 0.036	0.351 ± 0.065	0.376 ± 0.115	0.370 ± 0.086	0.394 ± 0.086	0.314 ± 0.089	0.260 ± 0.104	0.364 ± 0.114
2502	0.064 ± 0.043	0.020 ± 0.014	0.023 ± 0.008	0.081 ± 0.032	0.027 ± 0.009	0.018 ± 0.008	0.019 ± 0.011	0.065 ± 0.045	0.012 ± 0.003	0.019 ± 0.014	0.023 ± 0.005	0.084 ± 0.049	0.047 ± 0.053	0.115 ± 0.049	0.025 ± 0.024	0.060 ± 0.026	0.017 ± 0.013	0.055 ± 0.053
2511	0.293 ± 0.059	0.258 ± 0.032	0.318 ± 0.093	0.277 ± 0.073	0.238 ± 0.019	0.213 ± 0.035	0.333 ± 0.105	0.182 ± 0.046	0.275 ± 0.056	0.248 ± 0.069	0.322 ± 0.11	0.200 ± 0.031	0.272 ± 0.063	0.147 ± 0.033	0.345 ± 0.119	0.324 ± 0.089	0.232 ± 0.068	0.473 ± 0.175
2512	0.313 ± 0.066	0.350 ± 0.068	0.412 ± 0.094	0.379 ± 0.162	0.224 ± 0.016	0.544 ± 0.095	0.214 ± 0.118	0.263 ± 0.044	0.322 ± 0.105	0.266 ± 0.068	0.296 ± 0.086	0.310 ± 0.128	0.469 ± 0.079	0.289 ± 0.104	0.301 ± 0.183	0.142 ± 0.045	0.242 ± 0.187	0.165 ± 0.127
2515	0.130 ± 0.025	0.151 ± 0.02	0.140 ± 0.01	0.148 ± 0.067	0.112 ± 0.018	0.179 ± 0.052	0.126 ± 0.013	0.124 ± 0.03	0.159 ± 0.031	0.102 ± 0.016	0.129 ± 0.028	0.132 ± 0.038	0.119 ± 0.043	0.095 ± 0.013	0.140 ± 0.061	0.140 ± 0.051	0.145 ± 0.037	0.142 ± 0.023
2522	0.084 ± 0.028	0.078 ± 0.004	0.082 ± 0.005	0.077 ± 0.022	0.083 ± 0.009	0.054 ± 0.003	0.060 ± 0.021	0.079 ± 0.012	0.067 ± 0.021	0.068 ± 0.011	0.063 ± 0.015	0.070 ± 0.008	0.081 ± 0.011	0.059 ± 0.029	0.054 ± 0.042	0.072 ± 0.015	0.076 ± 0.014	0.068 ± 0.021
2523	0.210 ± 0.057	0.189 ± 0.051	0.161 ± 0.074	0.143 ± 0.033	0.181 ± 0.017	0.212 ± 0.054	0.123 ± 0.01	0.129 ± 0.007	0.221 ± 0.064	0.179 ± 0.039	0.174 ± 0.028	0.210 ± 0.071	0.159 ± 0.035	0.152 ± 0.032	0.263 ± 0.036	0.202 ± 0.058	0.245 ± 0.117	0.172 ± 0.034
2525	0.175 ± 0.054	0.161 ± 0.063	0.207 ± 0.088	0.206 ± 0.082	0.156 ± 0.021	0.187 ± 0.084	0.171 ± 0.086	0.149 ± 0.026	0.101 ± 0.058	0.162 ± 0.053	0.213 ± 0.066	0.190 ± 0.06	0.222 ± 0.018	0.238 ± 0.029	0.208 ± 0.084	0.206 ± 0.071	0.289 ± 0.117	0.246 ± 0.047
2532	0.033 ± 0.002	0.051 ± 0.02	0.062 ± 0.021	0.040 ± 0.014	0.040 ± 0.002	0.059 ± 0.01	0.055 ± 0.016	0.051 ± 0.008	0.057 ± 0.026	0.048 ± 0.018	0.102 ± 0.046	0.047 ± 0.024	0.044 ± 0.024	0.037 ± 0.005	0.073 ± 0.009	0.058 ± 0.015	0.059 ± 0.03	0.081 ± 0.037
2533	0.061 ± 0.024	0.042 ± 0.02	0.042 ± 0.013	0.047 ± 0.014	0.034 ± 0.018	0.036 ± 0.007	0.037 ± 0.009	0.038 ± 0.016	0.054 ± 0.004	0.021 ± 0.003	0.047 ± 0.005	0.025 ± 0.006	0.040 ± 0.018	0.053 ± 0.029	0.041 ± 0.008	0.054 ± 0.014	0.016 ± 0.008	0.045 ± 0.028
2534	0.142 ± 0.069	0.132 ± 0.04	0.078 ± 0.006	0.114 ± 0.033	0.094 ± 0.033	0.139 ± 0.004	0.087 ± 0.024	0.103 ± 0.011	0.094 ± 0.033	0.134 ± 0.049	0.100 ± 0.034	0.152 ± 0.105	0.128 ± 0.048	0.091 ± 0.026	0.128 ± 0.031	0.087 ± 0.047	0.098 ± 0.055	0.081 ± 0.041
2535	0.117 ± 0.011	0.069 ± 0.033	0.094 ± 0.021	0.021 ± 0.018	0.071 ± 0.054	0.042 ± 0.014	0.049 ± 0.028	0.092 ± 0.053	0.075 ± 0.024	0.081 ± 0.054	0.045 ± 0.031	0.051 ± 0.019	0.058 ± 0.002	0.032 ± 0.019	0.081 ± 0.029	0.037 ± 0.034	0.069 ± 0.021	0.115 ± 0.036
2601	0.070 ± 0.01	0.072 ± 0.014	0.066 ± 0.005	0.055 ± 0.003	0.056 ± 0.012	0.090 ± 0.009	0.053 ± 0.017	0.054 ± 0.012	0.065 ± 0.014	0.069 ± 0.013	0.062 ± 0.005	0.067 ± 0.017	0.064 ± 0.015	0.068 ± 0.013	0.060 ± 0.029	0.081 ± 0.011	0.080 ± 0.014	0.073 ± 0.027

Mean values (± sd, n=2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
2319		0.20	0.31	-	-0.09	0.65	-	=	=	=	=	=	=	=	=	=
2401	Ribose-phosphate pyrophosphokinase / GAPDH	0.58	0.001	↗↗↗	0.81	<0.001	↗↗↗↗	=	=	=	=	=	=	=	=	=
2405		0.10	0.63	-	0.00	1.00	-	=	=	=	=	=	=	=	=	=
2407		0.08	0.69	-	0.20	0.31	-	=	NM >	=	=	=	=	=	=	=
2412		0.15	0.46	-	0.04	0.83	-	=	=	=	=	=	=	=	=	=
2413		0.21	0.29	-	-0.26	0.19	-	=	=	=	=	=	=	=	=	=
2424	UDP-arabinopyranose mutase 1 EC=5.4.99.30	0.48	0.012	↗↗	0.51	0.006	↗↗↗	=	=	=	=	=	=	=	=	=
2425	Fructose-bisphosphate aldolase EC=4.1.2.13	-0.40	0.037	↘↘	0.10	0.62	-	=	=	=	=	=	=	=	=	=
2502	ND	-0.21	0.29	-	0.23	0.24	-	=	NM >	=	=	=	=	=	=	=
2511	Cinnamyl alcohol dehydrogenase EC=1.1.1.195	-0.08	0.69	-	0.45	0.018	↗↗	=	=	=	=	=	=	=	=	=
2512	Alcohol dehydrogenase EC=1.1.1.1	-0.06	0.78	-	-0.61	0.0008	↘↘↘↘	=	=	NM >	=	=	=	=	=	=
2515		0.13	0.51	-	-0.18	0.37	-	=	=	=	=	=	=	=	=	=
2522		-0.22	0.28	-	-0.13	0.53	-	=	=	=	=	=	=	=	=	=
2523		0.36	0.064	↗	0.07	0.72	-	=	=	=	=	=	=	=	=	=
2525	Isocitrate dehydrogenase [NADP], chloroplastic EC=1.1.1.42	0.39	0.042	↗↗	0.37	0.060	↗	=	=	=	=	=	=	=	=	=
2532		0.27	0.17	-	0.35	0.075	↗	=	=	=	=	=	=	=	=	=
2533	ND	-0.47	0.016	↘↘	0.16	0.42	-	=	=	=	=	M >>	=	=	=	=
2534		0.07	0.75	-	-0.34	0.10	-	=	=	=	=	=	=	=	=	=
2535		-0.29	0.14	-	0.23	0.24	-	=	M >	=	=	=	=	=	=	=
2601		0.20	0.33	-	0.14	0.47	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND=non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 2602 to 2717



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

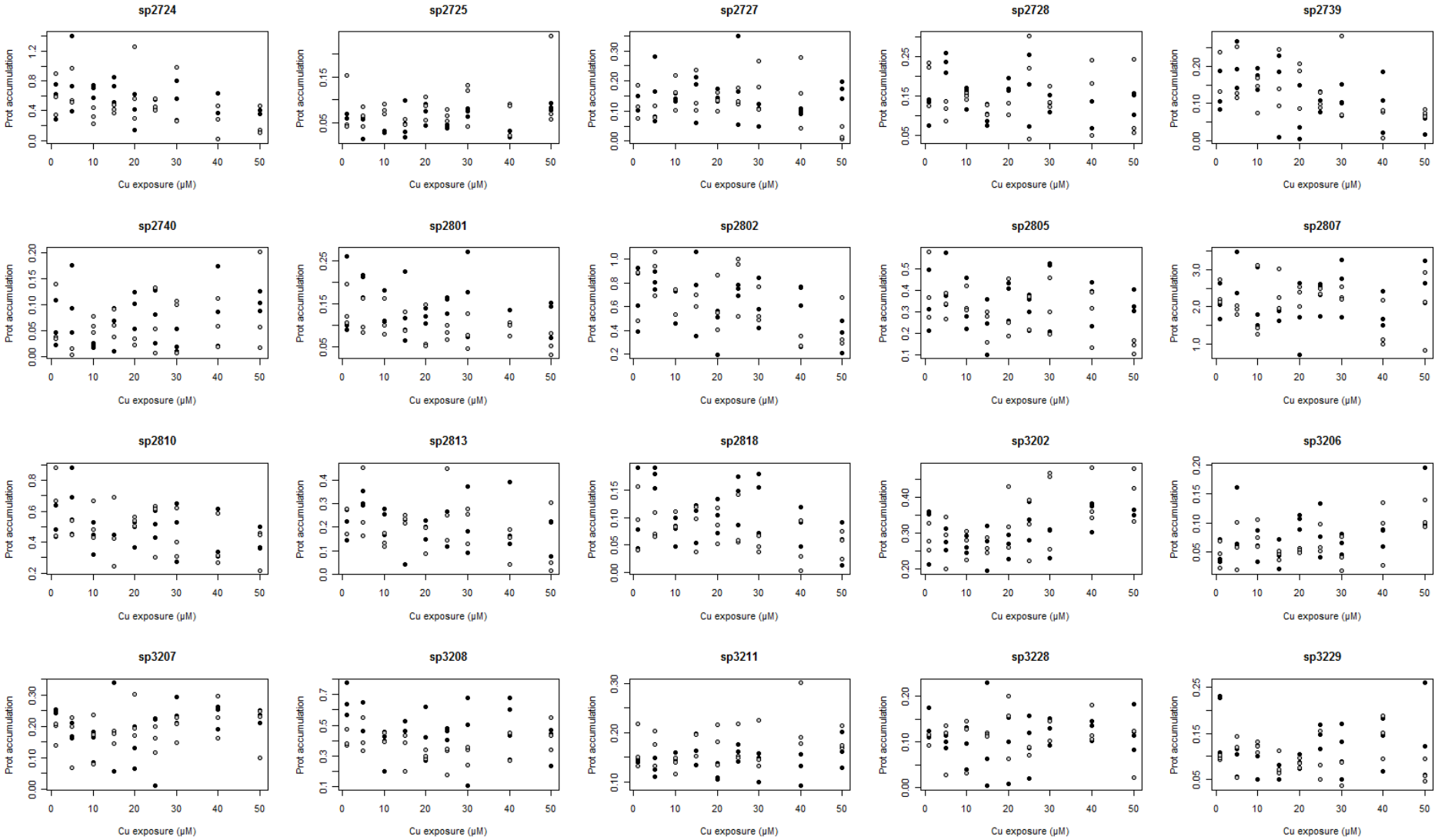
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
2602	0.055 ± 0.005	0.069 ± 0.013	0.092 ± 0.031	0.050 ± 0.012	0.058 ± 0.026	0.072 ± 0.007	0.089 ± 0.03	0.080 ± 0.008	0.071 ± 0.006	0.066 ± 0.014	0.106 ± 0.053	0.070 ± 0.032	0.066 ± 0.023	0.059 ± 0.031	0.048 ± 0.027	0.065 ± 0.008	0.079 ± 0.007	0.061 ± 0.028
2606	0.067 ± 0.01	0.035 ± 0.011	0.055 ± 0.003	0.042 ± 0.011	0.058 ± 0.006	0.054 ± 0.028	0.060 ± 0.014	0.045 ± 0.008	0.076 ± 0.016	0.037 ± 0.019	0.064 ± 0.006	0.052 ± 0.011	0.066 ± 0.023	0.032 ± 0.02	0.052 ± 0.027	0.051 ± 0.041	0.074 ± 0.006	0.036 ± 0.024
2607	0.089 ± 0.029	0.089 ± 0.019	0.081 ± 0.034	0.070 ± 0.021	0.098 ± 0.017	0.135 ± 0.022	0.092 ± 0.025	0.092 ± 0.009	0.086 ± 0.013	0.088 ± 0.028	0.100 ± 0.034	0.101 ± 0.028	0.097 ± 0.026	0.084 ± 0.033	0.091 ± 0.028	0.084 ± 0.037	0.085 ± 0.022	0.106 ± 0.02
2609	0.090 ± 0.017	0.075 ± 0.049	0.081 ± 0.05	0.069 ± 0.012	0.076 ± 0.011	0.072 ± 0.015	0.084 ± 0.026	0.041 ± 0.015	0.112 ± 0.072	0.080 ± 0.037	0.066 ± 0.05	0.082 ± 0.056	0.104 ± 0.03	0.078 ± 0.018	0.150 ± 0.038	0.074 ± 0.021	0.168 ± 0.039	0.072 ± 0.027
2614	0.051 ± 0.023	0.038 ± 0.041	0.048 ± 0.012	0.031 ± 0.014	0.038 ± 0.012	0.064 ± 0.009	0.021 ± 0.013	0.028 ± 0.005	0.036 ± 0.006	0.046 ± 0.029	0.046 ± 0.025	0.045 ± 0.027	0.043 ± 0.005	0.020 ± 0.012	0.045 ± 0.011	0.035 ± 0.008	0.040 ± 0.036	0.031 ± 0.012
2617	0.260 ± 0.03	0.354 ± 0.16	0.408 ± 0.112	0.331 ± 0.049	0.359 ± 0.149	0.324 ± 0.047	0.408 ± 0.037	0.275 ± 0.12	0.312 ± 0.114	0.213 ± 0.072	0.380 ± 0.061	0.398 ± 0.007	0.359 ± 0.137	0.316 ± 0.164	0.419 ± 0.145	0.291 ± 0.093	0.449 ± 0.109	0.406 ± 0.123
2618	0.123 ± 0.013	0.148 ± 0.039	0.215 ± 0.008	0.123 ± 0.04	0.161 ± 0.001	0.252 ± 0.021	0.249 ± 0.158	0.173 ± 0.057	0.309 ± 0.114	0.299 ± 0.159	0.145 ± 0.052	0.281 ± 0.166	0.147 ± 0.011	0.194 ± 0.041	0.170 ± 0.135	0.308 ± 0.102	0.236 ± 0.121	0.311 ± 0.127
2623	0.095 ± 0.017	0.093 ± 0.04	0.081 ± 0.009	0.055 ± 0.01	0.082 ± 0.017	0.088 ± 0.017	0.079 ± 0.021	0.060 ± 0.018	0.107 ± 0.039	0.070 ± 0.031	0.075 ± 0.046	0.043 ± 0.008	0.074 ± 0.023	0.033 ± 0.02	0.078 ± 0.055	0.086 ± 0.01	0.132 ± 0.039	0.047 ± 0.015
2627	0.160 ± 0.019	0.231 ± 0.076	0.211 ± 0.052	0.128 ± 0.039	0.146 ± 0.071	0.189 ± 0.007	0.148 ± 0.047	0.134 ± 0.034	0.200 ± 0.028	0.225 ± 0.085	0.204 ± 0.04	0.238 ± 0.036	0.173 ± 0.032	0.140 ± 0.006	0.182 ± 0.03	0.188 ± 0.093	0.187 ± 0.032	0.184 ± 0.097
2628	0.038 ± 0.015	0.039 ± 0.008	0.033 ± 0.007	0.023 ± 0.015	0.049 ± 0.004	0.043 ± 0.011	0.032 ± 0.016	0.038 ± 0.016	0.053 ± 0.008	0.046 ± 0.037	0.042 ± 0.007	0.048 ± 0.032	0.052 ± 0.026	0.026 ± 0.003	0.055 ± 0.019	0.037 ± 0.015	0.047 ± 0.016	0.034 ± 0.014
2629	0.191 ± 0.134	0.217 ± 0.04	0.132 ± 0.072	0.081 ± 0.054	0.070 ± 0.016	0.149 ± 0.078	0.091 ± 0.037	0.116 ± 0.072	0.057 ± 0.035	0.036 ± 0.019	0.085 ± 0.015	0.098 ± 0.087	0.099 ± 0.007	0.103 ± 0.075	0.117 ± 0.016	0.179 ± 0.155	0.185 ± 0.055	0.100 ± 0.082
2701	0.131 ± 0.02	0.121 ± 0.053	0.157 ± 0.037	0.074 ± 0.031	0.103 ± 0.027	0.116 ± 0.028	0.139 ± 0.038	0.116 ± 0.034	0.117 ± 0.042	0.118 ± 0.052	0.118 ± 0.018	0.104 ± 0.006	0.116 ± 0.073	0.129 ± 0.093	0.112 ± 0.035	0.106 ± 0.031	0.172 ± 0.027	0.118 ± 0.057
2702	0.054 ± 0.012	0.084 ± 0.026	0.097 ± 0.023	0.053 ± 0.006	0.041 ± 0.024	0.074 ± 0.012	0.045 ± 0.018	0.046 ± 0.023	0.102 ± 0.007	0.049 ± 0.004	0.078 ± 0.041	0.083 ± 0.047	0.048 ± 0.017	0.057 ± 0.005	0.071 ± 0.06	0.087 ± 0.03	0.091 ± 0.036	0.061 ± 0.044
2703	0.069 ± 0.021	0.086 ± 0.04	0.061 ± 0.014	0.066 ± 0.03	0.063 ± 0.027	0.064 ± 0.029	0.070 ± 0.021	0.105 ± 0.055	0.065 ± 0.017	0.072 ± 0.011	0.103 ± 0.041	0.088 ± 0.039	0.070 ± 0.046	0.077 ± 0.023	0.083 ± 0.011	0.099 ± 0.049	0.061 ± 0.033	0.109 ± 0.024
2708	0.044 ± 0.01	0.063 ± 0.038	0.047 ± 0.004	0.034 ± 0.01	0.034 ± 0.021	0.039 ± 0.011	0.038 ± 0.012	0.055 ± 0.023	0.074 ± 0.035	0.043 ± 0.014	0.062 ± 0.024	0.055 ± 0.044	0.035 ± 0.015	0.062 ± 0.025	0.041 ± 0.013	0.037 ± 0.022	0.049 ± 0.026	0.071 ± 0.026
2709	0.044 ± 0.013	0.062 ± 0.008	0.048 ± 0.009	0.050 ± 0.02	0.031 ± 0.008	0.031 ± 0.01	0.027 ± 0.009	0.046 ± 0.021	0.044 ± 0.017	0.050 ± 0.042	0.039 ± 0.016	0.053 ± 0.032	0.033 ± 0.014	0.044 ± 0.029	0.037 ± 0.027	0.059 ± 0.03	0.065 ± 0.016	0.062 ± 0.05
2710	0.041 ± 0.029	0.037 ± 0.024	0.063 ± 0.011	0.056 ± 0.022	0.066 ± 0.005	0.032 ± 0.013	0.048 ± 0.027	0.053 ± 0.015	0.063 ± 0.03	0.037 ± 0.013	0.046 ± 0.031	0.056 ± 0.016	0.043 ± 0.006	0.037 ± 0.03	0.063 ± 0.027	0.071 ± 0.029	0.049 ± 0.041	0.064 ± 0.022
2711	0.150 ± 0.023	0.165 ± 0.052	0.202 ± 0.042	0.099 ± 0.017	0.125 ± 0.03	0.159 ± 0.021	0.149 ± 0.054	0.110 ± 0.024	0.166 ± 0.018	0.112 ± 0.068	0.175 ± 0.031	0.199 ± 0.12	0.144 ± 0.051	0.170 ± 0.073	0.148 ± 0.078	0.161 ± 0.075	0.169 ± 0.058	0.186 ± 0.127
2716	0.090 ± 0.03	0.096 ± 0.018	0.081 ± 0.036	0.100 ± 0.022	0.095 ± 0.022	0.107 ± 0.02	0.082 ± 0.011	0.110 ± 0.03	0.080 ± 0.008	0.069 ± 0.015	0.082 ± 0.035	0.106 ± 0.014	0.086 ± 0.018	0.091 ± 0.028	0.068 ± 0.049	0.102 ± 0.037	0.086 ± 0.021	0.111 ± 0.044
2717	0.019 ± 0.002	0.034 ± 0.008	0.025 ± 0.008	0.016 ± 0.006	0.023 ± 0.01	0.033 ± 0.014	0.009 ± 0.003	0.025 ± 0.008	0.025 ± 0.003	0.032 ± 0.01	0.022 ± 0.008	0.031 ± 0.008	0.012 ± 0.011	0.025 ± 0.004	0.020 ± 0.006	0.033 ± 0.006	0.033 ± 0.005	0.044 ± 0.02

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
2602		-0.03	0.88	-	-0.06	0.77	-	=	=	=	=	=	=	=	=	=
2606		0.11	0.58	-	-0.02	0.92	-	=	=	=	=	=	=	=	=	=
2607		0.01	0.95	-	0.03	0.87	-	=	=	=	=	=	=	=	=	=
2609	Aldehyde dehydrogenase family 2 member B7, mito. EC=1.2.1.3	0.56	0.002	↗↗↗	0.08	0.69	-	=	=	=	=	=	=	=	=	=
2614		-0.03	0.88	-	-0.18	0.36	-	=	=	=	=	=	=	=	=	=
2617		0.33	0.096	↗	0.12	0.56	-	=	=	=	=	=	=	=	=	=
2618	Alanine aminotransferase 2 EC=2.6.1.2	0.09	0.66	-	0.48	0.011	↗↗	=	=	=	=	=	=	=	=	=
2623	Alanine aminotransferase 2 : ALAAT2 EC=2.6.1.2	0.22	0.28	-	-0.30	0.12	-	=	=	=	=	=	=	=	=	M >
2627		0.13	0.52	-	0.01	0.97	-	=	=	=	=	=	=	=	=	=
2628		0.33	0.089	↗	-0.02	0.93	-	=	=	=	=	=	=	=	=	=
2629		0.08	0.69	-	-0.11	0.59	-	=	=	=	=	=	=	=	=	=
2701		0.10	0.63	-	0.09	0.65	-	=	=	=	=	=	=	=	=	=
2702		0.17	0.39	-	0.04	0.86	-	=	=	=	=	M >	=	=	=	=
2703		0.10	0.63	-	0.29	0.15	-	=	=	=	=	=	=	=	=	=
2708		0.05	0.82	-	0.17	0.39	-	=	=	=	=	=	=	=	=	=
2709		0.25	0.22	-	0.13	0.53	-	=	=	=	=	=	=	=	=	=
2710		-0.03	0.88	-	0.34	0.086	↗	=	=	=	=	=	=	=	=	=
2711		-0.01	0.97	-	0.24	0.22	-	=	=	=	=	=	=	=	=	=
2716		-0.13	0.52	-	0.08	0.70	-	=	=	=	=	=	=	=	=	=
2717		0.22	0.26	-	0.36	0.063	↗	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 2724 to 3229



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

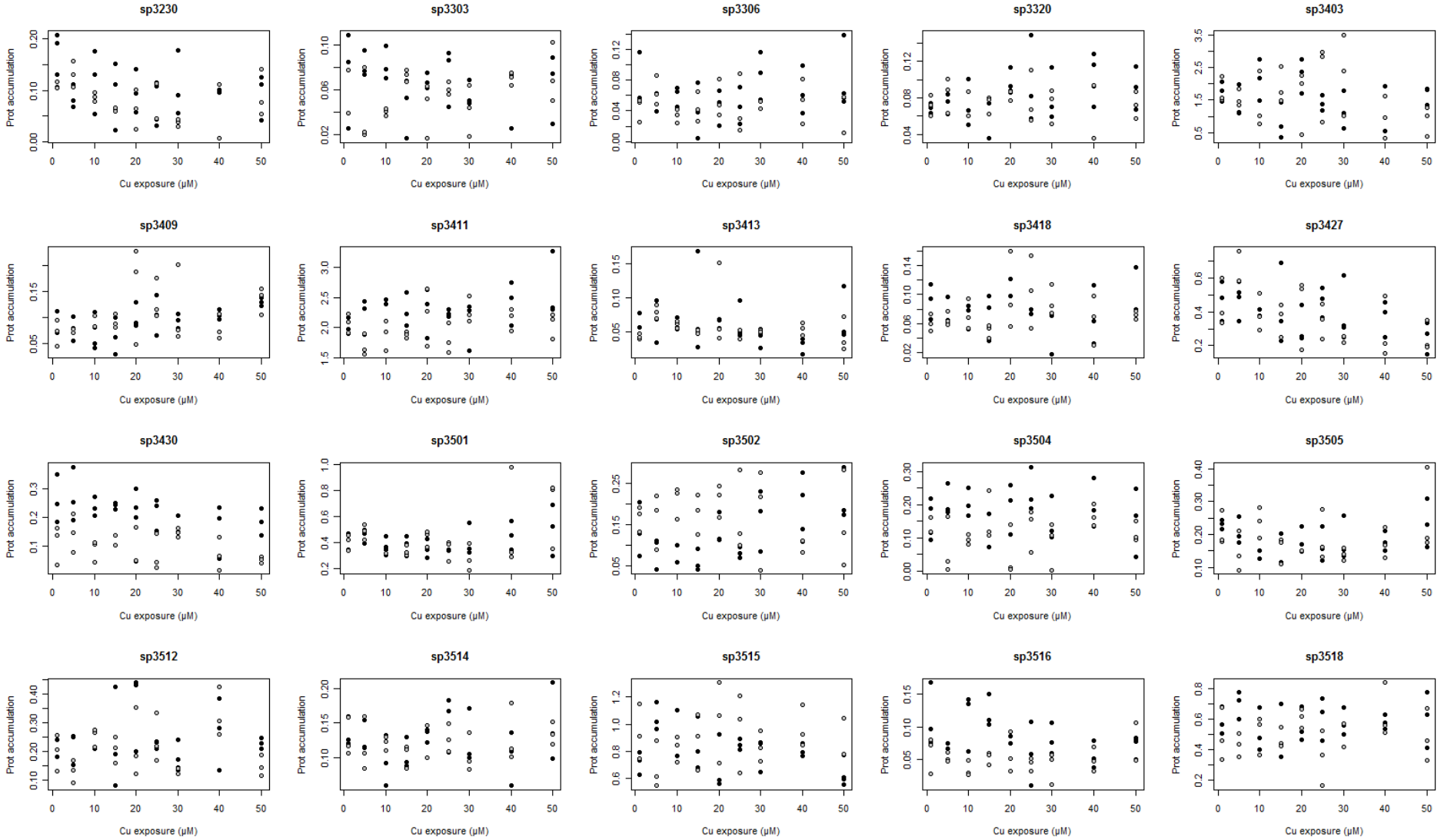
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
2724	0.554 ± 0.243	0.606 ± 0.278	0.844 ± 0.512	0.672 ± 0.259	0.673 ± 0.085	0.330 ± 0.11	0.698 ± 0.17	0.423 ± 0.056	0.393 ± 0.244	0.709 ± 0.499	0.471 ± 0.068	0.471 ± 0.085	0.548 ± 0.265	0.501 ± 0.417	0.490 ± 0.131	0.254 ± 0.222	0.385 ± 0.029	0.234 ± 0.198
2725	0.058 ± 0.013	0.080 ± 0.064	0.045 ± 0.027	0.064 ± 0.021	0.030 ± 0.001	0.079 ± 0.01	0.048 ± 0.043	0.050 ± 0.007	0.069 ± 0.024	0.083 ± 0.026	0.041 ± 0.004	0.066 ± 0.013	0.073 ± 0.009	0.098 ± 0.05	0.045 ± 0.036	0.067 ± 0.039	0.085 ± 0.008	0.122 ± 0.103
2727	0.118 ± 0.027	0.124 ± 0.056	0.171 ± 0.107	0.092 ± 0.022	0.144 ± 0.013	0.160 ± 0.058	0.154 ± 0.08	0.155 ± 0.071	0.150 ± 0.021	0.131 ± 0.031	0.190 ± 0.149	0.144 ± 0.03	0.093 ± 0.039	0.184 ± 0.081	0.100 ± 0.009	0.159 ± 0.118	0.171 ± 0.028	0.023 ± 0.023
2728	0.117 ± 0.037	0.194 ± 0.06	0.235 ± 0.025	0.113 ± 0.026	0.150 ± 0.03	0.149 ± 0.007	0.088 ± 0.015	0.119 ± 0.015	0.176 ± 0.018	0.112 ± 0.018	0.169 ± 0.091	0.188 ± 0.134	0.131 ± 0.021	0.144 ± 0.027	0.128 ± 0.058	0.158 ± 0.098	0.137 ± 0.031	0.123 ± 0.106
2739	0.125 ± 0.054	0.168 ± 0.061	0.200 ± 0.063	0.165 ± 0.076	0.169 ± 0.029	0.130 ± 0.049	0.140 ± 0.116	0.159 ± 0.079	0.064 ± 0.076	0.159 ± 0.065	0.106 ± 0.028	0.106 ± 0.022	0.119 ± 0.028	0.139 ± 0.122	0.104 ± 0.081	0.055 ± 0.042	0.049 ± 0.029	0.075 ± 0.009
2740	0.060 ± 0.044	0.071 ± 0.059	0.106 ± 0.065	0.012 ± 0.007	0.022 ± 0.005	0.061 ± 0.015	0.058 ± 0.043	0.064 ± 0.026	0.093 ± 0.036	0.027 ± 0.008	0.079 ± 0.051	0.064 ± 0.063	0.028 ± 0.023	0.072 ± 0.056	0.094 ± 0.077	0.064 ± 0.047	0.106 ± 0.019	0.092 ± 0.097
2801	0.150 ± 0.096	0.140 ± 0.048	0.198 ± 0.029	0.114 ± 0.042	0.134 ± 0.042	0.114 ± 0.044	0.136 ± 0.083	0.103 ± 0.025	0.122 ± 0.018	0.086 ± 0.053	0.150 ± 0.021	0.083 ± 0.017	0.174 ± 0.099	0.084 ± 0.041	0.124 ± 0.02	0.094 ± 0.017	0.123 ± 0.044	0.055 ± 0.025
2802	0.641 ± 0.273	0.750 ± 0.232	0.816 ± 0.074	0.898 ± 0.188	0.643 ± 0.164	0.674 ± 0.125	0.733 ± 0.36	0.665 ± 0.058	0.423 ± 0.201	0.607 ± 0.239	0.744 ± 0.047	0.825 ± 0.265	0.610 ± 0.214	0.591 ± 0.155	0.710 ± 0.089	0.290 ± 0.05	0.356 ± 0.14	0.429 ± 0.217
2805	0.340 ± 0.143	0.407 ± 0.157	0.428 ± 0.129	0.333 ± 0.06	0.319 ± 0.123	0.349 ± 0.061	0.236 ± 0.128	0.248 ± 0.075	0.366 ± 0.094	0.300 ± 0.14	0.346 ± 0.042	0.267 ± 0.09	0.416 ± 0.18	0.318 ± 0.131	0.356 ± 0.108	0.282 ± 0.134	0.345 ± 0.051	0.141 ± 0.032
2807	2.121 ± 0.484	2.354 ± 0.338	2.632 ± 0.763	1.920 ± 0.127	2.117 ± 0.837	1.932 ± 1.038	1.818 ± 0.171	2.419 ± 0.552	1.686 ± 0.963	2.315 ± 0.284	2.306 ± 0.49	2.391 ± 0.096	2.579 ± 0.794	2.329 ± 0.176	1.855 ± 0.49	1.423 ± 0.656	2.665 ± 0.574	1.954 ± 1.071
2810	0.519 ± 0.107	0.664 ± 0.221	0.705 ± 0.169	0.482 ± 0.052	0.432 ± 0.106	0.528 ± 0.125	0.446	0.452 ± 0.224	0.464 ± 0.084	0.535 ± 0.031	0.516 ± 0.087	0.515 ± 0.185	0.485 ± 0.189	0.445 ± 0.159	0.421 ± 0.168	0.390 ± 0.171	0.409 ± 0.08	0.375 ± 0.136
2813	0.197 ± 0.046	0.242 ± 0.06	0.317 ± 0.033	0.280 ± 0.155	0.233 ± 0.058	0.141 ± 0.03	0.164 ± 0.106	0.234 ± 0.017	0.191 ± 0.041	0.162 ± 0.065	0.212 ± 0.081	0.282 ± 0.156	0.215 ± 0.143	0.220 ± 0.081	0.228 ± 0.143	0.129 ± 0.078	0.173 ± 0.086	0.124 ± 0.159
2818	0.105 ± 0.077	0.098 ± 0.058	0.174 ± 0.019	0.081 ± 0.024	0.076 ± 0.026	0.093 ± 0.015	0.096 ± 0.037	0.085 ± 0.042	0.103 ± 0.031	0.085 ± 0.033	0.136 ± 0.045	0.085 ± 0.049	0.135 ± 0.056	0.050 ± 0.015	0.086 ± 0.036	0.042 ± 0.047	0.055 ± 0.04	0.053 ± 0.026
3202	0.309 ± 0.084	0.286 ± 0.039	0.280 ± 0.03	0.281 ± 0.075	0.267 ± 0.024	0.271 ± 0.04	0.265 ± 0.063	0.264 ± 0.022	0.264 ± 0.035	0.336 ± 0.086	0.335 ± 0.053	0.313 ± 0.086	0.284 ± 0.046	0.394 ± 0.12	0.354 ± 0.045	0.396 ± 0.078	0.356 ± 0.008	0.413 ± 0.076
3206	0.047 ± 0.021	0.046 ± 0.023	0.095 ± 0.057	0.059 ± 0.041	0.061 ± 0.026	0.080 ± 0.023	0.045 ± 0.025	0.045 ± 0.009	0.103 ± 0.013	0.053 ± 0.004	0.083 ± 0.047	0.070 ± 0.025	0.064 ± 0.017	0.045 ± 0.029	0.079 ± 0.016	0.088 ± 0.055	0.130 ± 0.057	0.112 ± 0.025
3207	0.248 ± 0.007	0.184 ± 0.039	0.181 ± 0.027	0.165 ± 0.086	0.145 ± 0.052	0.163 ± 0.079	0.191 ± 0.142	0.170 ± 0.021	0.132 ± 0.068	0.222 ± 0.07	0.153 ± 0.124	0.159 ± 0.042	0.247 ± 0.043	0.195 ± 0.041	0.235 ± 0.04	0.230 ± 0.067	0.233 ± 0.021	0.193 ± 0.081
3208	0.660 ± 0.105	0.408 ± 0.059	0.482 ± 0.158	0.422 ± 0.111	0.361 ± 0.143	0.411 ± 0.032	0.458 ± 0.071	0.339 ± 0.123	0.436 ± 0.174	0.306 ± 0.028	0.448 ± 0.042	0.287 ± 0.094	0.430 ± 0.295	0.314 ± 0.064	0.571 ± 0.124	0.331 ± 0.101	0.381 ± 0.128	0.442 ± 0.104
3211	0.144 ± 0.004	0.167 ± 0.045	0.128 ± 0.02	0.170 ± 0.035	0.151 ± 0.007	0.134 ± 0.017	0.144 ± 0.017	0.182 ± 0.026	0.118 ± 0.018	0.176 ± 0.041	0.160 ± 0.017	0.173 ± 0.039	0.135 ± 0.031	0.167 ± 0.05	0.127 ± 0.032	0.223 ± 0.068	0.164 ± 0.037	0.186 ± 0.025
3228	0.136 ± 0.034	0.107 ± 0.013	0.100 ± 0.014	0.094 ± 0.058	0.089 ± 0.047	0.101 ± 0.061	0.099 ± 0.117	0.116 ± 0.005	0.087 ± 0.074	0.140 ± 0.07	0.099 ± 0.071	0.082 ± 0.009	0.124 ± 0.03	0.131 ± 0.025	0.128 ± 0.022	0.133 ± 0.041	0.126 ± 0.051	0.089 ± 0.059
3229	0.189 ± 0.069	0.097 ± 0.005	0.093 ± 0.032	0.106 ± 0.046	0.092 ± 0.037	0.121 ± 0.012	0.066 ± 0.015	0.082 ± 0.026	0.088 ± 0.015	0.086 ± 0.011	0.143 ± 0.026	0.095 ± 0.053	0.118 ± 0.062	0.071 ± 0.03	0.132 ± 0.058	0.145 ± 0.046	0.147 ± 0.102	0.067 ± 0.025

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
2724	Phenylalanine ammonia-lyase / Phenylalanine/tyrosine ammonia-lyase EC=4.3.1.24/25	-0.40	0.040	↘↘	-0.39	0.043	↘↘	=	=	=	=	=	=	=	=	=
2725	Ketol-acid reductoisomerase, chloro. EC=1.1.1.86	0.35	0.075	↗	0.25	0.20	-	=	=	NM >>	=	=	=	=	=	=
2727	D-3-phosphoglycerate dehydrogenase, chloroplastic EC=1.1.1.95	-0.03	0.87	-	-0.19	0.34	-	=	=	=	=	=	=	=	=	M >>
2728		-0.17	0.41	-	-0.06	0.75	-	=	M >	=	=	=	=	=	=	=
2739	ND	-0.47	0.014	↘↘	-0.49	0.009	↘↘↘	=	=	=	=	=	=	=	=	=
2740		0.21	0.30	-	0.26	0.19	-	=	M >>	NM >	=	M >	=	=	=	=
2801	Aconitate hydratase, cytoplasmic EC=4.2.1.3	-0.20	0.31	-	-0.53	0.004	↘↘↘	=	=	=	=	=	=	=	=	=
2802	Methionine synthase: MetE EC=2.1.1.14	-0.33	0.089	↘	-0.60	0.0010	↘↘↘↘	=	=	=	=	=	=	=	M >	=
2805	Aconitate hydratase, cytoplasmic EC=4.2.1.3	0.03	0.87	-	-0.51	0.007	↘↘↘	=	=	=	=	=	=	=	=	M >
2807		0.10	0.61	-	-0.21	0.30	-	=	=	=	=	=	=	=	=	=
2810	Aconitate hydratase, cytoplasmic EC=4.2.1.3	-0.37	0.058	↘	-0.43	0.025	↘↘	=	=	=	=	=	=	=	=	=
2813		-0.18	0.37	-	-0.33	0.092	↘	=	=	=	=	=	=	=	=	=
2818	Aconitate hydratase, cytoplasmic EC=4.2.1.3	-0.32	0.11	-	-0.46	0.016	↘↘	=	M >	=	=	=	=	=	=	=
3202	Superoxide dismutase [Mn], mito. EC=1.15.1.1	0.46	0.015	↗↗	0.61	0.0008	↗↗↗	=	=	=	=	=	=	=	=	=
3206	ND	0.04	0.39	-	0.46	0.017	↗↗	=	=	=	=	M >	=	=	=	=
3207		0.19	0.34	-	0.22	0.27	-	=	=	=	=	=	=	=	=	=
3208		-0.18	0.37	-	-0.10	0.62	-	=	=	=	=	=	=	=	=	=
3211		0.14	0.48	-	0.32	0.099	↗	=	=	=	=	=	=	=	=	=
3228		0.12	0.55	-	0.04	0.83	-	=	=	=	=	=	=	=	=	=
3229		0.11	0.60	-	-0.11	0.59	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 3230 to 3518



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

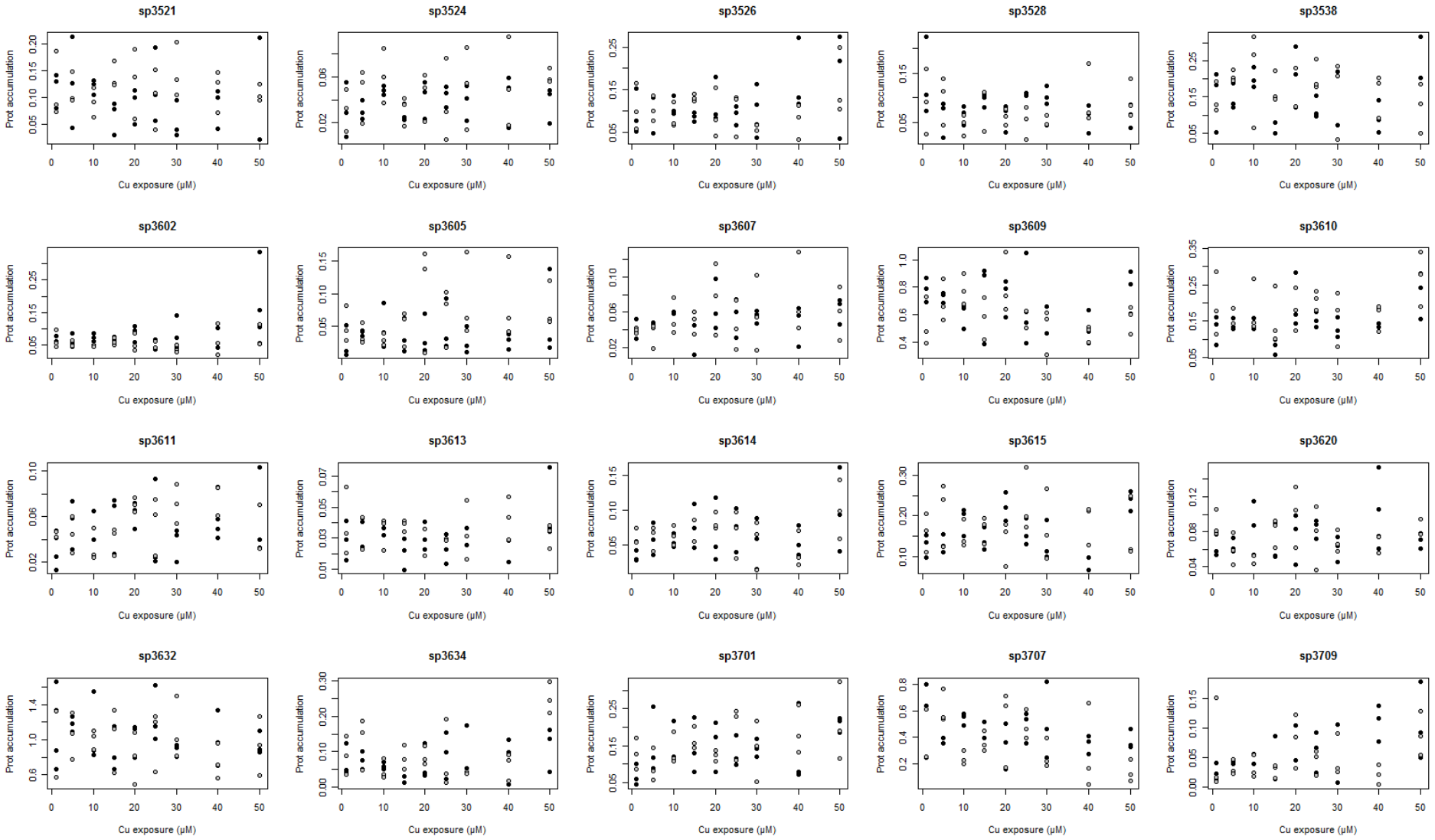
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
3230	0.176 ± 0.04	0.109 ± 0.007	0.087 ± 0.023	0.131 ± 0.025	0.120 ± 0.062	0.087 ± 0.009	0.095 ± 0.066	0.063 ± 0.004	0.097 ± 0.042	0.064 ± 0.038	0.085 ± 0.047	0.068 ± 0.039	0.108 ± 0.062	0.038 ± 0.007	0.099 ± 0.002	0.077 ± 0.06	0.093 ± 0.045	0.091 ± 0.045
3303	0.073 ± 0.042	0.065 ± 0.022	0.082 ± 0.012	0.041 ± 0.034	0.082 ± 0.015	0.040 ± 0.003	0.046 ± 0.026	0.073 ± 0.005	0.068 ± 0.007	0.044 ± 0.024	0.074 ± 0.026	0.061 ± 0.006	0.056 ± 0.012	0.042 ± 0.023	0.058 ± 0.027	0.070 ± 0.006	0.064 ± 0.031	0.074 ± 0.026
3306	0.076 ± 0.035	0.043 ± 0.016	0.050 ± 0.012	0.065 ± 0.019	0.060 ± 0.013	0.034 ± 0.009	0.040 ± 0.036	0.044 ± 0.019	0.046 ± 0.023	0.054 ± 0.024	0.046 ± 0.024	0.044 ± 0.038	0.087 ± 0.031	0.046 ± 0.006	0.065 ± 0.032	0.053 ± 0.029	0.084 ± 0.047	0.042 ± 0.027
3320	0.069 ± 0.006	0.072 ± 0.011	0.074 ± 0.011	0.084 ± 0.019	0.073 ± 0.025	0.078 ± 0.015	0.063 ± 0.023	0.074 ± 0.01	0.098 ± 0.014	0.083 ± 0.005	0.096 ± 0.047	0.078 ± 0.029	0.081 ± 0.028	0.073 ± 0.019	0.105 ± 0.031	0.074 ± 0.033	0.091 ± 0.024	0.072 ± 0.015
3403	1.771 ± 0.305	1.764 ± 0.391	1.404 ± 0.492	1.551 ± 0.254	2.130 ± 0.634	1.397 ± 0.878	0.830 ± 0.559	1.915 ± 0.535	2.277 ± 0.529	1.560 ± 0.98	1.402 ± 0.235	2.204 ± 1.196	1.170 ± 0.577	2.301 ± 1.244	1.460 ± 0.799	0.971 ± 0.652	1.662 ± 0.275	0.883 ± 0.444
3409	0.098 ± 0.023	0.070 ± 0.025	0.078 ± 0.024	0.075 ± 0.005	0.066 ± 0.037	0.089 ± 0.013	0.064 ± 0.036	0.091 ± 0.014	0.101 ± 0.024	0.154 ± 0.095	0.104 ± 0.039	0.131 ± 0.039	0.093 ± 0.014	0.114 ± 0.077	0.104 ± 0.01	0.080 ± 0.024	0.129 ± 0.008	0.134 ± 0.026
3411	2.013 ± 0.141	2.082 ± 0.159	2.208 ± 0.29	1.699 ± 0.178	2.319 ± 0.189	1.891 ± 0.251	2.282 ± 0.283	1.884 ± 0.058	2.280 ± 0.409	2.200 ± 0.478	2.239 ± 0.063	1.808 ± 0.242	2.086 ± 0.402	2.284 ± 0.214	2.425 ± 0.355	2.147 ± 0.181	2.633 ± 0.547	2.054 ± 0.211
3413	0.058 ± 0.019	0.043 ± 0.004	0.067 ± 0.031	0.080 ± 0.01	0.061 ± 0.009	0.062 ± 0.006	0.084 ± 0.075	0.050 ± 0.003	0.064 ± 0.007	0.082 ± 0.061	0.063 ± 0.028	0.046 ± 0.009	0.040 ± 0.011	0.053 ± 0.002	0.031 ± 0.012	0.055 ± 0.009	0.072 ± 0.04	0.044 ± 0.025
3418	0.091 ± 0.024	0.061 ± 0.012	0.074 ± 0.02	0.066 ± 0.01	0.072 ± 0.017	0.072 ± 0.021	0.072 ± 0.032	0.050 ± 0.009	0.092 ± 0.034	0.101 ± 0.053	0.076 ± 0.004	0.104 ± 0.05	0.055 ± 0.032	0.091 ± 0.021	0.069 ± 0.041	0.066 ± 0.034	0.096 ± 0.036	0.072 ± 0.006
3427	0.467 ± 0.118	0.442 ± 0.137	0.447 ± 0.091	0.639 ± 0.103	0.401 ± 0.022	0.389 ± 0.11	0.421 ± 0.239	0.359 ± 0.096	0.315 ± 0.109	0.423 ± 0.213	0.461 ± 0.089	0.348 ± 0.103	0.415 ± 0.175	0.242 ± 0.021	0.369 ± 0.106	0.287 ± 0.181	0.252 ± 0.094	0.248 ± 0.09
3430	0.261 ± 0.084	0.112 ± 0.067	0.273 ± 0.095	0.146 ± 0.067	0.236 ± 0.032	0.087 ± 0.039	0.240 ± 0.011	0.115 ± 0.02	0.245 ± 0.052	0.087 ± 0.067	0.217 ± 0.057	0.070 ± 0.064	0.188 ± 0.033	0.147 ± 0.016	0.162 ± 0.093	0.071 ± 0.057	0.185 ± 0.047	0.051 ± 0.012
3501	0.422 ± 0.068	0.403 ± 0.059	0.450 ± 0.055	0.481 ± 0.058	0.384 ± 0.054	0.310 ± 0.006	0.377 ± 0.078	0.334 ± 0.037	0.349 ± 0.071	0.435 ± 0.064	0.337 ± 0.005	0.345 ± 0.081	0.406 ± 0.123	0.281 ± 0.104	0.453 ± 0.112	0.531 ± 0.39	0.502 ± 0.198	0.660 ± 0.266
3502	0.135 ± 0.065	0.166 ± 0.03	0.086 ± 0.038	0.164 ± 0.067	0.086 ± 0.023	0.208 ± 0.039	0.061 ± 0.026	0.177 ± 0.048	0.136 ± 0.039	0.211 ± 0.038	0.083 ± 0.013	0.170 ± 0.097	0.166 ± 0.074	0.178 ± 0.122	0.212 ± 0.069	0.101 ± 0.017	0.216 ± 0.064	0.155 ± 0.117
3504	0.167 ± 0.065	0.133 ± 0.025	0.210 ± 0.048	0.067 ± 0.086	0.206 ± 0.043	0.095 ± 0.016	0.121 ± 0.049	0.156 ± 0.076	0.195 ± 0.075	0.052 ± 0.077	0.240 ± 0.066	0.131 ± 0.065	0.150 ± 0.067	0.083 ± 0.071	0.200 ± 0.075	0.167 ± 0.033	0.153 ± 0.104	0.116 ± 0.031
3505	0.231 ± 0.014	0.211 ± 0.054	0.208 ± 0.041	0.146 ± 0.06	0.156 ± 0.032	0.237 ± 0.047	0.165 ± 0.044	0.157 ± 0.04	0.183 ± 0.038	0.151 ± 0.002	0.168 ± 0.053	0.190 ± 0.076	0.182 ± 0.066	0.140 ± 0.018	0.179 ± 0.03	0.172 ± 0.047	0.233 ± 0.074	0.257 ± 0.129
3512	0.210 ± 0.03	0.199 ± 0.062	0.219 ± 0.056	0.133 ± 0.039	0.212 ± 0.003	0.252 ± 0.032	0.232 ± 0.175	0.208 ± 0.045	0.357 ± 0.135	0.221 ± 0.119	0.225 ± 0.012	0.240 ± 0.085	0.183 ± 0.054	0.136 ± 0.011	0.267 ± 0.126	0.331 ± 0.085	0.227 ± 0.019	0.150 ± 0.036
3514	0.136 ± 0.021	0.127 ± 0.028	0.128 ± 0.022	0.117 ± 0.038	0.095 ± 0.037	0.121 ± 0.01	0.104 ± 0.023	0.103 ± 0.017	0.133 ± 0.009	0.115 ± 0.027	0.153 ± 0.04	0.128 ± 0.02	0.125 ± 0.04	0.105 ± 0.028	0.102 ± 0.039	0.131 ± 0.042	0.147 ± 0.056	0.135 ± 0.016
3515	0.716 ± 0.086	0.935 ± 0.206	1.048 ± 0.104	0.677 ± 0.176	0.877 ± 0.198	0.822 ± 0.096	0.845 ± 0.191	0.880 ± 0.206	0.688 ± 0.205	1.027 ± 0.299	0.849 ± 0.038	0.962 ± 0.294	0.788 ± 0.124	0.834 ± 0.112	0.804 ± 0.049	0.971 ± 0.153	0.584 ± 0.027	0.865 ± 0.156
3516	0.114 ± 0.048	0.060 ± 0.029	0.072 ± 0.005	0.052 ± 0.007	0.113 ± 0.044	0.035 ± 0.012	0.121 ± 0.025	0.053 ± 0.009	0.078 ± 0.006	0.059 ± 0.031	0.059 ± 0.049	0.043 ± 0.01	0.081 ± 0.024	0.039 ± 0.024	0.056 ± 0.021	0.050 ± 0.019	0.079 ± 0.003	0.068 ± 0.033
3518	0.583 ± 0.091	0.490 ± 0.173	0.701 ± 0.089	0.431 ± 0.078	0.517 ± 0.142	0.509 ± 0.127	0.492 ± 0.184	0.470 ± 0.067	0.555 ± 0.113	0.608 ± 0.063	0.613 ± 0.143	0.350 ± 0.181	0.577 ± 0.09	0.468 ± 0.089	0.581 ± 0.049	0.637 ± 0.178	0.605 ± 0.184	0.485 ± 0.171

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
3230		-0.27	0.17	-	-0.30	0.13	-	=	=	=	=	=	=	=	=	=
3303		-0.23	0.25	-	0.28	0.16	-	=	=	M >	=	=	=	=	=	=
3306		0.23	0.24	-	-0.04	0.85	-	=	=	=	=	=	=	=	=	=
3320		0.38	0.050	↗	-0.10	0.60	-	=	=	=	=	=	=	=	=	=
3403		-0.09	0.66	-	-0.24	0.23	-	=	=	=	=	=	=	=	=	=
3409	Alpha-galactosidase EC=3.2.1.22	0.48	0.011	↗↗	0.30	0.13	-	=	=	=	=	=	=	=	=	=
3411	Malate dehydrogenase EC=1.1.1.37	0.39	0.044	↗↗	0.29	0.14	-	=	=	=	=	=	=	=	=	=
3413		-0.14	0.48	-	-0.19	0.34	-	=	=	=	=	=	=	=	=	=
3418		0.01	0.96	-	0.14	0.50	-	=	=	=	=	=	=	=	=	=
3427	Flavone 3'-O-methyltransferase 1	-0.38	0.048	↘↘	-0.57	0.002	↘↘↘	=	=	=	=	=	=	=	=	=
3430	Flavone 3'-O-methyltransferase / Tricetin 3',4',5'-O-trimethyltransferase / Malate DH	-0.52	0.006	↘↘↘	-0.35	0.071	↘	=	=	M >	M >	=	=	=	=	M >>
3501		0.22	0.28	-	0.35	0.075	↗	=	=	=	=	=	=	=	=	=
3502	GDP-mannose 3,5-epimerase 2 / Alcohol dehydrogenase 3	0.61	0.0008	↗↗↗	-0.21	0.28	-	=	=	NM >	=	=	=	=	=	=
3504		-0.07	0.73	-	0.15	0.45	-	=	=	M >	=	=	=	=	=	=
3505		0.05	0.80	-	0.13	0.51	-	=	=	=	=	=	=	=	=	=
3512		0.06	0.75	-	0.09	0.64	-	=	=	=	=	=	=	=	=	=
3514		0.11	0.59	-	0.16	0.43	-	=	=	=	=	=	=	=	=	=
3515	Isocitrate dehydrogenase [NADP] / GDP-mannose 3,5-epimerase 2	-0.42	0.031	↘↘	0.15	0.47	-	=	=	=	=	=	=	=	=	=
3516		-0.38	0.053	↘	0.11	0.60	-	=	=	M >	M >	=	=	=	=	=
3518		0.01	0.98	-	0.14	0.49	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 3521 to 3709



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

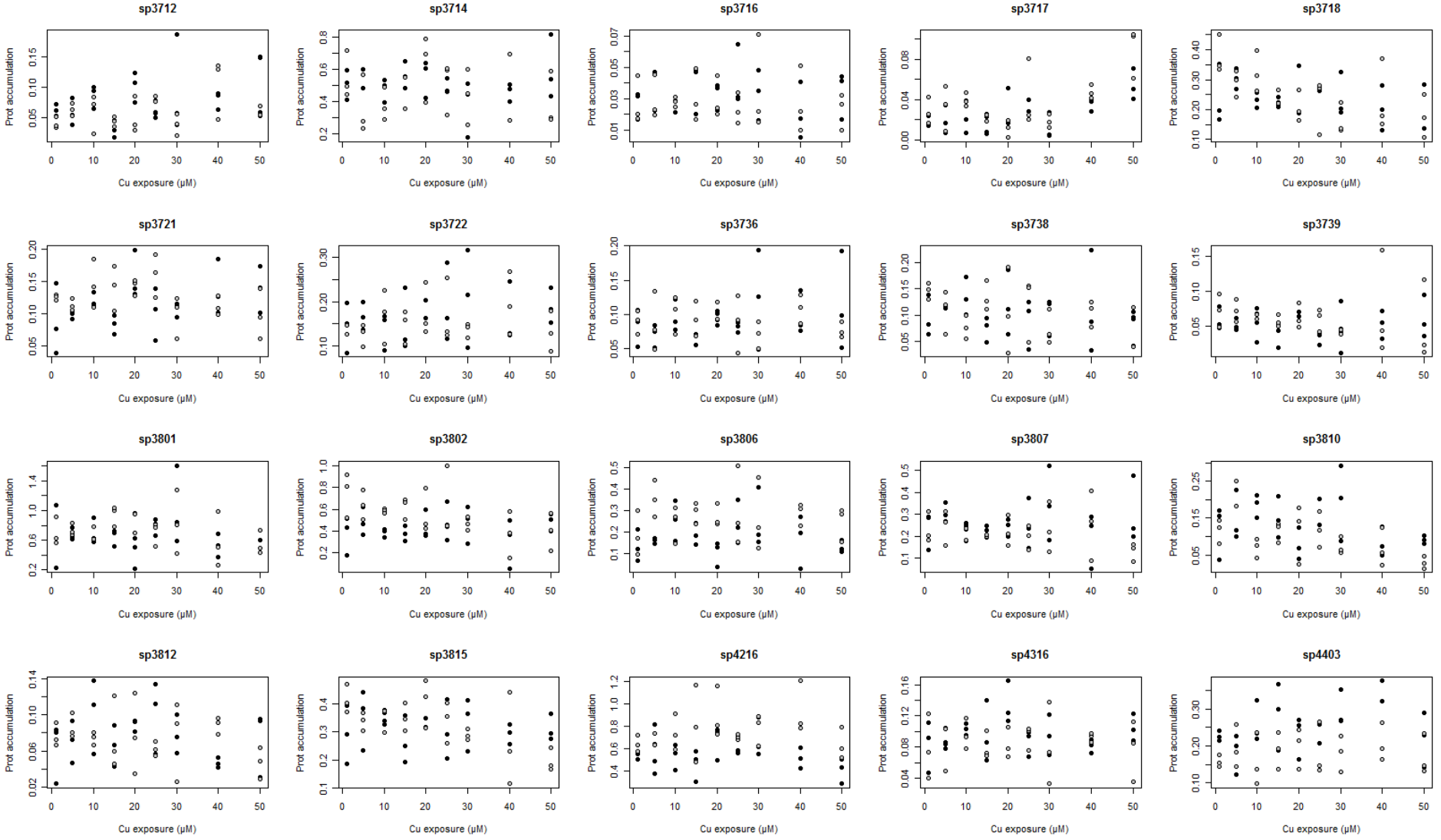
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
3521	0.117 ± 0.033	0.115 ± 0.062	0.127 ± 0.085	0.113 ± 0.03	0.120 ± 0.015	0.090 ± 0.028	0.065 ± 0.032	0.139 ± 0.026	0.087 ± 0.034	0.129 ± 0.066	0.118 ± 0.069	0.100 ± 0.057	0.054 ± 0.035	0.147 ± 0.051	0.084 ± 0.037	0.115 ± 0.039	0.111 ± 0.096	0.107 ± 0.016
3524	0.030 ± 0.024	0.031 ± 0.018	0.031 ± 0.008	0.046 ± 0.023	0.048 ± 0.004	0.061 ± 0.024	0.028 ± 0.007	0.032 ± 0.013	0.042 ± 0.017	0.044 ± 0.021	0.044 ± 0.009	0.037 ± 0.036	0.038 ± 0.015	0.051 ± 0.036	0.042 ± 0.023	0.054 ± 0.039	0.038 ± 0.015	0.060 ± 0.006
3526	0.093 ± 0.052	0.107 ± 0.054	0.093 ± 0.043	0.104 ± 0.03	0.110 ± 0.022	0.085 ± 0.031	0.085 ± 0.011	0.130 ± 0.009	0.118 ± 0.054	0.090 ± 0.058	0.090 ± 0.022	0.100 ± 0.052	0.105 ± 0.064	0.063 ± 0.009	0.173 ± 0.086	0.076 ± 0.041	0.176 ± 0.126	0.159 ± 0.079
3528	0.134 ± 0.079	0.092 ± 0.066	0.062 ± 0.037	0.099 ± 0.048	0.066 ± 0.02	0.046 ± 0.021	0.095 ± 0.013	0.059 ± 0.046	0.062 ± 0.028	0.062 ± 0.016	0.108 ± 0.002	0.051 ± 0.033	0.104 ± 0.018	0.052 ± 0.011	0.060 ± 0.029	0.100 ± 0.061	0.063 ± 0.024	0.096 ± 0.039
3538	0.150 ± 0.087	0.146 ± 0.042	0.146 ± 0.036	0.208 ± 0.015	0.203 ± 0.028	0.217 ± 0.134	0.093 ± 0.052	0.172 ± 0.044	0.208 ± 0.085	0.159 ± 0.062	0.119 ± 0.03	0.206 ± 0.043	0.122 ± 0.086	0.159 ± 0.111	0.093 ± 0.045	0.161 ± 0.059	0.218 ± 0.093	0.121 ± 0.068
3602	0.078 ± 0.017	0.067 ± 0.028	0.061 ± 0.021	0.058 ± 0.009	0.072 ± 0.012	0.046 ± 0.004	0.067 ± 0.007	0.060 ± 0.012	0.087 ± 0.025	0.055 ± 0.027	0.057 ± 0.017	0.054 ± 0.012	0.085 ± 0.052	0.036 ± 0.011	0.066 ± 0.031	0.064 ± 0.048	0.199 ± 0.122	0.074 ± 0.033
3605	0.024 ± 0.024	0.051 ± 0.027	0.041 ± 0.004	0.037 ± 0.016	0.049 ± 0.034	0.030 ± 0.009	0.034 ± 0.026	0.050 ± 0.027	0.036 ± 0.03	0.102 ± 0.081	0.048 ± 0.039	0.068 ± 0.044	0.027 ± 0.021	0.090 ± 0.064	0.028 ± 0.012	0.087 ± 0.061	0.061 ± 0.066	0.079 ± 0.035
3607	0.041 ± 0.011	0.040 ± 0.003	0.047 ± 0.002	0.035 ± 0.014	0.065 ± 0.01	0.054 ± 0.021	0.034 ± 0.019	0.049 ± 0.013	0.066 ± 0.029	0.076 ± 0.04	0.055 ± 0.022	0.044 ± 0.028	0.055 ± 0.008	0.058 ± 0.043	0.047 ± 0.023	0.077 ± 0.045	0.063 ± 0.015	0.060 ± 0.03
3609	0.781 ± 0.088	0.532 ± 0.178	0.729 ± 0.039	0.694 ± 0.151	0.600 ± 0.09	0.783 ± 0.111	0.731 ± 0.299	0.577 ± 0.152	0.736 ± 0.138	0.810 ± 0.219	0.661 ± 0.347	0.580 ± 0.069	0.578 ± 0.103	0.496 ± 0.164	0.500 ± 0.122	0.465 ± 0.059	0.781 ± 0.157	0.568 ± 0.1
3610	0.130 ± 0.04	0.192 ± 0.087	0.141 ± 0.015	0.171 ± 0.024	0.141 ± 0.015	0.183 ± 0.071	0.081 ± 0.021	0.158 ± 0.077	0.199 ± 0.075	0.181 ± 0.058	0.153 ± 0.021	0.208 ± 0.026	0.130 ± 0.028	0.163 ± 0.074	0.154 ± 0.027	0.164 ± 0.038	0.226 ± 0.064	0.269 ± 0.075
3611	0.029 ± 0.017	0.044 ± 0.003	0.055 ± 0.021	0.044 ± 0.016	0.043 ± 0.02	0.033 ± 0.014	0.057 ± 0.026	0.040 ± 0.012	0.062 ± 0.012	0.071 ± 0.006	0.046 ± 0.041	0.054 ± 0.026	0.037 ± 0.015	0.071 ± 0.017	0.050 ± 0.008	0.078 ± 0.014	0.059 ± 0.039	0.045 ± 0.022
3613	0.029 ± 0.013	0.039 ± 0.022	0.036 ± 0.01	0.036 ± 0.012	0.030 ± 0.007	0.034 ± 0.011	0.021 ± 0.01	0.038 ± 0.004	0.031 ± 0.009	0.025 ± 0.01	0.023 ± 0.01	0.029 ± 0.001	0.031 ± 0.006	0.034 ± 0.019	0.024 ± 0.008	0.043 ± 0.014	0.049 ± 0.023	0.032 ± 0.008
3614	0.033 ± 0.008	0.061 ± 0.012	0.058 ± 0.023	0.061 ± 0.017	0.055 ± 0.01	0.063 ± 0.015	0.076 ± 0.032	0.065 ± 0.018	0.064 ± 0.047	0.083 ± 0.013	0.073 ± 0.032	0.067 ± 0.033	0.054 ± 0.037	0.054 ± 0.035	0.054 ± 0.022	0.041 ± 0.026	0.099 ± 0.061	0.101 ± 0.043
3615	0.128 ± 0.028	0.160 ± 0.048	0.130 ± 0.022	0.213 ± 0.079	0.190 ± 0.035	0.152 ± 0.035	0.142 ± 0.028	0.168 ± 0.033	0.222 ± 0.036	0.138 ± 0.056	0.158 ± 0.034	0.231 ± 0.079	0.134 ± 0.049	0.171 ± 0.089	0.098 ± 0.031	0.185 ± 0.049	0.239 ± 0.025	0.160 ± 0.078
3620	0.063 ± 0.012	0.088 ± 0.015	0.064 ± 0.008	0.060 ± 0.018	0.097 ± 0.016	0.050 ± 0.006	0.065 ± 0.022	0.080 ± 0.017	0.075 ± 0.029	0.099 ± 0.035	0.084 ± 0.011	0.076 ± 0.036	0.061 ± 0.015	0.069 ± 0.013	0.107 ± 0.046	0.068 ± 0.01	0.075 ± 0.017	0.083 ± 0.009
3632	1.067 ± 0.523	1.078 ± 0.437	1.181 ± 0.085	1.049 ± 0.266	1.084 ± 0.4	1.011 ± 0.112	0.872 ± 0.254	1.025 ± 0.366	1.021 ± 0.193	0.795 ± 0.297	1.258 ± 0.319	1.033 ± 0.35	0.883 ± 0.07	1.105 ± 0.353	1.002 ± 0.315	0.747 ± 0.199	0.947 ± 0.13	0.929 ± 0.336
3634	0.070 ± 0.046	0.090 ± 0.054	0.075 ± 0.025	0.129 ± 0.073	0.059 ± 0.011	0.048 ± 0.029	0.040 ± 0.034	0.083 ± 0.034	0.065 ± 0.052	0.087 ± 0.026	0.092 ± 0.066	0.081 ± 0.098	0.089 ± 0.075	0.041 ± 0.004	0.080 ± 0.066	0.062 ± 0.039	0.113 ± 0.062	0.251 ± 0.045
3701	0.068 ± 0.029	0.128 ± 0.043	0.153 ± 0.089	0.093 ± 0.045	0.149 ± 0.058	0.137 ± 0.044	0.145 ± 0.076	0.168 ± 0.031	0.155 ± 0.07	0.123 ± 0.015	0.130 ± 0.042	0.194 ± 0.073	0.143 ± 0.023	0.140 ± 0.084	0.138 ± 0.11	0.189 ± 0.065	0.209 ± 0.021	0.209 ± 0.105
3707	0.561 ± 0.287	0.491 ± 0.207	0.383 ± 0.024	0.617 ± 0.128	0.540 ± 0.045	0.244 ± 0.051	0.434 ± 0.069	0.364 ± 0.078	0.341 ± 0.173	0.507 ± 0.293	0.490 ± 0.118	0.488 ± 0.111	0.507 ± 0.29	0.265 ± 0.114	0.349 ± 0.072	0.288 ± 0.327	0.377 ± 0.074	0.141 ± 0.084
3709	0.026 ± 0.014	0.060 ± 0.08	0.042 ± 0.002	0.033 ± 0.013	0.045 ± 0.009	0.033 ± 0.02	0.046 ± 0.037	0.029 ± 0.012	0.065 ± 0.034	0.080 ± 0.046	0.061 ± 0.034	0.044 ± 0.022	0.068 ± 0.053	0.049 ± 0.036	0.111 ± 0.031	0.021 ± 0.017	0.107 ± 0.065	0.090 ± 0.037

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
3521		-0.15	0.44	-	0.02	0.92	-	=	=	=	=	=	=	=	=	=
3524		0.15	0.44	-	0.24	0.23	-	=	=	=	=	=	=	=	=	=
3526	S-adenosylmethionine synthase EC=2.5.1.6	0.44	0.023	↗↗	0.11	0.59	-	=	=	=	=	=	=	=	=	=
3528		-0.24	0.22	-	0.09	0.67	-	=	=	=	=	=	=	=	=	=
3538		0.03	0.89	-	-0.24	0.23	-	=	=	=	=	=	=	=	=	=
3602	ND	0.46	0.015	↗↗	0.10	0.61	-	=	=	=	=	=	=	=	=	=
3605		0.14	0.48	-	0.36	0.064	↗	=	=	=	=	=	=	=	=	=
3607		0.22	0.28	-	0.31	0.12	-	=	=	=	=	=	=	=	=	=
3609		-0.16	0.44	-	-0.30	0.13	-	=	=	=	=	=	=	=	=	=
3610	Methylmalonate-semialdehyde dehydrogenase [acylating] / UDP-glucose 6-dehydrogenase 4	0.44	0.021	↗↗	0.26	0.19	-	=	=	=	=	=	=	=	=	=
3611		0.16	0.43	-	0.37	0.057	↗	=	=	=	=	=	=	=	=	=
3613		0.23	0.24	-	-0.03	0.87	-	=	=	=	=	=	=	=	=	=
3614		0.33	0.090	↗	0.17	0.39	-	=	=	=	=	=	=	=	=	=
3615		0.24	0.23	-	0.00	1.00	-	=	=	=	=	=	=	=	=	=
3620		0.23	0.25	-	0.08	0.69	-	=	=	M >	=	=	=	=	=	=
3632		-0.17	0.40	-	-0.21	0.30	-	=	=	=	=	=	=	=	=	=
3634		0.29	0.15	-	0.35	0.076	↗	=	=	=	=	=	=	=	=	=
3701	Ketol-acid reductoisomerase, chloroplastic EC=1.1.1.86	0.35	0.075	↗	0.46	0.017	↗↗	=	=	=	=	=	=	=	=	=
3707	Phenylalanine / Phenylalanine-tyrosine ammonia-lyase EC=4.3.1.24/25	-0.25	0.21	-	-0.48	0.011	↘↘	=	=	M >	=	=	=	=	=	=
3709	Ketol-acid reductoisomerase, chloroplastic EC=1.1.1.86	0.65	0.0003	↗↗↗	0.19	0.35	-	=	=	=	=	=	=	=	M >>	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 3712 to 4403



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

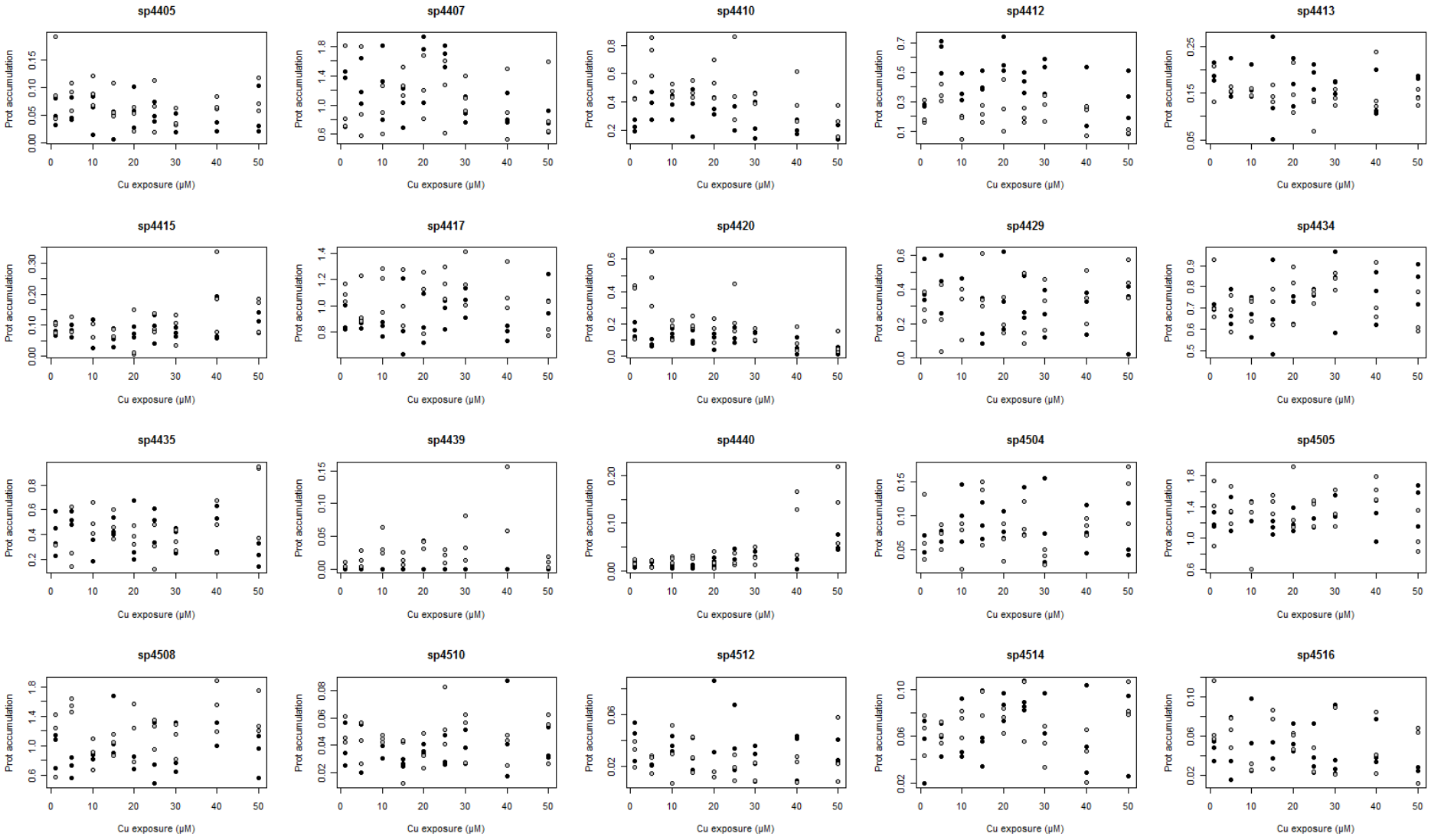
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
3712	0.063 ± 0.01	0.041 ± 0.009	0.059 ± 0.022	0.064 ± 0.011	0.087 ± 0.019	0.061 ± 0.032	0.032 ± 0.014	0.044 ± 0.008	0.103 ± 0.024	0.051 ± 0.031	0.056 ± 0.005	0.081 ± 0.005	0.095 ± 0.08	0.040 ± 0.017	0.081 ± 0.015	0.104 ± 0.05	0.119 ± 0.052	0.060 ± 0.009
3714	0.506 ± 0.09	0.552 ± 0.142	0.551 ± 0.06	0.358 ± 0.18	0.476 ± 0.071	0.377 ± 0.101	0.563 ± 0.082	0.483 ± 0.112	0.555 ± 0.117	0.624 ± 0.205	0.489 ± 0.045	0.504 ± 0.165	0.377 ± 0.176	0.436 ± 0.171	0.460 ± 0.056	0.419 ± 0.237	0.595 ± 0.197	0.392 ± 0.17
3716	0.027 ± 0.009	0.027 ± 0.015	0.031 ± 0.014	0.029 ± 0.014	0.027 ± 0.005	0.028 ± 0.003	0.038 ± 0.016	0.031 ± 0.017	0.033 ± 0.009	0.030 ± 0.013	0.042 ± 0.02	0.023 ± 0.01	0.033 ± 0.016	0.036 ± 0.031	0.021 ± 0.018	0.027 ± 0.021	0.034 ± 0.015	0.023 ± 0.012
3717	0.018 ± 0.005	0.028 ± 0.013	0.019 ± 0.014	0.032 ± 0.022	0.022 ± 0.016	0.040 ± 0.007	0.012 ± 0.009	0.023 ± 0.004	0.029 ± 0.02	0.011 ± 0.009	0.031 ± 0.008	0.042 ± 0.034	0.012 ± 0.013	0.019 ± 0.007	0.035 ± 0.006	0.048 ± 0.007	0.054 ± 0.016	0.090 ± 0.025
3718	0.239 ± 0.099	0.378 ± 0.064	0.304 ± 0.035	0.290 ± 0.044	0.231 ± 0.026	0.326 ± 0.068	0.221 ± 0.018	0.238 ± 0.025	0.267 ± 0.08	0.208 ± 0.052	0.217 ± 0.087	0.223 ± 0.092	0.240 ± 0.075	0.163 ± 0.052	0.203 ± 0.074	0.234 ± 0.119	0.176 ± 0.094	0.177 ± 0.071
3721	0.088 ± 0.055	0.126 ± 0.004	0.099 ± 0.006	0.114 ± 0.01	0.121 ± 0.011	0.146 ± 0.037	0.084 ± 0.015	0.141 ± 0.035	0.157 ± 0.037	0.143 ± 0.012	0.102 ± 0.04	0.160 ± 0.034	0.108 ± 0.011	0.099 ± 0.033	0.138 ± 0.043	0.112 ± 0.015	0.139 ± 0.036	0.099 ± 0.039
3722	0.144 ± 0.057	0.141 ± 0.012	0.167 ± 0.031	0.126 ± 0.025	0.139 ± 0.042	0.169 ± 0.062	0.149 ± 0.072	0.147 ± 0.038	0.173 ± 0.027	0.175 ± 0.059	0.189 ± 0.088	0.170 ± 0.073	0.209 ± 0.111	0.136 ± 0.017	0.166 ± 0.069	0.194 ± 0.071	0.188 ± 0.04	0.131 ± 0.046
3736	0.083 ± 0.027	0.090 ± 0.017	0.071 ± 0.017	0.087 ± 0.043	0.096 ± 0.023	0.101 ± 0.027	0.073 ± 0.018	0.094 ± 0.025	0.097 ± 0.011	0.101 ± 0.015	0.082 ± 0.007	0.088 ± 0.042	0.123 ± 0.073	0.071 ± 0.019	0.099 ± 0.032	0.109 ± 0.021	0.114 ± 0.071	0.077 ± 0.012
3738	0.095 ± 0.04	0.147 ± 0.016	0.124 ± 0.017	0.109 ± 0.041	0.135 ± 0.036	0.076 ± 0.022	0.074 ± 0.024	0.135 ± 0.028	0.120 ± 0.062	0.106 ± 0.083	0.089 ± 0.048	0.118 ± 0.061	0.103 ± 0.037	0.074 ± 0.033	0.115 ± 0.099	0.105 ± 0.025	0.098 ± 0.007	0.065 ± 0.043
3739	0.059 ± 0.017	0.073 ± 0.024	0.052 ± 0.009	0.072 ± 0.016	0.053 ± 0.024	0.065 ± 0.004	0.038 ± 0.017	0.058 ± 0.009	0.061 ± 0.011	0.064 ± 0.018	0.034 ± 0.01	0.061 ± 0.016	0.048 ± 0.037	0.042 ± 0.003	0.053 ± 0.02	0.074 ± 0.075	0.061 ± 0.031	0.051 ± 0.058
3801	0.640 ± 0.419	0.699 ± 0.188	0.672 ± 0.081	0.739 ± 0.082	0.753 ± 0.166	0.676 ± 0.097	0.646 ± 0.116	0.936 ± 0.137	0.451 ± 0.207	0.870 ± 0.151	0.787 ± 0.108	0.699 ± 0.155	1.010 ± 0.53	0.836 ± 0.425	0.528 ± 0.157	0.582 ± 0.364	0.533 ± 0.063	0.553 ± 0.156
3802	0.373 ± 0.174	0.751 ± 0.204	0.486 ± 0.127	0.639 ± 0.136	0.387 ± 0.038	0.584 ± 0.022	0.377 ± 0.067	0.618 ± 0.101	0.442 ± 0.135	0.561 ± 0.203	0.477 ± 0.181	0.636 ± 0.316	0.471 ± 0.172	0.468 ± 0.059	0.309 ± 0.226	0.374 ± 0.215	0.488 ± 0.083	0.396 ± 0.173
3806	0.133 ± 0.073	0.190 ± 0.103	0.160 ± 0.011	0.355 ± 0.084	0.255 ± 0.095	0.244 ± 0.087	0.189 ± 0.052	0.293 ± 0.051	0.106 ± 0.059	0.273 ± 0.053	0.242 ± 0.101	0.303 ± 0.186	0.251 ± 0.137	0.267 ± 0.17	0.166 ± 0.123	0.288 ± 0.051	0.130 ± 0.028	0.246 ± 0.08
3807	0.237 ± 0.085	0.233 ± 0.069	0.307 ± 0.043	0.245 ± 0.078	0.228 ± 0.044	0.218 ± 0.029	0.224 ± 0.026	0.205 ± 0.004	0.243 ± 0.04	0.223 ± 0.07	0.253 ± 0.115	0.197 ± 0.055	0.348 ± 0.17	0.235 ± 0.114	0.196 ± 0.124	0.254 ± 0.159	0.304 ± 0.149	0.130 ± 0.041
3810	0.122 ± 0.073	0.116 ± 0.033	0.148 ± 0.068	0.206 ± 0.04	0.185 ± 0.031	0.070 ± 0.027	0.151 ± 0.055	0.114 ± 0.027	0.077 ± 0.044	0.114 ± 0.08	0.155 ± 0.042	0.119 ± 0.049	0.195 ± 0.104	0.073 ± 0.023	0.083 ± 0.04	0.068 ± 0.051	0.091 ± 0.011	0.029 ± 0.016
3812	0.063 ± 0.034	0.077 ± 0.013	0.071 ± 0.024	0.086 ± 0.013	0.102 ± 0.041	0.074 ± 0.007	0.066 ± 0.023	0.076 ± 0.04	0.089 ± 0.007	0.078 ± 0.045	0.101 ± 0.039	0.062 ± 0.008	0.078 ± 0.021	0.076 ± 0.044	0.047 ± 0.006	0.089 ± 0.01	0.073 ± 0.037	0.047 ± 0.018
3815	0.290 ± 0.103	0.416 ± 0.049	0.354 ± 0.109	0.339 ± 0.031	0.345 ± 0.021	0.350 ± 0.046	0.267 ± 0.084	0.352 ± 0.05	0.327 ± 0.019	0.408 ± 0.085	0.304 ± 0.107	0.340 ± 0.073	0.336 ± 0.095	0.290 ± 0.021	0.294 ± 0.034	0.263 ± 0.166	0.312 ± 0.047	0.197 ± 0.041
4216	0.547 ± 0.037	0.645 ± 0.074	0.562 ± 0.228	0.671 ± 0.058	0.535 ± 0.112	0.743 ± 0.164	0.467 ± 0.14	0.817 ± 0.343	0.673 ± 0.152	0.901 ± 0.228	0.613 ± 0.065	0.704 ± 0.025	0.686 ± 0.172	0.781 ± 0.139	0.517 ± 0.093	0.941 ± 0.231	0.411 ± 0.111	0.641 ± 0.14
4316	0.084 ± 0.033	0.079 ± 0.042	0.083 ± 0.004	0.086 ± 0.031	0.103 ± 0.007	0.096 ± 0.02	0.097 ± 0.04	0.081 ± 0.018	0.134 ± 0.027	0.084 ± 0.02	0.087 ± 0.017	0.093 ± 0.015	0.095 ± 0.026	0.082 ± 0.053	0.082 ± 0.008	0.092 ± 0.006	0.105 ± 0.018	0.078 ± 0.039
4403	0.226 ± 0.014	0.159 ± 0.017	0.183 ± 0.054	0.195 ± 0.058	0.259 ± 0.055	0.157 ± 0.072	0.285 ± 0.09	0.189 ± 0.05	0.230 ± 0.058	0.199 ± 0.056	0.244 ± 0.031	0.182 ± 0.072	0.297 ± 0.049	0.180 ± 0.049	0.296 ± 0.094	0.206 ± 0.051	0.220 ± 0.074	0.171 ± 0.055

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
3712	Ketol-acid reductoisomerase, chloro. EC=1.1.1.86	0.39	0.043	↗↗	0.30	0.13	-	=	=	=	=	=	=	=	=	=
3714		-0.02	0.92	-	-0.11	0.59	-	=	=	=	=	=	=	=	=	=
3716		0.02	0.91	-	-0.07	0.73	-	=	=	=	=	=	=	=	=	=
3717	ND	0.57	0.002	↗↗↗	0.54	0.004	↗↗↗	=	=	=	=	=	=	=	=	=
3718	Succinate dehydrogenase [Ubi] flavoprotein subunit 1, mito. EC=1.3.5.1	-0.36	0.062	↘	-0.60	0.001	↘↘↘	=	=	=	=	=	=	=	=	=
3721	ND	0.38	0.048	↗↗	-0.31	0.12	-	=	=	=	=	=	=	=	=	=
3722		0.24	0.23	-	0.09	0.65	-	=	=	=	=	=	=	=	=	=
3736		0.32	0.10	-	-0.09	0.66	-	=	=	=	=	=	=	=	=	=
3738		-0.06	0.75	-	-0.35	0.073	↘	=	=	=	=	=	=	=	=	=
3739		0.04	0.84	-	-0.16	0.42	-	=	=	=	=	=	=	=	=	=
3801		-0.09	0.67	-	-0.25	0.22	-	=	=	=	=	=	=	=	=	=
3802	Aconitate hydratase, cytoplasmic EC=4.2.1.3	0.06	0.78	-	-0.56	0.002	↘↘↘	=	=	=	=	=	=	=	=	=
3806		-0.03	0.88	-	0.01	0.96	-	=	NM >	=	=	=	=	=	=	=
3807		0.08	0.68	-	-0.23	0.24	-	=	=	=	=	=	=	=	=	=
3810	ND	-0.25	0.20	-	-0.57	0.002	↘↘↘	=	=	M >	=	=	=	=	=	M >
3812		-0.11	0.60	-	-0.23	0.24	-	=	=	=	=	=	=	=	NM >	=
3815	NADH dehydrogenase [Ubi] iron-sulfur protein 1, mito. EC=1.6.5.3 - 1.6.99.3	-0.05	0.79	-	-0.63	0.0005	↘↘↘↘	=	=	=	=	=	=	=	=	=
4216		-0.15	0.46	-	0.14	0.50	-	=	=	=	=	=	=	=	=	=
4316		0.08	0.69	-	-0.02	0.94	-	=	=	=	=	=	=	=	=	=
4403		0.19	0.34	-	0.09	0.65	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 4405 to 4516



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

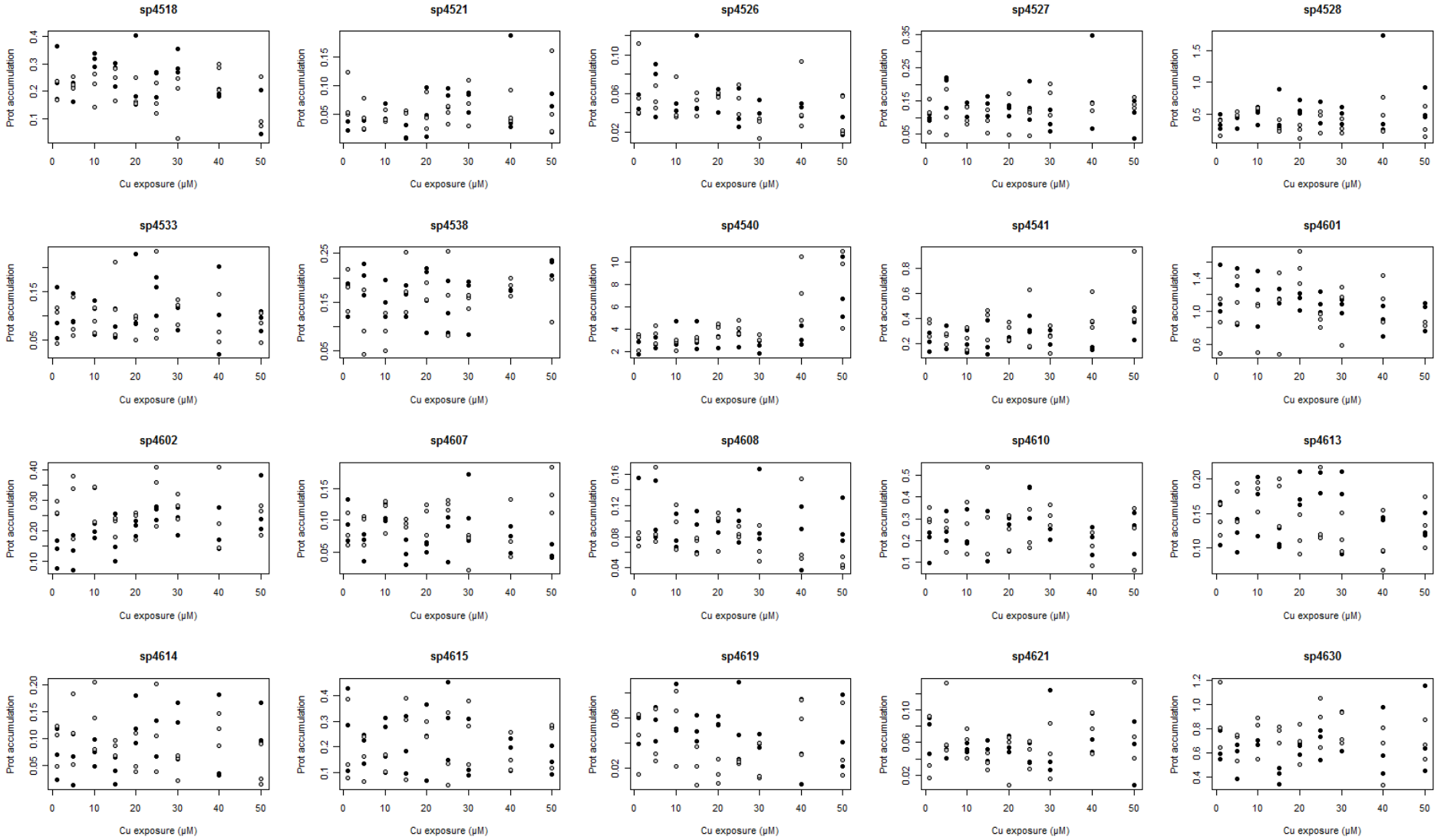
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
4405	0.054 ± 0.024	0.107 ± 0.076	0.057 ± 0.022	0.086 ± 0.025	0.054 ± 0.036	0.092 ± 0.027	0.038 ± 0.028	0.071 ± 0.032	0.062 ± 0.037	0.046 ± 0.023	0.054 ± 0.019	0.066 ± 0.047	0.036 ± 0.017	0.043 ± 0.017	0.041 ± 0.022	0.069 ± 0.012	0.051 ± 0.045	0.082 ± 0.031
4407	1.180 ± 0.415	1.113 ± 0.606	1.275 ± 0.325	1.089 ± 0.638	1.311 ± 0.505	0.923 ± 0.331	0.981 ± 0.271	1.302 ± 0.196	1.579 ± 0.481	1.232 ± 0.434	1.679 ± 0.15	1.165 ± 0.505	0.918 ± 0.18	1.138 ± 0.237	0.913 ± 0.222	0.975 ± 0.489	0.763 ± 0.146	1.001 ± 0.512
4410	0.229 ± 0.042	0.460 ± 0.066	0.381 ± 0.098	0.734 ± 0.138	0.366 ± 0.086	0.478 ± 0.05	0.342 ± 0.171	0.482 ± 0.062	0.364 ± 0.062	0.551 ± 0.134	0.337 ± 0.123	0.524 ± 0.302	0.250 ± 0.133	0.436 ± 0.041	0.215 ± 0.055	0.416 ± 0.18	0.175 ± 0.055	0.263 ± 0.108
4412	0.285 ± 0.022	0.214 ± 0.085	0.624 ± 0.118	0.355 ± 0.058	0.386 ± 0.096	0.143 ± 0.087	0.429 ± 0.069	0.216 ± 0.057	0.599 ± 0.124	0.264 ± 0.176	0.433 ± 0.07	0.201 ± 0.049	0.491 ± 0.123	0.262 ± 0.089	0.309 ± 0.205	0.192 ± 0.11	0.345 ± 0.161	0.090 ± 0.015
4413	0.193 ± 0.02	0.157 ± 0.044	0.174 ± 0.044	0.157 ± 0.006	0.175 ± 0.031	0.148 ± 0.009	0.146 ± 0.112	0.147 ± 0.018	0.172 ± 0.051	0.157 ± 0.054	0.187 ± 0.027	0.111 ± 0.038	0.165 ± 0.015	0.140 ± 0.017	0.140 ± 0.052	0.164 ± 0.064	0.169 ± 0.025	0.140 ± 0.017
4415	0.086 ± 0.022	0.095 ± 0.017	0.082 ± 0.021	0.094 ± 0.029	0.083 ± 0.05	0.076 ± 0.025	0.057 ± 0.03	0.080 ± 0.014	0.076 ± 0.017	0.056 ± 0.08	0.090 ± 0.047	0.101 ± 0.032	0.078 ± 0.014	0.091 ± 0.051	0.105 ± 0.076	0.200 ± 0.13	0.109 ± 0.033	0.145 ± 0.058
4417	0.886 ± 0.103	1.095 ± 0.066	0.865 ± 0.036	1.006 ± 0.195	0.832 ± 0.057	1.147 ± 0.176	0.884 ± 0.295	1.039 ± 0.217	0.881 ± 0.192	1.058 ± 0.24	0.947 ± 0.11	1.170 ± 0.121	1.029 ± 0.112	1.192 ± 0.206	0.798 ± 0.058	1.127 ± 0.183	1.074 ± 0.153	0.876 ± 0.139
4420	0.165 ± 0.045	0.321 ± 0.187	0.080 ± 0.025	0.480 ± 0.17	0.139 ± 0.028	0.170 ± 0.062	0.109 ± 0.043	0.204 ± 0.039	0.096 ± 0.053	0.162 ± 0.074	0.122 ± 0.049	0.268 ± 0.156	0.129 ± 0.029	0.122 ± 0.043	0.059 ± 0.053	0.099 ± 0.077	0.036 ± 0.021	0.073 ± 0.069
4429	0.428 ± 0.129	0.293 ± 0.086	0.435 ± 0.17	0.229 ± 0.196	0.304 ± 0.184	0.284 ± 0.156	0.192 ± 0.14	0.418 ± 0.166	0.373 ± 0.23	0.231 ± 0.109	0.327 ± 0.134	0.241 ± 0.221	0.257 ± 0.138	0.318 ± 0.15	0.281 ± 0.128	0.353 ± 0.156	0.267 ± 0.214	0.454 ± 0.114
4434	0.705 ± 0.013	0.761 ± 0.146	0.692 ± 0.086	0.680 ± 0.087	0.663 ± 0.095	0.703 ± 0.057	0.686 ± 0.226	0.715 ± 0.085	0.704 ± 0.068	0.778 ± 0.142	0.757 ± 0.034	0.758 ± 0.033	0.799 ± 0.196	0.830 ± 0.041	0.757 ± 0.125	0.758 ± 0.136	0.825 ± 0.096	0.659 ± 0.102
4435	0.422 ± 0.182	0.323 ± 0.005	0.527 ± 0.053	0.340 ± 0.251	0.345 ± 0.149	0.518 ± 0.128	0.456 ± 0.074	0.476 ± 0.119	0.379 ± 0.257	0.396 ± 0.077	0.490 ± 0.139	0.306 ± 0.179	0.376 ± 0.109	0.353 ± 0.083	0.477 ± 0.191	0.467 ± 0.211	0.237 ± 0.092	0.751 ± 0.33
4439		0.008 ± 0.004		0.015 ± 0.012		0.039 ± 0.021		0.015 ± 0.01		0.039 ± 0.007		0.020 ± 0.011		0.042 ± 0.036		0.091 ± 0.057		0.010 ± 0.008
4440	0.012 ± 0.007	0.019 ± 0.004	0.017 ± 0.008	0.013 ± 0.007	0.009 ± 0.003	0.024 ± 0.007	0.010 ± 0.004	0.029 ± 0.003	0.020 ± 0.009	0.021 ± 0.018	0.036 ± 0.011	0.022 ± 0.014	0.029 ± 0.014	0.030 ± 0.019	0.020 ± 0.014	0.110 ± 0.069	0.056 ± 0.017	0.140 ± 0.081
4504	0.055 ± 0.014	0.076 ± 0.05	0.072 ± 0.009	0.070 ± 0.019	0.103 ± 0.042	0.063 ± 0.036	0.090 ± 0.027	0.115 ± 0.051	0.084 ± 0.021	0.063 ± 0.028	0.096 ± 0.04	0.091 ± 0.026	0.087 ± 0.063	0.039 ± 0.011	0.079 ± 0.036	0.085 ± 0.013	0.070 ± 0.042	0.136 ± 0.044
4505	1.222 ± 0.098	1.352 ± 0.42	1.317 ± 0.219	1.399 ± 0.246	1.344 ± 0.126	1.133 ± 0.46	1.135 ± 0.09	1.443 ± 0.121	1.222 ± 0.152	1.433 ± 0.429	1.283 ± 0.157	1.359 ± 0.178	1.485 ± 0.183	1.364 ± 0.237	1.257 ± 0.268	1.636 ± 0.152	1.473 ± 0.287	1.051 ± 0.278
4508	0.971 ± 0.243	1.081 ± 0.442	0.712 ± 0.141	1.549 ± 0.089	0.863 ± 0.036	0.897 ± 0.208	1.201 ± 0.42	1.022 ± 0.146	0.772 ± 0.09	1.197 ± 0.396	0.851 ± 0.422	1.187 ± 0.213	0.908 ± 0.355	1.089 ± 0.241	1.102 ± 0.179	1.541 ± 0.345	0.884 ± 0.292	1.405 ± 0.301
4510	0.039 ± 0.016	0.050 ± 0.01	0.039 ± 0.018	0.042 ± 0.015	0.037 ± 0.006	0.045 ± 0.003	0.027 ± 0.002	0.032 ± 0.018	0.037 ± 0.003	0.035 ± 0.013	0.034 ± 0.012	0.058 ± 0.022	0.039 ± 0.012	0.049 ± 0.019	0.048 ± 0.036	0.039 ± 0.012	0.039 ± 0.012	0.048 ± 0.019
4512	0.041 ± 0.015	0.031 ± 0.011	0.019 ± 0.004	0.023 ± 0.008	0.037 ± 0.006	0.029 ± 0.023	0.028 ± 0.013	0.028 ± 0.014	0.043 ± 0.038	0.013 ± 0.003	0.040 ± 0.026	0.019 ± 0.01	0.029 ± 0.007	0.014 ± 0.008	0.031 ± 0.019	0.020 ± 0.01	0.030 ± 0.009	0.030 ± 0.026
4514	0.050 ± 0.028	0.063 ± 0.017	0.057 ± 0.014	0.063 ± 0.009	0.060 ± 0.028	0.072 ± 0.012	0.050 ± 0.013	0.092 ± 0.012	0.086 ± 0.012	0.074 ± 0.011	0.086 ± 0.004	0.090 ± 0.03	0.071 ± 0.023	0.052 ± 0.018	0.061 ± 0.038	0.044 ± 0.022	0.067 ± 0.036	0.089 ± 0.016
4516	0.046 ± 0.01	0.078 ± 0.033	0.043 ± 0.033	0.064 ± 0.015	0.061 ± 0.034	0.027 ± 0.004	0.059 ± 0.025	0.063 ± 0.033	0.056 ± 0.015	0.057 ± 0.009	0.047 ± 0.023	0.031 ± 0.014	0.051 ± 0.035	0.044 ± 0.039	0.049 ± 0.024	0.049 ± 0.032	0.039 ± 0.021	0.048 ± 0.031

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
4405		-0.12	0.54	-	-0.23	0.24	-	=	=	=	=	=	=	=	=	=
4407		-0.36	0.067	↘	-0.08	0.71	-	=	=	=	=	=	=	=	=	=
4410	Cinnamyl alcohol dehydrogenase / Tricetin 3',4',5'-O-trimethyltransferase	-0.42	0.028	↘↘	-0.50	0.008	↘↘↘	=	=	=	=	=	=	=	=	=
4412		-0.20	0.32	-	-0.36	0.061	↘	=	=	=	=	=	M >	=	=	M >
4413		-0.17	0.41	-	-0.10	0.61	-	=	=	=	=	=	=	=	=	=
4415	ND	0.26	0.19	-	0.42	0.028	↗↗	=	=	=	=	=	=	=	=	=
4417		0.30	0.13	-	-0.14	0.50	-	=	=	=	=	=	=	=	=	=
4420	Tricetin 3',4',5'-O-trimethyltransferase EC=2.1.1.169	-0.54	0.004	↘↘↘	-0.61	0.0008	↘↘↘↘	=	NM >>	=	=	=	=	=	=	=
4429		-0.27	0.17	-	0.30	0.13	-	=	=	=	=	=	=	=	=	=
4434	Alpha-1,4-glucan-protein synthase / UDP-arabinopyranose mutase 1	0.39	0.043	↗↗	0.00	0.98	-	=	=	=	=	=	=	=	=	=
4435	Alpha-1,4-glucan-protein synthase / UDP-arabinopyranose mutase / Phosphoglycerate kinase	-0.26	0.19	-	0.41	0.033	↗↗	=	=	=	=	=	=	=	=	=
4439	Glutamine synthetase cytosolic isozyme / S-adenosylmethionine synthase	-0.41	0.033	↘↘	0.30	0.13	-	NM >>	NM >>	NM >>	NM >>	NM >>	NM >>	NM >>	=	NM >>
4440	ATP phosphoribosyltransferase / Pyruvate dehydrogenase E1 component	0.68	<0.001	↗↗↗	0.71	<0.001	↗↗↗	=	=	=	NM >	=	=	=	=	=
4504		0.03	0.89	-	0.30	0.13	-	=	=	=	=	=	=	=	=	=
4505		0.30	0.14	-	-0.05	0.79	-	=	=	=	=	=	=	=	=	=
4508		0.09	0.64	-	0.28	0.15	-	=	NM >	=	=	=	=	=	=	=
4510		0.13	0.53	-	0.05	0.80	-	=	=	=	=	=	=	=	=	=
4512		-0.04	0.83	-	-0.13	0.53	-	=	=	=	=	=	=	=	=	=
4514		0.20	0.32	-	0.03	0.89	-	=	=	=	=	=	=	=	=	=
4516		-0.10	0.60	-	-0.23	0.24	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 4518 to 4630



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

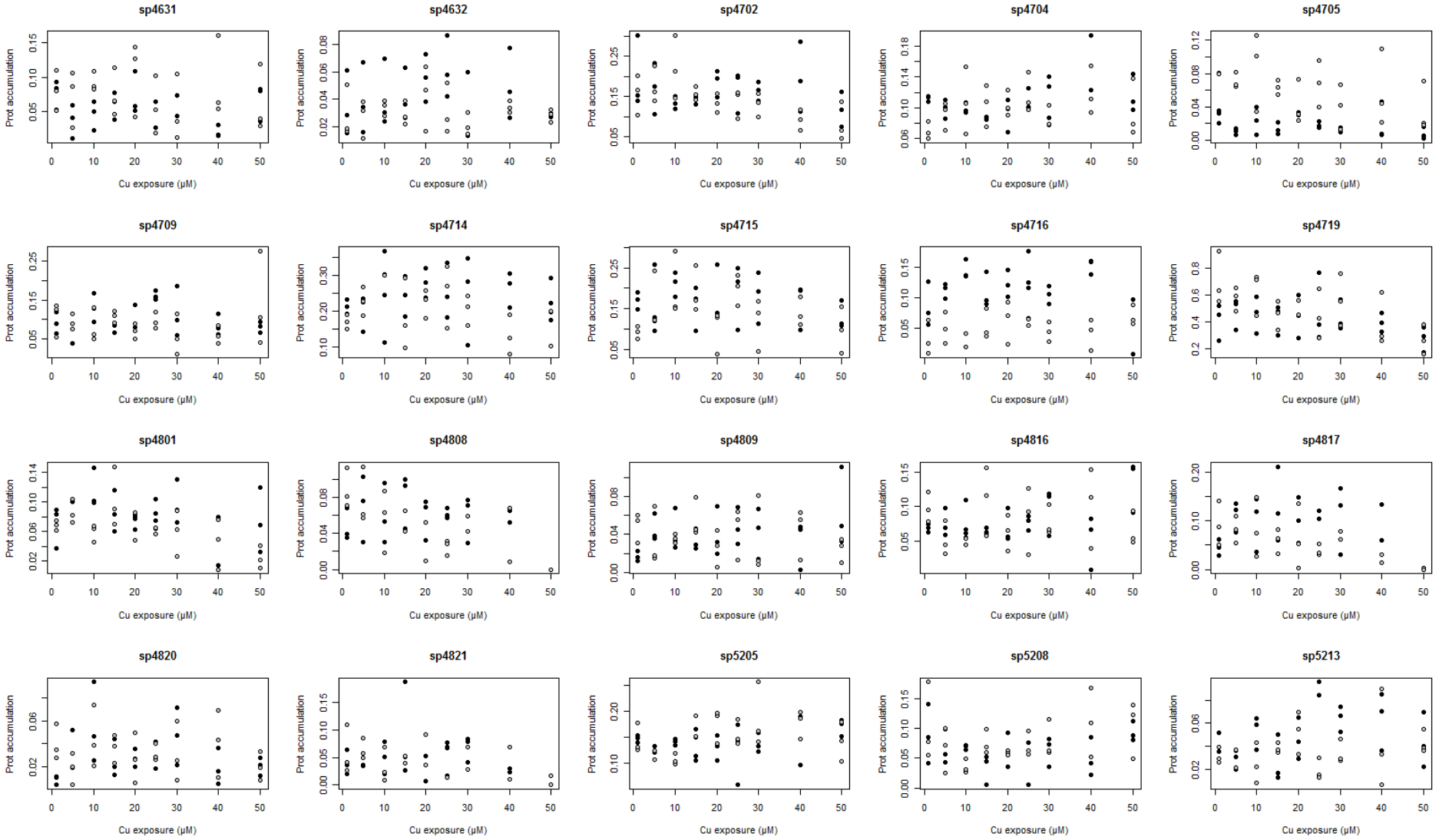
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
4518	0.277 ± 0.075	0.192 ± 0.038	0.204 ± 0.036	0.229 ± 0.022	0.315 ± 0.024	0.211 ± 0.062	0.269 ± 0.046	0.232 ± 0.061	0.246 ± 0.138	0.190 ± 0.053	0.238 ± 0.051	0.167 ± 0.056	0.302 ± 0.046	0.161 ± 0.118	0.193 ± 0.012	0.263 ± 0.051	0.168 ± 0.109	0.139 ± 0.099
4521	0.037 ± 0.015	0.076 ± 0.041	0.034 ± 0.009	0.049 ± 0.027	0.050 ± 0.016	0.046 ± 0.011	0.016 ± 0.013	0.053 ± 0.003	0.053 ± 0.043	0.053 ± 0.033	0.080 ± 0.017	0.051 ± 0.016	0.075 ± 0.019	0.069 ± 0.04	0.084 ± 0.09	0.059 ± 0.029	0.056 ± 0.035	0.077 ± 0.074
4526	0.047 ± 0.01	0.069 ± 0.037	0.069 ± 0.029	0.055 ± 0.012	0.043 ± 0.006	0.050 ± 0.023	0.070 ± 0.043	0.050 ± 0.013	0.055 ± 0.013	0.058 ± 0.002	0.042 ± 0.021	0.054 ± 0.015	0.041 ± 0.011	0.026 ± 0.011	0.044 ± 0.006	0.053 ± 0.035	0.037 ± 0.02	0.033 ± 0.021
4527	0.101 ± 0.01	0.110 ± 0.049	0.187 ± 0.05	0.112 ± 0.07	0.127 ± 0.022	0.101 ± 0.027	0.138 ± 0.03	0.090 ± 0.035	0.124 ± 0.016	0.132 ± 0.072	0.145 ± 0.059	0.095 ± 0.042	0.088 ± 0.034	0.161 ± 0.048	0.187 ± 0.144	0.128 ± 0.012	0.101 ± 0.058	0.144 ± 0.016
4528	0.376 ± 0.119	0.328 ± 0.142	0.396 ± 0.104	0.504 ± 0.039	0.477 ± 0.122	0.596 ± 0.015	0.504 ± 0.333	0.310 ± 0.102	0.599 ± 0.114	0.244 ± 0.108	0.517 ± 0.164	0.410 ± 0.18	0.494 ± 0.136	0.306 ± 0.116	0.786 ± 0.818	0.499 ± 0.272	0.622 ± 0.257	0.348 ± 0.247
4533	0.100 ± 0.054	0.089 ± 0.04	0.107 ± 0.034	0.091 ± 0.042	0.102 ± 0.037	0.091 ± 0.026	0.083 ± 0.03	0.128 ± 0.075	0.132 ± 0.083	0.082 ± 0.028	0.146 ± 0.042	0.119 ± 0.099	0.090 ± 0.023	0.112 ± 0.027	0.109 ± 0.09	0.086 ± 0.052	0.091 ± 0.021	0.079 ± 0.031
4538	0.164 ± 0.038	0.177 ± 0.043	0.199 ± 0.033	0.104 ± 0.067	0.156 ± 0.038	0.090 ± 0.038	0.158 ± 0.033	0.184 ± 0.063	0.173 ± 0.074	0.166 ± 0.021	0.136 ± 0.053	0.167 ± 0.087	0.153 ± 0.06	0.154 ± 0.014	0.171 ± 0.006	0.183 ± 0.019	0.225 ± 0.017	0.168 ± 0.051
4540	2.662 ± 0.791	2.962 ± 0.797	2.754 ± 0.488	3.554 ± 0.809	3.490 ± 1.119	2.658 ± 0.517	3.282 ± 1.3	3.095 ± 0.132	3.300 ± 0.969	3.981 ± 0.637	3.215 ± 0.697	4.169 ± 0.626	2.478 ± 0.58	3.370 ± 0.253	3.323 ± 0.883	7.499 ± 2.863	7.453 ± 2.733	8.289 ± 3.718
4541	0.210 ± 0.077	0.339 ± 0.073	0.253 ± 0.093	0.242 ± 0.046	0.209 ± 0.093	0.239 ± 0.088	0.224 ± 0.144	0.371 ± 0.129	0.265 ± 0.057	0.308 ± 0.075	0.338 ± 0.073	0.328 ± 0.265	0.252 ± 0.06	0.241 ± 0.111	0.230 ± 0.124	0.441 ± 0.156	0.352 ± 0.119	0.608 ± 0.292
4601	1.214 ± 0.303	0.831 ± 0.334	1.220 ± 0.351	1.127 ± 0.289	1.187 ± 0.34	0.880 ± 0.332	1.173 ± 0.089	1.026 ± 0.503	1.126 ± 0.108	1.527 ± 0.199	1.104 ± 0.129	0.888 ± 0.082	1.065 ± 0.083	1.013 ± 0.375	0.890 ± 0.186	1.150 ± 0.28	0.968 ± 0.183	0.837 ± 0.027
4602	0.128 ± 0.047	0.271 ± 0.024	0.131 ± 0.057	0.296 ± 0.109	0.199 ± 0.024	0.304 ± 0.065	0.167 ± 0.081	0.217 ± 0.034	0.209 ± 0.026	0.226 ± 0.048	0.261 ± 0.024	0.326 ± 0.101	0.234 ± 0.046	0.280 ± 0.041	0.196 ± 0.072	0.259 ± 0.137	0.275 ± 0.095	0.244 ± 0.052
4607	0.098 ± 0.033	0.083 ± 0.026	0.061 ± 0.023	0.089 ± 0.025	0.109 ± 0.014	0.110 ± 0.027	0.048 ± 0.02	0.095 ± 0.006	0.059 ± 0.009	0.105 ± 0.025	0.076 ± 0.037	0.124 ± 0.008	0.114 ± 0.053	0.056 ± 0.03	0.071 ± 0.021	0.080 ± 0.047	0.049 ± 0.012	0.145 ± 0.036
4608	0.103 ± 0.045	0.077 ± 0.009	0.107 ± 0.04	0.109 ± 0.053	0.083 ± 0.023	0.094 ± 0.029	0.089 ± 0.027	0.070 ± 0.011	0.096 ± 0.009	0.091 ± 0.027	0.095 ± 0.021	0.085 ± 0.007	0.110 ± 0.05	0.068 ± 0.024	0.082 ± 0.041	0.087 ± 0.058	0.096 ± 0.03	0.046 ± 0.007
4610	0.184 ± 0.074	0.313 ± 0.035	0.259 ± 0.068	0.231 ± 0.076	0.243 ± 0.088	0.265 ± 0.119	0.328 ± 0.217	0.327 ± 0.2	0.243 ± 0.081	0.242 ± 0.081	0.397 ± 0.082	0.234 ± 0.095	0.257 ± 0.056	0.316 ± 0.048	0.204 ± 0.065	0.166 ± 0.076	0.245 ± 0.097	0.224 ± 0.144
4613	0.144 ± 0.035	0.140 ± 0.023	0.120 ± 0.024	0.172 ± 0.029	0.166 ± 0.044	0.178 ± 0.022	0.112 ± 0.015	0.174 ± 0.037	0.182 ± 0.026	0.117 ± 0.029	0.169 ± 0.048	0.151 ± 0.058	0.160 ± 0.062	0.120 ± 0.029	0.127 ± 0.028	0.107 ± 0.044	0.131 ± 0.018	0.136 ± 0.037
4614	0.071 ± 0.047	0.093 ± 0.04	0.063 ± 0.047	0.115 ± 0.066	0.074 ± 0.026	0.140 ± 0.062	0.040 ± 0.025	0.084 ± 0.014	0.130 ± 0.045	0.066 ± 0.039	0.111 ± 0.038	0.115 ± 0.082	0.120 ± 0.053	0.051 ± 0.025	0.083 ± 0.086	0.117 ± 0.03	0.118 ± 0.042	0.044 ± 0.04
4615	0.274 ± 0.161	0.198 ± 0.166	0.203 ± 0.059	0.155 ± 0.086	0.251 ± 0.078	0.124 ± 0.04	0.199 ± 0.113	0.256 ± 0.167	0.226 ± 0.151	0.261 ± 0.033	0.304 ± 0.153	0.173 ± 0.147	0.169 ± 0.122	0.264 ± 0.126	0.179 ± 0.066	0.172 ± 0.076	0.145 ± 0.057	0.225 ± 0.095
4619	0.054 ± 0.012	0.042 ± 0.025	0.056 ± 0.014	0.042 ± 0.022	0.063 ± 0.021	0.056 ± 0.031	0.051 ± 0.01	0.021 ± 0.015	0.057 ± 0.004	0.017 ± 0.01	0.054 ± 0.031	0.025 ± 0.001	0.041 ± 0.006	0.022 ± 0.016	0.038 ± 0.034	0.055 ± 0.022	0.047 ± 0.029	0.038 ± 0.031
4621	0.073 ± 0.024	0.047 ± 0.04	0.049 ± 0.008	0.080 ± 0.046	0.053 ± 0.006	0.060 ± 0.018	0.051 ± 0.013	0.036 ± 0.011	0.057 ± 0.01	0.045 ± 0.032	0.044 ± 0.014	0.047 ± 0.017	0.062 ± 0.054	0.049 ± 0.034	0.069 ± 0.025	0.073 ± 0.026	0.050 ± 0.04	0.080 ± 0.048
4630	0.643 ± 0.128	0.879 ± 0.277	0.556 ± 0.15	0.673 ± 0.119	0.639 ± 0.078	0.756 ± 0.178	0.416 ± 0.069	0.761 ± 0.068	0.640 ± 0.051	0.682 ± 0.166	0.687 ± 0.128	0.863 ± 0.205	0.755 ± 0.165	0.776 ± 0.14	0.665 ± 0.285	0.606 ± 0.246	0.749 ± 0.365	0.698 ± 0.166

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
4518		-0.36	0.069	↘	-0.18	0.37	-	=	=	=	=	=	=	=	=	=
4521		0.36	0.067	↗	0.13	0.50	-	=	=	=	NM >	=	=	=	=	=
4526		-0.31	0.11	-	-0.37	0.054	↘	=	=	=	=	=	=	=	=	=
4527		-0.05	0.80	-	0.30	0.12	-	=	=	=	=	=	=	=	=	=
4528		0.33	0.096	↗	-0.09	0.66	-	=	=	=	=	=	=	=	=	=
4533		-0.02	0.94	-	-0.05	0.79	-	=	=	=	=	=	=	=	=	=
4538		0.20	0.32	-	0.26	0.19	-	=	=	=	=	=	=	=	=	=
4540	S-adenosylmethionine synthase / Enolase 2	0.55	0.003	↗↗↗	0.71	<0.001	↗↗↗↗	=	=	=	=	=	=	=	=	=
4541	S-adenosylmethionine synthase EC=2.5.1.6	0.33	0.097	↗	0.47	0.012	↗↗	=	=	=	=	=	=	=	=	=
4601	ATP synthase subunit alpha, mito. EC=3.6.3.14	-0.48	0.012	↘↘	-0.01	0.96	-	=	=	=	=	=	=	=	=	=
4602	Leucine aminopeptidase 2 / Methylmalonate-semialdehyde dehydrogenase [acylating]	0.58	0.002	↗↗↗	-0.11	0.58	-	=	=	=	=	=	=	=	=	=
4607		-0.20	0.32	-	0.23	0.25	-	=	=	=	=	=	=	=	=	NM >
4608		-0.07	0.73	-	-0.34	0.079	↘	=	=	=	=	=	=	=	=	=
4610		0.02	0.91	-	-0.25	0.22	-	=	=	=	=	=	=	=	=	=
4613	Succinate-semialdehyde dehydrogenase, mito. EC=1.2.1.24	-0.04	0.85	-	-0.38	0.049	↘↘	=	=	=	=	=	=	=	=	=
4614		0.34	0.083	↗	-0.29	0.14	-	=	=	=	=	=	=	=	=	=
4615		-0.28	0.16	-	0.13	0.53	-	=	=	=	=	=	=	=	=	=
4619	ND	-0.28	0.16	-	-0.02	0.90	-	=	=	=	=	M >>	=	=	=	=
4621		-0.02	0.91	-	0.19	0.34	-	=	=	=	=	=	=	=	=	=
4630		0.30	0.13	-	-0.21	0.30	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 4631 to 5213



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

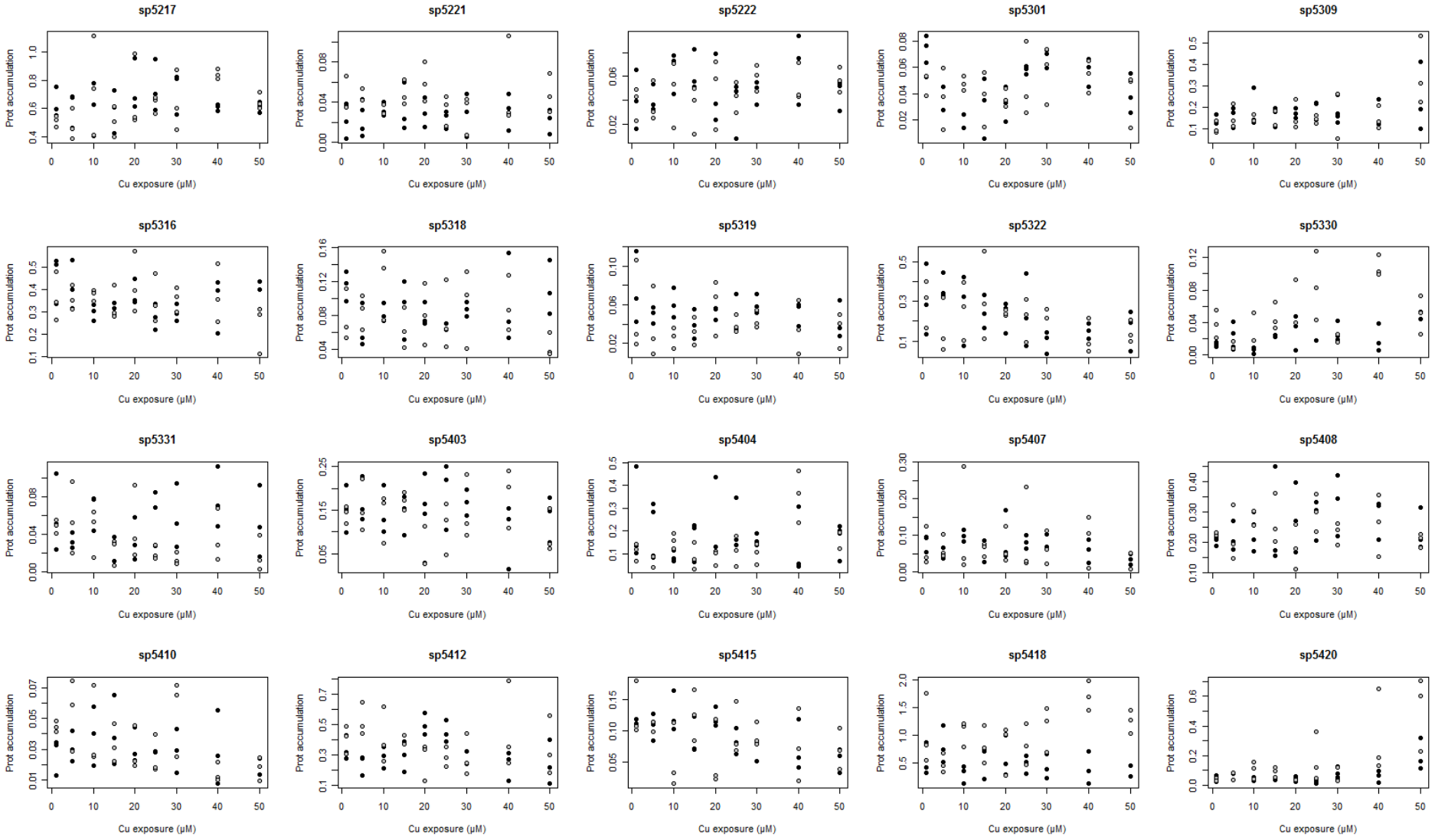
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
4631	0.077 ± 0.021	0.080 ± 0.028	0.037 ± 0.024	0.073 ± 0.041	0.046 ± 0.021	0.093 ± 0.014	0.060 ± 0.02	0.075 ± 0.035	0.073 ± 0.031	0.104 ± 0.054	0.037 ± 0.024	0.058 ± 0.041	0.051 ± 0.02	0.051 ± 0.048	0.021 ± 0.009	0.092 ± 0.059	0.066 ± 0.026	0.063 ± 0.048
4632	0.035 ± 0.024	0.029 ± 0.019	0.039 ± 0.026	0.027 ± 0.014	0.041 ± 0.025	0.034 ± 0.006	0.042 ± 0.019	0.029 ± 0.009	0.055 ± 0.017	0.042 ± 0.024	0.062 ± 0.022	0.031 ± 0.019	0.035 ± 0.023	0.021 ± 0.008	0.050 ± 0.026	0.034 ± 0.004	0.028 ± 0.001	0.028 ± 0.005
4702	0.198 ± 0.089	0.157 ± 0.049	0.171 ± 0.063	0.176 ± 0.044	0.134 ± 0.015	0.220 ± 0.078	0.141 ± 0.01	0.157 ± 0.017	0.185 ± 0.033	0.133 ± 0.023	0.169 ± 0.053	0.136 ± 0.036	0.163 ± 0.023	0.130 ± 0.029	0.196 ± 0.087	0.092 ± 0.026	0.118 ± 0.042	0.083 ± 0.048
4704	0.112 ± 0.003	0.070 ± 0.011	0.099 ± 0.013	0.091 ± 0.019	0.099 ± 0.007	0.108 ± 0.044	0.101 ± 0.025	0.104 ± 0.027	0.092 ± 0.022	0.104 ± 0.017	0.108 ± 0.015	0.117 ± 0.026	0.119 ± 0.028	0.087 ± 0.014	0.137 ± 0.052	0.120 ± 0.031	0.116 ± 0.024	0.095 ± 0.037
4705	0.029 ± 0.008	0.080 ± 0.001	0.010 ± 0.004	0.071 ± 0.009	0.023 ± 0.017	0.087 ± 0.048	0.013 ± 0.007	0.063 ± 0.009	0.032 ± 0.002	0.043 ± 0.027	0.018 ± 0.004	0.068 ± 0.028	0.013 ± 0.003	0.040 ± 0.027	0.020 ± 0.022	0.058 ± 0.046	0.008 ± 0.007	0.036 ± 0.03
4709	0.091 ± 0.028	0.106 ± 0.044	0.068 ± 0.026	0.092 ± 0.02	0.130 ± 0.037	0.081 ± 0.044	0.080 ± 0.013	0.108 ± 0.016	0.102 ± 0.03	0.070 ± 0.019	0.162 ± 0.012	0.097 ± 0.021	0.114 ± 0.065	0.059 ± 0.053	0.085 ± 0.027	0.060 ± 0.023	0.081 ± 0.014	0.141 ± 0.122
4714	0.213 ± 0.021	0.171 ± 0.02	0.202 ± 0.05	0.228 ± 0.04	0.241 ± 0.128	0.301 ± 0.003	0.243 ± 0.057	0.184 ± 0.099	0.280 ± 0.041	0.224 ± 0.039	0.253 ± 0.078	0.249 ± 0.088	0.245 ± 0.125	0.206 ± 0.042	0.265 ± 0.048	0.132 ± 0.055	0.230 ± 0.06	0.167 ± 0.056
4715	0.171 ± 0.021	0.093 ± 0.015	0.161 ± 0.086	0.162 ± 0.07	0.211 ± 0.029	0.199 ± 0.08	0.157 ± 0.054	0.191 ± 0.057	0.176 ± 0.07	0.099 ± 0.052	0.187 ± 0.08	0.198 ± 0.038	0.181 ± 0.063	0.118 ± 0.064	0.163 ± 0.056	0.140 ± 0.035	0.131 ± 0.034	0.097 ± 0.057
4716	0.086 ± 0.036	0.033 ± 0.028	0.113 ± 0.013	0.050 ± 0.026	0.145 ± 0.015	0.027 ± 0.013	0.110 ± 0.029	0.054 ± 0.025	0.123 ± 0.022	0.062 ± 0.035	0.139 ± 0.033	0.062 ± 0.006	0.105 ± 0.014	0.044 ± 0.016	0.152 ± 0.012	0.042 ± 0.025	0.056 ± 0.045	0.070 ± 0.016
4719	0.414 ± 0.134	0.705 ± 0.196	0.476 ± 0.117	0.576 ± 0.088	0.459 ± 0.137	0.632 ± 0.16	0.429 ± 0.112	0.455 ± 0.106	0.443 ± 0.16	0.489 ± 0.062	0.479 ± 0.252	0.453 ± 0.185	0.438 ± 0.113	0.561 ± 0.193	0.399 ± 0.069	0.394 ± 0.2	0.278 ± 0.093	0.268 ± 0.108
4801	0.070 ± 0.029	0.068 ± 0.007	0.094 ± 0.011	0.086 ± 0.016	0.115 ± 0.027	0.059 ± 0.012	0.086 ± 0.028	0.103 ± 0.04	0.073 ± 0.01	0.073 ± 0.021	0.088 ± 0.015	0.062 ± 0.005	0.097 ± 0.03	0.059 ± 0.031	0.057 ± 0.036	0.045 ± 0.034	0.074 ± 0.044	0.024 ± 0.015
4808	0.048 ± 0.018	0.088 ± 0.022	0.069 ± 0.037	0.078 ± 0.032	0.060 ± 0.033	0.056 ± 0.035	0.079 ± 0.03	0.054 ± 0.016	0.059 ± 0.023	0.031 ± 0.03	0.062 ± 0.006	0.025 ± 0.009	0.059 ± 0.026	0.051 ± 0.012	0.059 ± 0.009	0.039 ± 0.042		
4809	0.017 ± 0.006	0.049 ± 0.016	0.046 ± 0.015	0.034 ± 0.031	0.043 ± 0.022	0.036 ± 0.005	0.033 ± 0.011	0.056 ± 0.02	0.040 ± 0.026	0.026 ± 0.019	0.048 ± 0.02	0.045 ± 0.027	0.043 ± 0.027	0.034 ± 0.041	0.032 ± 0.025	0.044 ± 0.027	0.065 ± 0.042	0.024 ± 0.013
4816	0.069 ± 0.006	0.098 ± 0.022	0.075 ± 0.02	0.051 ± 0.025	0.079 ± 0.027	0.050 ± 0.006	0.064 ± 0.004	0.110 ± 0.05	0.069 ± 0.025	0.062 ± 0.027	0.077 ± 0.011	0.083 ± 0.049	0.097 ± 0.035	0.077 ± 0.023	0.052 ± 0.039	0.102 ± 0.059	0.135 ± 0.038	0.065 ± 0.025
4817	0.045 ± 0.017	0.093 ± 0.045	0.111 ± 0.031	0.082 ± 0.028	0.099 ± 0.056	0.083 ± 0.061	0.128 ± 0.077	0.059 ± 0.025	0.100 ± 0.047	0.063 ± 0.066	0.109 ± 0.009	0.039 ± 0.011	0.110 ± 0.071	0.070 ± 0.011	0.096 ± 0.051	0.022 ± 0.012		0.001 ± 0.001
4820	0.009 ± 0.004	0.040 ± 0.016	0.025 ± 0.025	0.018 ± 0.014	0.055 ± 0.035	0.044 ± 0.027	0.025 ± 0.016	0.036 ± 0.013	0.027 ± 0.008	0.027 ± 0.022	0.033 ± 0.013	0.031 ± 0.008	0.047 ± 0.025	0.031 ± 0.026	0.019 ± 0.016	0.041 ± 0.029	0.020 ± 0.008	0.020 ± 0.013
4821	0.040 ± 0.022	0.060 ± 0.045	0.041 ± 0.008	0.064 ± 0.019	0.049 ± 0.029	0.034 ± 0.032	0.088 ± 0.087	0.047 ± 0.009	0.030 ± 0.032	0.064 ± 0.039	0.072 ± 0.006	0.015 ± 0.001	0.068 ± 0.023	0.049 ± 0.03	0.027 ± 0.004	0.040 ± 0.042		0.005 ± 0.009
5205	0.148 ± 0.007	0.145 ± 0.029	0.126 ± 0.007	0.113 ± 0.009	0.141 ± 0.006	0.107 ± 0.011	0.129 ± 0.032	0.165 ± 0.024	0.131 ± 0.024	0.176 ± 0.032	0.125 ± 0.06	0.157 ± 0.025	0.138 ± 0.018	0.187 ± 0.062	0.158 ± 0.053	0.177 ± 0.027	0.170 ± 0.017	0.142 ± 0.037
5208	0.089 ± 0.049	0.103 ± 0.065	0.066 ± 0.029	0.065 ± 0.038	0.068 ± 0.004	0.036 ± 0.012	0.035 ± 0.025	0.075 ± 0.02	0.061 ± 0.029	0.059 ± 0.004	0.049 ± 0.038	0.072 ± 0.021	0.064 ± 0.024	0.079 ± 0.031	0.049 ± 0.032	0.110 ± 0.058	0.093 ± 0.017	0.104 ± 0.047
5213	0.042 ± 0.009	0.031 ± 0.007	0.029 ± 0.008	0.032 ± 0.009	0.053 ± 0.015	0.024 ± 0.018	0.027 ± 0.02	0.038 ± 0.005	0.046 ± 0.018	0.052 ± 0.019	0.090 ± 0.008	0.019 ± 0.009	0.064 ± 0.011	0.034 ± 0.01	0.064 ± 0.025	0.043 ± 0.043	0.044 ± 0.024	0.043 ± 0.01

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
4631		-0.15	0.46	-	-0.12	0.56	-	=	=	=	=	=	=	=	=	=
4632		-0.02	0.92	-	-0.01	0.95	-	=	=	=	=	=	=	=	=	=
4702	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1, mito. EC=1.3.5.1	-0.15	0.46	-	-0.63	0.0004	↘↘↘↘	=	=	=	=	=	=	=	=	=
4704		0.33	0.092	↗	0.22	0.26	-	=	=	=	=	=	=	=	=	=
4705	Phosphoglucomutase, cytoplasmic EC=5.4.2.2	-0.28	0.15	-	-0.42	0.033	↘↘	NM >>	NM >>	=	NM >>	=	NM >	=	=	=
4709		0.00	1.00	-	0.05	0.82	-	=	=	=	=	=	=	=	=	=
4714		0.15	0.46	-	-0.32	0.10	-	=	=	=	=	=	=	=	=	=
4715		-0.19	0.35	-	-0.18	0.37	-	=	=	=	=	=	=	=	=	=
4716	Heat shock 70 kDa protein 10, mitochondrial	-0.13	0.50	-	0.31	0.11	-	=	=	M >>	=	=	M >	M >	M >>	=
4719	Transketolase / 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	-0.31	0.12	-	-0.61	0.0006	↘↘↘↘	=	=	=	=	=	=	=	=	=
4801	NADH dehydrogenase [Ubi] iron-sulfur protein 1, mito. EC=1.6.5.3 - 1.6.99.3	-0.24	0.23	-	-0.56	0.003	↘↘↘	=	=	=	=	=	=	=	=	=
4808	ND	-0.46	0.017	↘↘	-0.73	<0.001	↘↘↘↘	=	=	=	=	=	M >	=	=	NM >>
4809		0.31	0.11	-	-0.17	0.40	-	=	=	=	=	=	=	=	=	=
4816	Cyanate hydratase / Chaperone protein ClpC1	0.38	0.049	↗↗	0.05	0.80	-	=	=	=	=	=	=	=	=	=
4817	ND	-0.29	0.15	-	-0.64	0.0005	↘↘↘↘	=	=	=	=	=	M >	=	=	=
4820		-0.02	0.92	-	-0.11	0.57	-	NM >	=	=	=	=	=	=	=	=
4821	ND	-0.27	0.18	-	-0.49	0.017	↘↘	=	=	=	=	=	M >>	=	=	=
5205		0.32	0.11	-	0.33	0.093	↗	=	=	=	=	=	=	=	=	=
5208		0.03	0.90	-	0.29	0.15	-	=	=	=	=	=	=	=	=	=
5213	ND	0.31	0.12	-	0.22	0.28	-	=	=	=	=	=	M >>	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 5217 to 5420



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

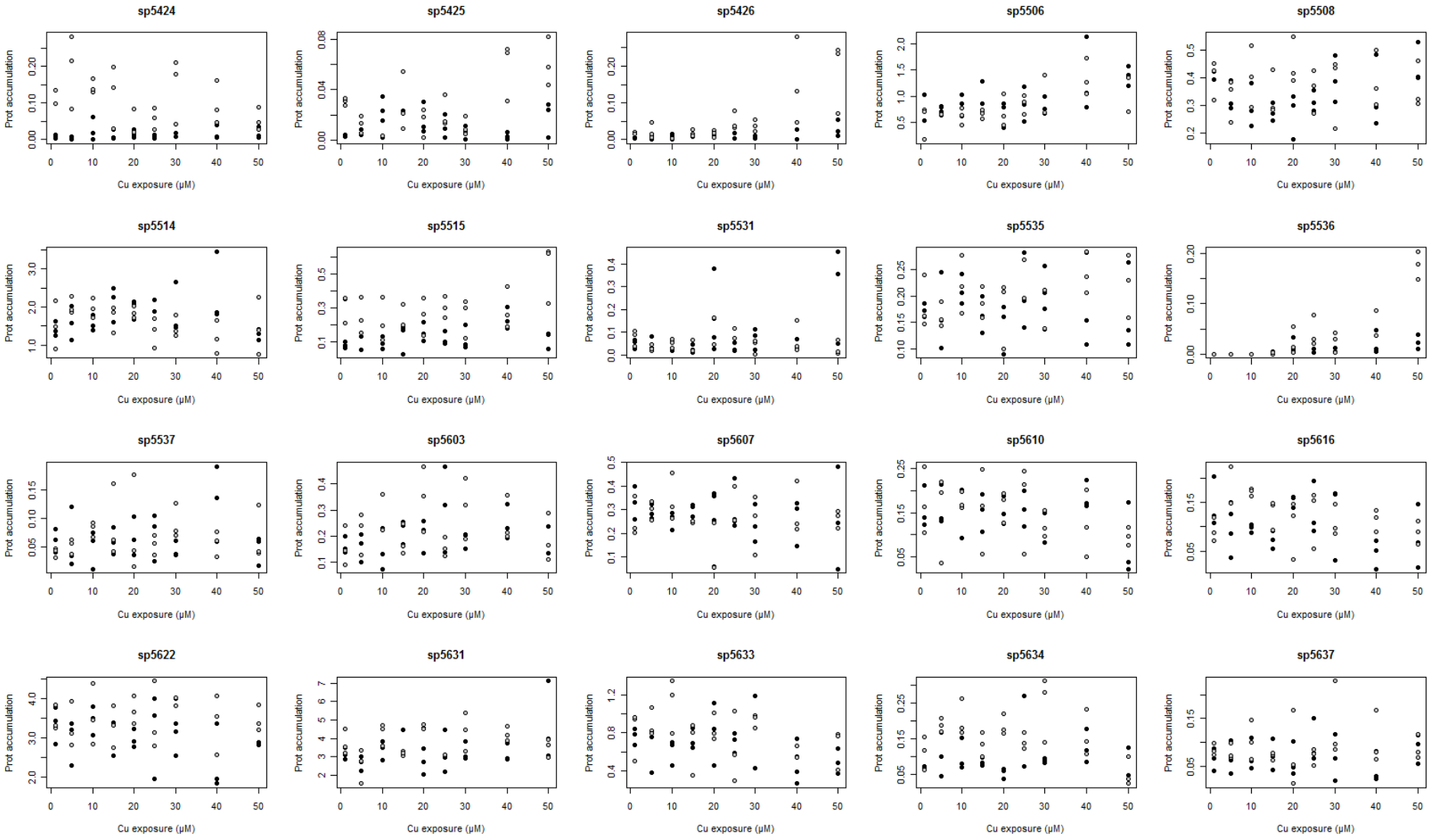
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
5217	0.634 ± 0.107	0.509 ± 0.039	0.607 ± 0.126	0.480 ± 0.112	0.602 ± 0.189	0.755 ± 0.353	0.585 ± 0.155	0.506 ± 0.107	0.746 ± 0.183	0.682 ± 0.266	0.746 ± 0.185	0.631 ± 0.059	0.730 ± 0.15	0.640 ± 0.213	0.607 ± 0.023	0.843 ± 0.035	0.611 ± 0.04	0.650 ± 0.059
5221	0.021 ± 0.017	0.046 ± 0.018	0.018 ± 0.013	0.046 ± 0.007	0.033 ± 0.007	0.032 ± 0.005	0.032 ± 0.024	0.049 ± 0.012	0.030 ± 0.015	0.060 ± 0.019	0.024 ± 0.009	0.033 ± 0.015	0.028 ± 0.021	0.030 ± 0.02	0.031 ± 0.018	0.054 ± 0.045	0.022 ± 0.012	0.048 ± 0.019
5222	0.040 ± 0.025	0.038 ± 0.014	0.041 ± 0.011	0.037 ± 0.017	0.065 ± 0.018	0.047 ± 0.028	0.063 ± 0.017	0.034 ± 0.02	0.047 ± 0.029	0.049 ± 0.03	0.036 ± 0.024	0.043 ± 0.013	0.047 ± 0.01	0.059 ± 0.011	0.069 ± 0.029	0.053 ± 0.016	0.046 ± 0.013	0.057 ± 0.01
5301	0.075 ± 0.01	0.048 ± 0.008	0.037 ± 0.009	0.037 ± 0.024	0.027 ± 0.015	0.048 ± 0.006	0.031 ± 0.023	0.037 ± 0.021	0.032 ± 0.013	0.036 ± 0.007	0.058 ± 0.003	0.048 ± 0.029	0.068 ± 0.007	0.056 ± 0.022	0.057 ± 0.011	0.054 ± 0.012	0.039 ± 0.015	0.038 ± 0.021
5309	0.127 ± 0.04	0.105 ± 0.028	0.158 ± 0.048	0.155 ± 0.054	0.186 ± 0.093	0.149 ± 0.016	0.163 ± 0.046	0.163 ± 0.039	0.174 ± 0.022	0.160 ± 0.069	0.195 ± 0.045	0.146 ± 0.019	0.154 ± 0.021	0.191 ± 0.118	0.162 ± 0.067	0.150 ± 0.053	0.236 ± 0.16	0.357 ± 0.159
5316	0.459 ± 0.108	0.364 ± 0.111	0.417 ± 0.109	0.362 ± 0.054	0.300 ± 0.037	0.377 ± 0.025	0.316 ± 0.025	0.332 ± 0.076	0.382 ± 0.059	0.425 ± 0.138	0.276 ± 0.06	0.354 ± 0.108	0.296 ± 0.038	0.360 ± 0.055	0.345 ± 0.124	0.377 ± 0.133	0.374 ± 0.078	0.238 ± 0.11
5318	0.116 ± 0.018	0.078 ± 0.03	0.065 ± 0.026	0.085 ± 0.02	0.083 ± 0.011	0.122 ± 0.042	0.090 ± 0.035	0.065 ± 0.024	0.080 ± 0.014	0.081 ± 0.036	0.086 ± 0.032	0.077 ± 0.041	0.088 ± 0.009	0.092 ± 0.046	0.093 ± 0.053	0.093 ± 0.032	0.112 ± 0.032	0.045 ± 0.014
5319	0.075 ± 0.037	0.052 ± 0.048	0.050 ± 0.008	0.038 ± 0.037	0.061 ± 0.015	0.026 ± 0.011	0.040 ± 0.015	0.033 ± 0.014	0.052 ± 0.007	0.060 ± 0.029	0.048 ± 0.021	0.040 ± 0.009	0.060 ± 0.01	0.044 ± 0.009	0.052 ± 0.012	0.036 ± 0.028	0.042 ± 0.019	0.035 ± 0.019
5322	0.302 ± 0.18	0.296 ± 0.118	0.374 ± 0.065	0.163 ± 0.138	0.273 ± 0.179	0.258 ± 0.148	0.247 ± 0.084	0.319 ± 0.224	0.230 ± 0.079	0.242 ± 0.015	0.245 ± 0.185	0.213 ± 0.11	0.098 ± 0.055	0.246 ± 0.025	0.151 ± 0.037	0.115 ± 0.087	0.162 ± 0.101	0.145 ± 0.056
5330	0.013 ± 0.003	0.038 ± 0.017	0.026 ± 0.016	0.011 ± 0.006	0.006 ± 0.004	0.029 ± 0.019	0.029 ± 0.01	0.046 ± 0.017	0.030 ± 0.022	0.057 ± 0.03	0.018	0.085 ± 0.042	0.028 ± 0.013	0.022 ± 0.006	0.019 ± 0.017	0.109 ± 0.013	0.041 ± 0.014	0.050 ± 0.024
5331	0.060 ± 0.041	0.048 ± 0.007	0.033 ± 0.008	0.056 ± 0.038	0.066 ± 0.02	0.044 ± 0.025	0.026 ± 0.014	0.022 ± 0.014	0.033 ± 0.023	0.048 ± 0.039	0.060 ± 0.029	0.019 ± 0.008	0.057 ± 0.034	0.013 ± 0.007	0.077 ± 0.033	0.036 ± 0.028	0.052 ± 0.039	0.018 ± 0.019
5403	0.153 ± 0.054	0.141 ± 0.02	0.169 ± 0.052	0.157 ± 0.06	0.145 ± 0.055	0.140 ± 0.056	0.142 ± 0.044	0.172 ± 0.021	0.180 ± 0.048	0.057 ± 0.048	0.192 ± 0.078	0.113 ± 0.059	0.167 ± 0.029	0.148 ± 0.074	0.100 ± 0.074	0.184 ± 0.068	0.135 ± 0.054	0.096 ± 0.051
5404	0.242 ± 0.211	0.110 ± 0.038	0.229 ± 0.126	0.072 ± 0.028	0.087 ± 0.025	0.157 ± 0.032	0.168 ± 0.09	0.087 ± 0.058	0.225 ± 0.184	0.087 ± 0.033	0.214 ± 0.113	0.113 ± 0.066	0.162 ± 0.026	0.100 ± 0.045	0.136 ± 0.15	0.356 ± 0.114	0.163 ± 0.082	0.169 ± 0.04
5407	0.081 ± 0.023	0.063 ± 0.052	0.056 ± 0.016	0.066 ± 0.031	0.099 ± 0.015	0.115 ± 0.151	0.062 ± 0.031	0.061 ± 0.017	0.088 ± 0.069	0.069 ± 0.048	0.082 ± 0.018	0.096 ± 0.119	0.079 ± 0.02	0.065 ± 0.045	0.058 ± 0.031	0.088 ± 0.071	0.035 ± 0.015	0.036 ± 0.024
5408	0.204 ± 0.014	0.227 ± 0.004	0.218 ± 0.048	0.222 ± 0.092	0.226 ± 0.066	0.273 ± 0.027	0.260 ± 0.166	0.269 ± 0.083	0.278 ± 0.114	0.184 ± 0.073	0.280 ± 0.067	0.298 ± 0.062	0.328 ± 0.101	0.231 ± 0.036	0.285 ± 0.067	0.259 ± 0.102	0.236 ± 0.069	0.208 ± 0.021
5410	0.027 ± 0.012	0.045 ± 0.003	0.031 ± 0.01	0.054 ± 0.023	0.039 ± 0.019	0.041 ± 0.026	0.041 ± 0.023	0.033 ± 0.012	0.031 ± 0.011	0.029 ± 0.014	0.028	0.025 ± 0.013	0.029 ± 0.014	0.054 ± 0.025	0.030 ± 0.024	0.015 ± 0.006	0.019 ± 0.005	0.018 ± 0.008
5412	0.343 ± 0.079	0.407 ± 0.09	0.243 ± 0.068	0.528 ± 0.108	0.288 ± 0.072	0.416 ± 0.182	0.293 ± 0.1	0.391 ± 0.032	0.502 ± 0.071	0.273 ± 0.126	0.450 ± 0.071	0.286 ± 0.064	0.273 ± 0.046	0.289 ± 0.135	0.240 ± 0.095	0.463 ± 0.285	0.244 ± 0.146	0.347 ± 0.194
5415	0.111 ± 0.008	0.130 ± 0.043	0.107 ± 0.022	0.106 ± 0.012	0.128 ± 0.032	0.053 ± 0.052	0.088 ± 0.03	0.125 ± 0.041	0.121 ± 0.016	0.056 ± 0.054	0.083 ± 0.021	0.098 ± 0.043	0.073 ± 0.018	0.093 ± 0.02	0.072 ± 0.041	0.076 ± 0.058	0.054 ± 0.019	0.071 ± 0.033
5418	0.544 ± 0.292	1.049 ± 0.633	0.814 ± 0.336	0.496 ± 0.175	0.312 ± 0.158	1.057 ± 0.232	0.565 ± 0.303	0.821 ± 0.343	0.592 ± 0.372	0.799 ± 0.453	0.486 ± 0.164	0.836 ± 0.373	0.430 ± 0.214	1.152 ± 0.407	0.399 ± 0.296	1.710 ± 0.269	0.588 ± 0.396	1.255 ± 0.206
5420	0.048 ± 0.019	0.036 ± 0.014	0.049 ± 0.024	0.054 ± 0.024	0.046 ± 0.013	0.103 ± 0.058	0.039 ± 0.006	0.090 ± 0.033	0.042 ± 0.017	0.036 ± 0.006	0.022 ± 0.013	0.176 ± 0.165	0.058 ± 0.022	0.092 ± 0.051	0.061 ± 0.04	0.324 ± 0.284	0.198 ± 0.105	0.511 ± 0.247

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
5217		0.05	0.79	-	0.35	0.072	↗	=	=	=	=	=	=	=	=	=
5221		0.06	0.77	-	0.06	0.76	-	=	=	=	=	=	=	=	=	=
5222		0.09	0.66	-	0.38	0.051	↗	=	=	=	=	=	=	=	=	=
5301		0.05	0.79	-	0.07	0.73	-	=	=	=	=	=	=	=	=	=
5309	Cysteine synthase EC=2.5.1.47	0.29	0.15	-	0.55	0.003	↗↗↗	=	=	=	=	=	=	=	=	=
5316		-0.23	0.25	-	-0.27	0.18	-	=	=	=	=	=	=	=	=	=
5318		0.16	0.42	-	-0.24	0.23	-	=	=	=	=	=	=	=	=	=
5319		-0.27	0.18	-	-0.06	0.76	-	=	=	=	=	=	=	=	=	=
5322	Remorin : DNA-binding protein	-0.51	0.006	↘↘↘	-0.35	0.076	↘	=	=	=	=	=	=	=	=	=
5330	ND	0.39	0.045	↗↗	0.45	0.019	↗↗	=	=	=	=	=	NM >>	=	NM >>	=
5331	ND	0.18	0.36	-	-0.40	0.040	↘↘	=	=	=	=	=	=	=	=	=
5403		-0.19	0.33	-	-0.10	0.61	-	=	=	=	=	=	=	=	=	=
5404	Glutamine synthetase EC=6.3.1.2	-0.15	0.46	-	0.49	0.010	↗↗↗	=	=	=	=	=	=	=	=	=
5407		-0.33	0.096	↘	-0.10	0.63	-	=	=	=	=	=	=	=	=	=
5408		0.23	0.24	-	-0.04	0.83	-	=	=	=	=	=	=	=	=	=
5410	ND	-0.25	0.21	-	-0.49	0.009	↘↘↘	=	=	=	=	=	=	=	=	=
5412		-0.16	0.42	-	-0.19	0.35	-	=	=	=	=	=	=	=	=	=
5415	Peroxidase 2 (Fragment) EC=1.11.1.7	-0.63	0.0004	↘↘↘↘	-0.26	0.20	-	=	=	=	=	=	=	=	=	=
5418	Glutamine synthetase cytosolic / Peroxidase 2	-0.12	0.55	-	0.48	0.012	↗↗	=	=	NM >	=	=	=	=	NM >>	=
5420	ND	0.56	0.003	↗↗↗	0.69	<0.001	↗↗↗↗	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 5424 to 5637



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

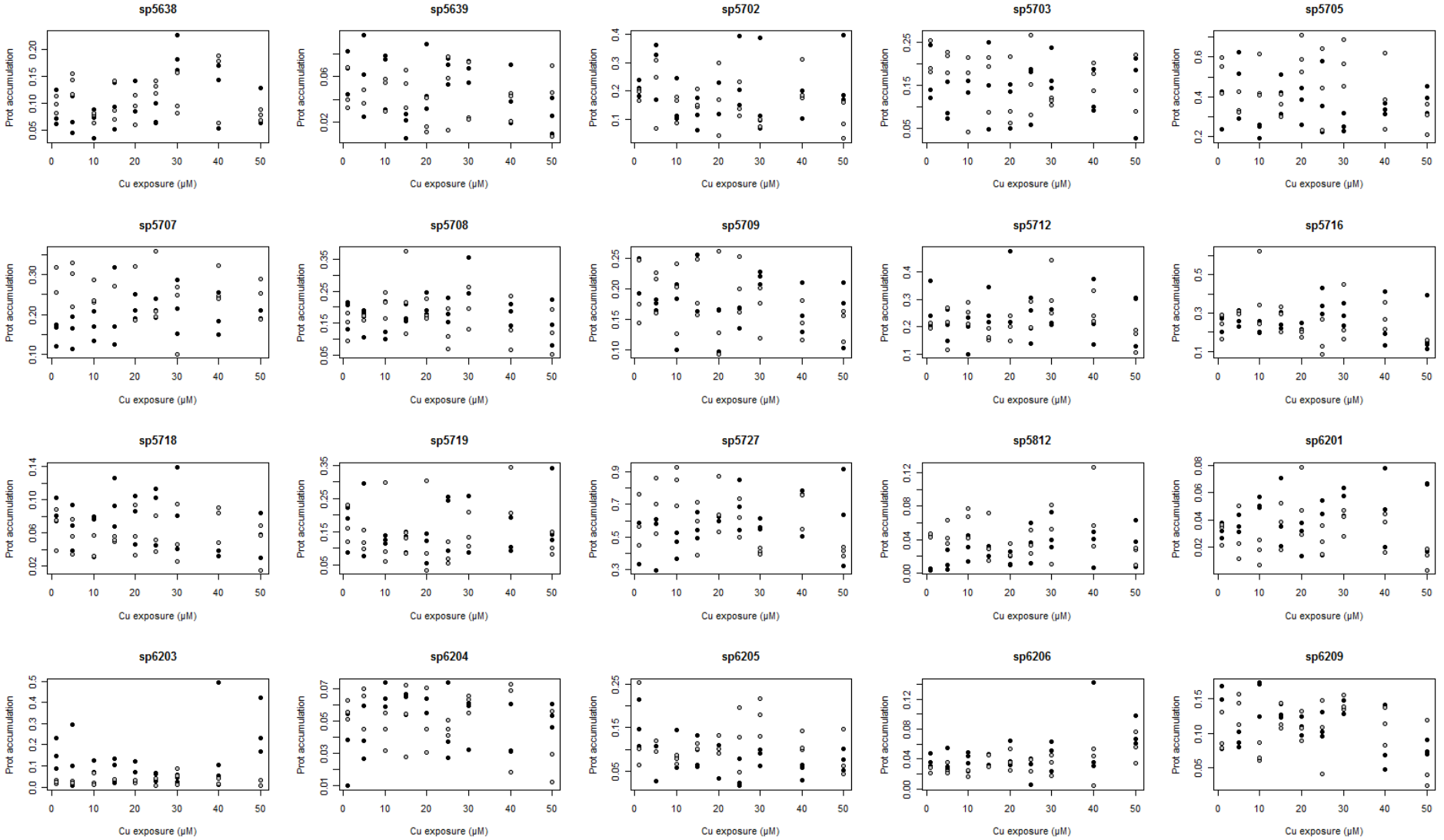
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
5424	0.008 ± 0.005	0.116 ± 0.027	0.003 ± 0.004	0.193 ± 0.1	0.027 ± 0.031	0.144 ± 0.019	0.012 ± 0.014	0.124 ± 0.085	0.014 ± 0.011	0.040 ± 0.036	0.007 ± 0.005	0.058 ± 0.029	0.011 ± 0.007	0.143 ± 0.09	0.017 ± 0.02	0.096 ± 0.059	0.017 ± 0.016	0.054 ± 0.032
5425	0.011 ± 0.014	0.031 ± 0.003	0.006 ± 0.002	0.013 ± 0.007	0.024 ± 0.01	0.003 ± 0.001	0.018 ± 0.008	0.028 ± 0.023	0.016 ± 0.013	0.015 ± 0.012	0.010 ± 0.01	0.021 ± 0.013	0.006 ± 0.005	0.010 ± 0.008	0.003 ± 0.003	0.058 ± 0.023	0.018 ± 0.014	0.062 ± 0.019
5426	0.009 ± 0.009	0.017 ± 0.003	0.006 ± 0.007	0.023 ± 0.022	0.012 ± 0.004	0.003 ± 0.002	0.009 ± 0.003	0.018 ± 0.009	0.015 ± 0.008	0.017 ± 0.01	0.007 ± 0.008	0.050 ± 0.025	0.007 ± 0.003	0.038 ± 0.015	0.010 ± 0.016	0.153 ± 0.118	0.030 ± 0.023	0.183 ± 0.098
5506	0.758 ± 0.256	0.537 ± 0.313	0.763 ± 0.053	0.647 ± 0.01	0.836 ± 0.202	0.616 ± 0.165	0.956 ± 0.288	0.660 ± 0.086	0.683 ± 0.248	0.699 ± 0.305	0.847 ± 0.331	0.849 ± 0.187	0.917 ± 0.136	0.919 ± 0.414	1.331 ± 0.708	1.352 ± 0.345	1.389 ± 0.192	1.135 ± 0.372
5508	0.414 ± 0.018	0.399 ± 0.07	0.330 ± 0.053	0.326 ± 0.077	0.297 ± 0.078	0.404 ± 0.11	0.277 ± 0.033	0.335 ± 0.082	0.271 ± 0.081	0.451 ± 0.084	0.315 ± 0.037	0.356 ± 0.078	0.393 ± 0.083	0.368 ± 0.129	0.338 ± 0.129	0.388 ± 0.101	0.443 ± 0.073	0.365 ± 0.084
5514	1.411 ± 0.195	1.513 ± 0.633	1.574 ± 0.44	2.025 ± 0.218	1.560 ± 0.201	1.972 ± 0.259	2.111 ± 0.464	1.717 ± 0.347	1.957 ± 0.254	1.859 ± 0.151	1.829 ± 0.395	1.346 ± 0.384	1.882 ± 0.676	1.464 ± 0.285	2.377 ± 0.938	1.195 ± 0.433	1.286 ± 0.14	1.472 ± 0.756
5515	0.079 ± 0.02	0.307 ± 0.085	0.077 ± 0.045	0.247 ± 0.106	0.092 ± 0.038	0.222 ± 0.132	0.126 ± 0.087	0.237 ± 0.072	0.156 ± 0.056	0.253 ± 0.111	0.116 ± 0.039	0.304 ± 0.063	0.116 ± 0.074	0.252 ± 0.116	0.236 ± 0.066	0.291 ± 0.123	0.115 ± 0.05	0.529 ± 0.174
5531	0.052 ± 0.02	0.078 ± 0.035	0.047 ± 0.031	0.032 ± 0.014	0.037 ± 0.022	0.053 ± 0.02	0.028 ± 0.019	0.052 ± 0.028	0.163 ± 0.19	0.123 ± 0.068	0.033 ± 0.019	0.096 ± 0.033	0.075 ± 0.046	0.041 ± 0.032	0.051 ± 0.031	0.071 ± 0.07	0.288 ± 0.21	0.030 ± 0.032
5535	0.173 ± 0.013	0.183 ± 0.05	0.166 ± 0.073	0.162 ± 0.023	0.211 ± 0.028	0.221 ± 0.056	0.164 ± 0.035	0.187 ± 0.03	0.143 ± 0.047	0.175 ± 0.065	0.204 ± 0.073	0.220 ± 0.042	0.213 ± 0.041	0.162 ± 0.043	0.181 ± 0.091	0.242 ± 0.039	0.169 ± 0.084	0.222 ± 0.06
5536								0.003 ± 0.003	0.018 ± 0.014	0.024 ± 0.028	0.006 ± 0.004	0.043 ± 0.031	0.006 ± 0.005	0.025 ± 0.02	0.021 ± 0.023	0.061 ± 0.035	0.024 ± 0.014	0.177 ± 0.028
5537	0.061 ± 0.02	0.040 ± 0.008	0.058 ± 0.054	0.052 ± 0.013	0.049 ± 0.033	0.081 ± 0.013	0.060 ± 0.024	0.088 ± 0.064	0.067 ± 0.034	0.078 ± 0.086	0.072 ± 0.041	0.054 ± 0.017	0.045 ± 0.014	0.091 ± 0.03	0.128 ± 0.066	0.056 ± 0.022	0.047 ± 0.026	0.068 ± 0.047
5603	0.164 ± 0.032	0.159 ± 0.077	0.159 ± 0.054	0.216 ± 0.079	0.145 ± 0.079	0.271 ± 0.078	0.221 ± 0.046	0.180 ± 0.058	0.206 ± 0.063	0.346 ± 0.125	0.307 ± 0.164	0.157 ± 0.035	0.187 ± 0.032	0.310 ± 0.116	0.248 ± 0.068	0.256 ± 0.087	0.160 ± 0.067	0.189 ± 0.09
5607	0.328 ± 0.07	0.260 ± 0.083	0.290 ± 0.032	0.296 ± 0.039	0.256 ± 0.037	0.343 ± 0.099	0.299 ± 0.026	0.249 ± 0.006	0.326 ± 0.061	0.118 ± 0.108	0.306 ± 0.109	0.304 ± 0.081	0.239 ± 0.079	0.246 ± 0.126	0.258 ± 0.099	0.293 ± 0.111	0.258 ± 0.217	0.264 ± 0.037
5610	0.158 ± 0.048	0.174 ± 0.076	0.161 ± 0.046	0.150 ± 0.099	0.153 ± 0.055	0.176 ± 0.02	0.152 ± 0.043	0.157 ± 0.097	0.153 ± 0.032	0.167 ± 0.035	0.159 ± 0.041	0.172 ± 0.101	0.115 ± 0.033	0.123 ± 0.03	0.188 ± 0.032	0.123 ± 0.076	0.079 ± 0.083	0.097 ± 0.02
5616	0.145 ± 0.051	0.092 ± 0.025	0.083 ± 0.045	0.175 ± 0.043	0.097 ± 0.009	0.172 ± 0.008	0.073 ± 0.018	0.129 ± 0.031	0.153 ± 0.012	0.100 ± 0.06	0.131 ± 0.055	0.124 ± 0.061	0.122 ± 0.079	0.110 ± 0.032	0.045 ± 0.029	0.114 ± 0.023	0.077 ± 0.066	0.088 ± 0.024
5622	3.352 ± 0.466	3.465 ± 0.327	2.956 ± 0.577	3.294 ± 0.584	3.459 ± 0.362	3.566 ± 0.789	3.257 ± 0.644	3.297 ± 0.531	2.973 ± 0.242	3.700 ± 0.346	3.179 ± 1.076	3.470 ± 0.869	3.021 ± 0.431	3.951 ± 0.118	2.388 ± 0.852	3.398 ± 0.755	2.855 ± 0.032	3.478 ± 0.327
5631	3.168 ± 0.323	3.771 ± 0.685	2.659 ± 0.393	2.571 ± 0.927	3.374 ± 0.522	4.284 ± 0.606	3.543 ± 0.804	3.188 ± 0.13	2.762 ± 0.696	4.625 ± 0.136	3.210 ± 1.173	3.060 ± 0.053	3.264 ± 0.5	4.399 ± 1.032	3.184 ± 0.498	4.238 ± 0.396	4.717 ± 2.133	3.521 ± 0.482
5633	0.764 ± 0.087	0.805 ± 0.258	0.654 ± 0.239	0.896 ± 0.147	0.610 ± 0.129	1.110 ± 0.285	0.728 ± 0.106	0.681 ± 0.283	0.802 ± 0.327	0.848 ± 0.143	0.707 ± 0.106	0.632 ± 0.365	0.864 ± 0.393	0.930 ± 0.068	0.467 ± 0.241	0.587 ± 0.07	0.499 ± 0.131	0.656 ± 0.208
5634	0.071 ± 0.002	0.111 ± 0.046	0.104 ± 0.062	0.188 ± 0.019	0.100 ± 0.046	0.203 ± 0.053	0.084 ± 0.013	0.134 ± 0.034	0.054 ± 0.014	0.187 ± 0.028	0.170 ± 0.099	0.142 ± 0.024	0.088 ± 0.007	0.244 ± 0.092	0.127 ± 0.047	0.161 ± 0.065	0.099 ± 0.045	0.054 ± 0.041
5637	0.064 ± 0.024	0.084 ± 0.012	0.066 ± 0.035	0.079 ± 0.016	0.072 ± 0.033	0.104 ± 0.041	0.074 ± 0.033	0.071 ± 0.008	0.061 ± 0.036	0.078 ± 0.08	0.098 ± 0.045	0.071 ± 0.017	0.068 ± 0.048	0.137 ± 0.08	0.045 ± 0.031	0.104 ± 0.056	0.089 ± 0.031	0.088 ± 0.026

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
5424	ND	0.14	0.48	-	-0.39	0.047	↘↘	NM >>	=	=	=	=	NM >>	NM >>	=	=
5425	Methylthioribose-1-phosphate isomerase EC=5.3.1.23	-0.09	0.67	-	0.59	0.001	↗↗↗	=	=	M >>	=	=	=	=	NM >>	=
5426	Methylthioribose-1-phosphate isomerase EC=5.3.1.23	0.37	0.055	↗	0.72	<0.001	↗↗↗↗	=	=	M >	=	=	=	NM >>	=	NM >
5506	S-adenosylmethionine synthase EC=2.5.1.6	0.57	0.002	↗↗↗	0.68	<0.001	↗↗↗↗	=	=	=	=	=	=	=	=	=
5508		0.26	0.20	-	0.00	0.98	-	=	=	=	=	=	=	=	=	=
5514	Actin	0.13	0.52	-	-0.39	0.043	↘↘	=	=	=	=	=	=	=	=	=
5515	S-adenosylmethionine synthase / Actin	0.42	0.031	↗↗	0.46	0.016	↗↗	NM >>	=	=	=	=	NM >	=	=	NM >>
5531	ND	0.47	0.014	↗↗	-0.10	0.64	-	=	=	=	=	=	=	=	=	=
5535		0.03	0.86	-	0.31	0.12	-	=	=	=	=	=	=	=	=	=
5536	ND	0.64	<0.001	↗↗↗↗	0.84	<0.001	↗↗↗↗				NM >>	=	=	=	=	NM >>
5537		0.17	0.40	-	0.09	0.66	-	=	=	=	=	=	=	=	=	=
5603		0.17	0.38	-	0.08	0.68	-	=	=	=	=	=	=	=	=	=
5607		-0.20	0.32	-	-0.04	0.83	-	=	=	=	=	=	=	=	=	=
5610		-0.29	0.14	-	-0.35	0.072	↘	=	=	=	=	=	=	=	=	=
5616		-0.27	0.18	-	-0.35	0.076	↘	=	=	NM >	=	=	=	=	=	=
5622		-0.36	0.068	↘	0.08	0.69	-	=	=	=	=	=	=	=	=	=
5631		0.37	0.054	↗	0.18	0.36	-	=	=	=	=	=	=	=	=	=
5633		-0.28	0.16	-	-0.36	0.066	↘	=	=	=	=	=	=	=	=	=
5634	ND	0.19	0.34	-	-0.24	0.24	-	=	=	=	=	NM >>	=	NM >	=	=
5637		0.06	0.76	-	0.14	0.49	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 5638 to 6209



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

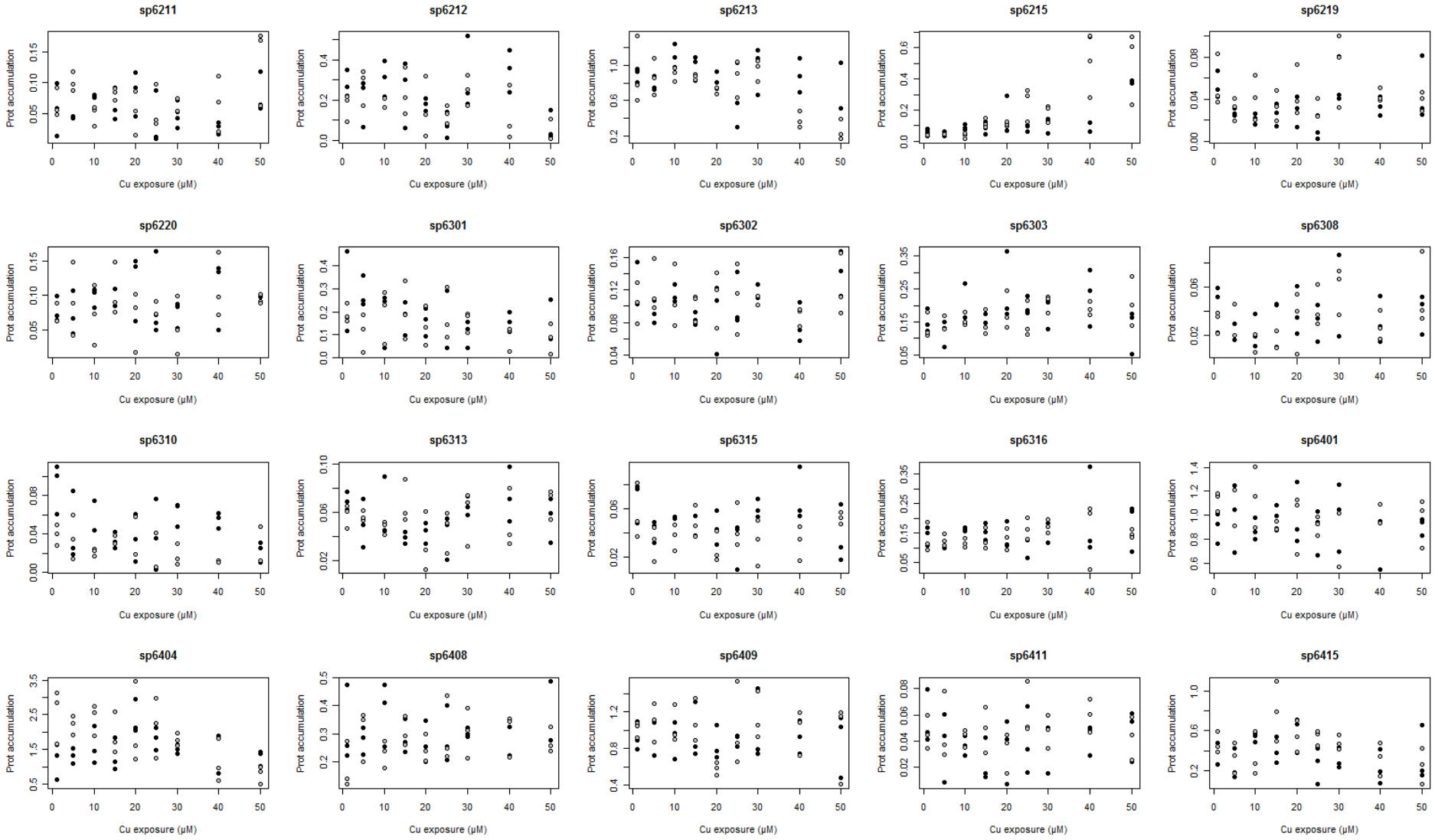
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
5638	0.087 ± 0.034	0.098 ± 0.015	0.075 ± 0.035	0.138 ± 0.02	0.066 ± 0.027	0.074 ± 0.01	0.095 ± 0.044	0.100 ± 0.037	0.115 ± 0.028	0.090 ± 0.028	0.077 ± 0.02	0.132 ± 0.012	0.191 ± 0.033	0.111 ± 0.041	0.123 ± 0.061	0.143 ± 0.07	0.086 ± 0.037	0.078 ± 0.01
5639	0.065 ± 0.019	0.047 ± 0.019	0.061 ± 0.035	0.041 ± 0.007	0.062 ± 0.026	0.047 ± 0.016	0.019 ± 0.011	0.051 ± 0.017	0.055 ± 0.03	0.023 ± 0.016	0.066 ± 0.012	0.050 ± 0.033	0.065 ± 0.01	0.040 ± 0.029	0.043 ± 0.026	0.037 ± 0.013	0.026 ± 0.016	0.041 ± 0.031
5702	0.211 ± 0.029	0.190 ± 0.02	0.287 ± 0.103	0.210 ± 0.126	0.154 ± 0.08	0.144 ± 0.05	0.119 ± 0.057	0.167 ± 0.036	0.156 ± 0.064	0.171 ± 0.127	0.251 ± 0.128	0.162 ± 0.063	0.190 ± 0.173	0.091 ± 0.014	0.162 ± 0.053	0.225 ± 0.075	0.251 ± 0.127	0.093 ± 0.064
5703	0.167 ± 0.066	0.207 ± 0.039	0.106 ± 0.046	0.207 ± 0.026	0.142 ± 0.016	0.145 ± 0.091	0.149 ± 0.1	0.165 ± 0.068	0.112 ± 0.055	0.123 ± 0.081	0.142 ± 0.072	0.166 ± 0.093	0.180 ± 0.05	0.113 ± 0.008	0.126 ± 0.052	0.172 ± 0.032	0.141 ± 0.099	0.149 ± 0.067
5705	0.361 ± 0.108	0.522 ± 0.094	0.477 ± 0.169	0.359 ± 0.056	0.234 ± 0.037	0.482 ± 0.118	0.414 ± 0.099	0.359 ± 0.056	0.364 ± 0.095	0.607 ± 0.095	0.385 ± 0.18	0.439 ± 0.206	0.265 ± 0.047	0.569 ± 0.119	0.339 ± 0.026	0.415 ± 0.194	0.389 ± 0.068	0.294 ± 0.077
5707	0.154 ± 0.03	0.277 ± 0.037	0.158 ± 0.042	0.284 ± 0.057	0.170 ± 0.038	0.251 ± 0.031	0.205 ± 0.101	0.271	0.217 ± 0.03	0.253 ± 0.097	0.214 ± 0.025	0.254 ± 0.092	0.218 ± 0.068	0.205 ± 0.092	0.196 ± 0.055	0.269 ± 0.046	0.199 ± 0.015	0.244 ± 0.051
5708	0.184 ± 0.047	0.143 ± 0.043	0.159 ± 0.046	0.167 ± 0.008	0.147 ± 0.062	0.209 ± 0.04	0.177 ± 0.029	0.235 ± 0.131	0.205 ± 0.035	0.188 ± 0.033	0.186 ± 0.038	0.125 ± 0.064	0.287 ± 0.06	0.196 ± 0.066	0.180 ± 0.035	0.143 ± 0.084	0.149 ± 0.072	0.122 ± 0.07
5709	0.212 ± 0.033	0.189 ± 0.053	0.175 ± 0.009	0.201 ± 0.036	0.164 ± 0.056	0.190 ± 0.058	0.198 ± 0.05	0.194 ± 0.048	0.143 ± 0.039	0.161 ± 0.089	0.157 ± 0.019	0.205 ± 0.046	0.218 ± 0.011	0.166 ± 0.043	0.165 ± 0.042	0.147 ± 0.032	0.163 ± 0.055	0.144 ± 0.027
5712	0.271 ± 0.088	0.207 ± 0.01	0.206 ± 0.058	0.201 ± 0.079	0.178 ± 0.07	0.251 ± 0.04	0.268 ± 0.069	0.169 ± 0.023	0.305 ± 0.15	0.196 ± 0.047	0.236 ± 0.088	0.229 ± 0.056	0.229 ± 0.031	0.331 ± 0.101	0.241 ± 0.123	0.265 ± 0.061	0.246 ± 0.102	0.155 ± 0.044
5716	0.249 ± 0.043	0.233 ± 0.064	0.267 ± 0.042	0.304 ± 0.008	0.219 ± 0.035	0.403 ± 0.197	0.255 ± 0.045	0.277 ± 0.067	0.224 ± 0.02	0.196 ± 0.018	0.354 ± 0.069	0.160 ± 0.094	0.291 ± 0.059	0.274 ± 0.153	0.245 ± 0.146	0.280 ± 0.073	0.215 ± 0.156	0.155 ± 0.005
5718	0.086 ± 0.015	0.067 ± 0.026	0.067 ± 0.028	0.056 ± 0.021	0.078 ± 0.002	0.040 ± 0.015	0.096 ± 0.03	0.053 ± 0.003	0.079 ± 0.03	0.061 ± 0.03	0.087 ± 0.037	0.057 ± 0.022	0.087 ± 0.05	0.056 ± 0.036	0.040 ± 0.008	0.074 ± 0.022	0.058 ± 0.027	0.047 ± 0.028
5719	0.167 ± 0.071	0.170 ± 0.055	0.176 ± 0.111	0.123 ± 0.029	0.126 ± 0.012	0.149 ± 0.13	0.124 ± 0.033	0.121 ± 0.032	0.108 ± 0.046	0.141 ± 0.143	0.196 ± 0.091	0.081 ± 0.034	0.150 ± 0.094	0.150 ± 0.053	0.130 ± 0.054	0.298 ± 0.08	0.202 ± 0.119	0.111 ± 0.035
5727	0.455 ± 0.126	0.593 ± 0.157	0.497 ± 0.173	0.696 ± 0.17	0.454 ± 0.081	0.824 ± 0.121	0.563 ± 0.083	0.567 ± 0.162	0.617 ± 0.019	0.681 ± 0.173	0.693 ± 0.153	0.619 ± 0.118	0.576 ± 0.036	0.413 ± 0.019	0.682 ± 0.155	0.690 ± 0.122	0.626 ± 0.298	0.413 ± 0.029
5812	0.018 ± 0.023	0.046 ± 0.002	0.014 ± 0.012	0.047 ± 0.014	0.030 ± 0.016	0.062 ± 0.018	0.022 ± 0.009	0.038 ± 0.03	0.015 ± 0.009	0.026 ± 0.008	0.036 ± 0.024	0.036 ± 0.015	0.048 ± 0.022	0.048 ± 0.036	0.032 ± 0.023	0.072 ± 0.05	0.036 ± 0.028	0.022 ± 0.011
6201	0.032 ± 0.006	0.031 ± 0.008	0.037 ± 0.006	0.028 ± 0.02	0.052 ± 0.004	0.017 ± 0.01	0.042 ± 0.026	0.036 ± 0.017	0.028 ± 0.013	0.052 ± 0.025	0.037 ± 0.021	0.025 ± 0.011	0.055 ± 0.01	0.039 ± 0.01	0.048 ± 0.029	0.033 ± 0.015	0.050 ± 0.029	0.012 ± 0.008
6203	0.155 ± 0.073	0.024 ± 0.01	0.133 ± 0.147	0.023 ± 0.006	0.070 ± 0.056	0.032 ± 0.029	0.085 ± 0.058	0.034 ± 0.003	0.073 ± 0.046	0.027 ± 0.005	0.053 ± 0.022	0.019 ± 0.019	0.042 ± 0.017	0.050 ± 0.039	0.217 ± 0.241	0.023 ± 0.015	0.274 ± 0.131	0.015 ± 0.013
6204	0.034 ± 0.022	0.057 ± 0.006	0.041 ± 0.017	0.060 ± 0.013	0.065 ± 0.007	0.044 ± 0.012	0.062 ± 0.007	0.051 ± 0.022	0.063 ± 0.008	0.049 ± 0.02	0.046 ± 0.024	0.046 ± 0.005	0.051 ± 0.016	0.061 ± 0.005	0.041 ± 0.017	0.053 ± 0.03	0.053 ± 0.007	0.033 ± 0.022
6205	0.156 ± 0.055	0.140 ± 0.101	0.076 ± 0.044	0.112 ± 0.015	0.095 ± 0.045	0.077 ± 0.01	0.086 ± 0.04	0.105 ± 0.008	0.091 ± 0.052	0.108 ± 0.022	0.039 ± 0.035	0.124 ± 0.074	0.084 ± 0.019	0.175 ± 0.044	0.051 ± 0.019	0.115 ± 0.024	0.077 ± 0.025	0.084 ± 0.056
6206	0.038 ± 0.009	0.026 ± 0.004	0.037 ± 0.016	0.027 ± 0.007	0.042 ± 0.007	0.022 ± 0.005	0.031 ± 0.001	0.040 ± 0.009	0.044 ± 0.017	0.038 ± 0.014	0.026 ± 0.019	0.034 ± 0.009	0.046 ± 0.021	0.033 ± 0.014	0.069 ± 0.063	0.034 ± 0.026	0.076 ± 0.02	0.056 ± 0.021
6209	0.132 ± 0.048	0.098 ± 0.028	0.090 ± 0.012	0.138 ± 0.023	0.157 ± 0.028	0.071 ± 0.014	0.131 ± 0.011	0.121 ± 0.02	0.111 ± 0.014	0.110 ± 0.021	0.110 ± 0.019	0.099 ± 0.053	0.141 ± 0.011	0.143 ± 0.011	0.086 ± 0.049	0.112 ± 0.028	0.078 ± 0.011	0.062 ± 0.051

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
5638		0.28	0.16	-	0.02	0.93	-	=	=	=	=	=	=	=	=	=
5639		-0.31	0.12	-	-0.11	0.59	-	=	=	=	=	=	=	=	=	=
5702		0.02	0.91	-	-0.25	0.21	-	=	=	=	=	=	=	=	=	=
5703		0.01	0.96	-	-0.24	0.22	-	=	=	=	=	=	=	=	=	=
5705		-0.07	0.71	-	-0.21	0.29	-	=	=	=	=	=	=	=	=	=
5707		0.32	0.11	-	-0.20	0.32	-	=	=	=	=	=	=	=	=	=
5708		0.09	0.67	-	-0.23	0.26	-	=	=	=	=	=	=	=	=	=
5709		-0.17	0.40	-	-0.36	0.061	↘	=	=	=	=	=	=	=	=	=
5712		0.04	0.86	-	0.06	0.78	-	=	=	=	=	=	=	=	=	=
5716		-0.03	0.89	-	-0.31	0.11	-	=	=	=	=	=	=	=	=	=
5718		-0.32	0.10	-	0.02	0.93	-	=	=	=	=	=	=	=	=	=
5719		0.10	0.61	-	0.14	0.50	-	=	=	=	=	=	=	=	=	=
5727	V-type proton ATPase catalytic subunit A / 70 kDa peptidyl-prolyl isomerase	0.44	0.021	↗↗	-0.40	0.039	↘↘	=	=	=	=	=	=	=	=	=
5812		0.37	0.056	↗	-0.10	0.62	-	=	=	=	=	=	=	=	=	=
6201		0.26	0.20	-	-0.14	0.49	-	=	=	M >	=	=	=	=	=	=
6203	L-ascorbate peroxidase 2, cytosolic EC=1.11.1.11	0.32	0.10	-	-0.09	0.67	-	M >>	=	=	=	=	=	=	=	M >>
6204		0.05	0.80	-	-0.26	0.19	-	=	=	=	=	=	=	=	=	=
6205	Protein IN2-1 homolog B = Glutathione S-transferase GSTZ5	-0.40	0.037	↘↘	-0.04	0.84	-	=	=	=	=	=	=	=	=	=
6206	ND	0.46	0.015	↗↗	0.48	0.011	↗↗	=	=	=	=	=	=	=	=	=
6209	Triosephosphate isomerase EC=5.3.1.1	-0.41	0.035	↘↘	-0.19	0.35	-	=	=	M >	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 6211 to 6415



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

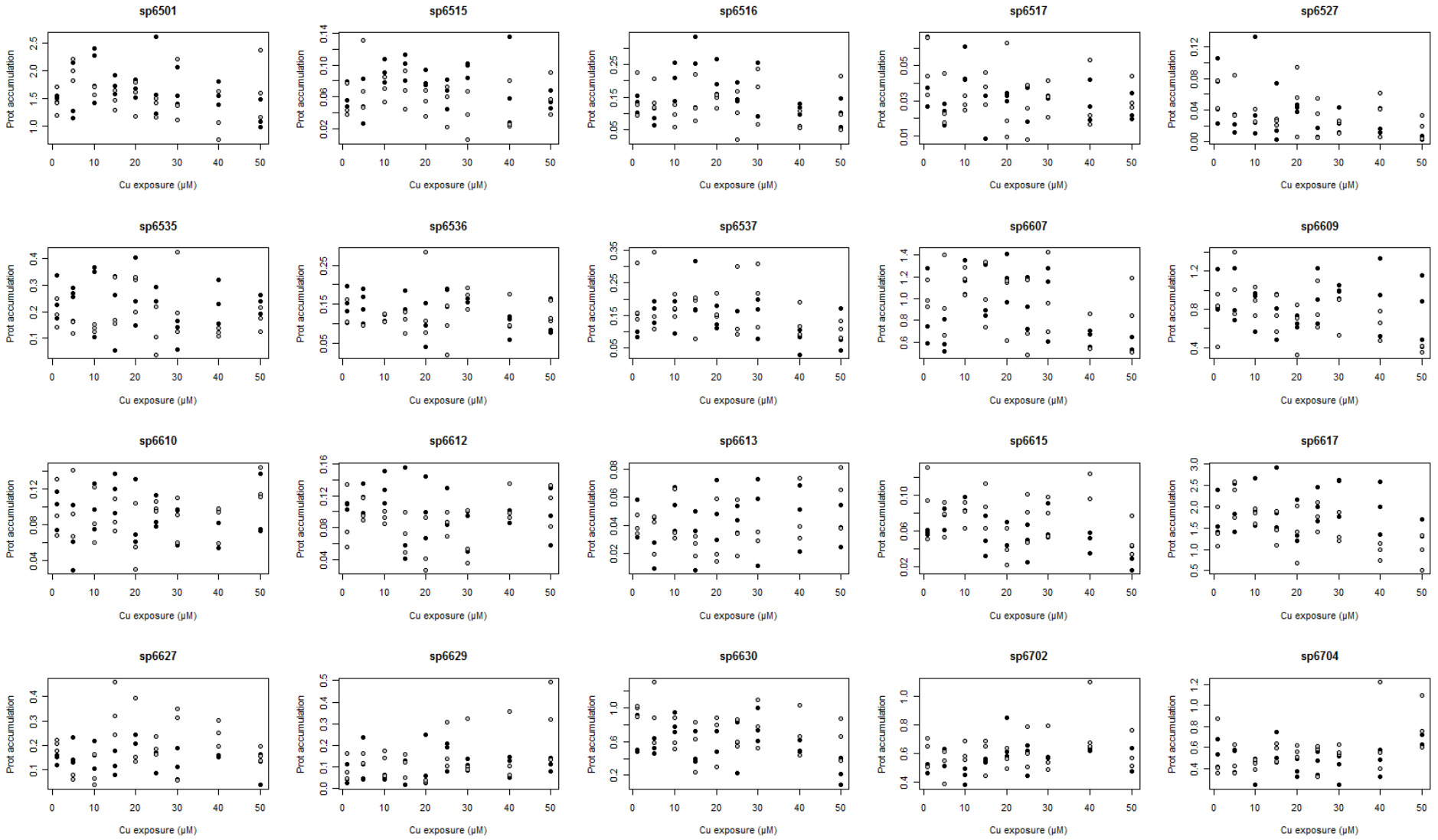
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
6211	0.057 ± 0.043	0.065 ± 0.024	0.045 ± 0.002	0.101 ± 0.016	0.071 ± 0.011	0.048 ± 0.016	0.063 ± 0.026	0.082 ± 0.01	0.085 ± 0.036	0.051 ± 0.036	0.036 ± 0.044	0.057 ± 0.036	0.047 ± 0.023	0.060 ± 0.012	0.027 ± 0.01	0.067 ± 0.045	0.080 ± 0.033	0.136 ± 0.063
6212	0.278 ± 0.066	0.171 ± 0.068	0.202 ± 0.121	0.274 ± 0.089	0.309 ± 0.089	0.178 ± 0.027	0.247 ± 0.167	0.236 ± 0.117	0.177 ± 0.033	0.155 ± 0.15	0.074 ± 0.066	0.129 ± 0.045	0.311 ± 0.182	0.249 ± 0.077	0.348 ± 0.105	0.119 ± 0.137	0.066 ± 0.073	0.040 ± 0.055
6213	0.900 ± 0.079	0.903 ± 0.382	0.779 ± 0.083	0.870 ± 0.208	1.105 ± 0.132	0.900 ± 0.075	0.983 ± 0.139	0.873 ± 0.033	0.827 ± 0.097	0.717 ± 0.036	0.633 ± 0.367	0.860 ± 0.205	0.970 ± 0.269	0.949 ± 0.122	0.885 ± 0.192	0.381 ± 0.096	0.684 ± 0.295	0.260 ± 0.117
6215	0.071 ± 0.009	0.040 ± 0.006	0.049 ± 0.014	0.041 ± 0.004	0.090 ± 0.019	0.036 ± 0.017	0.087 ± 0.04	0.116 ± 0.029	0.157 ± 0.118	0.112 ± 0.014	0.087 ± 0.022	0.248 ± 0.107	0.110 ± 0.051	0.185 ± 0.056	0.284 ± 0.336	0.490 ± 0.2	0.384 ± 0.012	0.505 ± 0.235
6219	0.053 ± 0.013	0.055 ± 0.025	0.027 ± 0.004	0.031 ± 0.011	0.021 ± 0.005	0.041 ± 0.021	0.025 ± 0.011	0.033 ± 0.014	0.029 ± 0.015	0.046 ± 0.024	0.012 ± 0.011	0.029 ± 0.009	0.055 ± 0.022	0.071 ± 0.035	0.033 ± 0.009	0.043 ± 0.006	0.046 ± 0.031	0.038 ± 0.009
6220	0.078 ± 0.019	0.071 ± 0.015	0.072 ± 0.032	0.093 ± 0.053	0.098 ± 0.013	0.072 ± 0.044	0.094 ± 0.013	0.105 ± 0.039	0.118 ± 0.048	0.067 ± 0.045	0.091 ± 0.063	0.078 ± 0.011	0.074 ± 0.019	0.055 ± 0.042	0.108 ± 0.051	0.110 ± 0.047	0.095 ± 0.006	0.094 ± 0.007
6301	0.251 ± 0.186	0.192 ± 0.04	0.280 ± 0.067	0.113 ± 0.083	0.184 ± 0.122	0.190 ± 0.118	0.177 ± 0.074	0.201 ± 0.126	0.157 ± 0.062	0.136 ± 0.086	0.142 ± 0.133	0.179 ± 0.114	0.107 ± 0.059	0.160 ± 0.045	0.156 ± 0.043	0.088 ± 0.054	0.142 ± 0.097	0.084 ± 0.065
6302	0.112 ± 0.039	0.104 ± 0.025	0.093 ± 0.014	0.122 ± 0.032	0.115 ± 0.011	0.110 ± 0.038	0.084 ± 0.008	0.101 ± 0.017	0.090 ± 0.043	0.111 ± 0.035	0.104 ± 0.033	0.111 ± 0.043	0.116 ± 0.009	0.108 ± 0.006	0.078 ± 0.025	0.088 ± 0.012	0.141 ± 0.027	0.123 ± 0.038
6303	0.152 ± 0.034	0.136 ± 0.039	0.118 ± 0.04	0.141 ± 0.023	0.203 ± 0.055	0.157 ± 0.02	0.169 ± 0.022	0.145 ± 0.038	0.244 ± 0.106	0.181 ± 0.057	0.196 ± 0.027	0.152 ± 0.055	0.185 ± 0.049	0.208 ± 0.026	0.230 ± 0.087	0.191 ± 0.021	0.131 ± 0.068	0.210 ± 0.075
6308	0.045 ± 0.02	0.032 ± 0.009	0.022 ± 0.007	0.038 ± 0.015	0.023 ± 0.014	0.016 ± 0.008	0.034 ± 0.02	0.019 ± 0.008	0.039 ± 0.02	0.033 ± 0.026	0.031 ± 0.015	0.043 ± 0.017	0.042 ± 0.039	0.059 ± 0.019	0.032 ± 0.019	0.028 ± 0.012	0.040 ± 0.016	0.055 ± 0.031
6310	0.090 ± 0.026	0.039 ± 0.011	0.043 ± 0.036	0.036 ± 0.022	0.048 ± 0.025	0.021 ± 0.003	0.033 ± 0.008	0.033 ± 0.005	0.036 ± 0.025	0.032 ± 0.022	0.039 ± 0.037	0.017 ± 0.02	0.062 ± 0.013	0.018 ± 0.011	0.055 ± 0.008	0.012 ± 0.001	0.022 ± 0.011	0.024 ± 0.02
6313	0.069 ± 0.007	0.057 ± 0.01	0.051 ± 0.02	0.057 ± 0.004	0.060 ± 0.026	0.048 ± 0.005	0.039 ± 0.005	0.067 ± 0.018	0.043 ± 0.009	0.034 ± 0.024	0.042 ± 0.018	0.046 ± 0.017	0.065 ± 0.008	0.058 ± 0.023	0.074 ± 0.023	0.052 ± 0.025	0.055 ± 0.019	0.068 ± 0.013
6315	0.067 ± 0.017	0.056 ± 0.023	0.042 ± 0.009	0.031 ± 0.014	0.050 ± 0.003	0.037 ± 0.011	0.043 ± 0.009	0.048 ± 0.013	0.043 ± 0.014	0.027 ± 0.013	0.032 ± 0.019	0.045 ± 0.018	0.060 ± 0.007	0.032 ± 0.019	0.069 ± 0.022	0.032 ± 0.014	0.036 ± 0.024	0.052 ± 0.005
6316	0.142 ± 0.031	0.130 ± 0.05	0.116 ± 0.027	0.126 ± 0.02	0.163 ± 0.006	0.117 ± 0.014	0.155 ± 0.029	0.128 ± 0.035	0.137 ± 0.045	0.131 ± 0.037	0.107 ± 0.036	0.165 ± 0.035	0.151 ± 0.033	0.172 ± 0.022	0.200 ± 0.153	0.159 ± 0.113	0.181 ± 0.081	0.148 ± 0.013
6401	0.898 ± 0.127	1.123 ± 0.082	0.993 ± 0.284	1.010 ± 0.172	0.877 ± 0.092	1.154 ± 0.257	0.988 ± 0.094	0.902 ± 0.043	0.980 ± 0.26	0.962 ± 0.25	0.879 ± 0.191	0.913 ± 0.082	1.000 ± 0.284	0.868 ± 0.259	0.815 ± 0.231	0.990 ± 0.089	0.911 ± 0.073	0.957 ± 0.208
6404	1.192 ± 0.515	2.545 ± 0.779	1.313 ± 0.222	2.211 ± 0.271	1.585 ± 0.53	2.401 ± 0.443	1.313 ± 0.468	1.918 ± 0.61	2.380 ± 0.499	2.101 ± 1.188	1.823 ± 0.322	2.157 ± 0.863	1.509 ± 0.124	1.780 ± 0.177	1.526 ± 0.611	1.134 ± 0.619	1.273 ± 0.218	0.783 ± 0.255
6408	0.319 ± 0.138	0.177 ± 0.084	0.277 ± 0.048	0.304 ± 0.092	0.380 ± 0.114	0.229 ± 0.049	0.286 ± 0.061	0.306 ± 0.052	0.266 ± 0.074	0.246 ± 0.048	0.288 ± 0.102	0.300 ± 0.118	0.303 ± 0.015	0.306 ± 0.09	0.298 ± 0.067	0.303 ± 0.077	0.346 ± 0.122	0.274 ± 0.046
6409	0.924 ± 0.151	1.010 ± 0.083	0.975 ± 0.219	1.094 ± 0.21	0.913 ± 0.21	1.042 ± 0.207	0.956 ± 0.307	1.097 ± 0.232	0.842 ± 0.188	0.580 ± 0.07	0.895 ± 0.064	1.016 ± 0.458	0.997 ± 0.401	1.138 ± 0.254	0.925 ± 0.185	1.000 ± 0.247	0.884 ± 0.356	0.916 ± 0.441
6411	0.056 ± 0.021	0.046 ± 0.013	0.038 ± 0.026	0.048 ± 0.026	0.036 ± 0.007	0.043 ± 0.007	0.024 ± 0.016	0.049 ± 0.017	0.035 ± 0.025	0.033 ± 0.015	0.039 ± 0.026	0.062 ± 0.02	0.042 ± 0.023	0.048 ± 0.013	0.043 ± 0.012	0.060 ± 0.012	0.047 ± 0.02	0.043 ± 0.017
6415	0.393 ± 0.113	0.476 ± 0.109	0.248 ± 0.156	0.332 ± 0.153	0.535 ± 0.041	0.346 ± 0.223	0.401 ± 0.134	0.795 ± 0.302	0.587 ± 0.179	0.544 ± 0.16	0.265 ± 0.183	0.539 ± 0.079	0.313 ± 0.109	0.481 ± 0.075	0.225 ± 0.173	0.322 ± 0.164	0.336 ± 0.277	0.248 ± 0.183

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
6211		-0.02	0.91	-	0.28	0.16	-	=	NM >	=	=	=	=	=	=	=
6212	L-ascorbate peroxidase 2, cytosolic EC=1.11.1.11	-0.21	0.30	-	-0.45	0.019	↘↘	=	=	=	=	=	=	=	=	=
6213	L-ascorbate peroxidase 2, cytosolic EC=1.11.1.11	-0.24	0.22	-	-0.69	<0.001	↘↘↘↘	=	=	=	=	=	=	=	M >	=
6215	Adenine phosphoribosyltransferase 1 EC=2.4.2.7	0.65	<0.001	↗↗↗↗	0.84	<0.001	↗↗↗↗	=	=	=	=	=	=	=	=	=
6219		0.14	0.49	-	0.03	0.86	-	=	=	=	=	=	=	=	=	=
6220		0.17	0.41	-	0.13	0.51	-	=	=	=	=	=	=	=	=	=
6301	ND	-0.40	0.041	↘↘	-0.34	0.087	↘	=	=	=	=	=	=	=	=	=
6302		0.19	0.35	-	-0.01	0.94	-	=	=	=	=	=	=	=	=	=
6303	Cysteine synthase EC=2.5.1.47	0.11	0.60	-	0.53	0.005	↗↗↗	=	=	=	=	=	=	=	=	=
6308		0.11	0.59	-	0.37	0.059	↗	=	=	=	=	=	=	=	=	=
6310	ND	-0.32	0.10	-	-0.40	0.036	↘↘	=	=	=	=	=	=	M >	M >>	=
6313		0.09	0.64	-	0.12	0.56	-	=	=	=	=	=	=	=	=	=
6315		-0.08	0.71	-	-0.02	0.92	-	=	=	=	=	=	=	=	=	=
6316		0.27	0.17	-	0.29	0.15	-	=	=	=	=	=	=	=	=	=
6401		-0.10	0.61	-	-0.28	0.16	-	=	=	=	=	=	=	=	=	=
6404	Glutamine synthetase cytosolic isozyme 1 / Peroxidase 2	0.05	0.80	-	-0.67	0.0001	↘↘↘↘	=	=	=	=	=	=	=	=	=
6408		0.04	0.83	-	0.26	0.19	-	=	=	=	=	=	=	=	=	=
6409		-0.05	0.81	-	-0.09	0.66	-	=	=	=	=	=	=	=	=	=
6411		0.05	0.81	-	0.10	0.61	-	=	=	=	=	=	=	=	=	=
6415		-0.22	0.27	-	-0.25	0.21	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 6501 to 6704



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

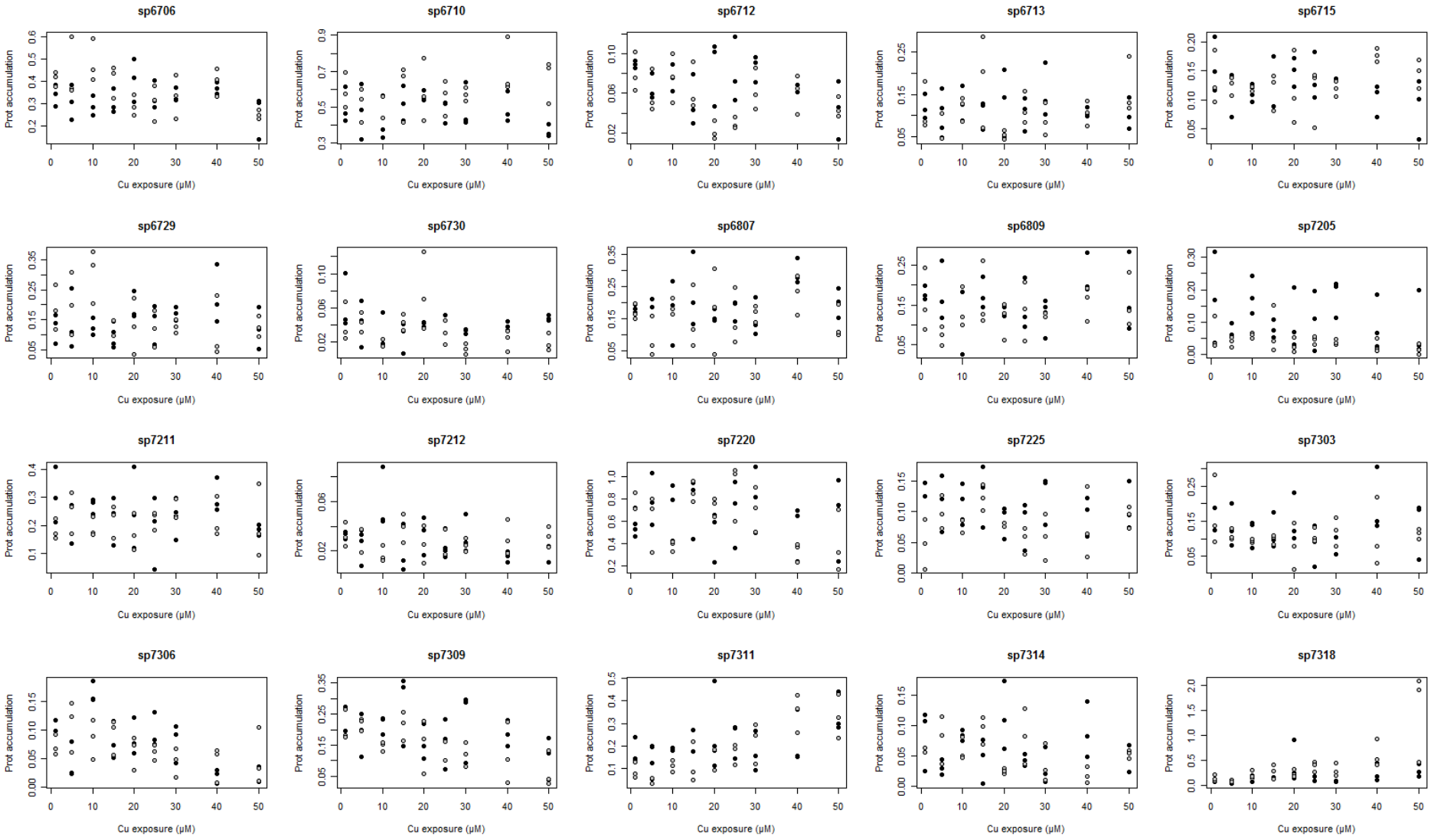
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
6501	1.496 ± 0.058	1.440 ± 0.259	1.521 ± 0.535	2.002 ± 0.195	2.030 ± 0.526	1.664 ± 0.083	1.739 ± 0.166	1.468 ± 0.18	1.673 ± 0.158	1.525 ± 0.308	1.800 ± 0.716	1.365 ± 0.175	1.671 ± 0.345	1.565 ± 0.572	1.577 ± 0.207	1.149 ± 0.436	1.180 ± 0.262	1.710 ± 0.614
6515	0.061 ± 0.016	0.053 ± 0.021	0.052 ± 0.028	0.081 ± 0.044	0.092 ± 0.015	0.070 ± 0.015	0.099 ± 0.016	0.069 ± 0.024	0.082 ± 0.01	0.053 ± 0.016	0.065 ± 0.019	0.052 ± 0.026	0.095 ± 0.01	0.037 ± 0.03	0.074 ± 0.055	0.043 ± 0.032	0.056 ± 0.011	0.062 ± 0.027
6516	0.131 ± 0.026	0.149 ± 0.068	0.089 ± 0.026	0.153 ± 0.047	0.202 ± 0.06	0.094 ± 0.034	0.236 ± 0.11	0.137 ± 0.073	0.206 ± 0.056	0.140 ± 0.022	0.159 ± 0.032	0.097 ± 0.075	0.176 ± 0.083	0.161 ± 0.086	0.115 ± 0.016	0.074 ± 0.028	0.102 ± 0.045	0.121 ± 0.086
6517	0.044 ± 0.021	0.048 ± 0.017	0.023 ± 0.006	0.029 ± 0.015	0.048 ± 0.011	0.029 ± 0.004	0.026 ± 0.016	0.037 ± 0.009	0.032 ± 0.002	0.030 ± 0.028	0.031 ± 0.011	0.024 ± 0.016	0.032 ± 0.001	0.032 ± 0.01	0.029 ± 0.012	0.030 ± 0.02	0.025 ± 0.008	0.033 ± 0.01
6527	0.068 ± 0.042	0.053 ± 0.021	0.015 ± 0.006	0.051 ± 0.029	0.059 ± 0.065	0.030 ± 0.01	0.030 ± 0.038	0.025 ± 0.004	0.043 ± 0.005	0.052 ± 0.044	0.020 ± 0.015	0.032 ± 0.025	0.026 ± 0.016	0.020 ± 0.008	0.023 ± 0.017	0.036 ± 0.028	0.005 ± 0.002	0.019 ± 0.014
6535	0.245 ± 0.083	0.192 ± 0.053	0.272 ± 0.016	0.148 ± 0.028	0.274 ± 0.146	0.137 ± 0.014	0.216 ± 0.144	0.217 ± 0.098	0.263 ± 0.13	0.283 ± 0.074	0.257 ± 0.031	0.120 ± 0.092	0.121 ± 0.058	0.247 ± 0.158	0.235 ± 0.082	0.122 ± 0.016	0.232 ± 0.036	0.172 ± 0.046
6536	0.161 ± 0.033	0.123 ± 0.034	0.165 ± 0.026	0.097 ± 0.003	0.111 ± 0.01	0.111 ± 0.012	0.151 ± 0.029	0.105 ± 0.029	0.096 ± 0.057	0.156 ± 0.112	0.173 ± 0.026	0.087 ± 0.063	0.152 ± 0.015	0.167 ± 0.028	0.095 ± 0.032	0.121 ± 0.047	0.108 ± 0.048	0.127 ± 0.029
6537	0.112 ± 0.038	0.203 ± 0.094	0.164 ± 0.034	0.200 ± 0.127	0.152 ± 0.052	0.178 ± 0.035	0.217 ± 0.086	0.160 ± 0.071	0.138 ± 0.037	0.172 ± 0.04	0.140 ± 0.041	0.166 ± 0.116	0.148 ± 0.063	0.213 ± 0.098	0.072 ± 0.039	0.133 ± 0.051	0.096 ± 0.067	0.107 ± 0.026
6607	0.869 ± 0.363	1.029 ± 0.129	0.636 ± 0.154	0.992 ± 0.38	1.184 ± 0.158	1.165 ± 0.129	1.018 ± 0.256	1.020 ± 0.302	1.187 ± 0.224	0.976 ± 0.313	0.948 ± 0.24	0.777 ± 0.357	1.012 ± 0.361	1.028 ± 0.373	0.642 ± 0.081	0.751 ± 0.182	0.564 ± 0.071	0.845 ± 0.341
6609	0.948 ± 0.239	0.733 ± 0.29	0.903 ± 0.29	1.052 ± 0.326	0.825 ± 0.224	0.889 ± 0.15	0.750 ± 0.242	0.749 ± 0.188	0.666 ± 0.064	0.627 ± 0.27	0.926 ± 0.293	0.819 ± 0.249	1.014 ± 0.034	0.778 ± 0.218	0.932 ± 0.409	0.638 ± 0.154	0.841 ± 0.338	0.392 ± 0.036
6610	0.098 ± 0.022	0.096 ± 0.032	0.064 ± 0.036	0.100 ± 0.038	0.099 ± 0.026	0.087 ± 0.031	0.116 ± 0.022	0.088 ± 0.019	0.087 ± 0.038	0.063 ± 0.038	0.091 ± 0.019	0.099 ± 0.006	0.083 ± 0.023	0.086 ± 0.025	0.077 ± 0.021	0.083 ± 0.021	0.095 ± 0.036	0.123 ± 0.019
6612	0.108 ± 0.004	0.088 ± 0.041	0.117 ± 0.018	0.100 ± 0.015	0.130 ± 0.02	0.093 ± 0.008	0.085 ± 0.062	0.074 ± 0.025	0.103 ± 0.039	0.054 ± 0.035	0.100 ± 0.026	0.085 ± 0.015	0.082 ± 0.027	0.064 ± 0.034	0.093 ± 0.008	0.109 ± 0.023	0.094 ± 0.036	0.110 ± 0.026
6613	0.043 ± 0.014	0.040 ± 0.007	0.027 ± 0.018	0.036 ± 0.015	0.053 ± 0.016	0.044 ± 0.019	0.031 ± 0.021	0.026 ± 0.007	0.050 ± 0.022	0.031 ± 0.024	0.044 ± 0.009	0.037 ± 0.02	0.048 ± 0.033	0.031 ± 0.004	0.047 ± 0.024	0.048 ± 0.023	0.039 ± 0.015	0.062 ± 0.022
6615	0.058 ± 0.003	0.092 ± 0.04	0.074 ± 0.013	0.074 ± 0.02	0.081 ± 0.018	0.079 ± 0.015	0.053 ± 0.023	0.088 ± 0.025	0.052 ± 0.015	0.041 ± 0.02	0.047 ± 0.021	0.076 ± 0.027	0.067 ± 0.021	0.077 ± 0.022	0.048 ± 0.012	0.105 ± 0.016	0.029 ± 0.013	0.051 ± 0.022
6617	1.776 ± 0.53	1.482 ± 0.474	1.925 ± 0.577	2.238 ± 0.441	2.039 ± 0.574	1.806 ± 0.182	2.101 ± 0.734	1.482 ± 0.409	1.566 ± 0.52	1.368 ± 0.668	2.037 ± 0.407	1.756 ± 0.35	2.331 ± 0.485	1.459 ± 0.368	1.973 ± 0.624	0.958 ± 0.206	1.330 ± 0.351	0.946 ± 0.405
6627	0.144 ± 0.021	0.202 ± 0.022	0.169 ± 0.056	0.067 ± 0.011	0.162 ± 0.056	0.090 ± 0.066	0.124 ± 0.049	0.341 ± 0.108	0.232 ± 0.023	0.227 ± 0.146	0.115 ± 0.049	0.196 ± 0.038	0.120 ± 0.064	0.241 ± 0.159	0.155 ± 0.003	0.250 ± 0.052	0.113 ± 0.066	0.163 ± 0.03
6629	0.058 ± 0.045	0.093 ± 0.061	0.108 ± 0.111	0.128 ± 0.028	0.049 ± 0.011	0.123 ± 0.06	0.063 ± 0.057	0.110 ± 0.055	0.112 ± 0.119	0.031 ± 0.007	0.159 ± 0.071	0.181 ± 0.109	0.109 ± 0.028	0.169 ± 0.132	0.108 ± 0.052	0.174 ± 0.16	0.110 ± 0.032	0.314 ± 0.181
6630	0.634 ± 0.243	0.972 ± 0.068	0.542 ± 0.092	0.924 ± 0.362	0.810 ± 0.121	0.661 ± 0.2	0.496 ± 0.201	0.568 ± 0.301	0.559 ± 0.14	0.661 ± 0.318	0.546 ± 0.309	0.667 ± 0.171	0.781 ± 0.201	0.799 ± 0.283	0.530 ± 0.077	0.709 ± 0.303	0.235 ± 0.161	0.638 ± 0.253
6702	0.503 ± 0.036	0.620 ± 0.103	0.583 ± 0.062	0.516 ± 0.115	0.438 ± 0.057	0.607 ± 0.071	0.552 ± 0.012	0.592 ± 0.132	0.679 ± 0.148	0.564 ± 0.072	0.569 ± 0.114	0.629 ± 0.145	0.557 ± 0.02	0.603 ± 0.163	0.632 ± 0.014	0.806 ± 0.252	0.625 ± 0.145	0.615 ± 0.131
6704	0.542 ± 0.132	0.546 ± 0.286	0.505 ± 0.12	0.472 ± 0.145	0.405 ± 0.14	0.444 ± 0.049	0.571 ± 0.159	0.565 ± 0.091	0.396 ± 0.086	0.562 ± 0.056	0.460 ± 0.11	0.509 ± 0.159	0.401 ± 0.138	0.572 ± 0.054	0.463 ± 0.128	0.723 ± 0.44	0.653 ± 0.059	0.820 ± 0.254

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
6501		-0.26	0.20	-	-0.18	0.36	-	=	=	=	=	=	=	=	=	=
6515		-0.03	0.87	-	-0.23	0.24	-	=	=	=	=	=	=	=	=	=
6516		-0.17	0.38	-	-0.19	0.33	-	=	=	=	=	=	=	=	=	=
6517		-0.28	0.15	-	-0.15	0.47	-	=	=	=	=	=	=	=	=	=
6527	4-hydroxy-3-methylbut-2-enyl diphosphate reductase, chloro. EC=1.17.1.2	-0.41	0.032	↘↘	-0.35	0.073	↘	=	=	=	=	=	=	=	=	=
6535		-0.18	0.37	-	-0.05	0.80	-	=	M >	=	=	=	=	=	=	=
6536		-0.36	0.065	↘	0.14	0.47	-	=	=	=	=	=	=	=	=	=
6537		-0.36	0.066	↘	-0.33	0.097	↘	=	=	=	=	=	=	=	=	=
6607		-0.31	0.11	-	-0.32	0.11	-	=	=	=	=	=	=	=	=	=
6609	ND	0.04	0.85	-	-0.51	0.006	↘↘↘	=	=	=	=	=	=	=	=	=
6610		-0.05	0.82	-	0.16	0.42	-	=	=	=	=	=	=	=	=	=
6612		-0.28	0.15	-	0.18	0.38	-	=	=	=	=	=	=	=	=	=
6613		0.09	0.65	-	0.34	0.083	↗	=	=	=	=	=	=	=	=	=
6615	ND	-0.53	0.004	↘↘↘	-0.14	0.50	-	=	=	=	=	=	=	=	=	=
6617	ATP synthase subunit alpha, mito. EC=3.6.3.14	-0.15	0.45	-	-0.57	0.002	↘↘↘	=	=	=	=	=	=	=	=	=
6627		-0.22	0.27	-	0.18	0.36	-	=	M >	=	=	=	=	=	=	=
6629	Chaperonin CPN60-2, mito. HSP60-2	0.25	0.20	-	0.49	0.009	↗↗↗	=	=	=	=	=	=	=	=	=
6630	Enolase / ATP synthase subunit beta / V-type proton ATPase subunit B 2	-0.39	0.046	↘↘	-0.26	0.20	-	=	=	=	=	=	=	=	=	=
6702	2,3-bisphosphoglycerate-independent phosphoglycerate mutase / Chaperonin CPN60-1	0.39	0.043	↗↗	0.28	0.15	-	=	=	=	=	=	=	=	=	=
6704	Chaperonin CPN60-2, mito. HSP60-2	0.14	0.47	-	0.47	0.014	↗↗	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 6706 to 7318



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

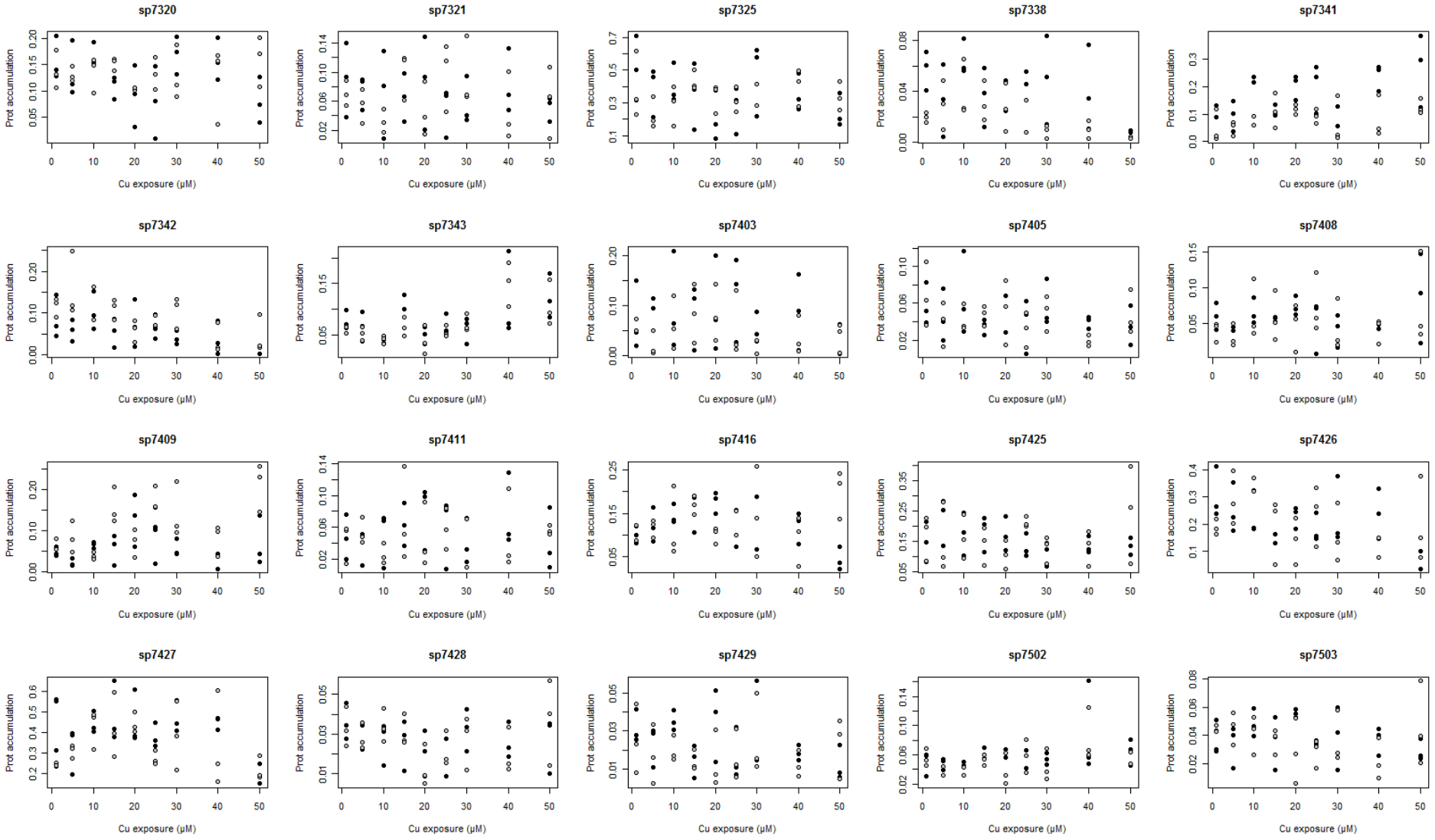
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
6706	0.340 ± 0.047	0.412 ± 0.034	0.309 ± 0.078	0.444 ± 0.135	0.291 ± 0.044	0.484 ± 0.097	0.305 ± 0.056	0.408 ± 0.073	0.409 ± 0.096	0.291 ± 0.046	0.334 ± 0.061	0.307 ± 0.079	0.336 ± 0.032	0.332 ± 0.098	0.370 ± 0.025	0.400 ± 0.062	0.252 ± 0.096	0.252 ± 0.019
6710	0.503 ± 0.099	0.591 ± 0.097	0.479 ± 0.155	0.521 ± 0.094	0.424 ± 0.125	0.520 ± 0.067	0.522 ± 0.095	0.599 ± 0.16	0.562 ± 0.03	0.586 ± 0.174	0.486 ± 0.065	0.559 ± 0.099	0.494 ± 0.125	0.571 ± 0.036	0.492 ± 0.086	0.712 ± 0.156	0.366 ± 0.034	0.658 ± 0.122
6712	0.089 ± 0.003	0.080 ± 0.02	0.065 ± 0.013	0.060 ± 0.022	0.076 ± 0.013	0.076 ± 0.025	0.051 ± 0.026	0.065 ± 0.024	0.086 ± 0.034	0.022 ± 0.009	0.081 ± 0.033	0.029 ± 0.006	0.086 ± 0.013	0.063 ± 0.021	0.065 ± 0.004	0.061 ± 0.02	0.044 ± 0.03	0.045 ± 0.01
6713	0.121 ± 0.029	0.114 ± 0.057	0.117 ± 0.046	0.066 ± 0.034	0.129 ± 0.041	0.118 ± 0.029	0.107 ± 0.035	0.187 ± 0.109	0.133 ± 0.081	0.055 ± 0.011	0.106 ± 0.04	0.117 ± 0.037	0.155 ± 0.064	0.090 ± 0.038	0.107 ± 0.011	0.106 ± 0.03	0.103 ± 0.037	0.163 ± 0.068
6715	0.158 ± 0.047	0.135 ± 0.047	0.114 ± 0.038	0.125 ± 0.016	0.110 ± 0.016	0.119 ± 0.005	0.115 ± 0.053	0.117 ± 0.032	0.149 ± 0.024	0.116 ± 0.063	0.138 ± 0.041	0.110 ± 0.051	0.130 ± 0.009	0.119 ± 0.013	0.102 ± 0.029	0.177 ± 0.011	0.088 ± 0.052	0.146 ± 0.026
6729	0.124 ± 0.049	0.188 ± 0.074	0.141 ± 0.102	0.202 ± 0.104	0.126 ± 0.027	0.304 ± 0.091	0.078 ± 0.027	0.129 ± 0.028	0.192 ± 0.046	0.127 ± 0.094	0.142 ± 0.067	0.119 ± 0.062	0.170 ± 0.022	0.127 ± 0.023	0.226 ± 0.097	0.111 ± 0.103	0.120 ± 0.07	0.126 ± 0.035
6730	0.064 ± 0.033	0.041 ± 0.023	0.043 ± 0.027	0.043 ± 0.011	0.032 ± 0.019	0.018 ± 0.005	0.027 ± 0.018	0.043 ± 0.009	0.041 ± 0.003	0.077 ± 0.045	0.050 ± 0.003	0.031 ± 0.014	0.033 ± 0.003	0.012 ± 0.006	0.039 ± 0.005	0.023 ± 0.012	0.048 ± 0.003	0.019 ± 0.01
6807	0.182 ± 0.013	0.172 ± 0.024	0.195 ± 0.015	0.089 ± 0.062	0.175 ± 0.1	0.187 ± 0.025	0.232 ± 0.115	0.147 ± 0.098	0.159 ± 0.019	0.178 ± 0.133	0.181 ± 0.033	0.150 ± 0.088	0.151 ± 0.058	0.168 ± 0.026	0.294 ± 0.04	0.227 ± 0.06	0.201 ± 0.045	0.136 ± 0.052
6809	0.179 ± 0.017	0.157 ± 0.079	0.180 ± 0.074	0.073 ± 0.024	0.131 ± 0.09	0.139 ± 0.05	0.178 ± 0.04	0.167 ± 0.083	0.137 ± 0.013	0.114 ± 0.046	0.144 ± 0.065	0.137 ± 0.074	0.124 ± 0.05	0.127 ± 0.006	0.223 ± 0.051	0.157 ± 0.043	0.172 ± 0.1	0.157 ± 0.068
7205	0.174 ± 0.141	0.062 ± 0.051	0.067 ± 0.029	0.040 ± 0.015	0.182 ± 0.057	0.059 ± 0.009	0.079 ± 0.028	0.070 ± 0.072	0.103 ± 0.092	0.029 ± 0.023	0.106 ± 0.092	0.045 ± 0.013	0.181 ± 0.058	0.039 ± 0.008	0.093 ± 0.083	0.027 ± 0.021	0.082 ± 0.101	0.017 ± 0.016
7211	0.306 ± 0.098	0.184 ± 0.037	0.225 ± 0.078	0.251 ± 0.074	0.270 ± 0.027	0.191 ± 0.033	0.224 ± 0.086	0.219 ± 0.057	0.255 ± 0.146	0.173 ± 0.066	0.186 ± 0.129	0.220 ± 0.033	0.230 ± 0.076	0.253 ± 0.037	0.301 ± 0.06	0.222 ± 0.072	0.185 ± 0.02	0.204 ± 0.13
7212	0.033 ± 0.003	0.033 ± 0.01	0.023 ± 0.013	0.031 ± 0.011	0.059 ± 0.025	0.017 ± 0.007	0.020 ± 0.019	0.039 ± 0.011	0.033 ± 0.016	0.025 ± 0.015	0.019 ± 0.004	0.031 ± 0.012	0.032 ± 0.015	0.025 ± 0.005	0.016 ± 0.004	0.031 ± 0.014	0.019 ± 0.008	0.032 ± 0.008
7220	0.521 ± 0.056	0.760 ± 0.082	0.786 ± 0.233	0.607 ± 0.252	0.710 ± 0.258	0.383 ± 0.046	0.751 ± 0.274	0.859 ± 0.094	0.493 ± 0.23	0.731 ± 0.082	0.689 ± 0.301	0.891 ± 0.253	0.798 ± 0.298	0.708 ± 0.201	0.526 ± 0.249	0.330 ± 0.084	0.648 ± 0.372	0.398 ± 0.276
7225	0.120 ± 0.03	0.047 ± 0.04	0.115 ± 0.046	0.098 ± 0.027	0.115 ± 0.033	0.079 ± 0.012	0.129 ± 0.051	0.122 ± 0.021	0.086 ± 0.027	0.078 ± 0.004	0.082 ± 0.04	0.054 ± 0.021	0.125 ± 0.041	0.058 ± 0.037	0.095 ± 0.032	0.077 ± 0.058	0.106 ± 0.039	0.092 ± 0.018
7303	0.146 ± 0.037	0.170 ± 0.1	0.134 ± 0.06	0.111 ± 0.016	0.120 ± 0.039	0.094 ± 0.005	0.117 ± 0.051	0.099 ± 0.013	0.152 ± 0.069	0.079 ± 0.065	0.082 ± 0.058	0.109 ± 0.019	0.080 ± 0.024	0.122 ± 0.041	0.198 ± 0.094	0.110 ± 0.098	0.137 ± 0.084	0.114 ± 0.013
7306	0.103 ± 0.013	0.072 ± 0.018	0.042 ± 0.032	0.111 ± 0.045	0.165 ± 0.018	0.085 ± 0.035	0.081 ± 0.033	0.092 ± 0.031	0.086 ± 0.033	0.063 ± 0.03	0.097 ± 0.031	0.061 ± 0.013	0.080 ± 0.034	0.044 ± 0.025	0.019 ± 0.012	0.043 ± 0.031	0.018 ± 0.015	0.049 ± 0.049
7309	0.216 ± 0.049	0.207 ± 0.05	0.187 ± 0.069	0.220 ± 0.02	0.218 ± 0.029	0.147 ± 0.016	0.279 ± 0.116	0.214 ± 0.046	0.157 ± 0.057	0.152 ± 0.087	0.158 ± 0.08	0.142 ± 0.037	0.225 ± 0.116	0.121 ± 0.039	0.187 ± 0.041	0.119 ± 0.098	0.111 ± 0.069	0.066 ± 0.058
7311	0.175 ± 0.057	0.088 ± 0.036	0.173 ± 0.043	0.047 ± 0.014	0.184 ± 0.007	0.110 ± 0.026	0.222 ± 0.047	0.118 ± 0.09	0.266 ± 0.196	0.152 ± 0.053	0.235 ± 0.078	0.168 ± 0.046	0.171 ± 0.088	0.221 ± 0.091	0.223 ± 0.118	0.349 ± 0.084	0.341 ± 0.088	0.331 ± 0.098
7314	0.083 ± 0.05	0.060 ± 0.005	0.031 ± 0.013	0.078 ± 0.04	0.084 ± 0.009	0.059 ± 0.019	0.044 ± 0.037	0.093 ± 0.022	0.114 ± 0.056	0.025 ± 0.005	0.043 ± 0.01	0.082 ± 0.046	0.031 ± 0.03	0.036 ± 0.031	0.090 ± 0.046	0.018 ± 0.013	0.050 ± 0.023	0.052 ± 0.007
7318	0.108 ± 0.034	0.150 ± 0.061	0.075 ± 0.032	0.103 ± 0.019	0.144 ± 0.056	0.215 ± 0.091	0.162 ± 0.015	0.282 ± 0.138	0.447 ± 0.416	0.244 ± 0.078	0.161 ± 0.05	0.392 ± 0.098	0.132 ± 0.067	0.305 ± 0.123	0.245 ± 0.176	0.626 ± 0.269	0.292 ± 0.128	1.488 ± 0.881

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
6706	Vacuolar proton ATPase catalytic subunit A EC=3.6.3.14	-0.08	0.69	-	-0.51	0.007	↘↘↘	=	=	=	=	=	=	=	=	=
6710		-0.23	0.24	-	0.37	0.057	↗	=	=	=	=	=	=	=	=	=
6712		-0.29	0.14	-	-0.31	0.11	-	=	=	=	=	M >	=	=	=	=
6713		-0.08	0.68	-	0.17	0.39	-	=	=	=	=	=	=	=	=	=
6715		-0.33	0.097	↘	0.29	0.14	-	=	=	=	=	=	=	=	=	=
6729	V-type proton ATPase / 70 kDa peptidyl-prolyl isomerase / Peptidyl-prolyl cis-trans isomerase FKBP62	0.23	0.25	-	-0.42	0.028	↘↘	=	=	=	=	=	=	=	=	=
6730		-0.07	0.73	-	-0.32	0.11	-	=	=	=	=	=	=	M >	=	M >
6807		0.21	0.29	-	0.13	0.51	-	=	=	=	=	=	=	=	=	=
6809		0.08	0.67	-	0.18	0.38	-	=	=	=	=	=	=	=	=	=
7205	L-ascorbate peroxidase 2, cytosolic EC=1.11.1.11	-0.16	0.43	-	-0.40	0.038	↘↘	=	=	M >	=	=	=	M >>	=	=
7211		-0.18	0.36	-	0.06	0.75	-	=	=	=	=	=	=	=	=	=
7212		-0.34	0.080	↘	0.03	0.89	-	=	=	=	=	=	=	=	=	=
7220		-0.05	0.80	-	-0.35	0.078	↘	=	=	=	=	=	=	=	=	=
7225		-0.16	0.43	-	0.03	0.88	-	=	=	=	=	=	=	=	=	=
7303		0.08	0.70	-	-0.11	0.59	-	=	=	=	=	=	=	=	=	=
7306	ND	-0.55	0.003	↘↘↘	-0.49	0.009	↘↘↘	=	=	=	=	=	=	=	=	=
7309	Caffeoyl-CoA O-methyltransferase EC=2.1.1.104	-0.33	0.096	↘	-0.65	0.0003	↘↘↘↘	=	=	=	=	=	=	=	=	=
7311	ND	0.40	0.039	↗↗	0.84	<0.001	↗↗↗↗	=	M >>	=	=	=	=	=	=	=
7314		-0.05	0.79	-	-0.36	0.071	↘	=	=	=	=	M >	=	=	=	=
7318	ND	0.31	0.12	-	0.72	<0.001	↗↗↗↗	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 7320 to 7503



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations.

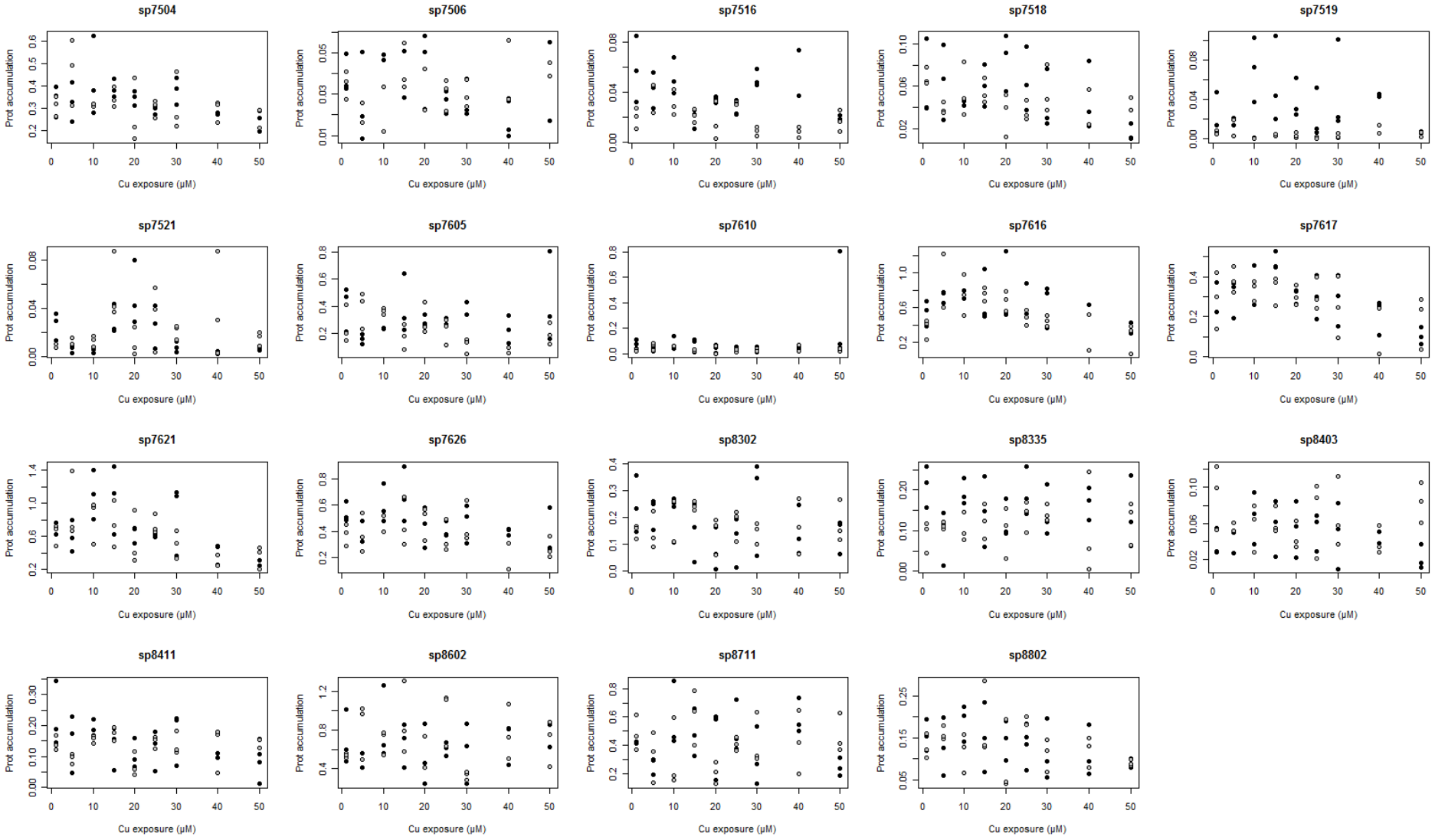
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
7320	0.157 ± 0.041	0.138 ± 0.036	0.135 ± 0.053	0.131 ± 0.015	0.165 ± 0.024	0.134 ± 0.034	0.109 ± 0.021	0.152 ± 0.011	0.092 ± 0.059	0.104 ± 0.003	0.079 ± 0.069	0.132 ± 0.03	0.169 ± 0.036	0.130 ± 0.052	0.159 ± 0.04	0.120 ± 0.073	0.080 ± 0.044	0.160 ± 0.048
7321	0.090 ± 0.051	0.070 ± 0.017	0.075 ± 0.023	0.054 ± 0.023	0.073 ± 0.061	0.032 ± 0.016	0.065 ± 0.033	0.099 ± 0.033	0.088 ± 0.064	0.047 ± 0.037	0.049 ± 0.034	0.099 ± 0.047	0.057 ± 0.033	0.095 ± 0.047	0.083 ± 0.044	0.047 ± 0.047	0.051 ± 0.017	0.061 ± 0.049
7325	0.509 ± 0.197	0.391 ± 0.204	0.388 ± 0.153	0.229 ± 0.096	0.405 ± 0.121	0.290 ± 0.12	0.354 ± 0.203	0.433 ± 0.063	0.209 ± 0.153	0.337 ± 0.09	0.270 ± 0.145	0.316 ± 0.079	0.475 ± 0.222	0.327 ± 0.075	0.355 ± 0.113	0.402 ± 0.114	0.243 ± 0.103	0.338 ± 0.089
7338	0.057 ± 0.016	0.020 ± 0.004	0.033 ± 0.029	0.029 ± 0.019	0.066 ± 0.014	0.039 ± 0.023	0.036 ± 0.024	0.032 ± 0.016	0.033 ± 0.014	0.027 ± 0.019	0.045 ± 0.011	0.016 ± 0.015	0.050 ± 0.035	0.008 ± 0.005	0.041 ± 0.034	0.010 ± 0.007	0.007 ± 0.003	0.003 ± 0.001
7341	0.117 ± 0.025	0.050 ± 0.059	0.096 ± 0.056	0.051 ± 0.026	0.222 ± 0.011	0.070 ± 0.019	0.111 ± 0.021	0.112 ± 0.062	0.202 ± 0.047	0.118 ± 0.018	0.203 ± 0.089	0.092 ± 0.028	0.104 ± 0.041	0.069 ± 0.086	0.238 ± 0.048	0.082 ± 0.076	0.269 ± 0.132	0.125 ± 0.028
7342	0.086 ± 0.051	0.115 ± 0.023	0.059 ± 0.025	0.158 ± 0.079	0.103 ± 0.046	0.137 ± 0.046	0.054 ± 0.035	0.111 ± 0.024	0.073 ± 0.057	0.058 ± 0.026	0.056 ± 0.014	0.087 ± 0.015	0.041 ± 0.016	0.105 ± 0.038	0.037 ± 0.04	0.036 ± 0.036	0.014 ± 0.01	0.046 ± 0.045
7343	0.077 ± 0.018	0.061 ± 0.006	0.066 ± 0.028	0.052 ± 0.013	0.041 ± 0.007	0.041 ± 0.009	0.091 ± 0.041	0.065 ± 0.018	0.050 ± 0.016	0.039 ± 0.029	0.068 ± 0.02	0.056 ± 0.011	0.062 ± 0.026	0.072 ± 0.017	0.116 ± 0.084	0.151 ± 0.043	0.124 ± 0.043	0.108 ± 0.045
7403	0.072 ± 0.07	0.062 ± 0.016	0.072 ± 0.058	0.021 ± 0.025	0.098 ± 0.099	0.063 ± 0.054	0.086 ± 0.066	0.084 ± 0.059	0.096 ± 0.096	0.083 ± 0.058	0.120 ± 0.085	0.055 ± 0.066	0.053 ± 0.031	0.022 ± 0.015	0.088 ± 0.076	0.038 ± 0.039	0.024 ± 0.034	0.038 ± 0.029
7405	0.058 ± 0.022	0.068 ± 0.035	0.045 ± 0.029	0.039 ± 0.024	0.066 ± 0.045	0.043 ± 0.014	0.035 ± 0.008	0.047 ± 0.011	0.051 ± 0.02	0.052 ± 0.035	0.038 ± 0.029	0.032 ± 0.019	0.057 ± 0.026	0.050 ± 0.019	0.040 ± 0.007	0.019 ± 0.006	0.036 ± 0.021	0.048 ± 0.024
7408	0.060 ± 0.019	0.040 ± 0.014	0.042 ± 0.003	0.032 ± 0.016	0.066 ± 0.018	0.065 ± 0.041	0.057 ± 0.003	0.058 ± 0.035	0.074 ± 0.014	0.047 ± 0.034	0.051 ± 0.038	0.074 ± 0.041	0.041 ± 0.023	0.044 ± 0.035	0.038 ± 0.015	0.041 ± 0.017	0.087 ± 0.063	0.077 ± 0.065
7409	0.047 ± 0.012	0.071 ± 0.015	0.021 ± 0.01	0.082 ± 0.038	0.065 ± 0.008	0.037 ± 0.008	0.055 ± 0.037	0.155 ± 0.044	0.127 ± 0.064	0.071 ± 0.035	0.076 ± 0.05	0.173 ± 0.03	0.056 ± 0.02	0.141 ± 0.067	0.029 ± 0.019	0.080 ± 0.038	0.068 ± 0.06	0.210 ± 0.059
7411	0.048 ± 0.028	0.043 ± 0.024	0.037 ± 0.022	0.054 ± 0.016	0.050 ± 0.035	0.026 ± 0.013	0.063 ± 0.027	0.070 ± 0.059	0.078 ± 0.041	0.046 ± 0.04	0.059 ± 0.044	0.058 ± 0.026	0.040 ± 0.028	0.030 ± 0.035	0.075 ± 0.046	0.050 ± 0.05	0.041 ± 0.039	0.056 ± 0.006
7416	0.088 ± 0.01	0.109 ± 0.019	0.121 ± 0.039	0.117 ± 0.021	0.145 ± 0.023	0.118 ± 0.082	0.146 ± 0.04	0.169 ± 0.022	0.176 ± 0.024	0.100 ± 0.019	0.129 ± 0.048	0.137 ± 0.032	0.102 ± 0.076	0.149 ± 0.105	0.120 ± 0.037	0.092 ± 0.058	0.044 ± 0.027	0.199 ± 0.056
7425	0.148 ± 0.066	0.169 ± 0.076	0.222 ± 0.077	0.146 ± 0.115	0.174 ± 0.071	0.161 ± 0.072	0.181 ± 0.059	0.139 ± 0.063	0.172 ± 0.057	0.106 ± 0.047	0.132 ± 0.039	0.212 ± 0.017	0.111 ± 0.04	0.125 ± 0.045	0.135 ± 0.028	0.131 ± 0.059	0.134 ± 0.028	0.245 ± 0.161
7426	0.305 ± 0.094	0.188 ± 0.028	0.253 ± 0.091	0.291 ± 0.097	0.246 ± 0.106	0.338 ± 0.027	0.189 ± 0.074	0.190 ± 0.121	0.228 ± 0.04	0.140 ± 0.086	0.182 ± 0.052	0.239 ± 0.11	0.232 ± 0.126	0.160 ± 0.106	0.269 ± 0.054	0.126 ± 0.041	0.072 ± 0.032	0.201 ± 0.155
7427	0.475 ± 0.14	0.244 ± 0.009	0.326 ± 0.111	0.313 ± 0.032	0.444 ± 0.053	0.427 ± 0.093	0.481 ± 0.147	0.424 ± 0.158	0.454 ± 0.131	0.443 ± 0.051	0.382 ± 0.06	0.276 ± 0.035	0.470 ± 0.077	0.383 ± 0.167	0.449 ± 0.029	0.338 ± 0.234	0.198 ± 0.049	0.223 ± 0.058
7428	0.036 ± 0.009	0.033 ± 0.01	0.030 ± 0.007	0.028 ± 0.007	0.026 ± 0.011	0.034 ± 0.009	0.025 ± 0.013	0.031 ± 0.008	0.020 ± 0.012	0.013 ± 0.011	0.018 ± 0.009	0.021 ± 0.009	0.032 ± 0.011	0.027 ± 0.013	0.026 ± 0.009	0.020 ± 0.012	0.027 ± 0.014	0.037 ± 0.022
7429	0.032 ± 0.009	0.025 ± 0.018	0.023 ± 0.011	0.017 ± 0.016	0.035 ± 0.005	0.020 ± 0.007	0.015 ± 0.009	0.014 ± 0.005	0.035 ± 0.019	0.013 ± 0.015	0.017 ± 0.013	0.016 ± 0.013	0.029 ± 0.024	0.026 ± 0.021	0.018 ± 0.004	0.012 ± 0.007	0.012 ± 0.009	0.023 ± 0.016
7502	0.050 ± 0.017	0.056 ± 0.012	0.048 ± 0.008	0.038 ± 0.009	0.048 ± 0.003	0.038 ± 0.008	0.062 ± 0.01	0.053 ± 0.007	0.062 ± 0.008	0.038 ± 0.022	0.048 ± 0.016	0.058 ± 0.023	0.054 ± 0.008	0.044 ± 0.022	0.088 ± 0.063	0.084 ± 0.036	0.064 ± 0.018	0.055 ± 0.011
7503	0.037 ± 0.012	0.044 ± 0.002	0.034 ± 0.015	0.046 ± 0.011	0.048 ± 0.01	0.041 ± 0.013	0.036 ± 0.019	0.036 ± 0.009	0.056 ± 0.003	0.028 ± 0.023	0.035 ± 0.001	0.028 ± 0.01	0.039 ± 0.022	0.037 ± 0.019	0.037 ± 0.01	0.022 ± 0.014	0.029 ± 0.008	0.046 ± 0.03

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
7320		-0.22	0.26	-	0.07	0.75	-	=	=	=	=	=	=	=	=	=
7321		-0.19	0.33	-	0.03	0.87	-	=	=	=	=	=	=	=	=	=
7325		-0.29	0.14	-	0.11	0.57	-	=	=	=	=	=	=	=	=	=
7338	ND	-0.40	0.038	↘↘	-0.55	0.003	↘↘↘	M >	=	=	=	=	=	=	=	=
7341	Phytepsin EC=3.4.23.40	0.51	0.007	↗↗↗	0.32	0.100	↗	=	=	M >>	=	=	=	=	=	=
7342	ND	-0.54	0.004	↘↘↘	-0.62	0.0005	↘↘↘↘	=	=	=	=	=	=	=	=	=
7343	ND	0.43	0.026	↗↗	0.63	0.0005	↗↗↗↗	=	=	=	=	=	=	=	=	=
7403		-0.17	0.39	-	-0.16	0.43	-	=	=	=	=	=	=	=	=	=
7405		-0.24	0.24	-	-0.25	0.21	-	=	=	=	=	=	=	=	=	=
7408		0.11	0.58	-	0.20	0.31	-	=	=	=	=	=	=	=	=	=
7409	ND	0.08	0.70	-	0.52	0.005	↗↗↗	=	=	=	=	=	=	=	=	=
7411		0.06	0.76	-	0.06	0.76	-	=	=	=	=	=	=	=	=	=
7416	ND	-0.33	0.093	↘	0.26	0.18	-	=	=	=	=	=	=	=	=	NM >>
7425		-0.37	0.057	↘	0.18	0.36	-	=	=	=	=	=	=	=	=	=
7426	40S ribosomal protein SA	-0.46	0.016	↘↘	-0.30	0.13	-	=	=	=	=	=	=	=	=	=
7427		-0.35	0.071	↘	-0.15	0.46	-	=	=	=	=	=	=	=	=	=
7428		-0.16	0.42	-	-0.05	0.80	-	=	=	=	=	=	=	=	=	=
7429		-0.35	0.072	↘	-0.02	0.93	-	=	=	=	=	=	=	=	=	=
7502		0.34	0.10	-	0.32	0.12	-	=	=	=	=	=	=	=	=	=
7503		-0.18	0.38	-	-0.17	0.40	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 7504 to 8802



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations.

SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
7504	0.339 ± 0.071	0.314 ± 0.044	0.329 ± 0.087	0.471 ± 0.148	0.429 ± 0.176	0.317 ± 0.008	0.389 ± 0.041	0.348 ± 0.045	0.348 ± 0.034	0.273 ± 0.143	0.294 ± 0.018	0.303 ± 0.041	0.381 ± 0.061	0.316 ± 0.129	0.290 ± 0.023	0.293 ± 0.049	0.249 ± 0.048	0.240 ± 0.046
7506	0.039 ± 0.01	0.035 ± 0.007	0.026 ± 0.022	0.021 ± 0.007	0.048 ± 0.002	0.023 ± 0.015	0.040 ± 0.016	0.042 ± 0.011	0.054 ± 0.005	0.029 ± 0.011	0.026 ± 0.006	0.030 ± 0.008	0.027 ± 0.009	0.030 ± 0.007	0.016 ± 0.009	0.037 ± 0.016	0.030 ± 0.022	0.042 ± 0.004
7516	0.058 ± 0.027	0.020 ± 0.008	0.042 ± 0.014	0.038 ± 0.013	0.052 ± 0.015	0.031 ± 0.01	0.020 ± 0.008	0.021 ± 0.006	0.034 ± 0.003	0.016 ± 0.015	0.027 ± 0.006	0.031 ± 0.001	0.051 ± 0.007	0.009 ± 0.004	0.056 ± 0.026	0.008 ± 0.005	0.020 ± 0.002	0.017 ± 0.008
7518	0.061 ± 0.038	0.068 ± 0.008	0.065 ± 0.035	0.039 ± 0.006	0.044 ± 0.003	0.055 ± 0.025	0.061 ± 0.02	0.055 ± 0.012	0.085 ± 0.027	0.035 ± 0.02	0.065 ± 0.03	0.036 ± 0.009	0.044 ± 0.028	0.055 ± 0.023	0.047 ± 0.032	0.035 ± 0.019	0.016 ± 0.008	0.043 ± 0.008
7519	0.022 ± 0.022	0.006 ± 0.002	0.013 ± 0.009	0.008 ± 0.009	0.070 ± 0.033	0.001 ± 0.001	0.056 ± 0.043	0.003 ± 0.001	0.039 ± 0.02	0.003 ± 0.003	0.023 ± 0.025	0.001 ± 0.001	0.047 ± 0.046	0.003 ± 0.002	0.031 ± 0.022	0.008 ± 0.005	0.005 ± 0.003	0.005 ± 0.002
7521	0.026 ± 0.011	0.008 ± 0.001	0.006 ± 0.003	0.012 ± 0.003	0.005 ± 0.002	0.013 ± 0.004	0.029 ± 0.012	0.055 ± 0.027	0.050 ± 0.026	0.011 ± 0.011	0.025 ± 0.018	0.033 ± 0.027	0.008 ± 0.004	0.021 ± 0.006	0.003 ± 0.001	0.040 ± 0.043	0.006 ± 0.001	0.015 ± 0.006
7605	0.403 ± 0.166	0.252 ± 0.141	0.161 ± 0.037	0.388 ± 0.138	0.235 ± 0.002	0.362 ± 0.023	0.391 ± 0.218	0.175 ± 0.095	0.294 ± 0.04	0.297 ± 0.118	0.281 ± 0.027	0.223 ± 0.095	0.305 ± 0.145	0.112 ± 0.054	0.228 ± 0.104	0.068 ± 0.023	0.430 ± 0.336	0.197 ± 0.078
7610	0.072 ± 0.043	0.031 ± 0.012	0.034 ± 0.015	0.059 ± 0.028	0.082 ± 0.051	0.053 ± 0.006	0.074 ± 0.056	0.029 ± 0.01	0.055 ± 0.014	0.023 ± 0.032	0.047 ± 0.006	0.024 ± 0.014	0.046 ± 0.011	0.023 ± 0.015	0.048 ± 0.01	0.038 ± 0.028	0.306 ± 0.435	0.037 ± 0.014
7616	0.545 ± 0.147	0.363 ± 0.115	0.735 ± 0.067	1.013 ± 0.355	0.825 ± 0.142	0.746 ± 0.233	0.694 ± 0.305	0.758 ± 0.077	0.774 ± 0.412	0.684 ± 0.111	0.659 ± 0.192	0.486 ± 0.084	0.648 ± 0.246	0.451 ± 0.063	0.557 ± 0.067	0.316 ± 0.291	0.348 ± 0.07	0.268 ± 0.172
7617	0.339 ± 0.102	0.286 ± 0.14	0.304 ± 0.096	0.383 ± 0.063	0.332 ± 0.108	0.336 ± 0.053	0.475 ± 0.046	0.339 ± 0.073	0.308 ± 0.038	0.303 ± 0.049	0.299 ± 0.11	0.309 ± 0.081	0.287 ± 0.128	0.247 ± 0.155	0.210 ± 0.089	0.128 ± 0.161	0.102 ± 0.043	0.186 ± 0.134
7621	0.698 ± 0.066	0.632 ± 0.129	0.603 ± 0.188	0.921 ± 0.411	1.105 ± 0.297	0.812 ± 0.265	1.064 ± 0.417	0.746 ± 0.278	0.638 ± 0.107	0.542 ± 0.329	0.624 ± 0.034	0.740 ± 0.115	0.864 ± 0.432	0.503 ± 0.166	0.408 ± 0.128	0.313 ± 0.085	0.323 ± 0.083	0.361 ± 0.133
7626	0.541 ± 0.076	0.376 ± 0.081	0.399 ± 0.114	0.381 ± 0.147	0.599 ± 0.151	0.438 ± 0.072	0.671 ± 0.213	0.459 ± 0.188	0.439 ± 0.155	0.476 ± 0.13	0.410 ± 0.063	0.352 ± 0.125	0.472 ± 0.147	0.454 ± 0.159	0.401 ± 0.028	0.211 ± 0.137	0.374 ± 0.18	0.272 ± 0.081
8302	0.245 ± 0.106	0.149 ± 0.025	0.220 ± 0.059	0.144 ± 0.069	0.254 ± 0.014	0.158 ± 0.088	0.148 ± 0.109	0.242 ± 0.017	0.078 ± 0.079	0.140 ± 0.071	0.115 ± 0.093	0.177 ± 0.059	0.263 ± 0.182	0.143 ± 0.039	0.144 ± 0.092	0.164 ± 0.103	0.139 ± 0.065	0.177 ± 0.078
8335	0.212 ± 0.051	0.089 ± 0.039	0.091 ± 0.069	0.112 ± 0.009	0.193 ± 0.032	0.106 ± 0.035	0.147 ± 0.086	0.123 ± 0.043	0.123 ± 0.048	0.100 ± 0.063	0.193 ± 0.06	0.139 ± 0.038	0.143 ± 0.064	0.143 ± 0.021	0.169 ± 0.04	0.103 ± 0.126	0.140 ± 0.089	0.125 ± 0.054
8403	0.038 ± 0.015	0.092 ± 0.036	0.046 ± 0.017	0.055 ± 0.005	0.067 ± 0.029	0.057 ± 0.027	0.057 ± 0.031	0.062 ± 0.016	0.054 ± 0.031	0.046 ± 0.015	0.053 ± 0.021	0.070 ± 0.043	0.049 ± 0.037	0.069 ± 0.039	0.047 ± 0.007	0.040 ± 0.016	0.022 ± 0.013	0.084 ± 0.022
8411	0.226 ± 0.104	0.142 ± 0.023	0.150 ± 0.093	0.095 ± 0.016	0.192 ± 0.026	0.156 ± 0.013	0.136 ± 0.07	0.174 ± 0.022	0.105 ± 0.047	0.073 ± 0.04	0.125 ± 0.064	0.148 ± 0.02	0.170 ± 0.086	0.139 ± 0.038	0.129 ± 0.044	0.132 ± 0.074	0.067 ± 0.05	0.146 ± 0.016
8602	0.695 ± 0.279	0.539 ± 0.022	0.488 ± 0.073	0.829 ± 0.291	0.822 ± 0.385	0.686 ± 0.128	0.659 ± 0.226	0.891 ± 0.372	0.520 ± 0.313	0.571 ± 0.231	0.606 ± 0.068	0.958 ± 0.284	0.584 ± 0.313	0.329 ± 0.042	0.688 ± 0.216	0.767 ± 0.287	0.741 ± 0.159	0.685 ± 0.237
8711	0.422 ± 0.006	0.486 ± 0.123	0.264 ± 0.059	0.330 ± 0.175	0.581 ± 0.235	0.315 ± 0.245	0.487 ± 0.164	0.611 ± 0.193	0.450 ± 0.252	0.211 ± 0.076	0.515 ± 0.182	0.412 ± 0.049	0.312 ± 0.203	0.423 ± 0.182	0.596 ± 0.12	0.423 ± 0.223	0.248 ± 0.063	0.472 ± 0.137
8802	0.156 ± 0.037	0.128 ± 0.029	0.128 ± 0.069	0.160 ± 0.017	0.189 ± 0.043	0.118 ± 0.047	0.150 ± 0.082	0.182 ± 0.089	0.146 ± 0.048	0.094 ± 0.087	0.120 ± 0.041	0.188 ± 0.011	0.116 ± 0.072	0.111 ± 0.038	0.113 ± 0.06	0.119 ± 0.036	0.090 ± 0.016	0.090 ± 0.008

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
7504	Adenosine kinase EC=2.7.1.20	-0.39	0.042	↘↘	-0.41	0.033	↘↘	=	=	=	=	=	=	=	=	=
7506		-0.33	0.11	-	0.27	0.20	-	=	=	=	=	=	=	=	=	=
7516	ND	-0.25	0.22	-	-0.47	0.013	↘↘	=	=	=	=	=	=	M >>	M >>	=
7518	Glutamine synthetase EC=6.3.1.2	-0.41	0.035	↘↘	-0.32	0.12	-	=	=	=	=	=	=	=	=	=
7519	Sucrose:sucrose 1-fructosyltransferase EC=2.4.1.99	-0.18	0.36	-	0.00	0.99	-	=	=	M >>	M >>	M >>	=	=	=	=
7521		-0.25	0.22	-	0.17	0.39	-	M >	=	=	=	=	=	=	=	=
7605	Alpha tubulin	0.13	0.53	-	-0.53	0.004	↘↘↘	=	=	=	=	=	=	=	=	=
7610		0.32	0.10	-	-0.18	0.38	-	=	=	=	=	=	=	=	=	=
7616	Tubulin beta-5 chain	-0.39	0.043	↘↘	-0.53	0.005	↘↘↘	=	=	=	=	=	=	=	=	=
7617	Beta-tubulin	-0.59	0.001	↘↘↘	-0.52	0.007	↘↘↘	=	=	=	=	=	=	=	=	=
7621	ND	-0.46	0.015	↘↘	-0.55	0.003	↘↘↘	=	=	=	=	=	=	=	=	=
7626	Beta-tubulin	-0.39	0.047	↘↘	-0.34	0.090	↘	=	=	=	=	=	=	=	=	=
8302		-0.27	0.17	-	0.79	0.055	↗	=	=	=	=	=	=	=	=	=
8335		-0.07	0.73	-	0.15	0.45	-	=	=	=	=	=	=	=	=	=
8403		-0.25	0.21	-	-0.03	0.88	-	=	=	=	=	=	=	=	=	NM >
8411	ND	-0.46	0.017	↘↘	0.08	0.68	-	=	=	=	=	=	=	=	=	=
8602		0.05	0.79	-	-0.03	0.87	-	=	=	=	=	=	=	=	=	=
8711		-0.08	0.68	-	0.08	0.68	-	=	=	=	=	=	=	=	=	=
8802		-0.38	0.059	↘	-0.26	0.19	-	=	=	=	=	=	=	=	=	=

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

All tables from Annexes 10 to 15 referred to the following legend:

Sp: spots number; ID: excised spots are referred as ‘excised’; rM/rNM: r coefficient of Pearson’s correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Annex 10 - Correlation with Cu in M and NM roots

Up-regulated spots (24 spots)

Sp	ID	rM	pval		rNM	pval	
4440	excised	0.68	**<0.001**	↗↗↗↗	0.71	**<0.001**	↗↗↗↗
5536	excised	0.64	**<0.001**	↗↗↗↗	0.84	**<0.001**	↗↗↗↗
6215	excised	0.65	**<0.001**	↗↗↗↗	0.84	**<0.001**	↗↗↗↗
3717	excised	0.57	**0.002**	↗↗↗	0.54	**0.004**	↗↗↗
5330	excised	0.39	**0.045**	↗↗	0.45	**0.019**	↗↗
5515	excised	0.42	**0.031**	↗↗	0.46	**0.016**	↗↗
6206	excised	0.46	**0.015**	↗↗	0.48	**0.011**	↗↗
2223	excised	0.59	**0.001**	↗↗↗	0.40	**0.037**	↗↗
7341	excised	0.51	**0.007**	↗↗↗	0.32	**0.100**	↗
2525	excised	0.39	**0.042**	↗↗	0.37	**0.060**	↗
513	excised	0.52	**0.006**	↗↗↗	0.77	**<0.001**	↗↗↗↗
2401	excised	0.58	**0.001**	↗↗↗	0.81	**<0.001**	↗↗↗↗
4540	excised	0.55	**0.003**	↗↗↗	0.71	**<0.001**	↗↗↗↗
5420	excised	0.56	**0.003**	↗↗↗	0.69	**<0.001**	↗↗↗↗
5506	excised	0.57	**0.002**	↗↗↗	0.68	**<0.001**	↗↗↗↗
1503	excised	0.40	**0.036**	↗↗	0.73	**<0.001**	↗↗↗↗
3202	excised	0.46	**0.015**	↗↗	0.61	**0.0008**	↗↗↗↗
7311	excised	0.40	**0.039**	↗↗	0.84	**<0.001**	↗↗↗↗
7343	excised	0.43	**0.026**	↗↗	0.63	**0.0005**	↗↗↗↗
5426	excised	0.37	**0.055**	↗	0.72	**<0.001**	↗↗↗↗
2210	excised	0.34	**0.080**	↗	0.53	**0.005**	↗↗↗
2424	excised	0.48	**0.012**	↗↗	0.51	**0.006**	↗↗↗
3701	excised	0.35	**0.075**	↗	0.46	**0.017**	↗↗
4541	excised	0.33	**0.097**	↗	0.47	**0.012**	↗↗

Down-regulated spots (26 spots)

With the exception of #5727 which was up-regulated in M and down-regulated in NM roots.

Sp	ID	rM	pval		rNM	pval	
5727	excised	0.44	**0.021**	↗↗	-0.40	**0.039**	↘↘
1211	excised	-0.66	**<0.001**	↘↘↘↘	-0.76	**<0.001**	↘↘↘↘
7306	excised	-0.55	**0.003**	↘↘↘	-0.49	**0.009**	↘↘↘
7617	excised	-0.59	**0.001**	↘↘↘	-0.52	**0.007**	↘↘↘
2724	excised	-0.40	**0.040**	↘↘	-0.39	**0.043**	↘↘
7504	excised	-0.39	**0.042**	↘↘	-0.41	**0.033**	↘↘
6537		-0.36	**0.066**	↘	-0.33	**0.097**	↘
1625	excised	-0.52	**0.005**	↘↘↘	-0.37	**0.054**	↘
3430	excised	-0.52	**0.006**	↘↘↘	-0.35	**0.071**	↘
5322	excised	-0.51	**0.006**	↘↘↘	-0.35	**0.076**	↘
6301	excised	-0.40	**0.041**	↘↘	-0.34	**0.087**	↘
6527	excised	-0.41	**0.032**	↘↘	-0.35	**0.073**	↘
7626	excised	-0.39	**0.047**	↘↘	-0.34	**0.090**	↘
1220	excised	-0.55	**0.003**	↘↘↘	-0.66	**0.0002**	↘↘↘↘
4420	excised	-0.54	**0.004**	↘↘↘	-0.61	**0.0008**	↘↘↘↘
7342	excised	-0.54	**0.004**	↘↘↘	-0.62	**0.0005**	↘↘↘↘
4808	excised	-0.46	**0.017**	↘↘	-0.73	**<0.001**	↘↘↘↘
2802	excised	-0.33	**0.089**	↘	-0.60	**0.0010**	↘↘↘↘
7309	excised	-0.33	**0.096**	↘	-0.65	**0.0003**	↘↘↘↘
2739	excised	-0.47	**0.014**	↘↘	-0.49	**0.009**	↘↘↘
3427	excised	-0.38	**0.048**	↘↘	-0.57	**0.002**	↘↘↘
4410	excised	-0.42	**0.028**	↘↘	-0.50	**0.008**	↘↘↘
7338	excised	-0.40	**0.038**	↘↘	-0.55	**0.003**	↘↘↘
7616	excised	-0.39	**0.043**	↘↘	-0.53	**0.005**	↘↘↘
7621	excised	-0.46	**0.015**	↘↘	-0.55	**0.003**	↘↘↘
3718	excised	-0.36	**0.062**	↘	-0.60	**0.001**	↘↘↘
2810	excised	-0.37	**0.058**	↘	-0.43	**0.025**	↘↘

Annex 11 - Correlation with Cu only in M roots

Up-regulated spots in M (32 spots)

Sp	ID	rM	pval		rNM	pval	
3502	excised	0.61	**0.0008**	↗↗↗↗	-0.21	0.28	-
3709	excised	0.65	**0.0003**	↗↗↗↗	0.19	0.35	-
2609	excised	0.56	**0.002**	↗↗↗	0.08	0.69	-
4602	excised	0.58	**0.002**	↗↗↗	-0.11	0.58	-
1315	excised	0.41	**0.032**	↗↗	0.30	0.13	-
1428	excised	0.45	**0.019**	↗↗	-0.27	0.17	-
2222	excised	0.41	**0.033**	↗↗	0.22	0.27	-
3409	excised	0.48	**0.011**	↗↗	0.30	0.13	-
3411	excised	0.39	**0.044**	↗↗	0.29	0.14	-
3526	excised	0.44	**0.023**	↗↗	0.11	0.59	-
3602	excised	0.46	**0.015**	↗↗	0.10	0.61	-
3610	excised	0.44	**0.021**	↗↗	0.26	0.19	-
3712	excised	0.39	**0.043**	↗↗	0.30	0.13	-
3721	excised	0.38	**0.048**	↗↗	-0.31	0.12	-
4434	excised	0.39	**0.043**	↗↗	0.00	0.98	-
4816	excised	0.38	**0.049**	↗↗	0.05	0.80	-
5531	excised	0.47	**0.014**	↗↗	-0.10	0.64	-
6702	excised	0.39	**0.043**	↗↗	0.28	0.15	-
2213		0.33	**0.094**	↗	-0.07	0.73	-
2316	excised	0.38	**0.052**	↗	-0.07	0.72	-
2523		0.36	**0.064**	↗	0.07	0.72	-
2617		0.33	**0.096**	↗	0.12	0.56	-
2628		0.33	**0.089**	↗	-0.02	0.93	-
2725	excised	0.35	**0.075**	↗	0.25	0.20	-
3320		0.38	**0.050**	↗	-0.10	0.60	-
3614		0.33	**0.090**	↗	0.17	0.39	-
4521		0.36	**0.067**	↗	0.13	0.50	-
4528		0.33	**0.096**	↗	-0.09	0.66	-
4614		0.34	**0.083**	↗	-0.29	0.14	-
4704		0.33	**0.092**	↗	0.22	0.26	-
5631		0.37	**0.054**	↗	0.18	0.36	-
5812		0.37	**0.056**	↗	-0.10	0.62	-

Down-regulated spots in M (35 spots)

Sp	ID	rM	pval		rNM	pval	
5415	excised	-0.63	**0.0004**	↘↘↘↘	-0.26	0.20	-
1626	excised	-0.54	**0.004**	↘↘↘	-0.16	0.43	-
6615	excised	-0.53	**0.004**	↘↘↘	-0.14	0.50	-
1214	excised	-0.38	**0.047**	↘↘	-0.25	0.21	-
1506	excised	-0.39	**0.045**	↘↘	0.20	0.32	-
1808	excised	-0.48	**0.012**	↘↘	-0.15	0.46	-
2425	excised	-0.40	**0.037**	↘↘	0.10	0.62	-
2533	excised	-0.47	**0.016**	↘↘	0.16	0.42	-
3515	excised	-0.42	**0.031**	↘↘	0.15	0.47	-
4439	excised	-0.41	**0.033**	↘↘	0.30	0.13	-
4601	excised	-0.48	**0.012**	↘↘	-0.01	0.96	-
6205	excised	-0.40	**0.037**	↘↘	-0.04	0.84	-
6209	excised	-0.41	**0.035**	↘↘	-0.19	0.35	-
6630	excised	-0.39	**0.046**	↘↘	-0.26	0.20	-
7426	excised	-0.46	**0.016**	↘↘	-0.30	0.13	-
7518	excised	-0.41	**0.035**	↘↘	-0.32	0.12	-
8411	excised	-0.46	**0.017**	↘↘	0.08	0.68	-
217	excised	-0.34	**0.082**	↘	-0.06	0.77	-
1521		-0.33	**0.094**	↘	-0.17	0.40	-
1708	excised	-0.32	**0.098**	↘	0.14	0.47	-
1803		-0.36	**0.063**	↘	-0.28	0.19	-
1813		-0.33	**0.091**	↘	-0.13	0.51	-
3516		-0.38	**0.053**	↘	0.11	0.60	-
4407		-0.36	**0.067**	↘	-0.08	0.71	-
4518		-0.36	**0.069**	↘	-0.18	0.37	-
5407		-0.33	**0.096**	↘	-0.10	0.63	-
5622		-0.36	**0.068**	↘	0.08	0.69	-
6536		-0.36	**0.065**	↘	0.14	0.47	-
6715		-0.33	**0.097**	↘	0.29	0.14	-
7212		-0.34	**0.080**	↘	0.03	0.89	-
7416	excised	-0.33	**0.093**	↘	0.26	0.18	-
7425		-0.37	**0.057**	↘	0.18	0.36	-
7427		-0.35	**0.071**	↘	-0.15	0.46	-
7429		-0.35	**0.072**	↘	-0.02	0.93	-
8802		-0.38	**0.059**	↘	-0.26	0.19	-

Annex 12 - Correlation with Cu only in NM roots

Up-regulated spots in NM (35 spots)

SSP	ID	cor M	pval		cor NM	pval	
1507	excised	0.16	0.41	-	0.61	**0.0008**	↗↗↗↗
1511	excised	-0.29	0.15	-	0.65	**0.0003**	↗↗↗↗
2312	excised	0.05	0.80	-	0.69	**<0.001**	↗↗↗↗
7318	excised	0.31	0.12	-	0.72	**<0.001**	↗↗↗↗
5309	excised	0.29	0.15	-	0.55	**0.003**	↗↗↗
5404	excised	-0.15	0.46	-	0.49	**0.010**	↗↗↗
5425	excised	-0.09	0.67	-	0.59	**0.001**	↗↗↗
6303	excised	0.11	0.60	-	0.53	**0.005**	↗↗↗
6629	excised	0.25	0.20	-	0.49	**0.009**	↗↗↗
7409	excised	0.08	0.70	-	0.52	**0.005**	↗↗↗
1504	excised	-0.21	0.29	-	0.42	**0.029**	↗↗
2511	excised	-0.08	0.69	-	0.45	**0.018**	↗↗
2618	excised	0.09	0.66	-	0.48	**0.011**	↗↗
3206	excised	0.04	0.39	-	0.46	**0.017**	↗↗
4415	excised	0.26	0.19	-	0.42	**0.028**	↗↗
4435	excised	-0.26	0.19	-	0.41	**0.033**	↗↗
5418	excised	-0.12	0.55	-	0.48	**0.012**	↗↗
6704	excised	0.14	0.47	-	0.47	**0.014**	↗↗
214		0.07	0.72	-	0.36	**0.075**	↗
1415		0.00	0.98	-	0.37	**0.060**	↗
2532		0.27	0.17	-	0.35	**0.075**	↗
2710		-0.03	0.88	-	0.34	**0.086**	↗
2717		0.22	0.26	-	0.36	**0.063**	↗
3211		0.14	0.48	-	0.32	**0.099**	↗
3501		0.22	0.28	-	0.35	**0.075**	↗
3605		0.14	0.48	-	0.36	**0.064**	↗
3611		0.16	0.43	-	0.37	**0.057**	↗
3634		0.29	0.15	-	0.35	**0.076**	↗
5205		0.32	0.11	-	0.33	**0.093**	↗
5217		0.05	0.79	-	0.35	**0.072**	↗
5222		0.09	0.66	-	0.38	**0.051**	↗
6308		0.11	0.59	-	0.37	**0.059**	↗
6613		0.09	0.65	-	0.34	**0.083**	↗
6710		-0.23	0.24	-	0.37	**0.057**	↗
8302		-0.27	0.17	-	0.79	**0.055**	↗

Down-regulated spots in NM (46 spots)

SSP	ID	cor M	pval		cor NM	pval	
2512	excised	-0.06	0.78	-	-0.61	**0.0008**	↘↘↘↘
3815	excised	-0.05	0.79	-	-0.63	**0.0005**	↘↘↘↘
4702	excised	-0.15	0.46	-	-0.63	**0.0004**	↘↘↘↘
4719	excised	-0.31	0.12	-	-0.61	**0.0006**	↘↘↘↘
4817	excised	-0.29	0.15	-	-0.64	**0.0005**	↘↘↘↘
6213	excised	-0.24	0.22	-	-0.69	**<0.001**	↘↘↘↘
6404	excised	0.05	0.80	-	-0.67	**0.0001**	↘↘↘↘
1505	excised	-0.26	0.19	-	-0.51	**0.007**	↘↘↘
2801	excised	-0.20	0.31	-	-0.53	**0.004**	↘↘↘
2805	excised	0.03	0.87	-	-0.51	**0.007**	↘↘↘
3802	excised	0.06	0.78	-	-0.56	**0.002**	↘↘↘
3810	excised	-0.25	0.20	-	-0.57	**0.002**	↘↘↘
4801	excised	-0.24	0.23	-	-0.56	**0.003**	↘↘↘
5410	excised	-0.25	0.21	-	-0.49	**0.009**	↘↘↘
6609	excised	0.04	0.85	-	-0.51	**0.006**	↘↘↘
6617	excised	-0.15	0.45	-	-0.57	**0.002**	↘↘↘
6706	excised	-0.08	0.69	-	-0.51	**0.007**	↘↘↘
7605	excised	0.13	0.53	-	-0.53	**0.004**	↘↘↘
1611	excised	-0.30	0.13	-	-0.39	**0.043**	↘↘
1742	excised	-0.11	0.59	-	-0.46	**0.015**	↘↘
2818	excised	-0.32	0.11	-	-0.46	**0.016**	↘↘
3707	excised	-0.25	0.21	-	-0.48	**0.011**	↘↘
4613	excised	-0.04	0.85	-	-0.38	**0.049**	↘↘
4705	excised	-0.28	0.15	-	-0.42	**0.033**	↘↘
4821	excised	-0.27	0.18	-	-0.49	**0.017**	↘↘
5331	excised	0.18	0.36	-	-0.40	**0.040**	↘↘
5424	excised	0.14	0.48	-	-0.39	**0.047**	↘↘
5514	excised	0.13	0.52	-	-0.39	**0.043**	↘↘
6212	excised	-0.21	0.30	-	-0.45	**0.019**	↘↘
6310	excised	-0.32	0.10	-	-0.40	**0.036**	↘↘
6729	excised	0.23	0.25	-	-0.42	**0.028**	↘↘
7205	excised	-0.16	0.43	-	-0.40	**0.038**	↘↘
7516	excised	-0.25	0.22	-	-0.47	**0.013**	↘↘
1328		-0.15	0.45	-	-0.38	**0.054**	↘
1414	excised	-0.11	0.58	-	-0.32	**0.099**	↘
2813		-0.18	0.37	-	-0.33	**0.092**	↘
3738		-0.06	0.75	-	-0.35	**0.073**	↘
4412		-0.20	0.32	-	-0.36	**0.061**	↘
4526		-0.31	0.11	-	-0.37	**0.054**	↘
4608		-0.07	0.73	-	-0.34	**0.079**	↘
5610		-0.29	0.14	-	-0.35	**0.072**	↘
5616		-0.27	0.18	-	-0.35	**0.076**	↘
5633		-0.28	0.16	-	-0.36	**0.066**	↘
5709		-0.17	0.40	-	-0.36	**0.061**	↘
7220		-0.05	0.80	-	-0.35	**0.078**	↘
7314		-0.05	0.79	-	-0.36	**0.071**	↘

Annex 13 - Over-expressed spots in roots

Spots over-expressed in M (60 spots)

SSP	ID	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
217	excised	M >	M >>	M >>	M >>	=	M >	M >	M >	=
314		=	=	=	=	=	=	M >	=	=
414		=	=	=	M >	=	=	=	=	=
1214	excised	=	=	=	M >	=	=	=	=	=
1216		=	=	=	=	=	=	=	M >	=
1403	excised	=	=	=	=	=	=	=	M >>	=
1414	excised	=	=	=	=	=	=	=	M >>	=
1511	excised	M >	M >	=	=	M >	=	=	=	=
1531	excised	=	M >>	=	=	=	=	=	=	=
1618	excised	M >>	M >>	M >>	M >>	M >>	M >>	=	M >	M >
1626	excised	=	=	=	=	M >	=	=	=	=
1703		=	=	=	=	M >	=	=	M >	=
1708	excised	=	M >>	=	=	=	M >	M >	=	=
1741	excised	=	M >>	=	=	M >	M >>	=	=	=
1808	excised	=	M >>	=	=	=	=	=	=	=
1813		=	M >	=	=	=	=	=	=	=
2207	excised	=	=	=	=	=	=	=	=	M >>
2221		=	=	M >	M >	=	=	=	=	=
2232		=	=	M >	=	=	=	=	=	=
2533	excised	=	=	=	=	M >>	=	=	=	=
2535		=	M >	=	=	=	=	=	=	=
2623	excised	=	=	=	=	=	=	=	=	M >
2702		=	=	=	=	M >	=	=	=	=
2727	excised	=	=	=	=	=	=	=	=	M >>
2728		=	M >	=	=	=	=	=	=	=
2802	excised	=	=	=	=	=	=	=	M >	=
2805	excised	=	=	=	=	=	=	=	=	M >
2818	excised	=	M >	=	=	=	=	=	=	=
3206	excised	=	=	=	=	M >	=	=	=	=
3303		=	=	M >	=	=	=	=	=	=

3430	excised	=	=	M >	M >	=	=	=	=	M >>
3504		=	=	M >	=	=	=	=	=	=
3516		=	=	M >	M >	=	=	=	=	=
3620		=	=	M >	=	=	=	=	=	=
3707	excised	=	=	M >	=	=	=	=	=	=
3709	excised	=	=	=	=	=	=	=	M >>	=
3810	excised	=	=	M >	=	=	=	=	=	M >
4412		=	=	=	=	=	M >	=	=	M >
4619	excised	=	=	=	=	M >>	=	=	=	=
4716	excised	=	=	M >>	=	=	M >	M >	M >>	=
4817	excised	=	=	=	=	=	M >	=	=	=
4821	excised	=	=	=	=	=	M >>	=	=	=
5213	excised	=	=	=	=	=	M >>	=	=	=
6201		=	=	M >	=	=	=	=	=	=
6203	excised	M >>	=	=	=	=	=	=	=	M >>
6209	excised	=	=	M >	=	=	=	=	=	=
6213	excised	=	=	=	=	=	=	=	M >	=
6310	excised	=	=	=	=	=	=	M >	M >>	=
6535		=	M >	=	=	=	=	=	=	=
6627		=	M >	=	=	=	=	=	=	=
6712		=	=	=	=	M >	=	=	=	=
6730		=	=	=	=	=	=	M >	=	M >
7205	excised	=	=	M >	=	=	=	M >>	=	=
7311	excised	=	M >>	=	=	=	=	=	=	=
7314		=	=	=	=	M >	=	=	=	=
7338	excised	M >	=	=	=	=	=	=	=	=
7341	excised	=	=	M >>	=	=	=	=	=	=
7516	excised	=	=	=	=	=	=	M >>	M >>	=
7519	excised	=	=	M >>	M >>	M >>	=	=	=	=
7521		M >	=	=	=	=	=	=	=	=

Spots over-expressed in NM (30 spots)

SSP	ID	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
1506	excised	=	=	=	=	=	=	=	=	NM >
1513		NM >	=	=	=	=	=	=	=	=
1603	excised	=	=	NM >>	=	=	=	=	=	=
1803		=	=	=	=	=	=	NM >	=	=
2213		=	=	NM >	=	=	=	=	=	=
2407		=	NM >	=	=	=	=	=	=	=
2502	excised	=	NM >	=	=	=	=	=	=	=
2512	excised	=	=	NM >	=	=	=	=	=	=
2725	excised	=	=	NM >>	=	=	=	=	=	=
3502	excised	=	=	NM >	=	=	=	=	=	=
3806		=	NM >	=	=	=	=	=	=	=
3812		=	=	=	=	=	=	=	NM >	=
4420	excised	=	NM >>	=	=	=	=	=	=	=
4439	excised	NM >>	NM >>	NM >>	NM >>	NM >>	NM >>	NM >>	=	NM >>
4440	excised	=	=	=	NM >	=	=	=	=	=
4508		=	NM >	=	=	=	=	=	=	=
4521		=	=	=	NM >	=	=	=	=	=
4607		=	=	=	=	=	=	=	=	NM >
4705	excised	NM >>	NM >>	=	NM >>	=	NM >	=	=	=
4820		NM >	=	=	=	=	=	=	=	=
5330	excised	=	=	=	=	=	NM >>	=	NM >>	=
5418	excised	=	=	NM >	=	=	=	=	NM >>	=
5424	excised	NM >>	=	=	=	=	NM >>	NM >>	=	=
5515	excised	NM >>	=	=	=	=	NM >	=	=	NM >>
5536	excised	=	=	=	NM >>	=	=	=	=	NM >>
5616		=	=	NM >	=	=	=	=	=	=
5634	excised	=	=	=	=	NM >>	=	NM >	=	=
6211		=	NM >	=	=	=	=	=	=	=
7416	excised	=	=	=	=	=	=	=	=	NM >>
8403		=	=	=	=	=	=	=	=	NM >

Spots over-expressed in one then the other population (5 spots)

SSP	ID	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
2316	excised	=	=	=	NM >>	=	=	=	=	M >
2740		=	M >>	NM >	=	M >	=	=	=	=
4808	excised	=	=	=	=	=	M >	=	=	NM >>
5425	excised	=	=	M >>	=	=	=	=	NM >>	=
5426	excised	=	=	M >	=	=	=	NM >>	=	NM >

Annex 14 - Over-expressed root spots correlated with Cu

Spots over-expressed in M and corelated in at least one population (30 spots)

Sp	ID	rM	rNM	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
3709	excised	↗↗↗↗	-	=	=	=	=	=	=	=	M >>	=
1626	excised	↘↘↘	-	=	=	=	=	M >	=	=	=	=
3430	excised	↘↘↘	↘	=	=	M >	M >	=	=	=	=	M >>
7341	excised	↗↗↗	↗	=	=	M >>	=	=	=	=	=	=
1214	excised	↘↘	-	=	=	=	M >	=	=	=	=	=
1808	excised	↘↘	-	=	M >>	=	=	=	=	=	=	=
2533	excised	↘↘	-	=	=	=	=	M >>	=	=	=	=
6209	excised	↘↘	-	=	=	M >	=	=	=	=	=	=
217	excised	↘	-	M >	M >>	M >>	M >>	=	M >	M >	M >	=
1708	excised	↘	-	=	M >>	=	=	=	M >	M >	=	=
1813		↘	-	=	M >	=	=	=	=	=	=	=
3516		↘	-	=	=	M >	M >	=	=	=	=	=
1511	excised	-	↗↗↗↗	M >	M >	=	=	M >	=	=	=	=
2802	excised	↘	↘↘↘↘	=	=	=	=	=	=	=	M >	=
4817	excised	-	↘↘↘↘	=	=	=	=	=	M >	=	=	=
6213	excised	-	↘↘↘↘	=	=	=	=	=	=	=	M >	=
7311	excised	↗↗	↗↗↗↗	=	M >>	=	=	=	=	=	=	=
2805	excised	-	↘↘↘	=	=	=	=	=	=	=	=	M >
3810	excised	-	↘↘↘	=	=	M >	=	=	=	=	=	M >
7338	excised	↘↘	↘↘↘	M >	=	=	=	=	=	=	=	=
2818	excised	-	↘↘	=	M >	=	=	=	=	=	=	=
3206	excised	-	↗↗	=	=	=	=	M >	=	=	=	=
3707	excised	-	↘↘	=	=	M >	=	=	=	=	=	=
4821	excised	-	↘↘	=	=	=	=	=	M >>	=	=	=
6310	excised	-	↘↘	=	=	=	=	=	=	M >	M >>	=
7205	excised	-	↘↘	=	=	M >	=	=	=	M >>	=	=
7516	excised	-	↘↘	=	=	=	=	=	=	M >>	M >>	=
1414	excised	-	↘	=	=	=	=	=	=	=	M >>	=
4412		-	↘	=	=	=	=	=	M >	=	=	M >
7314		-	↘	=	=	=	=	M >	=	=	=	=

Spots over-expressed in NM and corelated in at least one population (18 spots)

Sp	ID	rM	rNM	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
4440	excised	↗↗↗↗	↗↗↗↗	=	=	=	NM >	=	=	=	=	=
5536	excised	↗↗↗↗	↗↗↗↗	=	=	=	NM >>	=	=	=	=	NM >>
5330	excised	↗↗	↗↗	=	=	=	=	=	NM >>	=	NM >>	=
5515	excised	↗↗	↗↗	NM >>	=	=	=	=	NM >	=	=	NM >>
3502	excised	↗↗↗↗	-	=	=	NM >	=	=	=	=	=	=
1506	excised	↘↘	-	=	=	=	=	=	=	=	=	NM >
4439	excised	↘↘	-	NM >>	NM >>	NM >>	NM >>	NM >>	NM >>	NM >>	=	NM >>
1803		↘	-	=	=	=	=	=	=	NM >	=	=
2213		↗	-	=	=	NM >	=	=	=	=	=	=
2725	excised	↗	-	=	=	NM >>	=	=	=	=	=	=
4521		↗	-	=	=	=	NM >	=	=	=	=	=
7416	excised	↘	-	=	=	=	=	=	=	=	=	NM >>
2512	excised	-	↘↘↘↘	=	=	NM >	=	=	=	=	=	=
4420	excised	↘↘↘	↘↘↘↘	=	NM >>	=	=	=	=	=	=	=
4705	excised	-	↘↘	NM >>	NM >>	=	NM >>	=	NM >	=	=	=
5418	excised	-	↗↗	=	=	NM >	=	=	=	=	NM >>	=
5424	excised	-	↘↘	NM >>	=	=	=	=	NM >>	NM >>	=	=
5616		-	↘	=	=	NM >	=	=	=	=	=	=

Spots over-expressed in M and coreelated in at least one population (4 spots)

Sp	ID	rM	rNM	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
2316	excised	↗	-	=	=	=	NM >>	=	=	=	=	M >
4808	excised	↘↘	↘↘↘↘	=	=	=	=	=	M >	=	=	NM >>
5426	excised	↗	↗↗↗	=	=	M >	=	=	=	NM >>	=	NM >
5425	excised	-	↗↗↗	=	=	M >>	=	=	=	=	NM >>	=

Annex 15 - Root spots not influenced by treatments

Sp	rM	pval	rNM	pval
215	-0.06	0.78	-0.08	0.71
218	-0.24	0.22	-0.04	0.83
220	-0.13	0.54	-0.15	0.48
322	-0.06	0.78	0.15	0.48
412	-0.25	0.22	0.23	0.28
1206	0.04	0.85	-0.25	0.20
1207	0.03	0.90	0.23	0.24
1213	0.06	0.76	0.19	0.35
1215	-0.30	0.13	-0.15	0.47
1218	-0.12	0.56	-0.14	0.49
1227	0.29	0.16	-0.29	0.14
1229	-0.10	0.63	0.20	0.37
1302	0.08	0.69	0.20	0.34
1306	-0.26	0.18	-0.19	0.35
1309	-0.18	0.38	-0.04	0.86
1311	0.17	0.39	0.01	0.98
1408	-0.05	0.82	-0.20	0.31
1410	-0.29	0.14	0.06	0.75
1413	0.03	0.89	-0.22	0.27
1416	-0.21	0.29	-0.05	0.79
1502	-0.22	0.28	-0.28	0.15
1519	0.14	0.50	0.03	0.88
1522	0.16	0.42	0.08	0.69
1610	0.03	0.90	-0.27	0.18
1615	0.04	0.85	0.06	0.76
1616	0.08	0.69	0.12	0.56
1617	0.12	0.54	-0.08	0.71
1716	-0.09	0.65	0.06	0.77
1719	-0.07	0.74	0.30	0.13
1725	-0.18	0.37	-0.06	0.77
1817	-0.19	0.34	0.06	0.79
2208	-0.17	0.39	0.05	0.79
2209	0.00	0.99	0.20	0.32
2224	-0.27	0.17	-0.32	0.11
2307	0.09	0.65	-0.09	0.67
2319	0.20	0.31	-0.09	0.65
2405	0.10	0.63	0.00	1.00
2412	0.15	0.46	0.04	0.83
2413	0.21	0.29	-0.26	0.19
2515	0.13	0.51	-0.18	0.37
2522	-0.22	0.28	-0.13	0.53
2534	0.07	0.75	-0.34	0.10
2601	0.20	0.33	0.14	0.47

Sp	rM	pval	rNM	pval
4413	-0.17	0.41	-0.10	0.61
4417	0.30	0.13	-0.14	0.50
4429	-0.27	0.17	0.30	0.13
4504	0.03	0.89	0.30	0.13
4505	0.30	0.14	-0.05	0.79
4510	0.13	0.53	0.05	0.80
4512	-0.04	0.83	-0.13	0.53
4514	0.20	0.32	0.03	0.89
4516	-0.10	0.60	-0.23	0.24
4527	-0.05	0.80	0.30	0.12
4533	-0.02	0.94	-0.05	0.79
4538	0.20	0.32	0.26	0.19
4610	0.02	0.91	-0.25	0.22
4615	-0.28	0.16	0.13	0.53
4621	-0.02	0.91	0.19	0.34
4630	0.30	0.13	-0.21	0.30
4631	-0.15	0.46	-0.12	0.56
4632	-0.02	0.92	-0.01	0.95
4709	0.00	1.00	0.05	0.82
4714	0.15	0.46	-0.32	0.10
4715	-0.19	0.35	-0.18	0.37
4809	0.31	0.11	-0.17	0.40
5208	0.03	0.90	0.29	0.15
5221	0.06	0.77	0.06	0.76
5301	0.05	0.79	0.07	0.73
5316	-0.23	0.25	-0.27	0.18
5318	0.16	0.42	-0.24	0.23
5319	-0.27	0.18	-0.06	0.76
5403	-0.19	0.33	-0.10	0.61
5408	0.23	0.24	-0.04	0.83
5412	-0.16	0.42	-0.19	0.35
5508	0.26	0.20	0.00	0.98
5535	0.03	0.86	0.31	0.12
5537	0.17	0.40	0.09	0.66
5603	0.17	0.38	0.08	0.68
5607	-0.20	0.32	-0.04	0.83
5637	0.06	0.76	0.14	0.49
5638	0.28	0.16	0.02	0.93
5639	-0.31	0.12	-0.11	0.59
5702	0.02	0.91	-0.25	0.21
5703	0.01	0.96	-0.24	0.22
5705	-0.07	0.71	-0.21	0.29
5707	0.32	0.11	-0.20	0.32

2602	-0.03	0.88	-0.06	0.77
2606	0.11	0.58	-0.02	0.92
2607	0.01	0.95	0.03	0.87
2614	-0.03	0.88	-0.18	0.36
2627	0.13	0.52	0.01	0.97
2629	0.08	0.69	-0.11	0.59
2701	0.10	0.63	0.09	0.65
2703	0.10	0.63	0.29	0.15
2708	0.05	0.82	0.17	0.39
2709	0.25	0.22	0.13	0.53
2711	-0.01	0.97	0.24	0.22
2716	-0.13	0.52	0.08	0.70
2807	0.10	0.61	-0.21	0.30
3207	0.19	0.34	0.22	0.27
3208	-0.18	0.37	-0.10	0.62
3228	0.12	0.55	0.04	0.83
3229	0.11	0.60	-0.11	0.59
3230	-0.27	0.17	-0.30	0.13
3306	0.23	0.24	-0.04	0.85
3403	-0.09	0.66	-0.24	0.23
3413	-0.14	0.48	-0.19	0.34
3418	0.01	0.96	0.14	0.50
3505	0.05	0.80	0.13	0.51
3512	0.06	0.75	0.09	0.64
3514	0.11	0.59	0.16	0.43
3518	0.01	0.98	0.14	0.49
3521	-0.15	0.44	0.02	0.92
3524	0.15	0.44	0.24	0.23
3528	-0.24	0.22	0.09	0.67
3538	0.03	0.89	-0.24	0.23
3607	0.22	0.28	0.31	0.12
3609	-0.16	0.44	-0.30	0.13
3613	0.23	0.24	-0.03	0.87
3615	0.24	0.23	0.00	1.00
3632	-0.17	0.40	-0.21	0.30
3714	-0.02	0.92	-0.11	0.59
3716	0.02	0.91	-0.07	0.73
3722	0.24	0.23	0.09	0.65
3736	0.32	0.10	-0.09	0.66
3739	0.04	0.84	-0.16	0.42
3801	-0.09	0.67	-0.25	0.22
3807	0.08	0.68	-0.23	0.24
4216	-0.15	0.46	0.14	0.50
4316	0.08	0.69	-0.02	0.94
4403	0.19	0.34	0.09	0.65
4405	-0.12	0.54	-0.23	0.24

5708	0.09	0.67	-0.23	0.26
5712	0.04	0.86	0.06	0.78
5716	-0.03	0.89	-0.31	0.11
5718	-0.32	0.10	0.02	0.93
5719	0.10	0.61	0.14	0.50
6204	0.05	0.80	-0.26	0.19
6219	0.14	0.49	0.03	0.86
6220	0.17	0.41	0.13	0.51
6302	0.19	0.35	-0.01	0.94
6313	0.09	0.64	0.12	0.56
6315	-0.08	0.71	-0.02	0.92
6316	0.27	0.17	0.29	0.15
6401	-0.10	0.61	-0.28	0.16
6408	0.04	0.83	0.26	0.19
6409	-0.05	0.81	-0.09	0.66
6411	0.05	0.81	0.10	0.61
6415	-0.22	0.27	-0.25	0.21
6501	-0.26	0.20	-0.18	0.36
6515	-0.03	0.87	-0.23	0.24
6516	-0.17	0.38	-0.19	0.33
6517	-0.28	0.15	-0.15	0.47
6607	-0.31	0.11	-0.32	0.11
6610	-0.05	0.82	0.16	0.42
6612	-0.28	0.15	0.18	0.38
6713	-0.08	0.68	0.17	0.39
6807	0.21	0.29	0.13	0.51
6809	0.08	0.67	0.18	0.38
7211	-0.18	0.36	0.06	0.75
7225	-0.16	0.43	0.03	0.88
7303	0.08	0.70	-0.11	0.59
7320	-0.22	0.26	0.07	0.75
7321	-0.19	0.33	0.03	0.87
7325	-0.29	0.14	0.11	0.57
7403	-0.17	0.39	-0.16	0.43
7405	-0.24	0.24	-0.25	0.21
7408	0.11	0.58	0.20	0.31
7411	0.06	0.76	0.06	0.76
7428	-0.16	0.42	-0.05	0.80
7502	0.34	0.10	0.32	0.12
7503	-0.18	0.38	-0.17	0.40
7506	-0.33	0.11	0.27	0.20
7610	0.32	0.10	-0.18	0.38
8335	-0.07	0.73	0.15	0.45
8602	0.05	0.79	-0.03	0.87
8711	-0.08	0.68	0.08	0.68

Annex 16 - Identification details for the 85 root spots with a single protein identity

Sp: spot number; Db: consulted database, V: viridiplantae of Uniprot and A: *Agrostis spp.* EST database from NCBI; ID: Protein identity; Uniprot: Uniprot Accession; GenBank: Genbank Accession; e-value: e-value of the EST blastx on NCBI; Cov: % of coverage between experimental and database sequences; (nb): number of peptides matched between both sequences; Peptides: list of matched peptides.

Sp	Db	Cov (nb)	ID	Uniprot	GenBank / e-value	Peptides
217	A	11.7 (3)	Glutathione S-transferase (EC=2.5.1.18)	P12653	DV862008_2 / 2e-46	KVLDVYEAQLTK VLDVYEAQLTK VVEDNLVK
513	A	12.45 (3)	Formate dehydrogenase, mitochondrial (EC=1.2.1.2)	Q9ZRI8	DV856827_2 / 1e-97	cDVIVINTPLTEK GEDFPAENYIVK EGELASQYK
	V	21.28 (7)	Formate dehydrogenase 1, mitochondrial	Q9SXP2		cDVIVINTPLTEK GVIIVNNAR NPNFVGcVEGALGIR AYDLEGK LKPFNcNLLYHDR HIEDmHVLITTPFHPAYVSAER KGVIIVNNAR
		19.63 (8)	Formate dehydrogenase, mitochondrial	Q9ZRI8		FEEDLDAmLPK GVIIVNNAR EGELASQYK NPNFVGcVEGALGIR LQINPELEK AYDLEGK LKPFNcNLLYHDR KGVIIVNNAR
1211	A	28.71 (7)	L-ascorbate peroxidase 1 (EC=1.11.1.11)	Q10N21	DV857848_1 / 2e-135	FDNTYFTELLSGDK QMGLSDQDIVALSGGHTLGR TGGPFGTmK KPAEQAHAANAGLDIAVR SGFEGPWTK EGLLQLPSDK AFFEDYK
		7.41 (2)	L-ascorbate peroxidase 2, cytosolic	Q9FE01	GR281667_1 / 4e-108	TGGPFGTmK EDKPEPPPEGR
	V	16 (4)	L-ascorbate peroxidase 1, cytosolic	A2XFC7		TGGPFGTmK LAWHSAGTFDVSSK EGLLQLPSDK AFFEDYK
1220	A	15.84 (4)	L-ascorbate peroxidase 1, cytosolic	M7ZQM4	DV857848_1 / 2e-141	KPAEQAHAANAGLDIAVR FDNTYFTELLSGDK TGGPFGTmK AFFEDYK
	V	6.4 (2)	L-ascorbate peroxidase 1, cytosolic	A2XFC7		TGGPFGTmK AFFEDYK
1315	A	11.02 (2)	26S proteasome non-ATPase regulatory subunit 14	M7ZPJ4	DV860462_1 / 9e-97	AVQEEDELSPEK LINPQTmmLGQEPR
		16.9 (2)	26s proteasome non-ATPase regulatory subunit	G0Z6F1	DV857892_2 / 2e-142	LINPQTmmLGQEPR AGVPmEVmGLmLGEFVDDYTVR
	V	9.42 (3)	26S proteasome non-ATPase regulatory subunit 14 homolog	Q9LT08		AVQEEDELSPEK HYYSIAINYR VVIDAFR
1414	A	17.42 (2)	Probable voltage-gated potassium channel subunit beta	Q40648	GR278142_5 / 2e-82	ALEVIPLLTPEVLEK SLVDDTLR
	V	11.59 (3)	Probable voltage-gated potassium channel subunit beta	Q40648		LFWGGQGPNDK IEAVVQSKPK DAGVNFFDNAEVYANGR

1503	A	12.45 (3)	Formate dehydrogenase, mitochondrial	Q9ZRI8	DV856827_2 / 1e-97	cDVIVINTPLTEK GEDFPAENYIVK EGELASQYK
	V	30.85 (11)	Formate dehydrogenase 1, mitochondrial	Q9SXP2		cDVIVINTPLTEK KGVIIVNNAR GVIIVNNAR KIVGVFYK HIEDmHVLITTPFHPAYVSAER LKPFNcNLLYHDR AYDLEGK NPNFVGcVEGALGIR GHHYIVTDDKEGLNSELEK LKIDPELEK EGLNSELEK
		27.06 (10)	Formate dehydrogenase, mitochondrial	Q9ZRI8		FEEDLDAmLPK KGVIIVNNAR LQINPELEK GVIIVNNAR LKPFNcNLLYHDR DWLESK AYDLEGK NPNFVGcVEGALGIR NFLPGYQQVVKGEWNVAGIAHR EGELASQYK
1504	V	12.57 (4)	Protein disulfide isomerase-like 2-1	Q75M08		YGVSGYPTIQWFPK YGVSGFPTLK QDEGVVIANLDADK KLAPEYEK
1507	V	6.37 (3)	Formate dehydrogenase, mitochondrial	Q9ZRI8		FEEDLDAmLPK AYDLEGK DWLESK
1618	A	19.14 (4)	Mitochondrial-processing peptidase subunit alpha (EC=3.4.24.64)	P29677	DV855540_3 / 3e-41	DVHSTTGIFGIHTSTDAAFAPK SAILASLESK ELTSLATPGQVDQAQLDR KPVEHLLK
1626	A	21.12 (4)	mitochondrial processing peptidase alpha-chain precursor	Q9FNU9	DV855540_3 / 4e-77	SAILASLESK DVHSTTGIFGIHTSTDAAFAPK IISSPLTLASHGNVLNVPAYETVR KPVEHLLK
1708	V	9.42 (6)	Pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit beta	Q41141		EVPTSFGFDTAcK GQSHFFGYEGR AMVELEGAPFKK AmVELEGAPFK YYHFVR DKIETPEQFK
1808	A	17.51 (4)	Glycine dehydrogenase [decarboxylating], mitochondrial (EC=1.4.4.2)	O49852	DV857616_6 / 4e-177	IScADANAIAEEAR LNATVEmmPVTDPK IIGVSVDSSGKPALR LGTVTVQELPYFDTVK
		19.28 (4)	Glycine dehydrogenase [decarboxylating], mitochondrial	O49852	DV856328_4 / 0	IScADANAIAEEAR IIGVSVDSSGKPALR AAGFDLNVVVSDAK LGTVTVQELPYFDTVK
		6.46 (2)	Glycine dehydrogenase [decarbox]	P26969	DV853235_1 / 4e-140	IAILNANYmAK VDNVYGDR
		10.29 (2)	Glycine dehydrogenase [decarboxylating] A, mitochondrial	P49361	DY543450_5 / 7e-31	VDNVYGDR GAPHPPQLxmSDAWTKPYSR
	V	1.84 (2)	Glycine dehydrogenase [decarboxylating], mitochondrial	O49850		IAILNANYmAK VDNVYGDR
2207	V	10.4 (3)	Cysteine proteinase inhibitor 12	Q0JNR2		AKAEVVEDFAK ENALLEFVR ELQEFR
2210	A	47.83 (7)	superoxide dismutase EC=1.15.1.1	I1HKJ7	DV859502_4 / 2e-105	LGWAIDEDFGSFDK GDASAVVQLQGAIK LSVETTANQDPLVTK ALEQLDAAVSK GANLVPLLGIDVWEHAY NLKPTNEGGGEPPHGK NVRPDYLNNIWK
	V	18.1 (4)	Superoxide dismutase [Mn] 3.2, mitochondrial	P41978		GDASAVVQLQGAIK LSVETTANQDPLVTK KLSVETTANQDPLVTK NVRPDYLNNIWK
2222	A	23.11 (4)	Proteasome subunit beta type EC=3.4.25.1	I1H1Q7	DV860130_6 / 3e-122	ISQLTDNVYVcR SGSAADTQVISDYVR SmLQAGmIVGGWDK YEGGQIYSVPLGGTILR

2223	A	27.96 (6)	Glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic (EC=1.2.1.12)	P26517	DV857802_3 / 8e-155	LVSWYDNEWGYSNR AGIALNDNFVK VPTVDVSVVDLTVR IINDNFGIVEGLmTTVHAITATQK KVVISAPSK DAPmFVVGVNEDK
	V	16.91 (6)	Glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic	P08735		LVSWYDNEWGYSNR KVVISAPSK DAPmFVVGVNEDK VPTVDVSVVDLTVR VVISAPSK SSIFDAK
		12.17 (5)	Glyceraldehyde-3-phosphate dehydrogenase 1, cytosolic	Q0J8A4		KVVISAPSK AGIALNDNFVK VPTVDVSVVDLTVR VVISAPSK SSIFDAK
		15.54 (4)	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic	P26518		VPTVDVSVVDLTVR VINDKFGIVEGLMTTVHSITATQK AASYEEIK SSIFDAK
2312	V	23.62 (6)	Probable L-ascorbate peroxidase 6, chloroplastic	P0C0L1		EIVALSGAHTLGR HAANAGLVNALK LPAAGPPSPAEHLR LGWHDAGTYDK FEIELK NGPGAPGGQSWTSQWLK
		4.46 (2)	L-ascorbate peroxidase T, chloro.	Q42593		EIVALSGAHTLGR mGLDDKEIVALSGAHTLGR
2424	V	6.44 (2)	UDP-arabinopyranose mutase 1	Q9SRT9		YIFTIDDDcFVAK YVDAVmTIPK
2425	A	33.56 (7)	Fructose-bisphosphate aldolase EC=4.1.2.13	Q9XGH5	DV853997_1 / 5e-142	cAYVTEIVLAAcYK ANSEATLGTYQGDAVLGEGAAESLHVK AQAAFLVR KVAPEVIAEYTVR ALNDQHVLLEGSLLKPNmVTPGSDAK VAPEVIAEYTVR KPWSLSFSFGR
	V	23.46 (7)	Fructose-bisphosphate aldolase cytoplasmic isozyme	P17784		GILAADESTGTIGK NAAYIGTPGK YYEAGAR ANSEATLGTYKGDAVLGEGASESLHVK ALQQSTLK KPWSLSFSFGR YKDELIK
2511	V	22.44 (7)	Probable cinnamyl alcohol dehydrogenase	O22380		GGILGLGGVGHmGVK YPmVPGHEVVGEVVEVGPEVSK GLTSQIEVVK SmGHHVTVISSSDK ANVEQYcNK ANVEQYcNKK HFGLMTPGLR
		12.95 (6)	Cinnamyl alcohol dehydrogenase 2	Q6ZHS4		TGPEDVVVK mDYVNQALER GLTSQIEVVK ANVEQYcNK KTGPEDVVVK TVTGWAAR
		12.4 (4)	Alcohol dehydrogenase 2	Q4R1E8		GTFFGNYKPR FGcTDFVNPK GVmIGDGKSR ILYTALcHTDVYFWEAK
2512	A	24.24 (2)	Alcohol dehydrogenase ADH2D	A9U8G1	DV859576_5 / 5e-49	FITHSVPFSQINTAFDLmLK TDLPEVVEmYMR
	V	21.11 (6)	Alcohol dehydrogenase 3	P10848		ILYTALcHTDVYFWEAK TDLPEVVEmYmR FGcTDFVNPK FITHSVPFSQINTAFDLmLK GVmIGDGQSR SEESNLcDLLR
2525	V	9.01 (3)	Isocitrate dehydrogenase [NADP], chloroplastic (Fragment)	Q40345		TIEAEAAHGTVTR VANPIVEmDGDEmTR LIFPFVELDIK
2609	A	38.96 (5)	Aldehyde dehydrogenase family 2 member B7, mitochondrial (EC=1.2.1.3)	Q8S528	GR279156_6 / 1e-92	SNLKPVTLELGGK TAEQTPLSALYVSK VGPALAcGNTVVLK IAFTGSTDTGK IImELSAR
		25.82 (5)	Aldehyde dehydrogenase family 2 member B7, mitochondrial	Q8S528	DY543427_4 / 2e-91	SGVDSGATLVTGGDK IAQEEIFGPVQSILK GVEQGPQIDGEQFNK FNDLNEVIK GYYIQPTVFSDVQDDmK

2618	V	21.58 (6)	Alanine aminotransferase 2	P52894		ALVVINPGNPTGQVLAEENQYDIVK LLESTGIVVVPGSGFGQVPGTWHFR ATGAYSHSQGIK GGYFEITGFSAPVR APDAFYALR SLGYGEEDLPLVSYQSVSK
2623	V	19.5 (6)	Alanine aminotransferase 2	P52894		GGYFEITGFSAPVR ALVVINPGNPTGQVLAEENQYDIVK ATGAYSHSQGIK LLESTGIVVVPGSGFGQVPGTWHFR APDAFYALR GEIVIHAQR
2724	V	15.41 (11)	Phenylalanine ammonia-lyase	P14717		FEILEAITK VLTmNPTGDLSSAR DGPALQVELLR INTLLQGYSGIR HLEENIK VFLGISQGK NPSLDYGFK TKDGPALQVELLR EVNSVNDNPVIDVHR FEEELR TSPQWLGPQIEVIR
		13.09 (9)	Phenylalanine/tyrosine ammonia-lyase	Q8VXG7		FEILEAITK LLNTGVSPcLPLR DGPALQVELLR INTLLQGYSGIR HLEENIK NPSLDYGFK TKDGPALQVELLR EVNSVNDNPVIDVHR TSPQWLGPQIEVIR
		9.7 (7)	Phenylalanine ammonia-lyase	Q42667		HLEENLK TAEAVDILK NPSLDYGFK LIDPMLEcLK ALHGGNFQGTPIGVSMDNTR KTAEAVDILK TSPQWLGPQIEVIR
2725	A	8.13 (2)	Ketol-acid reductoisomerase, chloroplastic EC=1.1.1.86	Q65XK0	DV854412_1 / 0	GVAFmVDNcSTTAR NTVEcITGIVSK
	V	11.25 (5)	Ketol-acid reductoisomerase, chloroplastic	Q65XK0		GVAFmVDNcSTTAR NISVIAVcPK GmLEVYNSLTEEGKK EGLPAFPmGNIDQTR NLFPLLPEAFK
		7.06 (3)	Ketol-acid reductoisomerase, chloroplastic	Q01292		NTVEcITGVISK NISVIAVcPK EVNGAGINSSFAVHQDVDGR
2727	V	4.81 (3)	D-3-phosphoglycerate dehydrogenase, chloroplastic	O04130		GGVIDEDALVR NVAQADASIK YVGVSLVGK
2801	A	36.55 (8)	Putative aconitate hydratase, cytoplasmic EC=4.2.1.3	Q6YZX6	GR280935_4 / 9e-167	ANNmFVDYNEPQIDR FVEFHGEGmGK TSLAPGSGVVTK SDETVSmIEAYLR FDFHGQPAELK SDWHAcLDNK SGLQEYFNK GFAVPK
		28.19 (5)	Putative aconitate hydratase, cytoplasmic	Q6YZX6	DV853500_3 / 3e-110	AGEDADSLGLTGHER SEGHDTIILAGAEYGSGSSR SNLVGmGIIPLcFK LSVFDAATK FTINLPTDVSEIRPGQDVTITTDNGK
	V	10.13 (8)	Putative aconitate hydratase, cytoplasmic	Q6YZX6		AGEDADSLGLTGHER IIDWENTSPK ILLESAIR SNLVGmGIIPLcFK LAEIPFKPAR TSLAPGSGVVTK FDFHGQPAELK FYSLPALNDPR
		5.23 (4)	Aconitate hydratase, cytoplasmic	P49608		ILLESAIR SNLVGmGIIPLcFK TSLAPGSGVVTK SDETVSmIEAYLR
		4.38 (2)	Aconitate hydratase, cytoplasmic	O04916		ANNmFVDYNEPQQEK TSLAPGSGVVTK

2802	V	7.19 (5)	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	Q42699		GmLTGPVTILNWSFVR YLFAGVVDGR FALESFWDKK IPSTEEIADR VVEVNALAK
		7.32 (5)	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	P93263		GmLTGPVTILNWSFVR YLFAGVVDGR KISEEEYVK LNLPILPTTTIGSFPQTVELR ISEEEYVK
2805	A	29.83 (6)	Putative aconitate hydratase, cytoplasmic	Q6YZX6	GR280935_4 / 9e-167	ANNmFVDYNEPQIDR AcELGLEVKPWVK SGLQEYFNK FDFHGQPAELK SDETVSmIEAYLR SDWHAcLDNK
		11.74 (2)	Putative aconitate hydratase, cyto.	Q6YZX6	DV853500_3 / 3e-110	AGEDADSLGLTGHER SEGHDTIILAGAEYGSGSSR
	V	7.46 (6)	Putative aconitate hydratase, cytoplasmic	Q6YZX6		AGEDADSLGLTGHER IIDWENTSPK AcELGLEVKPWVK ILLESAIR LAEIPFKPAR FDFHGQPAELK
2810	A	14.91 (2)	Putative aconitate hydratase, cyto.	M8CZ57	FD932947_3 / 3e-60	AGEDADSLGLTGHER FTINLPTDVSEIRPGQDVTITTDNGK
	V	4.19 (2)	Aconitate hydratase (Fragment)	Q42669		NGVTATDLVLTVTQmLR AGEDADSLGLTGHER
2818	V	4.19 (2)	Aconitate hydratase (Fragment)	Q42669		AGEDADSLGLTGHER NGVTATDLVLTVTQmLR
		2.67 (2)	Putative aconitate hydratase, cyto.	Q6YZX6		AGEDADSLGLTGHER KDFNSYGSR
3202	A	33.33 (6)	superoxide dismutase EC=1.15.1.1	I1HKJ7	DV859502_4 / 2e-105	ALEQLDAAVSK AIDEDFGSFDK NVRPDYLNNIWK LGWAIDEDFGSFDK KLSVETTANQDPLVTK NLKPTNEGGGEPPHGK
	V	21.7 (4)	Superoxide dismutase [Mn] 3.1, mitochondrial	P09233		GDASAVVQLQAAIK HHATYVANYNK NVRPDYLNNIWK FNGGGHVNHSIFWK
		11.69 (3)	Superoxide dismutase [Mn], mitochondrial	Q43008		LSVETTANQDPLVTK HHATYVANYNK KLSVETTANQDPLVTK
3409	V	17.03 (6)	Alpha-galactosidase	Q9FXT4		ALADYVHAK ETADALVNTGLAK APLLIGcDVR mPGSLDHEEQDVK TFASWGVDYLK TTGDIADNWGSmTSR
3411	A	27.16 (7)	Malate dehydrogenase EC=1.1.1.37	C3VNF1	DV856531_3 / 1e-119	KmDATAQELSEEK mDATAQELSEEK LSSALSAASSAcDHIR LNVQVSDVK ALGQISER ELVKDDEWLNTEFIATVQQR NAIIWGNHSSSQYPDVNHATVK
	V	19.58 (6)	Malate dehydrogenase, cytoplasmic	Q7XDC8		SQASALEAHAAPNcK mELVDAAFPLLK VLVVANPANTNALILK KmDATAQELSEEK mDATAQELSEEK EFAPSIPEK
		34.64 (7)	Malate dehydrogenase	Q9FSF0		mELVDAAFPLLK VLVVANPANTNALILK LSSALSAASSAcDHIR LNVQVSDVK GVVATTDAVEAcTGVNVAVmVGGFPR ALGQISER GVmLGADQPVILHmLDIPPAAEALNGVK

3427	V	38.61 (11)	Flavone O-methyltransferase 1	Q84N28		NAIELGLLETLVAAGGK DAVLDGGIPFNK FLTPNEDGVSmAALALmNQDK VLmESWYYLK LLASYNVVScTmEEGK AYGmSAFEYHGTDPR NcYDALPAHGK NHSIIITK VPSGDAILmK LLASYNVVScTmEEGKDGR WILHDWSDEHcATLLK
		25.56 (9)	Tricetin 3',4',5'-O-trimethyltransferase	Q38J50		DAVLDGGIPFNK YGAAPVcK VLmESWYYLK AYGmSAFEYHGTDPR NcYDALPAHGK NHSIIITK VPSGDAILmK RYGAAPVcK WILHDWSDEHcATLLK
3526	A	21.45 (5)	S-adenosylmethionine synthase 1 (EC=2.5.1.6)	A6XMY9	DV858225_3 / 1e-145	TAAYGHFGR FVIGGPHGDAGLTGR EHVIKPVIPAQYLDEK DDADFTWEVVKPLK TQVTVEYR
	V	33.84 (9)	S-adenosylmethionine synthase	B0LXM0		GIGFVSNDVGLDADHcK IVRDTcRGIGFVSNDVGLDADHcK TAAYGHFGR FVIGGPHGDAGLTGR TNmVMVFGEITTK VHTVLISTQHDETVTNDEIAADLK VLVNIEQQSPDIAQGVHGHFTK SIVASGIAR TQVTVEYHNDNGAmVPIR
		27.99 (8)	S-adenosylmethionine synthase 2	Q9FUZ1		TAAYGHFGR FVIGGPHGDAGLTGR TNmVMVFGEITTK VHTVLISTQHDETVTNDEIAADLK SIVASGLAR EHVIKPVIPEKYLDEK DDADFTWEVVKPLK TIFHLNPSGR
		24.81 (7)	S-adenosylmethionine synthase	Q944U4		TAAYGHFGR FVIGGPHGDAGLTGR VHTVLISTQHDETVTNDEIAADLK VLVNIEQQSPDIAQGVHGHFTK NDGGAmVPIR TQVTVEYR TIFHLNPSGR
3701	V	4.5 (2)	Ketol-acid reductoisomerase, chloro.	Q65XK0		GVAFmVDNcSTTAR VSLAGHEEYIVR
3707	A	26.98 (2)	Phenylalanine/tyrosine ammonia-lyase (EC=4.3.1.25)	Q8VXG7	GR280853_5 / 7e-37	NPSLDYGFK LAIANIGK
		41.05 (2)	Phenylalanine ammonia-lyase (EC=4.3.1.24)	P14717	GR280711_5 / 2e-39	AVLVDHALTTGAAETEGEATVFSK VAFESGTAPIPNLIK
	V	19.54 (11)	Phenylalanine ammonia-lyase	P14717		INTLLQGYSGIR FEILEAITK EVNSVNDNPVIDVHR DGPALQVELLR VFLGISQGK TSPQWLGPQIEVIR NPSLDYGFK KVDAAEAFK LAIANIGK VGQVAAVAQAKDAAGVAVELDEEARPR VLTmNPTGDLSSAR
		14.22 (9)	Phenylalanine/tyrosine ammonia-lyase	Q8VXG7		INTLLQGYSGIR FEILEAITK EVNSVNDNPVIDVHR DGPALQVELLR TSPQWLGPQIEVIR LLNTGVSPcLPLR NPSLDYGFK KVDAAEAFK LAIANIGK
		5.96 (5)	Phenylalanine ammonia-lyase	Q42667		TAEAVDILK KTAEAVDILK TSPQWLGPQIEVIR LIDPmLEcLK NPSLDYGFK
3709	V	4.5 (2)	Ketol-acid reductoisomerase, chloro.	Q65XK0		GVAFmVDNcSTTAR VSLAGHEEYIVR

3712	V	5.02 (2)	Ketol-acid reductoisomerase, chloro.	Q65XK0		GVAFmVDNcSTTAR EGLPAFPmGNIDQTR
		5.38 (2)	Ketol-acid reductoisomerase, chloro.	Q01292		NTVEcITGVISK EVNGAGINSSFAVHQDVDGR
3718	V	16.4 (7)	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1, mitochondrial	O82663		LGANSLLDIVVFGR SSQTILATGGYGR AFGGQSLDFGK AYFSATSAHTcTGDGNAmVAR AAIGLSEHGFNTAcITK ImQNNAAVFR LPGISETAAIFAGVDVTK
3802	A	15.13 (3)	Putative aconitate hydratase, cyto.	Q6YZX6	GR280935_4 / 1e-163	ANNmFVDYNEPQIDR FVEFHGEGmGK SDWHAcLDNK
		11.74 (2)	Putative aconitate hydratase, cyto.	Q6YZX6	DV853500_3 / 2e-113	AGEDADSLGLTGHER SEGHDTIILAGAEYGSGSSR
	V	2.9 (2)	Putative aconitate hydratase, cyto.	Q6YZX6		AGEDADSLGLTGHER FDFHGQPAELK
		4.23 (2)	Aconitate hydratase, cyto.	P49608		DAYcLLNFGDSITTDHISPAGSIHK SDETVSmIEAYLR
3815	A	37.8 (6)	NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, mitochondrial (EC=1.6.5.3 , 1.6.99.3)	Q9FGI6	DV868571_4 / 4e-87	TVVENFYmTDSITR ANVILPSSAFSEK IMAQcSATLLK EPSTISPEVKPPVK ALSEVAGAPLPYDSVAGVR EGTYENTEGcTQWTIPAVPTVGDAR
		32.08 (5)	NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, mitochondrial	Q43644	DV862455_3 / 3e-74	ANVILPSSAFSEK IMAQcSATLLK TAVENFYmTDSITR EGTYENTEGcTQWTIPAVPTVGDAR EPSTISAEVKPPVK
		14.21 (2)	NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, mitochondrial	Q43644	GR282331_6 / 3e-81	NPVIIAGAGLFER ANVILPSSAFSEK
	V	9.89 (6)	NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, mitochondrial	Q43644		LNEDINEEWISDK FASEVAGVEDLGmLGR LSDAESmmALK GSGEEIGTYVEK NPVIIVGAGVFDR LSIAGNcR
		9.76 (6)	NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, mitochondrial	Q9FGI6		GFTVLQAcEVAGVDIPR LNEDINEEWISDK GSGEEIGTYVEK ATETIDVSDAVGSNIR FcYDGLK LSIAGNcR
4420	V	8.71 (4)	Tricetin 3',4',5'-O-trimethyltransferase	Q38J50		DAVLDGGIPFNK YGAAPVcK VLmESWYYLK RYGAAPVcK

4541	A	43.25 (9)	S-adenosylmethionine synthase 1	A6XMY9	DV858225_3 / 3e-148	FVIGGPHGDAGLTGR SIVASGLAR NDGGAmVPIR EHVIKPVIPAQYLDEK DDADFTWEVVKPLK VHTVLISTQHDETVTNDEIATDLK TAAYGHFGR TQVTVEYR IIIDTYGGWGAHGGGAFSGK
	V	34.01 (8)	S-adenosylmethionine synthase 2	Q4LB23		FVIGGPHGDAGLTGR TNmVmVFGEITTK DDADFTWEVVKPLK TAAYGHFGR VHTVLISTQHDETVTNDEIAADLK VLVNIEQQSPDIAQGVHGHFTK DIGFISDDVGLDADHcK IIIDTYGGWGAHGGGAFSGK
		26.46 (7)	S-adenosylmethionine synthase 2	Q9FUZ1		FVIGGPHGDAGLTGR SIVASGLAR TNmVmVFGEITTK DDADFTWEVVKPLK TAAYGHFGR VHTVLISTQHDETVTNDEIAADLK IIIDTYGGWGAHGGGAFSGK
		35.53 (8)	S-adenosylmethionine synthase 3	Q4LB22		FVIGGPHGDAGLTGR LcDQVSDAVLDAcLAQDADSK DDADFTWEVVKPLK TAAYGHFGR VHTVLISTQHDETVTNDEIAADLK VLVNIEQQSPDIAQGVHGHFTK NIGFISDDVGLDADR IIIDTYGGWGAHGGGAFSGK
		27.34 (7)	S-adenosylmethionine synthase	Q944U4		FVIGGPHGDAGLTGR NDGGAmVPIR TAAYGHFGR TQVTVEYR VHTVLISTQHDETVTNDEIAADLK VLVNIEQQSPDIAQGVHGHFTK IIIDTYGGWGAHGGGAFSGK
4601	V	36.54 (14)	ATP synthase subunit alpha, mitochondrial	P0C520		mTNFYTNFQVDEIGR GIRPAINVGLSVSR AAELTTLLESR TAIAIDTILNQK VVSVGDGIAR VVDALGVPIDGK TGSIVDVPAGKAmLGR AVDSLVPIGR DNGmHALIIYDDLSK LELAQYR EVAAFAQFGSDLDAATQALLNR QPQYEPLPIEK SVHEPmQTGLK VYGLNEIQAGEmVEFASGVK
4613	V	20.49 (8)	Succinate-semialdehyde dehydrogenase, mitochondrial	P51649		GANIMLGGK KITFTGSTAVGK ITFTGSTAVGK ILVQEGIYEK VSEALEYGLVGVNEGIISTEVAPFGGVK AVQSLKVGNGLEESTSQGPLINEAAVQK KWHDLIISHK NSGQTcVcANR
4702	A	25.19 (2)	Succinate dehydrogenase flavoprotein subunit, mitochondrial	B6U124	GR280547_4 / 1e-80	PGLLAAGEAAcASVHGANR LGANSLLDIVVFGR
	V	27.44 (11)	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1, mitochondrial	O82663		LGANSLLDIVVFGR ImQNNAAVFR AYFSATSAHTcTGDGNAmVAR LPGISETAAIFAGVDVTK AVIELENYGLPFSR AAIGLSEHGFNTAcITK AFGGQSLDFGK SSQTILATGGYGR GSDWLGDQDAIQYmcR AGLPLQDLEFVQFHPTGIYGAGcLITEGSR HTLGYWEDEK

4705	A	36.43 (4)	Phosphoglucomutase, cytoplasmic (EC=5.4.2.2)	Q9SNX2	GR280735_5 / 2e-86	YDYENVDAEAAK LSGTGSVGATIR IYIEQYEK ESSDALSPLVDVALK
	V	23.75 (10)	Phosphoglucomutase, cytoplasmic	Q9SNX2		YDYENVDAEAAK GATIVVSGDGR LSGTGSVGATIR YNmGNGGPAPESVTDK IYIEQYEK ESSDALSPLVDVALK SmPTSAALDVVAK YLFGDGSR EDFGGGHPDPNLTYAK FFGNLmDAGmcSVcGEESFGTGSDHIR
		15.95 (8)	Phosphoglucomutase, cytoplasmic 1	P93804		GATIVVSGDGR LSGTGSVGATIR YNmGNGGPAPESVTDK SmPTSAALDVVAK DAVQIITK YLFGDGSR EDFGGGHPDPNLTYAK DPVDGSVSK
4716	V	3.67 (2)	Heat shock 70 kDa protein 10, mito.	Q9LDZ0		EVDEVLLVGGmTR NTADTTIYSIEK
4801	A	18.11 (3)	NADH-ubiquinone oxidoreductase 75 kDa subunit	B6U2J0	DV868571_4 / 8e-99	ANVILPSSAFSEK ALSEVAGAPLPYDSVAGVR TVVENFYmTDSITR
		14.21 (2)	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	M7Z8I2	GR282331_6 / 6e-102	NPVIIAGAGLFER ANVILPSSAFSEK
	V	9.08 (4)	NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, mitochondrial	Q43644		FASEVAGVEDLGmLGR LSDAESmmALK LmTSELSGNVIDIcPVGALTSKPFAFK LNEDINEEWISDK
		9.76 (4)	NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, mitochondrial	Q9FGI6		GFTVLQAcEVAGVDIPR LmTSELSGNVIDIcPVGALTSKPFAFK LNEDINEEWISDK ATETIDVSDAVGSNIR
5309	A	15.98 (4)	Cysteine synthase (EC=2.5.1.47)	P38076	DV853802_2 / 6e-93	LFVVVFPSFGER IDGLISGIGTGGTITGTGR IHYETTGPEIWK YLSSVLFQSFR
		20.45 (3)	Cysteine synthase	P38076	GR279047_4 / 1e-82	AFGAELILTDPLLGmK TPNSYILQQFENAANPK IHYETTGPEIWK
	V	24.62 (6)	Cysteine synthase	P38076		LFVVVFPSFGER AFGAELILTDPLLGmK IGYSmITDAEEK TPNSYILQQFENAANPK IHYETTGPEIWK LESmEPcSSVK
		11.38 (3)	Cysteine synthase	Q9XEA8		IGYSmITDAEEK IHYETTGPEIWK LILTmPASmSmER
5322	A	17.26 (4)	Remorin	B4G1B0	DV856161_3 / 2e-37	KVEVEAAPEPEAPAVPAAEPEAPSKDVTEEK VPAEEEKPAVDDSK KVEVEAAPEPEAPAVPAAEPEAPSK ANIEAQLK
5404	A	5.22 (3)	Glutamine synthetase EC=6.3.1.2	C5IW59	GR282200_2 / 1e-124	HKEHIAAYGEGNER EHIAAYGEGNER IAAYGEGNER
	V	6.21 (3)	Glutamine synthetase cytosolic isozyme 1-3	Q9LVI8		HKEHIAAYGEGNER EHIAAYGEGNER DIVDAHYK
		2.79 (2)	Glutamine synthetase, chloro./mito.	Q43127		EEGGFEVIK SmREEGGFEVIK
5415	V	9.41 (2)	Peroxidase 2 (Fragment)	Q01548		TPDVFDNK YYFDLIAR
5425	V	8.45 (3)	Methylthioribose-1-phosphate isomerase	Q9AYT7		ELLNSEGGLGK ALHSGGVLEK LTAFELVHDK
5426	V	14.71 (4)	Methylthioribose-1-phosphate isomerase	Q9AYT7		LTAFELVHDK ELLNSEGGLGK KLEYLVSSRPTAVNLSDAATK AIGLHGAEFLQR
		9.63 (2)	Methylthioribose-1-phosphate isomerase	A2ZCP0		LTAFELVHDK DISVLTHcNTGSLATAGYGTALGVIR

5506	A	23.88 (5)	S-adenosylmethionine synthase 1	A6XMY9	DV858225_3 / 1e-145	FVIGGPHGDAGLTGR IIIDTYGGWGAHGGGAFSGK TAAYGHFGR TQVTVEYR ENFDFRPGmISINLDLK
		37.84 (3)	S-adenosylmethionine synthase 3	Q4LB22	GR281508_3 / 4e-52	AIGVPEPLSVFVDSYGTGK TAAYGHFGR DDPDFTWEVVKPLK
	V	27.53 (7)	S-adenosylmethionine synthase 1	A2Y053		FVIGGPHGDAGLTGR LcDQVSDAVLDAcLAEDPDSK IIIDTYGGWGAHGGGAFSGK DDPDFTWEVVKPLK VLVNIEQQSPDIAQGVHGHFTK TAAYGHFGR TQVTVEYR
		20.87 (5)	S-adenosylmethionine synthase 2	Q4H1G3		FVIGGPHGDAGLTGR VHTVLISTQHDETVSNDEIAADLK IIIDTYGGWGAHGGGAFSGK DDPDFTWEVVKPLK TAAYGHFGR
		24.37 (6)	S-adenosylmethionine synthase 3	Q4LB22		TNmVmVLGEITTK FVIGGPHGDAGLTGR IIIDTYGGWGAHGGGAFSGK VLVNIEQQSPDIAQGVHGHFTK TAAYGHFGR ENFDFRPGmISINLDLK
		15.94 (5)	S-adenosylmethionine synthase 3	A7QJG1		FVIGGPHGDAGLTGR IIIDTYGGWGAHGGGAFSGK NEGGAmVPIR TAAYGHFGR TQVTVEYR
		17.86 (5)	S-adenosylmethionine synthase	Q8W3Y4		FVIGGPHGDAGLTGR IIIDTYGGWGAHGGGAFSGK SIVASELAR TAAYGHFGR ENFDFRPGmISINLDLK
		16.16 (4)	S-adenosylmethionine synthase	A4ULF8		FVIGGPHGDAGLTGR IIIDTYGGWGAHGGGAFSGK TAAYGHFGR MATETFLYTSESVNEGHPDK
5514	A	26.83 (5)	Actin	O23951	DV859467_4 / 2e-110	SYELPDGQVITIGAER LAYVALDYEQELESAK GEYDESGPAIVHR GYSFTTTAER EITALAPSSmK
	V	33.42 (10)	Actin-1	A2XLF2		SYELPDGQVITIGAER DAYVGDEAQSK AGFAGDDAPR AEYDESGPSIVHR GYSFTTTAER KDLYGNIVLSGGTTmFPGIADR EITALAPSSmK VAPEEHPVLLTEAPLNPK RGILTLK DLTDYLmK
6203	A	24.81 (5)	L-ascorbate peroxidase 2, cytosolic (EC=1.11.1.11)	Q9FE01	GR281667_1 / 4e-108	cPAELAHGANAGLDIAVR YAADxDAFFADYAEAHLK LPNATLGSDHLR TGGPFGTmK EGLLQLPTDK
		33.8 (2)	L-ascorbate peroxidase 2, cytosolic	Q9FE01	DV860161_6 / 1e-18	VLLTDESFRPFVDK EGLLQLPTDK
	V	10.76 (2)	L-ascorbate peroxidase 2, cytosolic	Q9FE01		YAADEDAFFADYAEAHLK TGGPFGTmK
6205	A	20.47 (7)	Protein IN2-1 homolog B	Q8H8U5	DV854188_1 / 1e-103	VPSLEHNNQVK GDVSEETVAALDK FIEEVNKIDAYTQTK NYDITKGKPNLQK FQIFFSGIK IDAYTQTK FIEEVNK
	V	18.85 (4)	Protein IN2-1 homolog B	A1XBB7		IVAIDLADRPAWYK LYVAYHcPYAQR VPSLEHNNQVK FQIFFSGIK

6209	A	29.82 (11)	Triosephosphate isomerase EC=5.3.1.1	M7Z1M4	DV853744_1 / 4e-133	IIYGGSVNAANSAELAK RHVIGEDDQFIGK KEDIDGFLVGGASLK VASPEQAQEVHAAVR GPDFATIcNSVTSK HVIGEDDQFIGKK VmAcIGELLEER PEQAQEVHAAVR HVIGEDDQFIGK TNVSADVASAVR VASPEQAQEVH
	V	45.64 (13)	Triosephosphate isomerase, chloroplastic	P46225		VmAcIGELLEER IEVSAQNTWIGK IIYGGSVNAANcAELAK TNVSADVASTVR KEDIDGFLVGGASLK VASPEQAQEVHAAVR HVIGEDDEFIGKK GPDFATIcNSVTSK AAYALSQNLK FFVGGNWK RHVIGEDDEFIGK HVIGEDDEFIGK TFDVcFK
6212	A	11.11 (2)	L-ascorbate peroxidase 2, cytosolic	M8C1W9	GR281667_1 / 9e-118	cPAELAHGANAGLDIAVR LPNATLGSDHLR
	V	20.72 (3)	L-ascorbate peroxidase 2, cytosolic	Q9FE01		YAADEDAFFADYAEAHLK QVFSAQMGLSDKDIVALSGGHTLGR TGGPFGTmK
6213	A	30.37 (6)	L-ascorbate peroxidase 2, cytosolic	M8C1W9	GR281667_1 / 9e-118	YAADxDAFFADYAEAHLK LPNATLGSDHLR cPAELAHGANAGLDIAVR TGGPFGTmK QVFSAQmGLSDQDIVALSGGHTLGR TGGPFGTmKcPAELAHGANAGLDIAVR
	V	23.9 (4)	L-ascorbate peroxidase 2, cytosolic	Q9FE01		YAADEDAFFADYAEAHLK TGGPFGTmK QVFSAQmGLSDKDIVALSGGHTLGR NcAPLmLR
6215	A	23.46 (3)	Adenine phosphoribosyltransferase 1 (EC=2.4.2.7)	Q43199	GR281579_5 / 1e-69	LPGEVISEEYSLEYGTDK IEMHVGAVQPNDR LGNRPVFVLVK
	V	30.94 (5)	Adenine phosphoribosyltransferase 1	Q43199		LPGEVISEEYSLEYGTDK IEMHVGAVQPNDR GFIFGPPIALAIGAK DTTDLFVER KLPGEVISEEYSLEYGTDK
6303	A	18.05 (4)	Cysteine synthase	M8CF13	DV853802_2 / 1e-90	EGLLVGISSGAAAAAAIK IDGLISGIGTGGTITGTGR IHYETTGPEIWK LFVVVFPSFGER
		23.18 (3)	Cysteine synthase EC=2.5.1.47	P38076	GR279047_4 / 1e-79	TPNSYILQQFENAANPK IHYETTGPEIWK SVLIEPTSGNTGIGLAFmAAAK
	V	37.54 (8)	Cysteine synthase	P38076		EGLLVGISSGAAAAAAIK LFVVVFPSFGER IGYSmITDAEEK DVTELIGNTPLVYLNK TPNSYILQQFENAANPK IHYETTGPEIWK LVLTmPASmSmER SVLIEPTSGNTGIGLAFmAAAK
6527	V	9.01 (4)	4-hydroxy-3-methylbut-2-enyl diphosphate reductase, chloroplastic	Q94B35		VGIANQTTmLK AVQIAYEAR LWITNEIIHNPTVNK VWNTVEK
6617	V	28.29 (9)	ATP synthase subunit alpha, mitochondrial	P0C520		mTNFYTNFQVDEIGR GIALNLENENVGIVVFGSDTAIK GIRPAINVGLSVSR QIVVIYAAVNGFcDR TAIAIDTILNQK AAELTTLLESR VYGLNEIQAGEmVEFASGVK EVAAFAQFGSDLDAATQALLNR VVDALGVPIDGK
6629	V	6.43 (3)	Chaperonin CPN60-2, mitochondrial	Q05046		IGGASEAEVGEK SVAAGmNAmDLR DDTVILDGAGDKK

6704	V	19.48 (11)	Chaperonin CPN60-2, mitochondrial	Q05046		GISmAVDSVVTNLK SVAAGmNAmDLR IGGASEAEVGEK GYISPYFITNQK IGVQIIQNALK NVVIEQSYGAPK AGIIDPLK DDTVILDGAGDKK VTDALNATK APGFGENR APGFGENRK
		19.41 (13)	Chaperonin CPN60-1, mitochondrial	P29185		SVAAGmNAmDLR IGGASEAEVGEK IGVQIIQNALK GVEELADAVK DDTVILDGAGDKK VTDALNATK APGFGENR GEYVDMVK LQTANFDQK APGFGENRK cELEDPLILIHDK cELEDPLILIHDKK VTVSKDDTVILDGAGDKK
6706	A	26.46 (7)	Vacuolar proton-ATPase subunit A	Q1W681	FE527958_5 / 1e-116	YATALEGFYDKFDSDFIDmR NIIHFNTLANQAVER EDDLNEIVQLVGK FEDPAEGEDVLVAK NTLANQAVER LYDDLTTGFR EDYLAQNAFTPYDK
	V	34.31 (15)	V-type proton ATPase catalytic subunit A (Fragment)	Q40002		TTLVANTSNmPVAAR FEDPAEGEDVLVAK DmGYNVSMmADSTSR LAEMPADSGYPAYLASR VGHDSLIGEIIR LAADTPLLTGQR EDDLNEIVQLVGK TVISQALSK EASIYTGITIAEYFR mGDLFYR EDYLAQNAFTPYDK ISYIAPAGQYSLQDTVLELEFQGIK YSNSDTVVYVGcGER VQcLGSPDR NLEDEAR
		27.29 (12)	V-type proton ATPase catalytic subunit A	P09469		TTLVANTSNmPVAAR DmGYNVSMmADSTSR VSGPVVVADGmGGAAmYELVR LAADTPLLTGQR EDDLNEIVQLVGK TVISQALSK EASIYTGITIAEYFR EDYLAQNAFTPYDK SGDVYIPR YSNSDTVVYVGcGER LEGDSATIQVYEETAGLmVNDPVLR ESEYGYVR
7205	A	34.07 (6)	L-ascorbate peroxidase 2, cytosolic	Q9FE01	GR281667_1 / 4e-108	YAADxDAFFADYAEAHLK cPAELAHGANAGLDIAVR QVFSAQmGLSDQDIVALSGGHTLGR LPNATLGSDHLR TGGPFGTmK EGLLQLPTDK
	V	20.72 (3)	L-ascorbate peroxidase 2, cytosolic	Q9FE01		YAADEDAFFADYAEAHLK QVFSAQmGLSDKDIVALSGGHTLGR TGGPFGTmK
7309	A	32.42 (8)	Caffeoyl-CoA O-methyltransferase	M4GQ75	DV856154_2 / 5e-163	VGGLLGYDNTLWNGSVVLPDDAPmR EQTTTNGAAASGTEQVTR DFVFVDADKDNYLNYHER DNYLNYHER ENYETIGLPcIEK VGGLLGYDNTLWNGSVVLPDDAPmRK KTmEIGVYTGY SLLQSDALYQYILETTVYPR
	V	6.59 (2)	Caffeoyl-CoA O-methyltransferase 1	Q9XGD6		DFVLVLNK DNYLNYHER
7341	V	13.78 (6)	Phytepsin	P42210		FDGILGLGFKEISVGK IGAAGVVSQEcK HYVGEHTYVPVTQK cYFSIAcYLHSR FDGILGLGFKEISVGKAVPVWYK DQEFIEATK

7426	V	9.09 (3)	40S ribosomal protein SA	O80377		LLILTDPR YVDIGIPANNK NcDFQMER
7504	A	22.47 (4)	Adenosine kinase	Q8L5P6	DV866906_3 / 5e-65	IAVITQGADPVVVAEDGK GGcYGANVIIQR ISQLPLAAGK PVVVAEDGK
		11.34 (2)	Adenosine kinase, putative EC=2.7.1.20	B9T0A9	DV865243_2 / 3e-102	GWETENVEEIALK PYVDYIFGNETEAR
	V	13.08 (4)	Adenosine kinase 1	Q9SF85		SLIANLSAANcYK LNNAILAEDK SGcTYPEKPDFN KPENWALVEK
7518	A	16.5 (2)	Glutamine synthetase EC=6.3.1.2	I1J2T4	GR278149_5 / 5e-105	HDLHISEYGEGNER LTGLHETASISDFSWGVANR
	V	7.57 (2)	Glutamine synthetase, chloroplastic	P25462		GGNNVLVIcDTYTPQGEPLPTNK AAQIFSDPK
7519	A	15.38 (3)	Sucrose:sucrose 1-fructosyltransferase (EC=2.4.1.99)	Q9FSV7	GR279352_5 / 4e-63	LYASTSFYDPAK VILGYVGETDSR GWASIQSIPR
7605	A	22.75 (5)	Alpha tubulin	Q08333	DV858436_1 / 6e-152	IHFmLSSYAPVISAEK AFVHWYVGEGmEEGEFSEAR TIQFVDWcPTGFK DVNAAVATIK AVcmISNSTSVVEVFSR
	V	37.69 (13)	Tubulin alpha-1 chain	O22347		IHFmLSSYAPVISAEK AVFVDLEPTVIDEVR DVNAAVATIK TIQFVDWcPTGFK AVcmISNSTSVVEVFSR EDLAALEK EDAANNFAR TIGGGDDAFNTFFSETGAGK SLDIERPTYTNLNR FDLmYAK EIVDLcLDR AFVHWYVGEGmEEGEFSEAR QLFHPEQLISGK
		36 (12)	Tubulin alpha-6 chain	P29511		IHFmLSSYAPVISAEK AVFVDLEPTVIDEVR TIQFVDWcPTGFK cGINYQPPTVVPGGDLAK EDLAALEK EDAANNFAR SLNIERPTYTNLNR FDLmYAK EIVDLcLDR FDGALNVDVTEFQTNLVPYPR AFVHWYVGEGmEEGEFSEAR QLFHPEQLISGK
7616	A	19.87 (5)	Tubulin beta-4 chain	Q9ZRA9	DV855836_1 / 4e-125	VSEQFTAmFR LAVNLIPFPR EVDEQMLNVQNK LHFFmVGFAPLTSR NSSYFVEWIPNNVK
		38.02 (4)	Tubulin beta chain	Q39445	GR282115_3 / 2e-86	mmLTFSVFPSPK LAVNLIPFPR LHFFmVGFAPLTSR FPGQLNSDLR
	V	41.39 (15)	Tubulin beta-5 chain	Q9ZRA8		FWEVVcDEHGIDPTGR YVGTSDLQLER AVLmDLEPGTmDSVR VSEQFTAmFR mmLTFSVFPSPK LAVNLIPFPR EVDEQMINVQNK VNVYYNEAScGR SSVcDIAPR LHFFmVGFAPLTSR NSSYFVEWIPNNVK SLTVPELTQQmWDSK FPGQLNSDLR EILHIQGGQcGNQIGSK IREEYPDR

7617	A	31.03 (3)	Beta-tubulin	F6K2D0	GR282174_5 / 5e-80	EVDEQMLNVQNK VSEQFTAMFR NSSYFVEWIPNNVK
	V	51.01 (17)	Tubulin beta-5 chain	Q9ZRA8		VNVYYNEAScGR VSEQFTAmFR AVLmDLEPGTmDSVR EVDEQmINVQNK LTTPSFGDLNHLISATmSGVTccLR YLTASAMFR NSSYFVEWIPNNVK LHFFmVGFAPLTSR LAVNLIPFPR FWEVVcDEHGIDPTGR FPGQLNSDLR NmmcAADPR IREEYPDR GHYTEGAELIDSVLDVVR YVGTSDLQLER TGPYGQIFRPDNFVFGQSGAGNNWAK SSVcDIAPR
7626	A	65.29 (6)	Beta-tubulin	M9ZNH1	GR282115_3 / 2e-83	VSDTVVEPYNATLSVH LAVNLIPFPR LTTPSFGDLNHLISATm mmLTFSVFPSPK LHFFmVGFAPLTSR FPGQLNSDLR
		40.52 (3)	Beta-tubulin	F6K2D0	GR282174_5 / 5e-80	EVDEQmLNVQNK ALTVPELTQQmWDSK GLSmSSTFVGNSTSIQEmFR
		26.23 (2)	Beta-tubulin	V5NSU2	GR279087_3 / 3e-69	AVLmDLEPGTmDSVR SLGGGTGSGmGTLLISK
	V	48.2 (15)	Tubulin beta-2 chain	P18026		VNVYYNEAScGR SSVcDIPPR VSEQFTAmFR EVDEQmINVQNK FPGQLNSDLR GLSmSSTFVGNSTSIQEmFR LAVNLIPFPR EILHIQGGQcGNQIGSK YLTASAMFR NmmcAADPR LHFFmVGFAPLTSR FWEVVcDEHGIDPTGR AVLMDLEPGTmDAVR TGPYGQIFRPDNFVFGQSGAGNNWAK LTTPSFGDLNHLISATmSGVTccLR
		43.88 (14)	Tubulin beta-6 chain	P29514		VNVYYNEAScGR VSEQFTAmFR EVDEQmINVQNK FPGQLNSDLR ALTVPELTQQmWDSK mmLTFSVFPSPK LAVNLIPFPR EILHIQGGQcGNQIGSK YLTASAMFR NmmcAADPR LHFFmVGFAPLTSR FWEVVcDEHGIDPTGR TGPYGQIFRPDNFVFGQSGAGNNWAK LTTPSFGDLNHLISATmSGVTccLR

Annex 17 - Identification details for the 24 root spots with multiple identifications

Sp: spot number; Dtb: consulted database, V: viridiplantae of Uniprot and A: Agrostis spp. EST database; ID: Protein identity; Uniprot: Uniprot Accession; gb Access: Genbank Accession; e-value: e-value of the blastx on NCBI; Cov: % of coverage between experimental and database sequences; (nb): number of peptides matched between both sequences; peptids: list of matched peptides

Sp	Db	Cov (nb)	ID	Uniprot	GenBank / e-val	Peptides
1428	A	23 (3)	Glyceraldehyde-3-phosphate dehydrogenase 1	C9EAC1	DV867339_1 / 3e-76	VPTVDVSVVDLTVR LVSWYDNEWGYSTR AGIALNDNFVK
	V	14.57 (4)	UDP-arabinopyranose mutase 1	Q9SRT9		YVDAVmTIPK ELIGPAMYFGLmGDGQPIGR YIFTIDDDcFVAK ASNPFVNLK
		22.3 (5)	Glyceraldehyde-3-phosphate dehydrogenase 2, cytosolic (Fragment)	P08477		VPTVDVSVVDLTVR AGIALNDNFVK GILGYVDEDLVSTDFQGDSR KVIISAPSK LVSWYDNEWGYSTR
		16.57 (5)	UDP-arabinopyranose mutase 3	O22666		YVDAVmTIPK ELIGPAMYFGLmGDGQPIGR ASNPFVNLK TGLPYIWHSK YDDmWAGWcVK
1511	A	21.79 (6)	Protein disulfide isomerase-like 2-1 (EC=5.3.4.1)	Q75M08	GR280817_5 / 3E-132	QDEGVVIANLDADK YGVSGFPTLK ADEFVIK DVLVEFYAPWcGHcK IYVNVAK SLAPVYEK
		9.31 (4)	Protein disulfide isomerase-like 2-1	Q75M08	DV853132_3 / 2E-79	DFQSAADDK YGVSGFPTLK DFQSAADDKR IYVNVAK
	V	14.48 (5)	Protein disulfide isomerase-like 2-1	Q75M08		QDEGVVIANLDADK YGVSGFPTLK ADEFVIK KLAPEYEK YGVSGYPTIQWFPK
		5.92 (2)	Fructose-bisphosphate aldolase, cytoplasmic isozyme	P08440		GILAADESTGTIGK YKDELIK
1611	A	24.42 (4)	mitochondrial processing peptidase alpha-chain precursor	Q9FNU9	DV855540_3 / 4e-77	IISSPLTLASHGNVLNVPAYETVR DVHSTTGIFGIHTSTDAAFAPK SAILASLESK ELTSLATPGQVDQAQLDR
	V	13.78 (5)	6-phosphogluconate dehydrogenase, decarboxylating 2, chloroplastic	Q2R480		LPANLIQAQR GILYLGmGVSGGEEGAR TVVLLVQAGR NPELANLIVDR AVEAGISTPGmSASLSYFDTYR
2401	V	14.77 (3)	Ribose-phosphate pyrophosphokinase 4	Q6ZFT5		HVVIVDDLVQSGGTLR VEEEGDVATAFTLAR GGPTSVVIYDIHALQER
		7.54 (2)	Glyceraldehyde-3-phosphate dehydrogenase 2, cytosolic (Fragment)	P08477		VPTVDVSVVDLTVR KVIISAPSK

3430	A	20 (4)	Tricetin 3',4',5'-O-trimethyltransferase (EC=2.1.1.169)	Q38J50	GR281675_2 / 1E-77	NHSIIITK VPSGDAxLmK NcYDALPAHGK KVPSGDAxLmK
	V	24.44 (7)	Flavone O-methyltransferase 1	Q84N28		FLTPNEDGVSmAALALmNQDK DAVLDGGIPFNK VPSGDAILmK VLmESWYYLK NHSIIITK LLASYNVVScTmEEGK NcYDALPAHGK
		16.57 (6)	Tricetin 3',4',5'-O-trimethyltransferase	Q38J50		VPSGDAILmK VLmESWYYLK YGAAPVcK NHSIIITK DAVLDGGIPFNK NcYDALPAHGK
		12.05 (3)	Malate dehydrogenase, cytoplasmic	Q7XDC8		mDATAQELSEEK VLVVANPANTNALILK mELVDAAFPLLK
3502	A	31.29 (9)	GDP-mannose 3,5-epimerase 2 (EC=5.1.3.18)	Q2R1V8	GR282296_6 / 2E-159	FEmWGDGLQTR EADAWPAEPQDAYGLEK SFTFIDEcVEGVLR FHNIYGPYGTWK ALTSTDRFEmWGDGLQTR DFDIEcR LATEELcK ITYLWIK QLETVVSLK
		15.79 (4)	GDP-mannose 3,5-epimerase 2	Q2R1V8	DV854035_1 / 2E-43	AEGGNVSDYGSSK VcTTmAPVQLGSLR EKAEGGNVSDYGSSK ITYLWIK
		52.13 (4)	GDP-mannose 3,5-epimerase 2	Q2R1V8	GR281601_5 / 2E-60	ISITGAGGFIASHLAR GEGHYIIASDWK LKGEGHYIIASDWK NEHmEEDmFcHEFHLADLR
		16.36 (5)	GDP-mannose 3,5-epimerase 2	Q2R1V8	DV853791_3 / 2E-105	FEmWGDGLQTR SFTFIDEcVEGVLR ALTSTDRFEmWGDGLQTR ELPIHHIPGPEGVR ITYLWIK
		15.15 (2)	Alcohol dehydrogenase 3 (EC=1.1.1.1)	P10848	DV859576_5 / 5E-49	THPmNFLNER GTFFGNYKPR
	V	23.18 (8)	GDP-mannose 3,5-epimerase 2	Q2R1V8		FFYASSAcIYPEFK ISITGAGGFIASHIAR FEmWGDGLQTR LATEELcK SFTFIDEcVEGVLR ALTSTDRFEmWGDGLQTR EKAPAAFcR VMDNcLK
		6.83 (2)	Alcohol dehydrogenase 1 (Fragment)	Q07264		THPmNFLNER GVmIGDGKSR
3515	A	40.75 (9)	Isocitrate dehydrogenase [NADP] EC=1.1.1.42	M7YI34	DV867425_1 / 8e-119	GGETSTNSIASIFAWTR DLALLVHGSSK TIEAEAAHGTVTR LEEAcVGTVESGK LIDDmVAYALK SEGGYVWAcK YEAAGIWYEHR GDYLNTEEFIDAVAAELQSR SKYEAAGIWYEHR
	V	24.48 (10)	Isocitrate dehydrogenase [NADP], chloroplastic	Q40345		VANPIVEmDGDEMTR GGETSTNSIASIFAWTR TIEAEAAHGTVTR LIDDmVAYALK HAFGDQYR YEAAGIWYEHR SEGGYVWAcK SKYEAAGIWYEHR LIFPFVELDIK WPLYLSTK
		21.71 (8)	Cytosolic isocitrate dehydrogenase [NADP]	Q9SRZ6		VANPIVEmDGDEMTR GGETSTNSIASIFAWTR TIEAEAAHGTVTR LIDDmVAYALK MAFEKIKVANPIVEmDGDEMTR HAFGDQYR SEGGYVWAcK WPLYLSTK
		7.55 (2)	GDP-mannose 3,5-epimerase 2	Q2R1V8		SFTFIDEcVEGVLR FFYASSAcIYPEFK

3610	V	6.59 (3)	Methylmalonate-semialdehyde dehydrogenase [acylating], mito.	Q0WM29		ASFAGDLNFYGK LAMNITTEQGK AVSFVGSNTAGmHIYAR
		5 (2)	UDP-glucose 6-dehydrogenase 4	Q2QS14		VVSSmFNTVSGK AADLTYWESAAR
4410	A	12.55 (3)	Probable cinnamyl alcohol dehydrogenase 8A (EC=1.1.1.195)	Q6ERX1	DV859534_5 / 1E-71	HGVTADVEVVK LGADGFLVSK MDYVNTAIER
	V	7.34 (2)	Putative cinnamyl alcohol dehydrogenase 5	Q0J6T3		LGADAFVVSK HVGVLGLGGLGHVAVK
		7.58 (3)	Tricetin 3',4',5'-O-trimethyltransferase	Q38J50		YGAAPVcK NHSIIITK NcYDALPAHGK
4434	A	27.64 (7)	Alpha-1,4-glucan-protein synthase	A6N1F0	DV858285_2 / 5e-133	YVDAVLTIPK cYISLSEQVR GTLFPmcGMNLAFDR YDDMWAGWcVK TGLPYLWHSK VIcDHLSLGVK ASNPFVNLK
		52.54 (3)	Alpha-1,4-glucan-protein synthase [UDP-forming] 1 EC=2.4.1.-	Q9SC19	GR280939_5 / 1e-34	HLIIVQDGDPSK VPEGFDYELYNR AScISFK
	V	32.14 (10)	UDP-arabinopyranose mutase 1	Q8H8T0		YVFTIDDDcFVAK DINALEQHIK VPEGFDYELYNR DELDIVIPTIR ASNPFVNLK GTLFPmcGMNLAFDR VIcDHLSLGVK TGLPYIWHSK NLLSPSTPFFFNTLYDPYR AScISFK
		34.07 (11)	Alpha-1,4-glucan-protein synthase [UDP-forming]	P80607		DINALEQHIK VPEGFDYELYNR DELDIVIPTIR ASNPFVNLK GTLFPmcGMNLAFDR NLDFLEmWR VIcDHLSLGVK YDDmWAGWcVK TGLPYIWHSK NLLSPSTPFFFNTLYDPYR AScISFK
4435	A	40.68 (2)	Alpha-1,4-glucan-protein synthase [UDP-forming] 1 EC=2.4.1.-	Q9SC19	GR280939_5 / 1e-34	HLIIVQDGDPSK VPEGFDYELYNR
		7.27 (2)	Alpha-1,4-glucan-protein synthase	A6N1F0	DV858285_2 / 5e-133	YVDAVLTIPK cYISLSEQVR
	V	17.31 (5)	UDP-arabinopyranose mutase 1	Q8H8T0		YVFTIDDDcFVAK VPEGFDYELYNR ASNPFVNLK NLLSPSTPFFFNTLYDPYR DINALEQHIK
		5.99 (2)	Phosphoglycerate kinase, cytosolic	P12783		ELDYLVGAVANPK SVGTLGEADLK
		51.67 (3)	Alpha-1,4-glucan-protein synthase [UDP-forming] (Fragments)	P85413		YVDAVLTIPK VPEGFDYELYNR ASNPFVNLK
4439	A	7.46 (2)	Glutamine synthetase cytosolic isozyme 1 EC=6.3.1.2	P24099	GR282200_2 / 2E-125	EHIAAYGEGNER DIVDAHYK
		12.46 (2)	S-adenosylmethionine synthase 1	A6XMY9	DV858225_3 / 1E-145	EHVIKPVIPAQYLDEK IIIDTYGGWGAHGGGAFSGK
	V	18.27 (4)	S-adenosylmethionine synthase 1	A6XMY9		TNmVmVFGEITTK VHTVLISTQHDETVTNDEIAADLK IIIDTYGGWGAHGGGAFSGK NIGFISDDVGLDADR
		12.43 (4)	Glutamine synthetase cytosolic isozyme 1-3	Q9LVI8		EHIAAYGEGNER RPASNmDPYVVTSmIAETTILG HKEHIAAYGEGNER DIVDAHYK
4440	A	8.59 (2)	ATP phosphoribosyltransferase, chloroplastic (EC=2.4.2.17)	Q10S55	GR279455_5 / 2E-145	LTYIFNEETPR VVTGFGYVGAK
	V	6.59 (2)	Pyruvate dehydrogenase E1 component subunit alpha-3, chloroplastic	Q7XTJ3		KGPAFGmPGVHVDGmDVLK SVmAELFGK

4540	A	52.6 (11)	S-adenosylmethionine synthase 1	A6XMY9	DV858225_3 / 1E-145	FVIGGPHGDAGLTGR NDGGAmVPIR DDADFTWEVVKPLK IIIDTYGGWGAHGGGAFSGK TAAYGHFGR SIVASGLAR EHVIKPVIPAQYLDEK TIFHLNPSGR VHTVLISTQHDETVTNDEIATDLK TQVTVEYR ENFDFRPGmISINLDLK
		44.16 (2)	S-adenosylmethionine synthase	Q944U4	GR280992_5 / 7E-26	NDGGAmVPIR VHTVLISTQHDETVTNDEIAADLK
		11.7 (2)	Enolase 2 (EC=4.2.1.11)	P42895	DV859464_4 / 1E-151	IEEELGAAAVYAGLK VVIGmDVAASEFYGEK
		15.32 (2)	S-adenosylmethionine synthase 3	Q4LB22	GR281508_3 / 4E-52	TAAYGHFGR IPDKEILK
	V	46.45 (12)	S-adenosylmethionine synthase 3	Q4LB22		NIGFISDDVGLDADR FVIGGPHGDAGLTGR TNmVmVLGEITTK LcDQVSDAVLDAcLAQDADSK VHTVLISTQHDETVTNDEIAADLK DDADFTWEVVKPLK IIIDTYGGWGAHGGGAFSGK TAAYGHFGR VLVNIEQQSPDIAQGVHGHFTK NGTcAWVRPDGK ENFDFRPGmISINLDLK KNGTcAWVRPDGK
		50.76 (11)	S-adenosylmethionine synthase 1	A6XMY9		NIGFISDDVGLDADR FVIGGPHGDAGLTGR TNmVmVFGEITTK LcDQVSDAVLDAcLAQDADSK VHTVLISTQHDETVTNDEIAADLK IIIDTYGGWGAHGGGAFSGK TAAYGHFGR VLVNIEQQSPDIAQGVHGHFTK ENFDFRPGmISINLDLK TIFHLNPSGR RPEDIGAGDQGImFGYATDETPELmPLSHVLATK
		29.87 (8)	S-adenosylmethionine synthase	Q944U4		FVIGGPHGDAGLTGR NDGGAmVPIR VHTVLISTQHDETVTNDEIAADLK IIIDTYGGWGAHGGGAFSGK TAAYGHFGR VLVNIEQQSPDIAQGVHGHFTK TQVTVEYR TIFHLNPSGR
		33.16 (8)	S-adenosylmethionine synthase 1	A9P822		FVIGGPHGDAGLTGR TNmVmVFGEITTK VHTVLISTQHDETVTNDEIAADLK IIIDTYGGWGAHGGGAFSGK TAAYGHFGR VLVNIEQQSPDIAQGVHGHFTK NIGFISDDVGLDADKcK TIFHLNPSGR
		23.53 (7)	S-adenosylmethionine synthase 1	A7PQS0		FVIGGPHGDAGLTGR TNmVmVFGEITTK IIIDTYGGWGAHGGGAFSGK TAAYGHFGR NEGGAmVPIR TQVTVEYR KNFDFRPGmISINLDLK
		19.95 (5)	S-adenosylmethionine synthase 2	P24260		FVIGGPHGDAGLTGR VHTVLISTQHDETVTNDEIAADLK TAAYGHFGR SIVASGLAR MAAAADTFLFTSESVNEGHPDK
		12.33 (3)	Enolase 2	P42895		AAVPSGASTGVYEALELR IEEELGAIAVYAGAK SGETEDTFIADLAVGLSTGQIK

4602	V	20.4 (11)	Leucine aminopeptidase 2, chloroplastic	Q6K669		FDmGGSAAVFGAAK LTLADALVYAcNQGVDK AGQSVVLR TIEVNNTDAEGR SGVADmVNTGGR GIGESVASVAK TGPGcSIELmK GLTFDSGGYNIK FENAVLK QGGSITAALFLK LAIVGK
		3.79 (2)	Methylmalonate-semialdehyde dehydrogenase [acylating], mito.	Q0WM29		LAMNITTEQGK ASFAGDLNFYGK
4719	A	21.35 (3)	Transketolase, chloroplastic (EC=2.2.1.1)	Q7SIC9	DV863383_1 / 3E-57	EYGITAEAVVAAAK ISIEAGSTLGWQK ESVLPAAVTAR
	V	6.67 (4)	Transketolase, chloroplastic	Q7SIC9		ISIEAGSTLGWQK VTTTIGFGSPNK FLAIDAVEK ESVLPAAVTAR
		2.16 (2)	Transketolase-2, chloroplastic	F4IW47		FLAIDAVEK FAAYEKK
		5.51 (4)	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase, chloroplastic	Q6K8J4		GmVESALEFAR ImSYYGDSPR VNPGNFADR TEYVScPScGR
4816	A	9.37 (2)	Cyanate hydratase EC=4.2.1.104	B6TTW1	DV857698_4 / 3e-92	AIDLIDEAGSR RFQPVKVPEPTVDESIQILR
	V	19.17 (14)	Chaperone protein ClpC1, chloroplastic	Q7F9I1		mVGESTEAVGAGVGGGSSGQK AIDLIDEAGSR LIGSPPGYVGYTEGGQLTEAVR TAIAEGLAQR VLESLGADPNNIR VITLDmGLLVAGTK IIGQDEAVK VLELSLEEAR GSGFVAVEIPFTPR HAQLPDEAK LDEmIVFR NNPcLIGEPGVGK LLEDSLAEK mPTLEEYGTNLTK
5418	A	8.21 (3)	Glutamine synthetase cytosolic isozyme 1	P24099	GR282200_2 / 2E-125	HKEHIAAYGEGNER EHIAAYGEGNER DIVDAHYK
		5.86 (2)	Glutamine synthetase cytosolic isozyme 1-1	P14656	DV856149_1 / 9E-118	GIEQEYTLLQK DIVDAHYK
	V	5.06 (3)	Glutamine synthetase	P12424		HKEHIAAYGEGNER EHIAAYGEGNER LGLKHKEHIAAYGEGNER
		6.23 (3)	Glutamine synthetase cytosolic isozyme 1-5	Q8GXW5		QHIAAYGEGNER DIVDAHYK HKQHIAAYGEGNER
		18.24 (3)	Peroxidase 2 (Fragment)	Q01548		YYFDLIAR mSNmDILTGTKGEIR TPDVFDNK
		5.6 (3)	Glutamine synthetase cytosolic isozyme	P52783		DIVDAHYK KEGGFEVIK SmRKEGGFEVIK

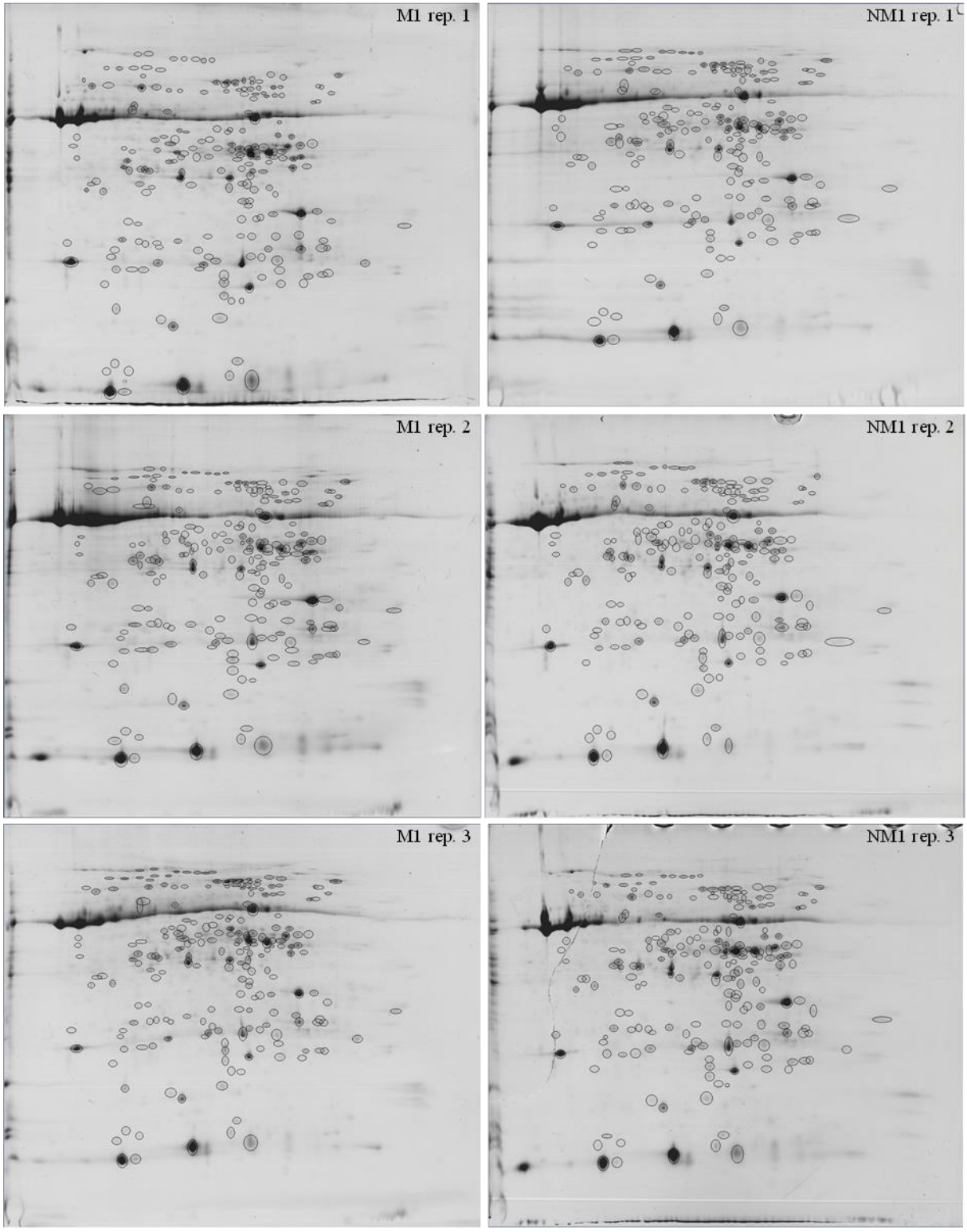
5515	A	17.99 (4)	S-adenosylmethionine synthase 1	A6XMY9	DV858225_3 / 1E-145	FVIGGPHGDAGLTGR TQVTVEYR IIIDTYGGWGAHGGGAFSGK TAAYGHFGR
		45.05 (4)	S-adenosylmethionine synthase 3	Q4LB22	GR281508_3 / 4E-52	AIGVPEPLSVFVDSYGTGK TAAYGHFGR DDPDFTWEVVKPLK IPDKEILK
		10.57 (2)	Actin-3	Q10AZ4	DV859467_4 / 5E-110	SYELPDGQVITIGAER GYSFTTTAER
	V	20.05 (5)	S-adenosylmethionine synthase 3	Q4LB22		FVIGGPHGDAGLTGR TNmVmVLGEITTK IIIDTYGGWGAHGGGAFSGK TAAYGHFGR VLVNIEQQSPDIAQGVHGHFTK
		29.55 (8)	S-adenosylmethionine synthase 1	A2Y053		FVIGGPHGDAGLTGR LcDQVSDAVLDAcLAEDPDSK DDPDFTWEVVKPLK TQVTVEYR IIIDTYGGWGAHGGGAFSGK TAAYGHFGR IPDKEILK VLVNIEQQSPDIAQGVHGHFTK
		22.9 (6)	S-adenosylmethionine synthase 2	Q4H1G3		FVIGGPHGDAGLTGR DDPDFTWEVVKPLK VHTVLISTQHDETVSNDEIAADLK IIIDTYGGWGAHGGGAFSGK TAAYGHFGR IPDKEILK
		15.94 (5)	S-adenosylmethionine synthase 3	A7QJG1		FVIGGPHGDAGLTGR TQVTVEYR IIIDTYGGWGAHGGGAFSGK TAAYGHFGR NEGGAmVPIR
		23.16 (7)	S-adenosylmethionine synthase	O22338		FVIGGPHGDAGLTGR TQVTVEYR IIIDTYGGWGAHGGGAFSGK SIVASELAR TAAYGHFGR IPDKEILK VLVNIEQQSPDIAQGVHGHFTK
		27.85 (8)	Actin-1	A2XLF2		SYELPDGQVITIGAER DAYVGDEAQSK VAPEEHPVLLTEAPLNPK AEYDESGPSIVHR AGFAGDDAPR GYSFTTTAER YPIEHGIVSNWDDmEK EITALAPSSmK
		24.11 (6)	S-adenosylmethionine synthase 2	P93438		FVIGGPHGDAGLTGR LcDQVSDAVLDAcLAQDPDSK IIIDTYGGWGAHGGGAFSGK TAAYGHFGR IPDKEILK VLVNIEQQSPDIAQGVHGHFTK

5727	A	31.08 (7)	Vacuolar proton-ATPase subunit A	Q1W681	FE527958_5 / 1e-116	FEDPAEGEDVLVAK EDYLAQNAFTPYDK EDDLNEIVQLVGK LYDDLTTGFR YATALEGFYDKFDSDFIDmR DALGEGDKITLETAK NIIHFNTLANQAVER
	V	45.86 (21)	V-type proton ATPase catalytic subunit A (Fragment)	Q40002		TTLVANTSNmPVAAR FEDPAEGEDVLVAK DmGYNVSmmADSTSR YSNSDTVVYVGcGER LAEmPADSGYPAYLASR EASIYTGITIAEYFR EDYLAQNAFTPYDK GNEmAEVLmDFPQLTmTLPDGR EDDLNEIVQLVGK ISYIAPAGQYSLQDTVLELEFQGIK LAADTPLLTGQR GVSVPALDKDQLWEFQPNK TVISQALSK VQcLGSPDR VGHDSLIGEIIR mGDLFYR NLEDEAR LASFYER EFTMLHTWPVR LLREDYLAQNAFTPYDK EVLQREDDLNEIVQLVGK
		33.39 (17)	V-type proton ATPase catalytic subunit A	P09469		TTLVANTSNmPVAAR DmGYNVSmmADSTSR YSNSDTVVYVGcGER EASIYTGITIAEYFR EDYLAQNAFTPYDK GNEmAEVLmDFPQLTmTLPDGR LEGDSATIQVYEETAGLmVNDPVLR EDDLNEIVQLVGK VSGPVVVADGmGGAAmYELVR SGDVYIPR LAADTPLLTGQR TVISQALSK KVSGPVVVADGmGGAAmYELVR ESEYGYVR LASFYER LLREDYLAQNAFTPYDK EVLQREDDLNEIVQLVGK
		7.16 (4)	70 kDa peptidyl-prolyl isomerase	Q43207		LEDGTVVSK TVTEIGDDKK TDEEAVIEGLDR SEGVEFTVK
6404	A	5.22 (2)	Glutamine synthetase cytosolic isozyme 1	P24099	GR282200_2 / 2E-125	HKEHIAAYGEGNER EHIAAYGEGNER
	V	18.24 (3)	Peroxidase 2 (Fragment)	Q01548		YYFDLIAR TPDVFDNK mSNmDILTGTKGEIR
		27.45 (2)	Glutamine synthetase (Fragments)	P85087		EHIAAYGEGNER HKEHIAAYGEGNER
6630	A	23.32 (9)	Enolase 1	P26301		GAVPSGASTGIYEALELR YGQDATNVGDEGGFAPNIQENK mGVEVYHNLK DGGSDYLGK YNQLLR KYGQDATNVGDEGGFAPNIQENK IPLYQHIANLAGNK DKTYDLNFK VNQIGSVTESIEAVR
		22.65 (8)	Enolase 2	P42895		AAVPSGASTGVYEALELR MTEEIGEQVQIVGDDLLVTNPTR YGQDATNVGDEGGFAPNIQENK YNQLLR KYGQDATNVGDEGGFAPNIQENK IPLYQHIANLAGNK ScNALLLK DQTYDLNFK
		7.07 (3)	ATP synthase subunit beta, mitochondrial	Q01859		TVLImELINNVAK TIAmDGTEGLVR VLNTGSPITVPVGR
		4.35 (2)	V-type proton ATPase subunit B 2	Q40079		TPVSLDmLGR RGQVLEVDGEK

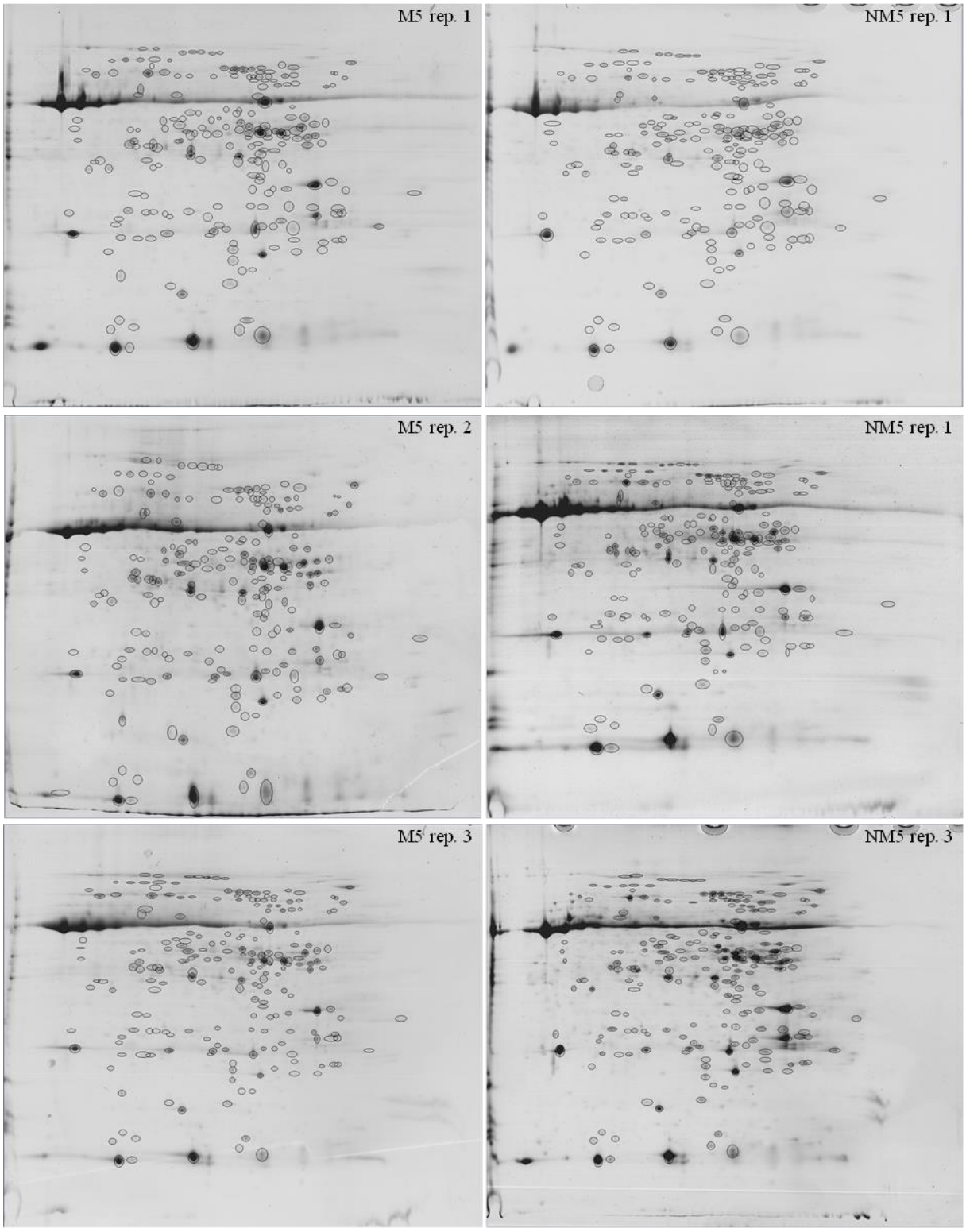
6702	A	16.18 (3)	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	M7YLI9	GR277914_4 / 8e-50	LVDAALESGK IWEDEGFNYIK IFAQGAK
		5.67 (2)	Phosphoglycerate mutase	S5TM29	DV862103_5 / 2e-46	SGYFDETK TSGEYLVK
	V	18.07 (10)	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	P30792		GWDAQVLGEAPYK RGWDAQVLGEAPYK YAGmLQYDGELK AHGTAVGLPSDDDmGNSEVGHNALGAGR TFAcSETVK DALLSGK IFAQGAK TSGEYLVK FKSALEAVK mYVTMDR
		10.89 (7)	Probable 2,3-bisphosphoglycerate-independent phosphoglycerate mutase 2	Q9M9K1		YAGmLQYDGELK YENDWSVVK TFAcSETVK DAILSGK FKSALEAVK mYVTMDR SGKPALDK
		10.75 (6)	Chaperonin CPN60-1, mitochondrial	P29185		SVAAGmNAmDLR IGGASEAEVGEK DDTVILDGAGDKK APGFGENR VTDALNATK GEYVDmVK
6729	A	15.38 (4)	Vacuolar proton-ATPase subunit A	Q1W681	FE527958_5 / 1e-116	FEDPAEGEDVLVAK LYDDLTTGFR YATALEGFYDK DALGEGDKITLETAK
	V	26.55 (13)	V-type proton ATPase catalytic subunit A (Fragment)	Q40002		TTLVANTSNmPVAAR LAADTPLLTGQR LAEmPADSGYPAYLASR FEDPAEGEDVLVAK DmGYNVSmMADSTSR YSNSDTVVYVGcGER EDDLNEIVQLVGK mGDLFYR EDYLAQNAFTPYDK VQcLGSPDR TVISQALSK LASFYER NLEDEAR
		20.71 (10)	V-type proton ATPase catalytic subunit A	P09469		TTLVANTSNmPVAAR LAADTPLLTGQR DmGYNVSmMADSTSR YSNSDTVVYVGcGER EDDLNEIVQLVGK VSGPVVVADGmGGAAmYELVR SGDVYIPR EDYLAQNAFTPYDK TVISQALSK LASFYER
		8.77 (5)	70 kDa peptidyl-prolyl isomerase	Q43207		LGQGQVIK LEDGTVVSK ITcNLNNAAcK LQDGTVFLK TDEEAVIEGLDR
		3.27 (2)	Peptidyl-prolyl cis-trans isomerase FKBP62	Q38931		LQDGTVFLK SDGVEFTVK

Annex 18 - 2D-gels from leaf soluble proteome

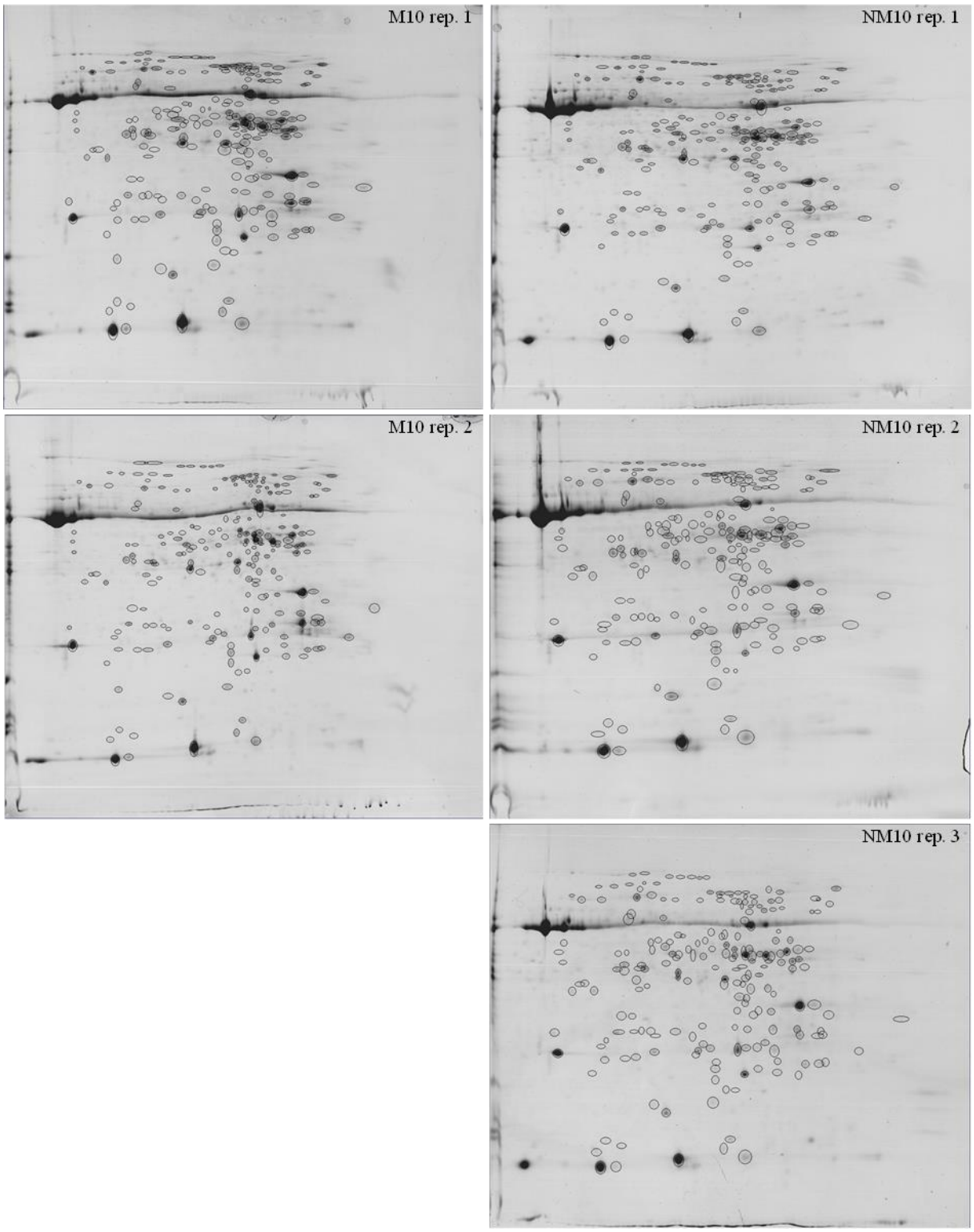
Distribution of soluble protein spots from *Agrostis capillaris* roots, for M and NM populations exposed to nine Cu exposures (1, 5, 10, 15, 20, 25, 30, 40 and 50 µM). Linear pI from 4 to 7.



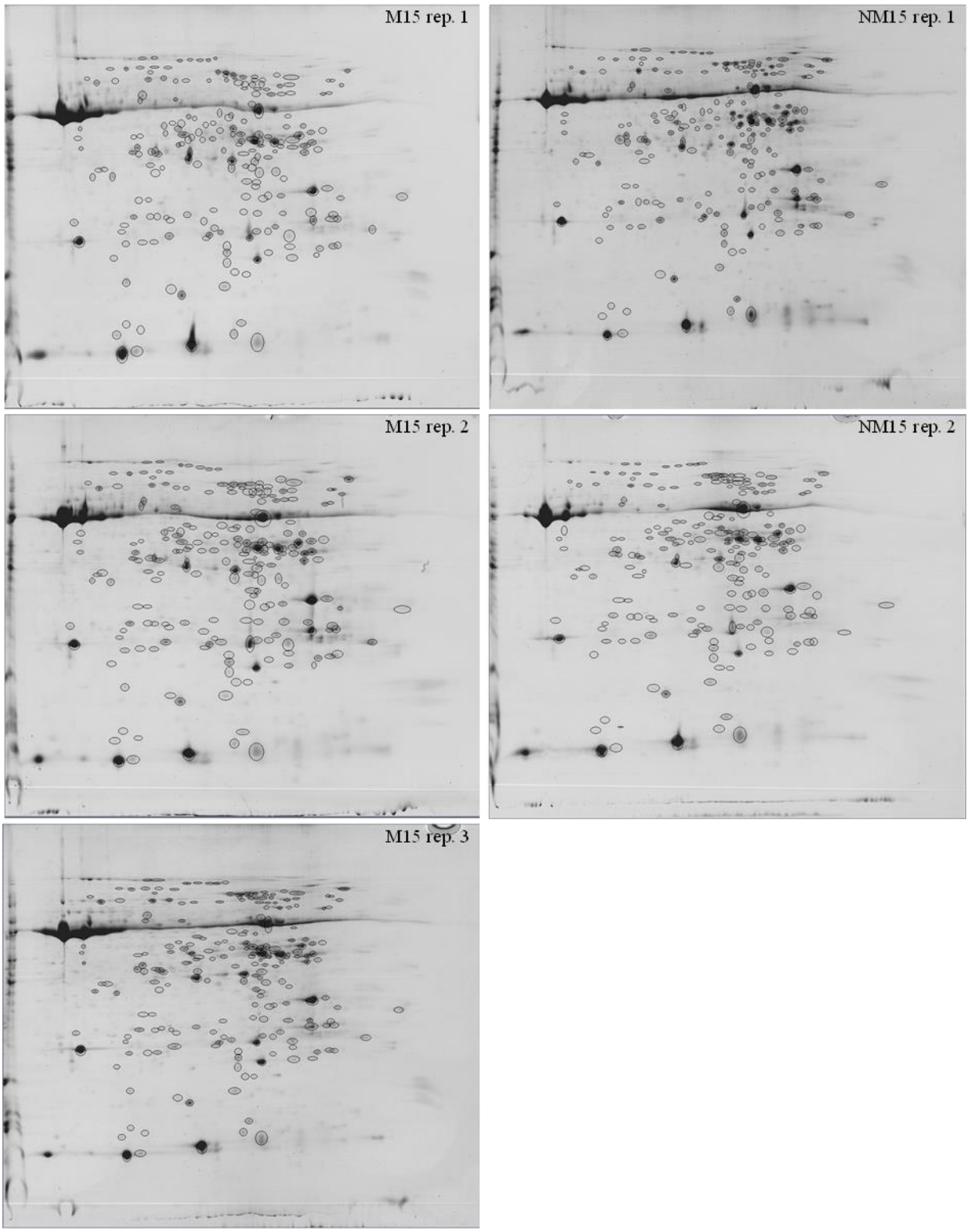
Leaf replicates at 1µM



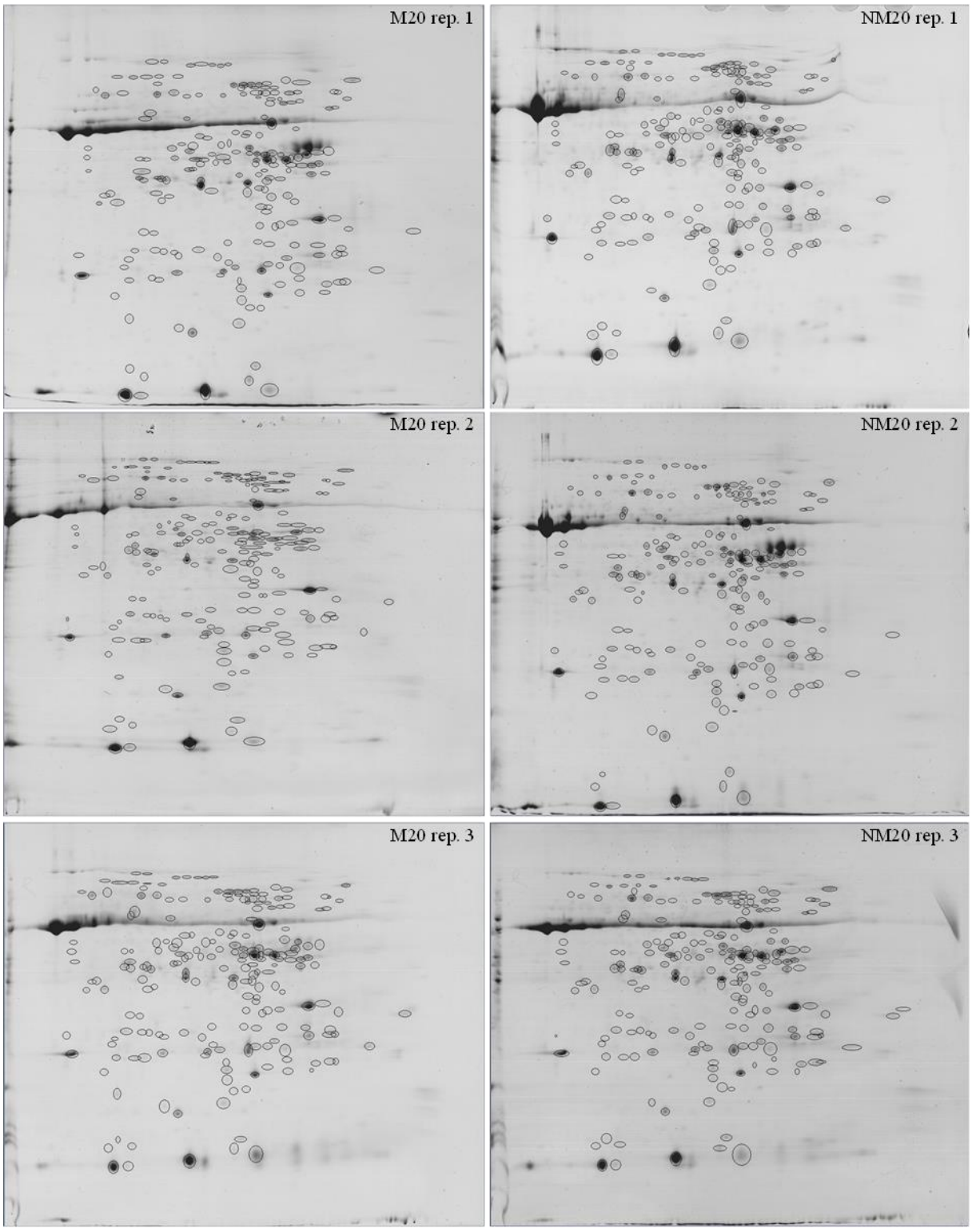
Leaf replicates at 5µM



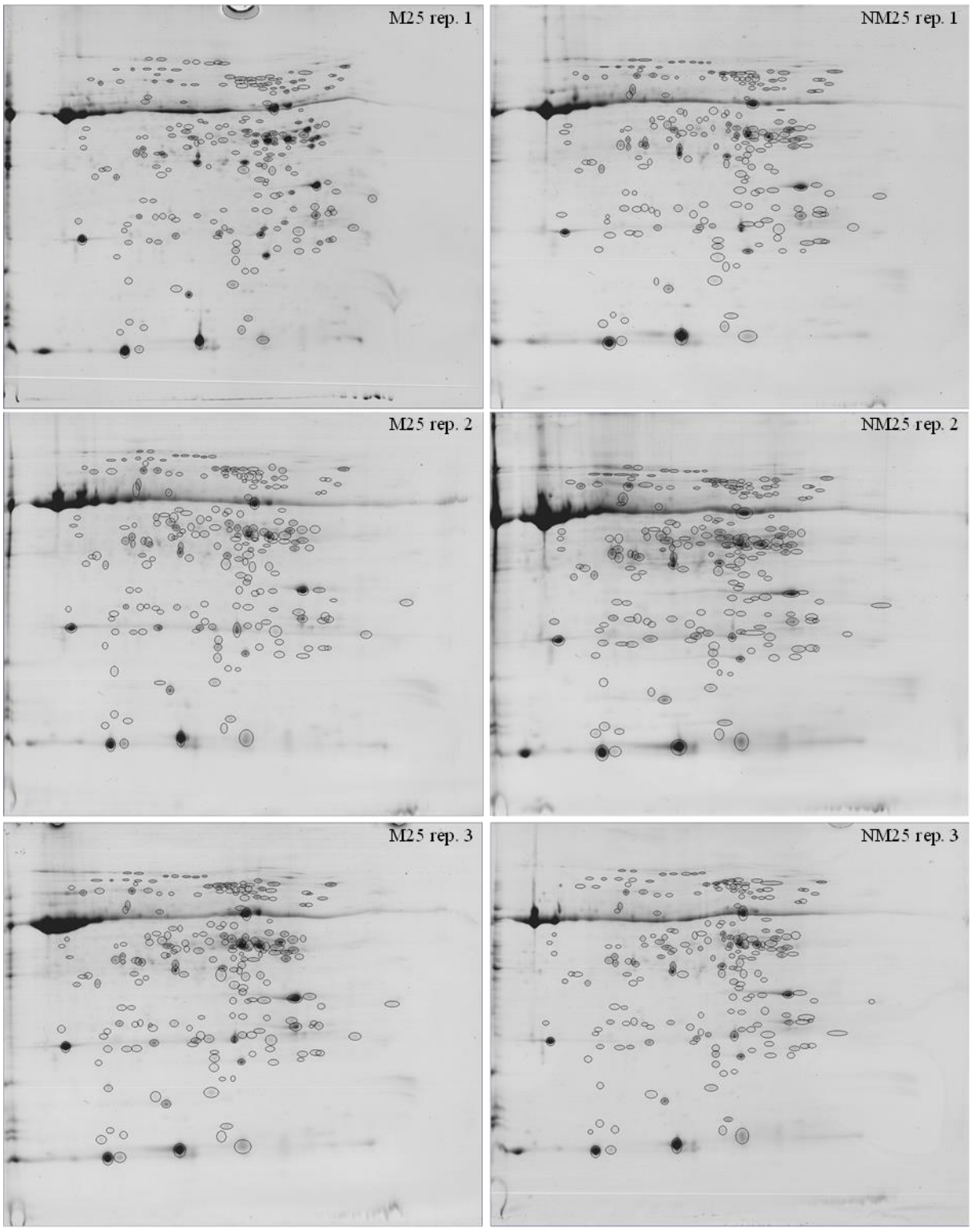
Leaf replicates at 10µM



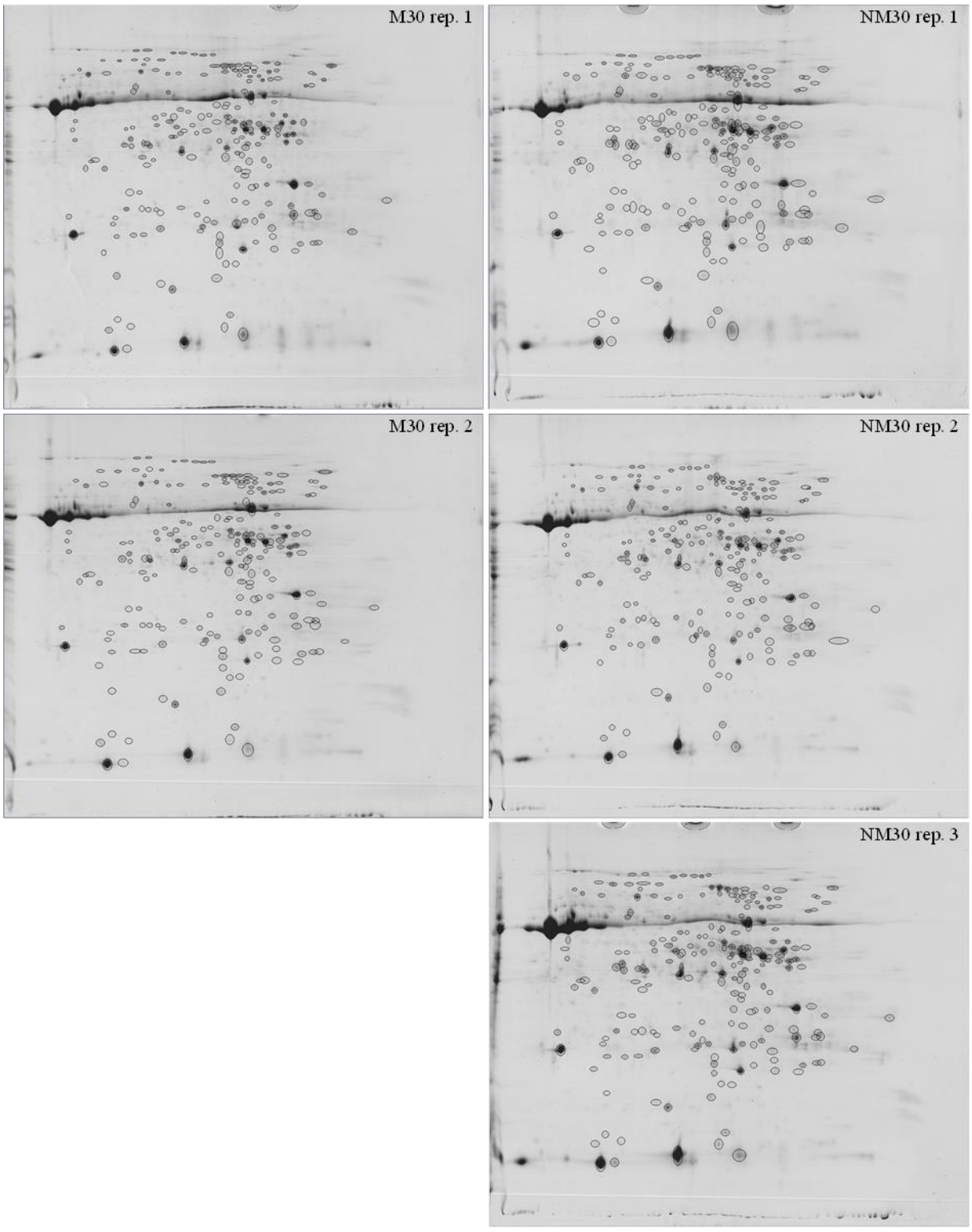
Leaf replicates at 15µM



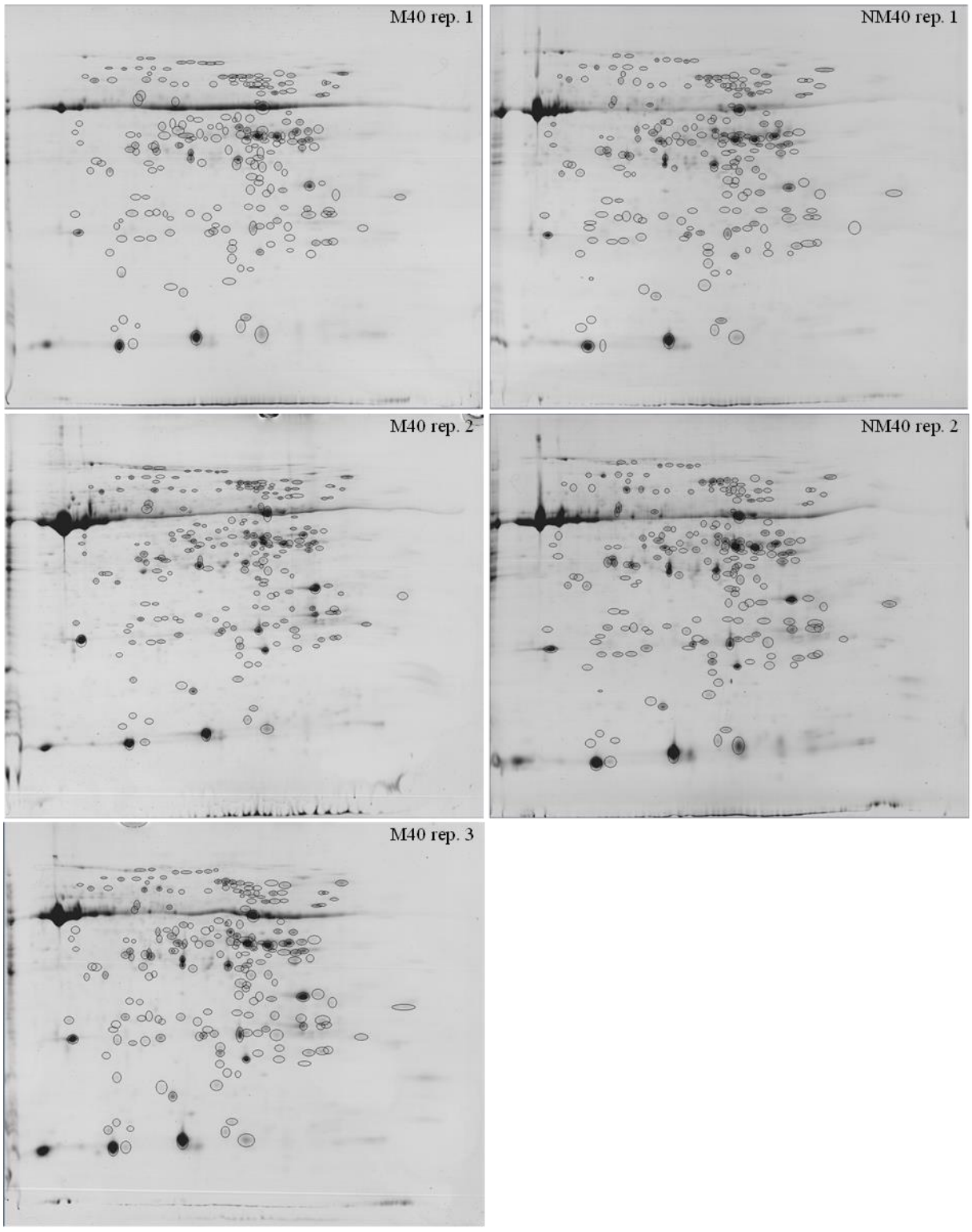
Leaf replicates at 20µM



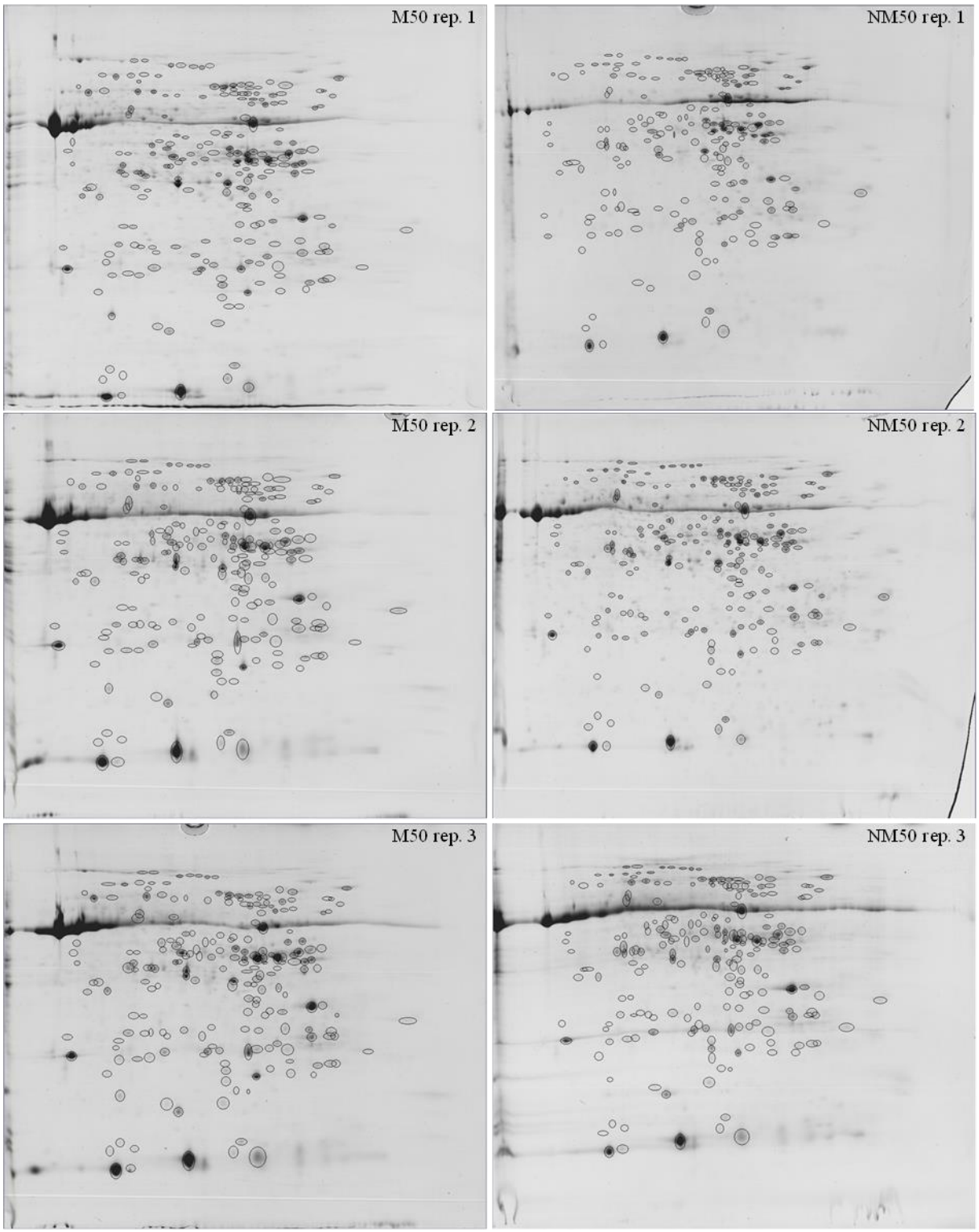
Leaf replicates at 25µM



Leaf replicates at 30µM



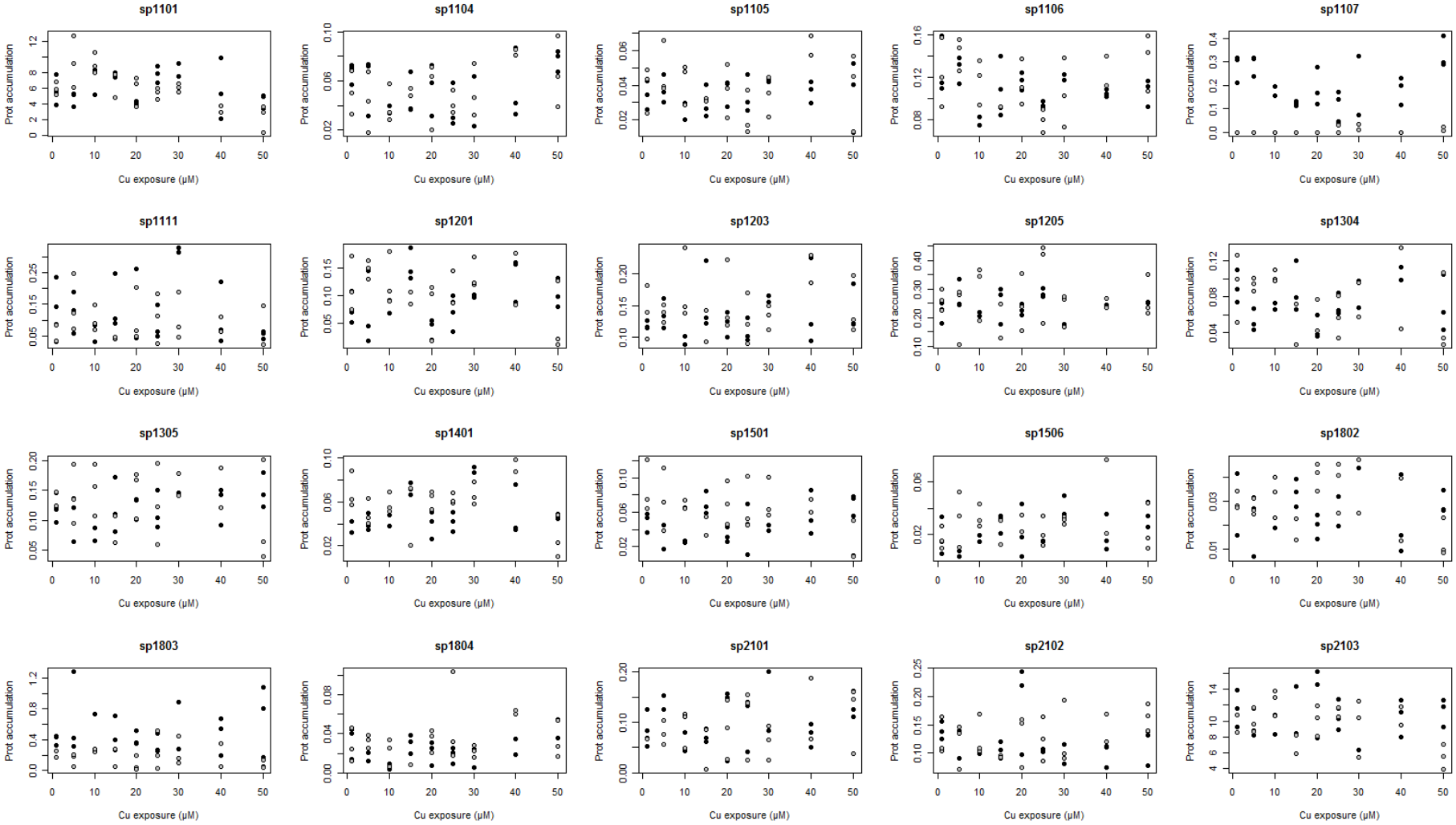
Leaf replicates at 40µM



Leaf replicates at 50µM

Annex 19 - Description of the 214 leaf spots

Spots 1101 to 2103



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

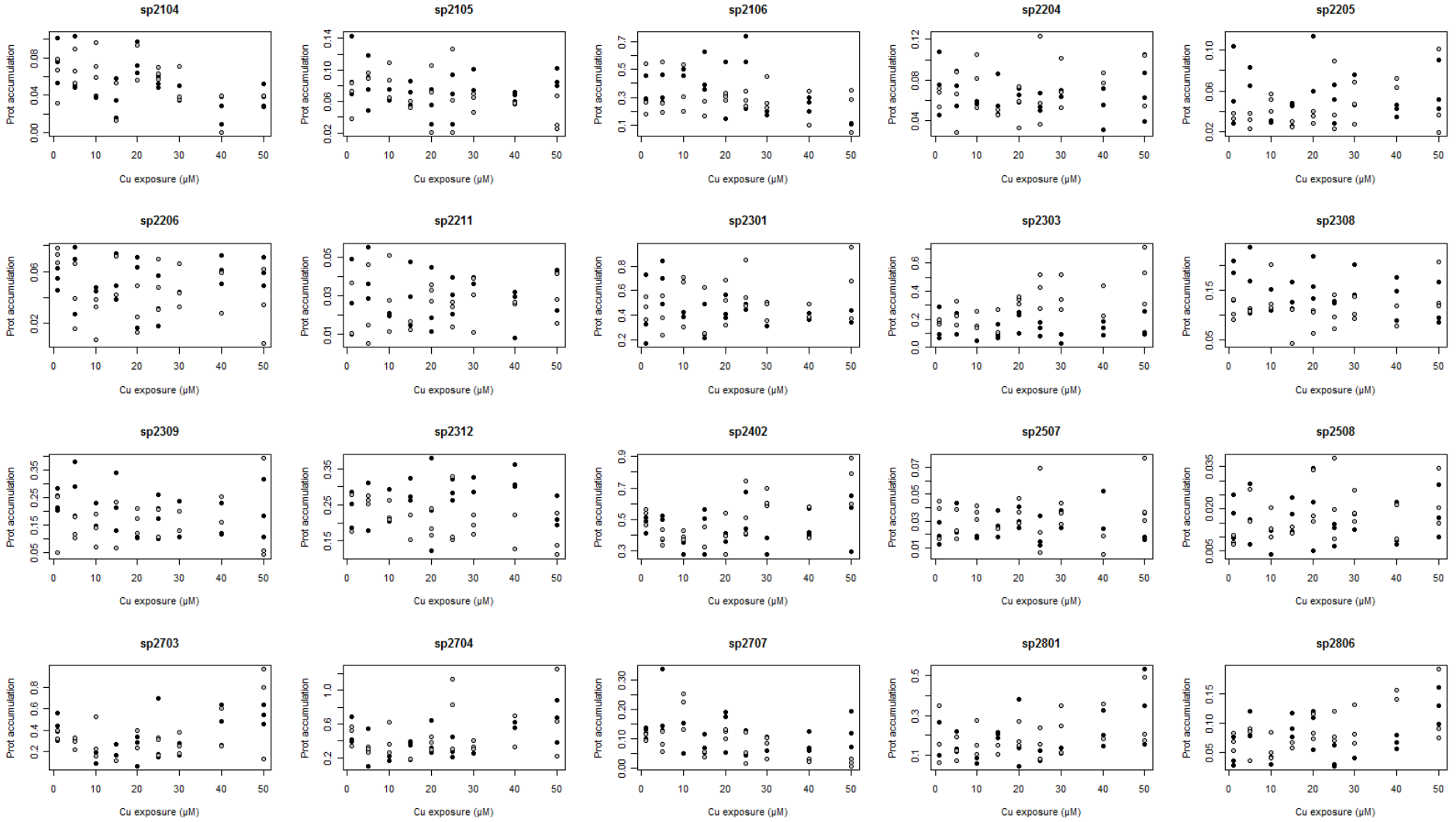
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
1101	5.709 ± 1.984	5.932 ± 0.795	4.674 ± 0.913	9.349 ± 3.344	6.731 ± 2.264	9.129 ± 1.328	7.659 ± 0.304	6.298 ± 2.154	4.05 ± 0.291	5.843 ± 1.917	7.788 ± 1.034	5.35 ± 0.712	8.393 ± 1.202	6.054 ± 0.527	5.787 ± 3.923	3.331 ± 0.531	4.438 ± 0.948	2.294 ± 1.734
1104	0.067 ± 0.009	0.05 ± 0.018	0.059 ± 0.024	0.043 ± 0.025	0.037 ± 0.004	0.04 ± 0.015	0.047 ± 0.018	0.051 ± 0.004	0.054 ± 0.021	0.051 ± 0.028	0.038 ± 0.018	0.042 ± 0.009	0.043 ± 0.028	0.051 ± 0.022	0.054 ± 0.029	0.083 ± 0.004	0.077 ± 0.009	0.066 ± 0.029
1105	0.034 ± 0.008	0.039 ± 0.013	0.038 ± 0.008	0.048 ± 0.016	0.025 ± 0.007	0.042 ± 0.012	0.03 ± 0.009	0.032 ± 0.001	0.037 ± 0.008	0.037 ± 0.016	0.034 ± 0.011	0.022 ± 0.013	0.043 ± 0.001	0.034 ± 0.011	0.036 ± 0.006	0.063 ± 0.008	0.035 ± 0.02	0.038 ± 0.022
1106	0.128 ± 0.027	0.123 ± 0.033	0.128 ± 0.013	0.143 ± 0.015	0.078 ± 0.005	0.117 ± 0.021	0.111 ± 0.028	0.091 ± 0.001	0.116 ± 0.008	0.114 ± 0.022	0.093 ± 0.003	0.079 ± 0.011	0.12 ± 0.004	0.104 ± 0.033	0.105 ± 0.004	0.126 ± 0.02	0.106 ± 0.013	0.136 ± 0.027
1107	0.279 ± 0.06	0	0.29 ± 0.045	0	0.176 ± 0.027	0	0.123 ± 0.011	0	0.19 ± 0.08	0	0.121 ± 0.065	0.022 ± 0.019	0.199 ± 0.179	0.024 ± 0.016	0.183 ± 0.058	0	0.333 ± 0.07	0.015 ± 0.01
1111	0.136 ± 0.102	0.069 ± 0.03	0.126 ± 0.066	0.148 ± 0.091	0.058 ± 0.038	0.103 ± 0.041	0.148 ± 0.086	0.043 ± 0.003	0.152 ± 0.154	0.107 ± 0.086	0.087 ± 0.053	0.108 ± 0.08	0.322 ± 0.011	0.105 ± 0.075	0.109 ± 0.099	0.087 ± 0.032	0.053 ± 0.013	0.064 ± 0.071
1201	0.077 ± 0.029	0.119 ± 0.049	0.07 ± 0.067	0.149 ± 0.017	0.08 ± 0.016	0.127 ± 0.048	0.154 ± 0.029	0.096 ± 0.015	0.041 ± 0.018	0.079 ± 0.053	0.068 ± 0.033	0.107 ± 0.034	0.1 ± 0.003	0.138 ± 0.028	0.136 ± 0.04	0.131 ± 0.066	0.104 ± 0.026	0.053 ± 0.064
1203	0.119 ± 0.006	0.139 ± 0.042	0.137 ± 0.023	0.138 ± 0.013	0.095 ± 0.009	0.175 ± 0.056	0.157 ± 0.054	0.117 ± 0.034	0.121 ± 0.02	0.157 ± 0.056	0.11 ± 0.018	0.127 ± 0.04	0.16 ± 0.007	0.132 ± 0.019	0.146 ± 0.069	0.207 ± 0.031	0.142 ± 0.036	0.146 ± 0.045
1205	0.221 ± 0.036	0.262 ± 0.037	0.275 ± 0.051	0.225 ± 0.104	0.213 ± 0.008	0.301 ± 0.097	0.252 ± 0.066	0.188 ± 0.086	0.227 ± 0.019	0.25 ± 0.1	0.286 ± 0.016	0.349 ± 0.145	0.22 ± 0.061	0.235 ± 0.06	0.242 ± 0.005	0.251 ± 0.021	0.252 ± 0.003	0.267 ± 0.074
1304	0.091 ± 0.018	0.092 ± 0.038	0.053 ± 0.012	0.094 ± 0.007	0.07 ± 0.005	0.102 ± 0.007	0.088 ± 0.028	0.049 ± 0.032	0.045 ± 0.013	0.065 ± 0.02	0.07 ± 0.012	0.058 ± 0.024	0.082 ± 0.021	0.083 ± 0.022	0.115 ± 0.018	0.09 ± 0.064	0.07 ± 0.032	0.056 ± 0.044
1305	0.111 ± 0.013	0.139 ± 0.013	0.107 ± 0.038	0.141 ± 0.05	0.076 ± 0.016	0.152 ± 0.043	0.121 ± 0.046	0.085 ± 0.032	0.123 ± 0.019	0.149 ± 0.04	0.114 ± 0.032	0.125 ± 0.067	0.145 ± 0.001	0.165 ± 0.021	0.128 ± 0.032	0.153 ± 0.047	0.148 ± 0.029	0.101 ± 0.087
1401	0.035 ± 0.006	0.069 ± 0.017	0.041 ± 0.008	0.05 ± 0.012	0.043 ± 0.007	0.058 ± 0.009	0.072 ± 0.005	0.046 ± 0.037	0.039 ± 0.012	0.062 ± 0.008	0.042 ± 0.009	0.062 ± 0.005	0.089 ± 0.003	0.067 ± 0.01	0.049 ± 0.023	0.093 ± 0.007	0.046 ± 0.002	0.027 ± 0.019
1501	0.049 ± 0.011	0.087 ± 0.03	0.045 ± 0.027	0.074 ± 0.036	0.026 ± 0.002	0.068 ± 0.005	0.07 ± 0.013	0.044 ± 0.015	0.033 ± 0.008	0.071 ± 0.025	0.042 ± 0.029	0.067 ± 0.031	0.042 ± 0.004	0.073 ± 0.024	0.057 ± 0.026	0.068 ± 0.011	0.07 ± 0.012	0.023 ± 0.024
1506	0.018 ± 0.014	0.017 ± 0.009	0.015 ± 0.017	0.032 ± 0.021	0.017 ± 0.004	0.033 ± 0.009	0.029 ± 0.007	0.021 ± 0.013	0.022 ± 0.02	0.026 ± 0.007	0.016 ± 0.003	0.022 ± 0.012	0.043 ± 0.01	0.031 ± 0.003	0.02 ± 0.014	0.049 ± 0.039	0.035 ± 0.009	0.024 ± 0.018
1802	0.028 ± 0.013	0.03 ± 0.004	0.022 ± 0.013	0.027 ± 0.003	0.021 ± 0.003	0.032 ± 0.009	0.033 ± 0.006	0.018 ± 0.006	0.019 ± 0.005	0.041 ± 0.006	0.024 ± 0.007	0.037 ± 0.011	0.046 ± 0.002	0.032 ± 0.013	0.022 ± 0.017	0.026 ± 0.018	0.029 ± 0.005	0.014 ± 0.008
1803	0.403 ± 0.071	0.196 ± 0.048	0.67 ± 0.53	0.146 ± 0.081	0.504 ± 0.332	0.257 ± 0.019	0.459 ± 0.223	0.167 ± 0.158	0.408 ± 0.094	0.083 ± 0.097	0.334 ± 0.127	0.246 ± 0.25	0.581 ± 0.436	0.236 ± 0.188	0.467 ± 0.248	0.203 ± 0.207	0.685 ± 0.462	0.075 ± 0.047
1804	0.033 ± 0.017	0.028 ± 0.018	0.018 ± 0.005	0.032 ± 0.007	0.006 ± 0.004	0.022 ± 0.014	0.03 ± 0.01	0.008	0.021 ± 0.012	0.034 ± 0.012	0.018 ± 0.008	0.051 ± 0.046	0.015 ± 0.014	0.022 ± 0.006	0.029 ± 0.009	0.062 ± 0.002	0.042 ± 0.011	0.033 ± 0.019
2101	0.087 ± 0.036	0.068	0.117 ± 0.039	0.078 ± 0.024	0.061 ± 0.025	0.092 ± 0.038	0.072 ± 0.013	0.047 ± 0.055	0.109 ± 0.074	0.087 ± 0.059	0.105 ± 0.055	0.106 ± 0.071	0.142 ± 0.082	0.061 ± 0.034	0.075 ± 0.023	0.127 ± 0.085	0.133 ± 0.026	0.114 ± 0.067
2102	0.139 ± 0.015	0.125 ± 0.033	0.125 ± 0.03	0.117 ± 0.04	0.099 ± 0.001	0.127 ± 0.036	0.106 ± 0.015	0.094 ± 0.003	0.187 ± 0.079	0.129 ± 0.048	0.098 ± 0.011	0.125 ± 0.039	0.098 ± 0.025	0.128 ± 0.057	0.099 ± 0.021	0.144 ± 0.035	0.115 ± 0.033	0.164 ± 0.023
2103	11.599 ± 2.351	10.068 ± 1.272	9.516 ± 1.846	10.005 ± 1.579	9.527 ± 1.743	12.456 ± 1.609	10.366 ± 3.499	7.056 ± 1.671	12.879 ± 4.452	10.126 ± 1.903	10.672 ± 1.978	11.277 ± 0.651	8.415 ± 2.87	9.457 ± 3.676	10.574 ± 2.377	10.673 ± 1.618	11.253 ± 1.727	5.472 ± 1.566

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
1101	Oxygen-evolving enhancer protein 2, chloroplastic	-0.05	0.80	-	-0.70	0.0001	↘↘↘↘	-	-	-	-	-	-	-	-	-
1104	50S ribosomal protein L10, chloroplastic	0.15	0.48	-	0.41	0.04	↗↗	-	-	-	-	-	-	-	-	-
1105		0.13	0.54	-	0.02	0.93	-	-	-	-	-	-	-	-	-	-
1106		-0.26	0.22	-	0.00	0.99	-	-	-	-	-	-	-	-	-	-
1107	ND	0.06	0.772	-	0.45	0.03	↗↗	M >>	M >>	M >>	M >>	M >>	-	-	M >>	M >>
1111		-0.11	0.62	-	-0.14	0.51	-	-	-	-	-	-	-	M >	-	-
1201		0.27	0.20	-	-0.34	0.10	↘	-	-	-	-	-	-	-	-	-
1203		0.21	0.31	-	0.12	0.58	-	-	-	-	-	-	-	-	-	-
1205		0.08	0.72	-	0.06	0.79	-	-	-	-	-	-	-	-	-	-
1304		0.20	0.35	-	-0.31	0.13	-	-	-	-	-	-	-	-	-	-
1305	Cysteine synthase / Malate dehydrogenase 1	0.46	0.021	↗↗	-0.12	0.58	-	-	-	-	-	-	-	-	-	-
1401		0.20	0.35	-	-0.16	0.45	-	-	-	-	-	-	-	-	-	-
1501	ND	0.30	0.14	-	-0.47	0.02	↘↘	-	-	NM >>	-	-	-	-	-	-
1506		0.36	0.078	↗	0.13	0.54	-	-	-	-	-	-	-	-	-	-
1802		0.10	0.63	-	-0.29	0.15	-	-	-	-	-	-	-	-	-	-
1803	Polyphenol oxidase EC=1.10.3.1	0.11	0.59	-	-0.15	0.48	-	-	-	-	-	M >	-	-	-	M >
1804	Methionine synthase : MetE EC=2.1.1.14	0.33	0.11	-	0.25	0.23	-	-	-	-	M >>	-	-	-	NM >	-
2101		0.20	0.33	-	0.29	0.16	-	-	-	-	-	-	-	-	-	-
2102		-0.20	0.33	-	0.35	0.08	↗	-	-	-	-	-	-	-	-	-
2103	RuBisCO small subunit EC=4.1.1.39	0.03	0.89	-	-0.43	0.034	↘↘	-	-	-	-	-	-	-	-	-

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 2104 to 2806



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

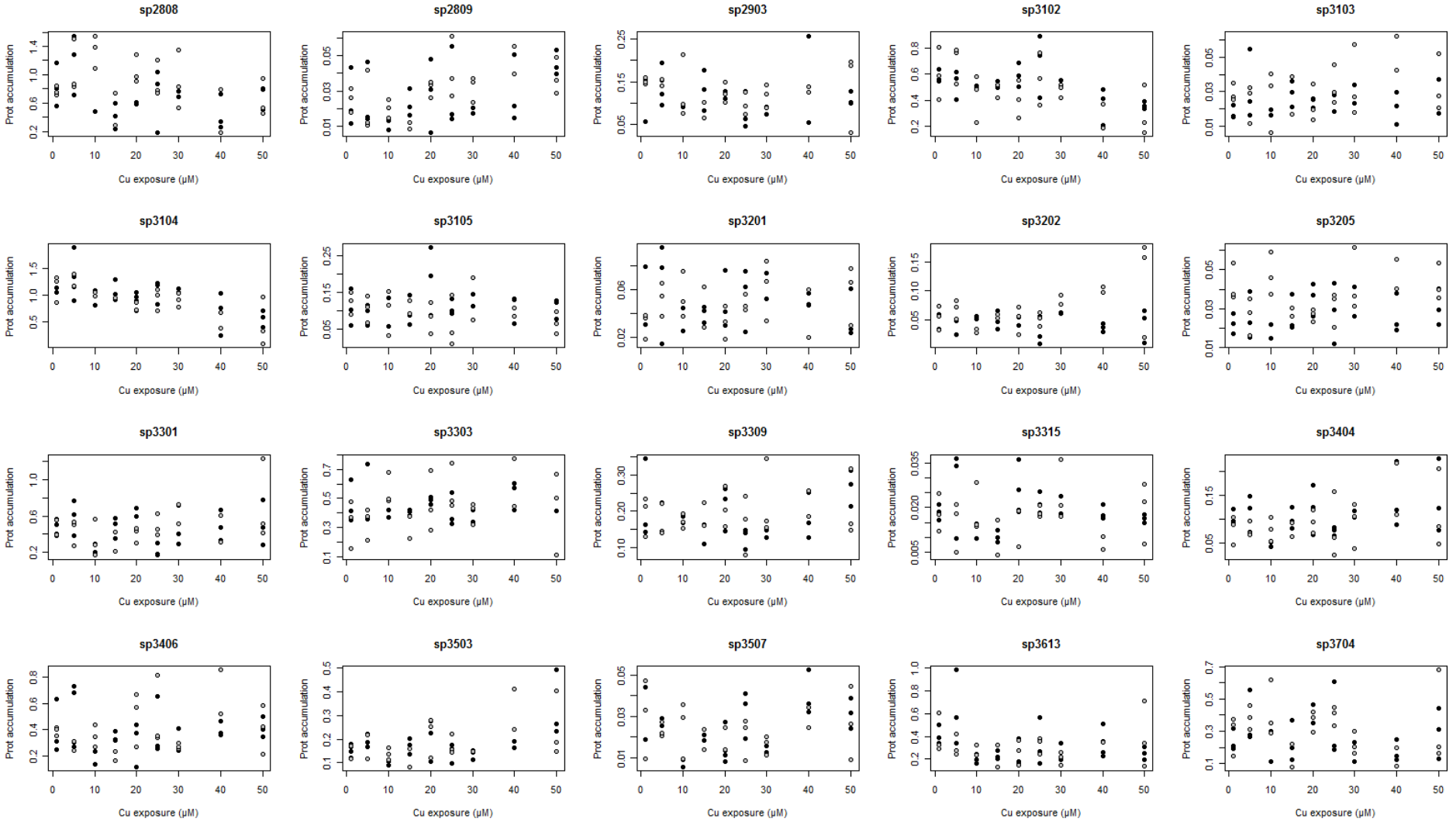
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
2104	0.076 ± 0.024	0.059 ± 0.024	0.068 ± 0.031	0.069 ± 0.019	0.038 ± 0.002	0.075 ± 0.019	0.036 ± 0.021	0.033 ± 0.029	0.078 ± 0.018	0.075 ± 0.026	0.053 ± 0.006	0.063 ± 0.006	0.042 ± 0.011	0.048 ± 0.019	0.025 ± 0.015	0.02 ± 0.028	0.036 ± 0.014	0.039
2105	0.099 ± 0.039	0.065 ± 0.024	0.081 ± 0.035	0.092 ± 0.004	0.068 ± 0.01	0.087 ± 0.023	0.071 ± 0.015	0.056 ± 0.006	0.054 ± 0.022	0.066 ± 0.043	0.065 ± 0.031	0.07 ± 0.053	0.087 ± 0.019	0.06 ± 0.012	0.067 ± 0.006	0.057 ± 0.001	0.089 ± 0.012	0.04 ± 0.023
2106	0.342 ± 0.099	0.329 ± 0.189	0.344 ± 0.106	0.336 ± 0.19	0.48 ± 0.033	0.347 ± 0.169	0.458 ± 0.147	0.219 ± 0.073	0.335 ± 0.203	0.305 ± 0.026	0.505 ± 0.262	0.285 ± 0.052	0.184 ± 0.018	0.31 ± 0.12	0.254 ± 0.051	0.221 ± 0.169	0.169 ± 0.101	0.228 ± 0.158
2204	0.076 ± 0.031	0.064 ± 0.009	0.073 ± 0.017	0.061 ± 0.03	0.058 ± 0.002	0.08 ± 0.026	0.063 ± 0.021	0.049 ± 0.004	0.065 ± 0.007	0.055 ± 0.021	0.057 ± 0.009	0.072 ± 0.045	0.067 ± 0.004	0.074 ± 0.025	0.053 ± 0.02	0.082 ± 0.008	0.063 ± 0.024	0.088 ± 0.029
2205	0.061 ± 0.039	0.035 ± 0.003	0.062 ± 0.023	0.031 ± 0.008	0.03 ± 0.001	0.049 ± 0.009	0.04 ± 0.012	0.028 ± 0.004	0.078 ± 0.031	0.034 ± 0.006	0.049 ± 0.019	0.05 ± 0.035	0.061 ± 0.021	0.048 ± 0.021	0.041 ± 0.006	0.068 ± 0.007	0.062 ± 0.025	0.052 ± 0.043
2206	0.054 ± 0.009	0.072 ± 0.006	0.058 ± 0.027	0.04 ± 0.025	0.046 ± 0.002	0.026 ± 0.017	0.054 ± 0.018	0.057 ± 0.021	0.05 ± 0.029	0.029 ± 0.018	0.035 ± 0.02	0.049 ± 0.02	0.044	0.047 ± 0.017	0.061 ± 0.011	0.043 ± 0.022	0.06 ± 0.011	0.034 ± 0.029
2211	0.037 ± 0.011	0.019 ± 0.015	0.04 ± 0.014	0.022 ± 0.021	0.021 ± 0.001	0.03 ± 0.02	0.031 ± 0.016	0.015 ± 0.003	0.025 ± 0.017	0.032 ± 0.004	0.03 ± 0.009	0.022 ± 0.007	0.038 ± 0.003	0.027 ± 0.014	0.023 ± 0.013	0.026 ± 0.001	0.036 ± 0.011	0.029 ± 0.013
2301	0.406 ± 0.292	0.46 ± 0.093	0.678 ± 0.18	0.392 ± 0.162	0.402 ± 0.024	0.561 ± 0.222	0.316 ± 0.152	0.437 ± 0.269	0.451 ± 0.103	0.507 ± 0.186	0.47 ± 0.021	0.621 ± 0.199	0.405 ± 0.134	0.45 ± 0.08	0.379 ± 0.032	0.435 ± 0.072	0.484 ± 0.175	0.668 ± 0.295
2303	0.151 ± 0.121	0.183 ± 0.017	0.164 ± 0.076	0.239 ± 0.086	0.098 ± 0.069	0.182 ± 0.063	0.107 ± 0.051	0.188 ± 0.114	0.191 ± 0.083	0.338 ± 0.03	0.132 ± 0.049	0.405 ± 0.12	0.059 ± 0.046	0.376 ± 0.126	0.137 ± 0.052	0.33 ± 0.154	0.15 ± 0.089	0.517 ± 0.204
2308	0.174 ± 0.04	0.108 ± 0.021	0.169 ± 0.067	0.109 ± 0.003	0.13 ± 0.029	0.146 ± 0.048	0.135 ± 0.027	0.077 ± 0.049	0.169 ± 0.044	0.092 ± 0.026	0.126 ± 0.002	0.103 ± 0.035	0.171 ± 0.043	0.11 ± 0.023	0.137 ± 0.044	0.098 ± 0.029	0.115 ± 0.045	0.15 ± 0.049
2309	0.234 ± 0.044	0.187 ± 0.117	0.283 ± 0.098	0.134 ± 0.04	0.188 ± 0.059	0.134 ± 0.061	0.227 ± 0.106	0.151 ± 0.118	0.128 ± 0.039	0.167 ± 0.045	0.178 ± 0.08	0.176 ± 0.059	0.172 ± 0.09	0.177 ± 0.04	0.157 ± 0.064	0.207 ± 0.068	0.201 ± 0.106	0.164 ± 0.197
2312	0.241 ± 0.05	0.245 ± 0.061	0.251 ± 0.066	0.263 ± 0.011	0.248 ± 0.062	0.229 ± 0.029	0.286 ± 0.032	0.187 ± 0.05	0.246 ± 0.129	0.197 ± 0.039	0.289 ± 0.03	0.214 ± 0.1	0.306 ± 0.028	0.195 ± 0.028	0.322 ± 0.033	0.174 ± 0.067	0.226 ± 0.043	0.158 ± 0.061
2402	0.47 ± 0.05	0.522 ± 0.052	0.463 ± 0.082	0.383 ± 0.051	0.317 ± 0.052	0.398 ± 0.031	0.45 ± 0.15	0.389 ± 0.088	0.395 ± 0.031	0.405 ± 0.132	0.507 ± 0.146	0.556 ± 0.173	0.33 ± 0.075	0.629 ± 0.06	0.463 ± 0.091	0.481 ± 0.138	0.507 ± 0.185	0.761 ± 0.147
2507	0.02 ± 0.008	0.034 ± 0.015	0.029 ± 0.012	0.026 ± 0.011	0.018 ± 0.001	0.036 ± 0.005	0.028 ± 0.01	0.024	0.032 ± 0.008	0.037 ± 0.009	0.02 ± 0.012	0.032 ± 0.033	0.031 ± 0.009	0.036 ± 0.008	0.032 ± 0.018	0.012 ± 0.01	0.023 ± 0.011	0.048 ± 0.025
2508	0.018 ± 0.008	0.009 ± 0.002	0.017 ± 0.011	0.021 ± 0.008	0.008 ± 0.006	0.014 ± 0.005	0.018 ± 0.006	0.012 ± 0.002	0.021 ± 0.015	0.022 ± 0.01	0.012 ± 0.004	0.022 ± 0.015	0.015 ± 0.004	0.02 ± 0.006	0.013 ± 0.008	0.015 ± 0.008	0.018 ± 0.009	0.023 ± 0.01
2703	0.434 ± 0.127	0.367 ± 0.04	0.281 ± 0.052	0.281 ± 0.058	0.141 ± 0.071	0.301 ± 0.198	0.198 ± 0.059	0.114 ± 0.001	0.231 ± 0.144	0.341 ± 0.089	0.336 ± 0.308	0.268 ± 0.09	0.218 ± 0.079	0.273 ± 0.099	0.456 ± 0.187	0.425 ± 0.245	0.543 ± 0.087	0.63 ± 0.441
2704	0.5 ± 0.166	0.473 ± 0.122	0.313 ± 0.224	0.3 ± 0.034	0.19 ± 0.042	0.412 ± 0.183	0.373 ± 0.025	0.178 ± 0.008	0.41 ± 0.206	0.373 ± 0.079	0.31 ± 0.126	0.756 ± 0.416	0.282 ± 0.043	0.347 ± 0.051	0.503 ± 0.152	0.513 ± 0.262	0.648 ± 0.249	0.7 ± 0.518
2707	0.121 ± 0.02	0.109 ± 0.013	0.203 ± 0.116	0.085 ± 0.035	0.102 ± 0.072	0.201 ± 0.064	0.078 ± 0.033	0.045 ± 0.012	0.138 ± 0.075	0.118 ± 0.017	0.099 ± 0.048	0.063 ± 0.055	0.082 ± 0.033	0.071 ± 0.037	0.083 ± 0.035	0.026 ± 0.008	0.127 ± 0.062	0.017 ± 0.011
2801	0.142 ± 0.108	0.188 ± 0.146	0.158 ± 0.053	0.129 ± 0.062	0.075 ± 0.02	0.177 ± 0.09	0.203 ± 0.014	0.128 ± 0.032	0.189 ± 0.173	0.197 ± 0.065	0.09 ± 0.029	0.16 ± 0.078	0.124 ± 0.02	0.238 ± 0.118	0.225 ± 0.094	0.27 ± 0.125	0.345 ± 0.189	0.291 ± 0.175
2806	0.046 ± 0.026	0.068 ± 0.014	0.092 ± 0.024	0.071 ± 0.03	0.036 ± 0.009	0.058 ± 0.023	0.094 ± 0.021	0.062 ± 0.006	0.094 ± 0.035	0.09 ± 0.022	0.039 ± 0.02	0.088 ± 0.027	0.04	0.092 ± 0.034	0.067 ± 0.012	0.148 ± 0.011	0.129 ± 0.031	0.118 ± 0.063

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

SSP	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
2104	ND	-0.51	0.009	↘↘↘	-0.46	0.03	↘↘	-	-	-	-	-	-	-	-	-
2105	Nucleoside diphosphate kinase 2 EC=2.7.4.6	-0.07	0.75	-	-0.42	0.04	↘↘	-	-	-	-	-	-	-	-	-
2106	RuBisCO small subunit EC=4.1.1.39	-0.41	0.040	↘↘	-0.28	0.18	-	-	-	-	-	-	-	-	-	-
2204		-0.27	0.20	-	0.31	0.13	-	-	-	-	-	-	-	-	-	-
2205		0.00	0.98	-	0.38	0.06	↗	-	-	-	-	-	-	-	-	-
2206		0.08	0.70	-	-0.22	0.29	-	-	-	-	-	-	-	-	-	-
2211		-0.10	0.63	-	0.17	0.42	-	-	-	-	-	-	-	-	-	-
2301		-0.10	0.62	-	0.27	0.19	-	-	-	-	-	-	-	-	-	-
2303	Bark storage protein A / Glutelin type-A 1	-0.04	0.84	-	0.69	0.0001	↗↗↗↗	-	-	-	-	-	NM >	NM >>	-	-
2308		-0.34	0.098	↘	0.17	0.42	-	-	-	-	-	-	-	-	-	-
2309		-0.30	0.15	-	0.11	0.60	-	-	-	-	-	-	-	-	-	-
2312	Putative L-ascorbate peroxidase, chloroplastic EC=1.11.1.11	0.13	0.53	-	-0.53	0.01	↘↘↘	-	-	-	-	-	-	-	-	-
2402	Fructose-bisphosphate aldolase EC=4.1.2.13	0.13	0.53	-	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
2507		0.11	0.61	-	0.13	0.52	-	-	-	NM >	-	-	-	-	-	-
2508		-0.02	0.92	-	0.35	0.091	↗	-	-	-	-	-	-	-	-	-
2703	ATP synthase sub. alpha / RuBisCO large subunit / 60 kDa chaperonin sub. Beta	0.39	0.055	↗	0.40	0.05	↗↗	-	-	-	-	-	-	-	-	-
2704	FBP aldolase / ATP synthase sub. alpha / Ketol-acid reductoisomerase	0.38	0.062	↗	0.34	0.09	↗	-	-	-	M >	-	-	-	-	-
2707	Polyphenol oxidase EC=1.10.3.1	-0.22	0.30	-	-0.59	0.002	↘↘↘	-	-	-	-	-	-	-	-	M >>
2801	Methionine synthase : MetE EC=2.1.1.14	0.45	0.025	↗↗	0.43	0.03	↗↗	-	-	-	-	-	-	-	-	-
2806	Methionine synthase : MetE EC=2.1.1.14	0.33	0.112	-	0.60	0.001	↗↗↗	-	-	-	-	-	-	-	NM >	-

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 2808 to 3704



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

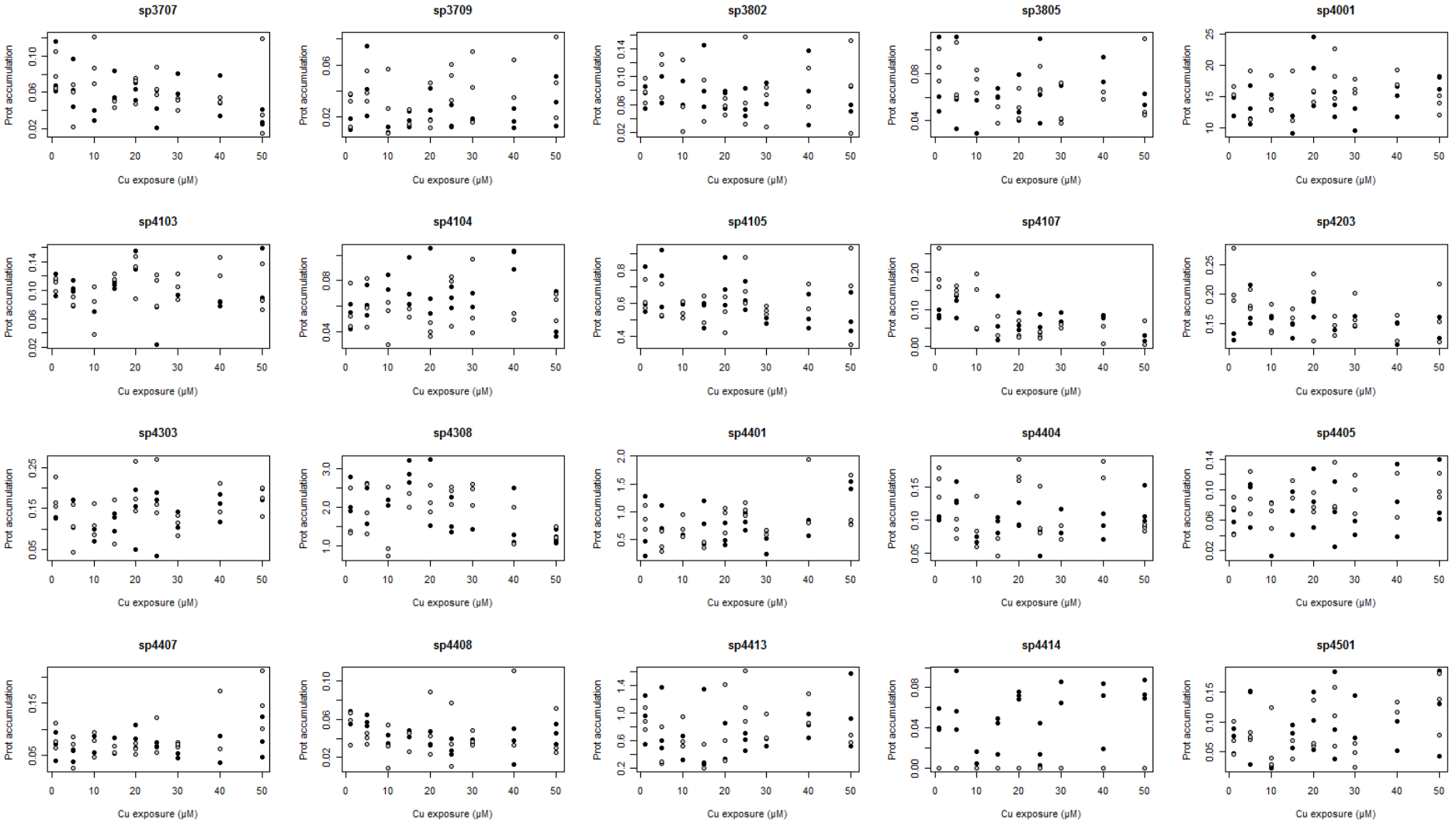
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
2808	0.846 ± 0.304	0.769 ± 0.068	1.182 ± 0.427	1.064 ± 0.378	0.48 ± 0.001	1.339 ± 0.228	0.42 ± 0.181	0.513 ± 0.321	0.7 ± 0.177	1.052 ± 0.201	0.698 ± 0.454	0.907 ± 0.262	0.725 ± 0.058	0.903 ± 0.408	0.439 ± 0.253	0.49 ± 0.434	0.701 ± 0.165	0.645 ± 0.267
2809	0.025 ± 0.016	0.025 ± 0.007	0.025 ± 0.018	0.021 ± 0.017	0.011 ± 0.004	0.02 ± 0.005	0.023 ± 0.008	0.01 ± 0.002	0.028 ± 0.021	0.031 ± 0.005	0.029 ± 0.023	0.042 ± 0.018	0.019 ± 0.002	0.032 ± 0.007	0.029 ± 0.019	0.047 ± 0.011	0.045 ± 0.007	0.038 ± 0.01
2903	0.119 ± 0.055	0.151 ± 0.008	0.138 ± 0.051	0.151 ± 0.008	0.083 ± 0.011	0.13 ± 0.074	0.13 ± 0.047	0.084 ± 0.026	0.114 ± 0.013	0.124 ± 0.024	0.079 ± 0.042	0.099 ± 0.026	0.082 ± 0.012	0.118 ± 0.028	0.146 ± 0.102	0.132 ± 0.008	0.11 ± 0.016	0.138 ± 0.092
3102	0.578 ± 0.049	0.601 ± 0.202	0.53 ± 0.112	0.69 ± 0.143	0.506 ± 0.004	0.433 ± 0.181	0.515 ± 0.028	0.469 ± 0.071	0.591 ± 0.092	0.409 ± 0.143	0.686 ± 0.241	0.565 ± 0.198	0.486 ± 0.091	0.48 ± 0.051	0.367 ± 0.142	0.281 ± 0.128	0.358 ± 0.028	0.301 ± 0.194
3103	0.018 ± 0.004	0.029 ± 0.005	0.032 ± 0.02	0.024 ± 0.011	0.018 ± 0.002	0.027 ± 0.018	0.029 ± 0.007	0.028 ± 0.015	0.024 ± 0.003	0.023 ± 0.011	0.025 ± 0.006	0.033 ± 0.011	0.029 ± 0.007	0.034 ± 0.02	0.021 ± 0.009	0.052 ± 0.014	0.025 ± 0.01	0.034 ± 0.016
3104	1.149 ± 0.099	1.149 ± 0.253	1.377 ± 0.492	1.241 ± 0.134	0.949 ± 0.201	1.006 ± 0.034	1.078 ± 0.193	0.942 ± 0.021	0.971 ± 0.07	0.765 ± 0.083	1.079 ± 0.214	0.942 ± 0.197	1.086 ± 0.048	0.911 ± 0.127	0.69 ± 0.395	0.532 ± 0.214	0.569 ± 0.149	0.463 ± 0.447
3105	0.109 ± 0.05	0.123 ± 0.03	0.091 ± 0.028	0.106 ± 0.035	0.097 ± 0.055	0.101 ± 0.061	0.098 ± 0.041	0.111 ± 0.024	0.185 ± 0.093	0.083 ± 0.042	0.109 ± 0.022	0.065 ± 0.069	0.129 ± 0.022	0.114 ± 0.066	0.11 ± 0.038	0.097 ± 0.016	0.11 ± 0.027	0.068 ± 0.03
3201	0.049 ± 0.026	0.031 ± 0.011	0.063 ± 0.042	0.053 ± 0.014	0.035 ± 0.014	0.054 ± 0.019	0.04 ± 0.007	0.046 ± 0.024	0.049 ± 0.024	0.033 ± 0.013	0.054 ± 0.026	0.049 ± 0.007	0.063 ± 0.015	0.062 ± 0.025	0.051 ± 0.005	0.04 ± 0.028	0.037 ± 0.02	0.058 ± 0.025
3202	0.043 ± 0.015	0.054 ± 0.02	0.032 ± 0.013	0.069 ± 0.016	0.053 ± 0.004	0.03 ± 0.004	0.049 ± 0.016	0.056 ± 0.005	0.045 ± 0.008	0.051 ± 0.024	0.028 ± 0.025	0.052 ± 0.012	0.061 ± 0.001	0.082 ± 0.009	0.037 ± 0.008	0.103 ± 0.007	0.043 ± 0.029	0.118 ± 0.086
3205	0.022 ± 0.005	0.042 ± 0.01	0.025 ± 0.012	0.027 ± 0.009	0.018 ± 0.005	0.048 ± 0.011	0.026 ± 0.009	0.028 ± 0.003	0.035 ± 0.009	0.027 ± 0.003	0.028 ± 0.016	0.031 ± 0.009	0.034 ± 0.011	0.043 ± 0.016	0.026 ± 0.01	0.048 ± 0.011	0.03 ± 0.009	0.043 ± 0.009
3301	0.491 ± 0.086	0.448 ± 0.097	0.586 ± 0.192	0.439 ± 0.146	0.249 ± 0.064	0.341 ± 0.199	0.482 ± 0.116	0.321 ± 0.148	0.574 ± 0.125	0.403 ± 0.089	0.22 ± 0.077	0.494 ± 0.121	0.348 ± 0.078	0.651 ± 0.122	0.494 ± 0.168	0.459 ± 0.205	0.512 ± 0.248	0.718 ± 0.442
3303	0.466 ± 0.143	0.334 ± 0.164	0.61 ± 0.217	0.336 ± 0.108	0.395 ± 0.039	0.552 ± 0.111	0.403 ± 0.019	0.303 ± 0.107	0.484 ± 0.026	0.465 ± 0.211	0.409 ± 0.114	0.561 ± 0.16	0.38 ± 0.059	0.404 ± 0.072	0.532 ± 0.097	0.61 ± 0.231	0.475 ± 0.051	0.426 ± 0.288
3309	0.217 ± 0.11	0.192 ± 0.054	0.196 ± 0.048	0.169 ± 0.045	0.177 ± 0.012	0.173 ± 0.02	0.166 ± 0.056	0.192 ± 0.045	0.214 ± 0.061	0.21 ± 0.055	0.128 ± 0.029	0.166 ± 0.082	0.137 ± 0.015	0.224 ± 0.104	0.182 ± 0.063	0.22 ± 0.05	0.266 ± 0.05	0.209 ± 0.093
3315	0.018 ± 0.003	0.018 ± 0.006	0.027 ± 0.015	0.015 ± 0.009	0.012 ± 0.003	0.019 ± 0.008	0.01 ± 0.002	0.01 ± 0.008	0.027 ± 0.009	0.015 ± 0.007	0.022 ± 0.004	0.019 ± 0.002	0.021 ± 0.004	0.025 ± 0.01	0.018 ± 0.002	0.008 ± 0.003	0.016 ± 0.001	0.019 ± 0.01
3404	0.104 ± 0.014	0.08 ± 0.031	0.114 ± 0.038	0.078 ± 0.016	0.047 ± 0.007	0.079 ± 0.025	0.101 ± 0.022	0.079 ± 0.021	0.121 ± 0.052	0.095 ± 0.024	0.075 ± 0.008	0.082 ± 0.068	0.111 ± 0.009	0.092 ± 0.048	0.143 ± 0.069	0.163 ± 0.077	0.142 ± 0.076	0.113 ± 0.082
3406	0.396 ± 0.204	0.391 ± 0.035	0.559 ± 0.253	0.287 ± 0.037	0.187 ± 0.065	0.35 ± 0.081	0.342 ± 0.038	0.201 ± 0.05	0.311 ± 0.169	0.501 ± 0.208	0.397 ± 0.222	0.503 ± 0.267	0.326 ± 0.117	0.274 ± 0.02	0.399 ± 0.055	0.685 ± 0.234	0.416 ± 0.079	0.409 ± 0.186
3503	0.15 ± 0.03	0.151 ± 0.031	0.191 ± 0.027	0.152 ± 0.058	0.097 ± 0.012	0.138 ± 0.026	0.172 ± 0.033	0.122 ± 0.055	0.201 ± 0.087	0.217 ± 0.084	0.144 ± 0.04	0.175 ± 0.042	0.13 ± 0.021	0.148 ± 0.004	0.2 ± 0.039	0.326 ± 0.122	0.33 ± 0.14	0.246 ± 0.139
3507	0.027 ± 0.015	0.03 ± 0.019	0.028 ± 0.002	0.023 ± 0.004	0.007 ± 0.002	0.025 ± 0.014	0.021 ± 0.003	0.019 ± 0.007	0.016 ± 0.01	0.017 ± 0.006	0.032 ± 0.012	0.02 ± 0.01	0.014 ± 0.002	0.016 ± 0.005	0.04 ± 0.011	0.029 ± 0.007	0.032 ± 0.007	0.027 ± 0.018
3613	0.408 ± 0.081	0.407 ± 0.172	0.628 ± 0.325	0.313 ± 0.095	0.179 ± 0.022	0.269 ± 0.05	0.235 ± 0.038	0.227 ± 0.132	0.241 ± 0.121	0.264 ± 0.109	0.332 ± 0.211	0.328 ± 0.071	0.267 ± 0.103	0.21 ± 0.057	0.334 ± 0.155	0.353 ± 0.004	0.252 ± 0.058	0.395 ± 0.29
3704	0.238 ± 0.069	0.286 ± 0.124	0.365 ± 0.164	0.385 ± 0.073	0.204 ± 0.133	0.419 ± 0.175	0.229 ± 0.127	0.15 ± 0.099	0.372 ± 0.088	0.366 ± 0.066	0.335 ± 0.239	0.398 ± 0.059	0.173 ± 0.083	0.221 ± 0.071	0.174 ± 0.067	0.139 ± 0.08	0.295 ± 0.157	0.349 ± 0.287

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

SSP	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
2808	Polyphenol oxidase : PPO EC=1.10.3.1	-0.29	0.16	-	-0.36	0.08	↘	-	-	NM >>	-	-	-	-	-	-
2809	GTP-binding protein TypA	0.37	0.065	↗	0.55	0.005	↗↗↗	-	-	-	-	-	-	-	-	-
2903		-0.03	0.89	-	-0.10	0.64	-	-	-	-	-	-	-	-	-	-
3102	ND	-0.47	0.019	↘↘	-0.54	0.01	↘↘↘	-	-	-	-	-	-	-	-	-
3103		0.01	0.95	-	0.34	0.10	↗	-	-	-	-	-	-	-	-	-
3104	Cytochrome b6-f complex Fe/S subunit EC=1.10.9.1	-0.65	0.0005	↘↘↘↘	-0.75	< 0.0001	↘↘↘↘	-	-	-	-	-	-	-	-	-
3105		0.08	0.72	-	-0.29	0.16	-	-	-	-	-	-	-	-	-	-
3201		-0.10	0.64	-	0.23	0.28	-	-	-	-	-	-	-	-	-	-
3202	ND	0.00	0.98	-	0.56	0.004	↗↗↗	-	-	M >	-	-	-	-	NM >>	-
3205		0.25	0.23	-	0.22	0.29	-	-	-	NM >	-	-	-	-	-	-
3301	ATP synthase subunit gamma / Malate dehydrogenase	-0.03	0.87	-	0.44	0.026	↗↗	-	-	-	-	-	-	-	-	-
3303		-0.05	0.83	-	0.23	0.28	-	-	-	-	-	-	-	-	-	-
3309		0.13	0.53	-	0.21	0.30	-	-	-	-	-	-	-	-	-	-
3315		-0.09	0.67	-	0.05	0.80	-	-	-	-	-	-	-	-	M >	-
3404		0.34	0.092	↗	0.36	0.08	↗	-	-	-	-	-	-	-	-	-
3406		-0.02	0.94	-	0.28	0.17	-	-	-	-	-	-	-	-	-	-
3503	Isocitrate dehydrogenase [NADP], chloro. EC=1.1.1.42	0.50	0.010	↗↗	0.49	0.01	↗↗	-	-	-	-	-	-	-	-	-
3507		0.34	0.098	↗	-0.02	0.94	-	-	-	-	-	-	-	-	-	-
3613		-0.30	0.14	-	0.06	0.77	-	-	-	-	-	-	-	-	-	-
3704		-0.10	0.64	-	-0.15	0.47	-	-	-	-	-	-	-	-	-	-

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 3707 to 4501



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

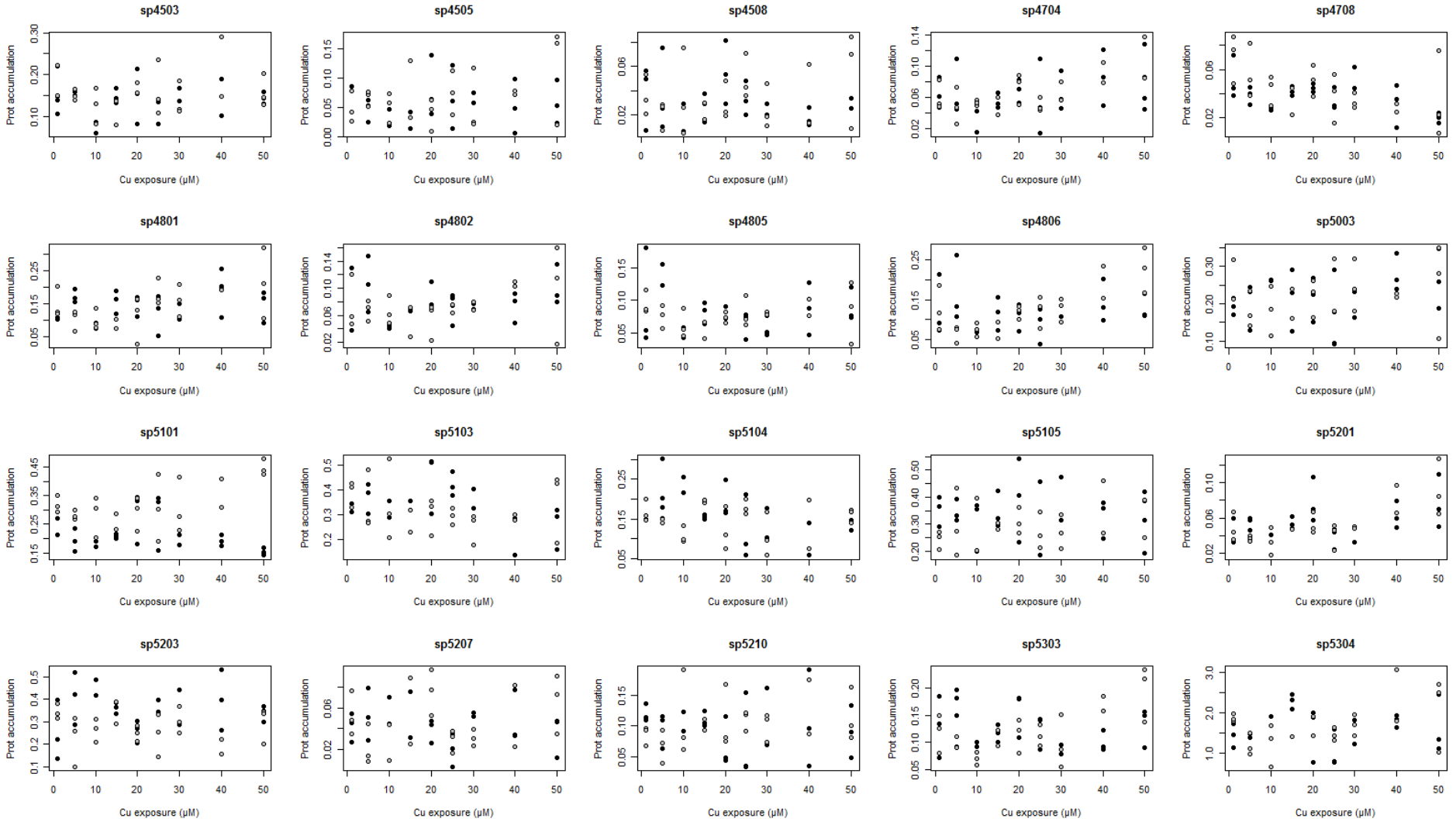
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
3707	0.082 ± 0.03	0.082 ± 0.02	0.068 ± 0.026	0.05 ± 0.025	0.035 ± 0.008	0.092 ± 0.027	0.063 ± 0.017	0.047 ± 0.005	0.062 ± 0.01	0.065 ± 0.016	0.042 ± 0.021	0.069 ± 0.016	0.07 ± 0.016	0.048 ± 0.007	0.054 ± 0.023	0.052 ± 0.003	0.031 ± 0.009	0.056 ± 0.055
3709	0.022 ± 0.014	0.027 ± 0.013	0.045 ± 0.027	0.042 ± 0.012	0.01 ± 0.003	0.03 ± 0.025	0.018 ± 0.006	0.02 ± 0.008	0.028 ± 0.012	0.025 ± 0.019	0.018 ± 0.01	0.048 ± 0.014	0.018	0.043 ± 0.027	0.018 ± 0.008	0.049 ± 0.021	0.032 ± 0.019	0.049 ± 0.032
3802	0.072 ± 0.016	0.079 ± 0.017	0.077 ± 0.02	0.107 ± 0.032	0.077 ± 0.024	0.067 ± 0.052	0.093 ± 0.046	0.065 ± 0.042	0.07 ± 0.014	0.057 ± 0.012	0.06 ± 0.021	0.084 ± 0.066	0.076 ± 0.022	0.062 ± 0.031	0.082 ± 0.054	0.085 ± 0.039	0.065 ± 0.019	0.086 ± 0.067
3805	0.073 ± 0.034	0.087 ± 0.014	0.067 ± 0.04	0.076 ± 0.027	0.043 ± 0.02	0.074 ± 0.01	0.063 ± 0.004	0.045 ± 0.01	0.055 ± 0.021	0.053 ± 0.013	0.07 ± 0.037	0.073 ± 0.012	0.055 ± 0.02	0.05 ± 0.019	0.075 ± 0.018	0.061 ± 0.005	0.054 ± 0.009	0.067 ± 0.037
4001	14.007 ± 1.818	16.169 ± 0.783	13.502 ± 3.143	13.982 ± 4.403	14.189 ± 1.683	15.359 ± 2.862	10.79 ± 1.446	15.157 ± 5.587	19.229 ± 5.543	15.24 ± 0.984	13.757 ± 2.003	18.55 ± 3.92	11.359 ± 2.562	16.538 ± 1.148	14.509 ± 2.501	18.148 ± 1.67	17.504 ± 1.167	13.739 ± 1.516
4103	0.109 ± 0.016	0.109 ± 0.009	0.105 ± 0.008	0.083 ± 0.007	0.077 ± 0.01	0.075 ± 0.034	0.107 ± 0.004	0.119 ± 0.005	0.138 ± 0.015	0.123 ± 0.031	0.059 ± 0.031	0.104 ± 0.023	0.09 ± 0.004	0.105 ± 0.018	0.081 ± 0.003	0.133 ± 0.018	0.107 ± 0.046	0.099 ± 0.034
4104	0.053 ± 0.01	0.058 ± 0.018	0.063 ± 0.012	0.061 ± 0.019	0.078 ± 0.008	0.049 ± 0.018	0.077 ± 0.019	0.054 ± 0.005	0.075 ± 0.027	0.041 ± 0.006	0.067 ± 0.008	0.069 ± 0.022	0.065 ± 0.008	0.062 ± 0.03	0.098 ± 0.008	0.052 ± 0.004	0.049 ± 0.02	0.061 ± 0.011
4105	0.655 ± 0.143	0.637 ± 0.093	0.755 ± 0.172	0.586 ± 0.111	0.551 ± 0.055	0.552 ± 0.051	0.544 ± 0.082	0.562 ± 0.118	0.716 ± 0.148	0.535 ± 0.108	0.635 ± 0.088	0.714 ± 0.144	0.492 ± 0.025	0.557 ± 0.025	0.536 ± 0.108	0.641 ± 0.105	0.529 ± 0.123	0.661 ± 0.295
4107	0.087 ± 0.011	0.203 ± 0.055	0.112 ± 0.031	0.153 ± 0.011	0.048 ± 0.003	0.133 ± 0.075	0.069 ± 0.06	0.056 ± 0.037	0.064 ± 0.025	0.04 ± 0.025	0.059 ± 0.026	0.028 ± 0.009	0.079 ± 0.017	0.056 ± 0.006	0.056 ± 0.043	0.03 ± 0.034	0.017 ± 0.013	0.025 ± 0.037
4203	0.151 ± 0.042	0.222 ± 0.05	0.174 ± 0.036	0.188 ± 0.017	0.161 ± 0.002	0.151 ± 0.028	0.14 ± 0.015	0.167 ± 0.011	0.18 ± 0.018	0.185 ± 0.06	0.141 ± 0.005	0.146 ± 0.017	0.153 ± 0.013	0.168 ± 0.03	0.138 ± 0.022	0.141 ± 0.031	0.134 ± 0.023	0.163 ± 0.051
4303	0.139 ± 0.021	0.182 ± 0.039	0.149 ± 0.039	0.103 ± 0.059	0.084 ± 0.021	0.119 ± 0.038	0.121 ± 0.023	0.117 ± 0.075	0.134 ± 0.076	0.195 ± 0.063	0.131 ± 0.085	0.189 ± 0.07	0.123 ± 0.027	0.11 ± 0.025	0.154 ± 0.034	0.176 ± 0.049	0.18 ± 0.015	0.168 ± 0.035
4308	2.244 ± 0.489	1.752 ± 0.662	2.238 ± 0.569	1.937 ± 0.644	2.129 ± 0.09	1.402 ± 0.988	2.91 ± 0.281	2.194 ± 0.248	2.223 ± 0.902	2.194 ± 0.35	1.712 ± 0.486	2.346 ± 0.236	1.971 ± 0.737	2.385 ± 0.289	1.632 ± 0.756	1.532 ± 0.683	1.222 ± 0.181	1.333 ± 0.164
4401	0.641 ± 0.564	0.884 ± 0.215	0.697 ± 0.413	0.429 ± 0.199	0.571 ± 0.021	0.721 ± 0.201	0.791 ± 0.389	0.396 ± 0.078	0.555 ± 0.208	0.887 ± 0.243	0.813 ± 0.161	1.037 ± 0.119	0.376 ± 0.198	0.631 ± 0.039	0.742 ± 0.16	1.368 ± 0.806	1.238 ± 0.409	1.094 ± 0.493
4404	0.103 ± 0.003	0.159 ± 0.022	0.138 ± 0.018	0.086 ± 0.014	0.071 ± 0.006	0.093 ± 0.039	0.095 ± 0.012	0.059 ± 0.019	0.104 ± 0.019	0.172 ± 0.017	0.072 ± 0.023	0.106 ± 0.039	0.099 ± 0.026	0.077 ± 0.012	0.091 ± 0.02	0.177 ± 0.017	0.119 ± 0.03	0.089 ± 0.005
4405	0.058 ± 0.015	0.069 ± 0.026	0.087 ± 0.032	0.093 ± 0.028	0.048 ± 0.05	0.068 ± 0.016	0.07 ± 0.028	0.1 ± 0.016	0.087 ± 0.039	0.081 ± 0.013	0.069 ± 0.043	0.097 ± 0.034	0.05 ± 0.013	0.096 ± 0.026	0.085 ± 0.048	0.093 ± 0.041	0.09 ± 0.043	0.104 ± 0.016
4407	0.067 ± 0.028	0.084 ± 0.025	0.052 ± 0.012	0.06 ± 0.031	0.071 ± 0.023	0.073 ± 0.024	0.068 ± 0.016	0.061 ± 0.009	0.084 ± 0.023	0.063 ± 0.011	0.069 ± 0.005	0.077 ± 0.038	0.049 ± 0.007	0.07 ± 0.005	0.062 ± 0.026	0.118 ± 0.078	0.082 ± 0.04	0.152 ± 0.056
4408	0.052 ± 0.018	0.052 ± 0.018	0.058 ± 0.006	0.04 ± 0.006	0.039 ± 0.006	0.032 ± 0.022	0.045 ± 0.003	0.035 ± 0.014	0.038 ± 0.008	0.052 ± 0.034	0.03 ± 0.009	0.04 ± 0.034	0.037 ± 0.003	0.039 ± 0.008	0.034 ± 0.019	0.072 ± 0.055	0.045 ± 0.01	0.042 ± 0.025
4413	0.912 ± 0.352	0.901 ± 0.156	0.819 ± 0.481	0.45 ± 0.301	0.49 ± 0.244	0.682 ± 0.232	0.624 ± 0.628	0.369 ± 0.24	0.862 ± 0.545	0.769 ± 0.574	0.586 ± 0.126	1.183 ± 0.379	0.568 ± 0.076	0.744 ± 0.201	0.812 ± 0.173	1.061 ± 0.306	1.001 ± 0.53	0.641 ± 0.061
4414	0.046 ± 0.012	0	0.064 ± 0.03	0	0.01 ± 0.009	0	0.036 ± 0.019	0	0.072 ± 0.003	0	0.02 ± 0.022	0	0.075 ± 0.014	0	0.058 ± 0.035		0.077 ± 0.009	
4501	0.071 ± 0.021	0.071 ± 0.028	0.111 ± 0.07	0.077 ± 0.006	0.024 ± 0.001	0.064 ± 0.052	0.077 ± 0.019	0.053 ± 0.022	0.102 ± 0.048	0.087 ± 0.043	0.102 ± 0.074	0.109 ± 0.048	0.109 ± 0.05	0.046 ± 0.02	0.095 ± 0.04	0.125 ± 0.012	0.119 ± 0.072	0.132 ± 0.052

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
3707	Succinate dehydrogenase [Ubi] flavoprotein subunit 1, mito.	-0.46	0.022	↘↘	-0.27	0.19	-	-	-	NM >	-	-	-	-	-	-
3709		-0.06	0.772	-	0.34	0.09	↗	-	-	-	-	-	-	-	-	-
3802		-0.09	0.67	-	-0.02	0.93	-	-	-	-	-	-	-	-	-	-
3805		-0.06	0.79	-	-0.27	0.19	-	-	-	-	-	-	-	-	-	-
4001		0.25	0.22	-	0.05	0.82	-	-	-	-	-	-	-	-	-	-
4103		-0.15	0.49	-	0.22	0.29	-	-	-	-	-	-	-	-	-	-
4104		0.10	0.65	-	0.08	0.71	-	-	-	-	-	-	-	-	M >	-
4105	Ribulose-phosphate 3-epimerase EC=5.1.3.1	-0.40	0.045	↘↘	0.16	0.45	-	-	-	-	-	-	-	-	-	-
4107	Ferritin / Chlorophyll a-b binding protein	-0.56	0.004	↘↘↘	-0.75	< 0.0001	↘↘↘↘	NM >	-	-	-	-	-	-	-	-
4203		-0.36	0.077	↘	-0.36	0.07	↘	-	-	-	-	-	-	-	-	-
4303		0.30	0.145	-	0.17	0.41	-	-	-	-	-	-	-	-	-	-
4308	FBP aldolase / Oxidoreductase	-0.55	0.005	↘↘↘	-0.11	0.59	-	-	-	-	-	-	-	-	-	-
4401	Glyceraldehyde-3-phosphate dehydrogenase / Phosphoglycerate kinase	0.36	0.077	↗	0.45	0.02	↗↗	-	-	-	-	-	-	-	-	-
4404		-0.06	0.793	-	-0.06	0.76	-	-	-	-	-	-	-	-	-	-
4405		0.20	0.35	-	0.36	0.077	↗	-	-	-	-	-	-	-	-	-
4407	GAPDH B / Aspartate aminotransferase	0.14	0.49	-	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-
4408		-0.39	0.057	↘	0.10	0.63	-	-	-	-	-	-	-	-	-	-
4413		0.10	0.63	-	0.15	0.49	-	-	-	-	-	-	-	-	-	-
4414	FBP aldolase / RuBisCO small subunit	0.33	0.109	-	NA	NA		M >>	M >>	M >>	M >>	M >>	M >>	M >>	M >>	M >>
4501	Apyrase EC=3.6.1.5	0.27	0.20	-	0.45	0.02	↗↗	-	-	-	-	-	-	-	-	-

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 4503 to 5304



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

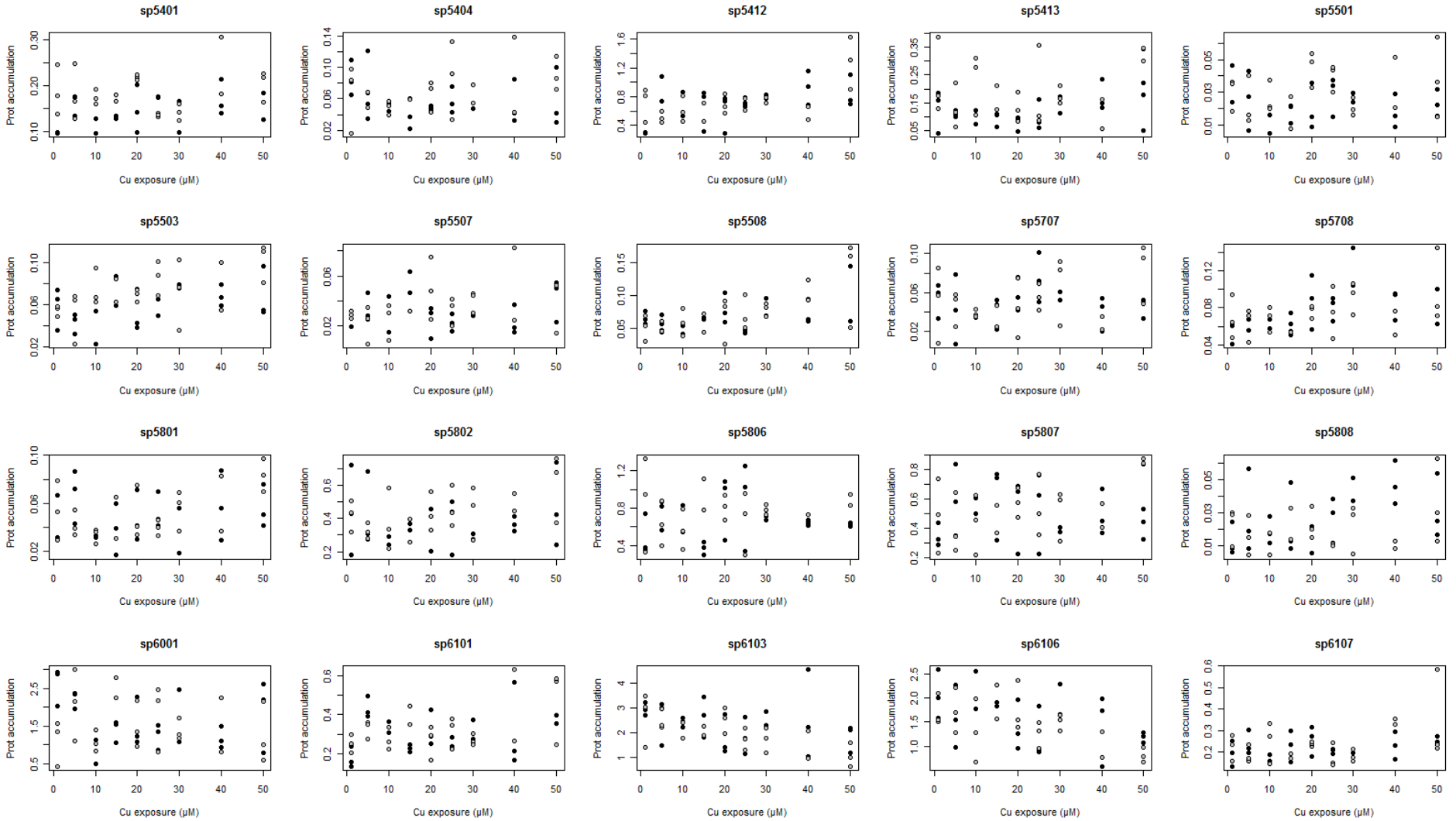
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
4503	0.131 ± 0.022	0.198 ± 0.041	0.15 ± 0.01	0.151 ± 0.013	0.073 ± 0.018	0.126 ± 0.043	0.148 ± 0.018	0.109 ± 0.04	0.149 ± 0.066	0.174 ± 0.014	0.117 ± 0.03	0.162 ± 0.067	0.152 ± 0.023	0.138 ± 0.04	0.147 ± 0.044	0.22 ± 0.101	0.144 ± 0.015	0.159 ± 0.039
4505	0.064 ± 0.032	0.049 ± 0.027	0.047 ± 0.019	0.067 ± 0.013	0.033 ± 0.02	0.052 ± 0.026	0.03 ± 0.014	0.082 ± 0.069	0.081 ± 0.052	0.04 ± 0.027	0.066 ± 0.054	0.075 ± 0.037	0.066 ± 0.013	0.055 ± 0.054	0.051 ± 0.046	0.075 ± 0.004	0.058 ± 0.037	0.117 ± 0.084
4508	0.038 ± 0.027	0.035 ± 0.017	0.037 ± 0.034	0.021 ± 0.012	0.018 ± 0.016	0.035 ± 0.036	0.027 ± 0.012	0.023 ± 0.01	0.055 ± 0.026	0.03 ± 0.016	0.033 ± 0.014	0.05 ± 0.018	0.025 ± 0.006	0.025 ± 0.018	0.018 ± 0.008	0.037 ± 0.034	0.031 ± 0.005	0.055 ± 0.04
4704	0.065 ± 0.02	0.061 ± 0.019	0.069 ± 0.036	0.049 ± 0.024	0.03 ± 0.019	0.053 ± 0.003	0.055 ± 0.01	0.049 ± 0.016	0.069 ± 0.015	0.073 ± 0.02	0.057 ± 0.048	0.05 ± 0.009	0.07 ± 0.034	0.065 ± 0.013	0.086 ± 0.036	0.092 ± 0.019	0.078 ± 0.045	0.103 ± 0.031
4708	0.052 ± 0.018	0.071 ± 0.02	0.039 ± 0.007	0.057 ± 0.022	0.027 ± 0.001	0.044 ± 0.012	0.042 ± 0.004	0.034 ± 0.015	0.045 ± 0.004	0.051 ± 0.013	0.035 ± 0.009	0.038 ± 0.02	0.053 ± 0.012	0.034 ± 0.006	0.031 ± 0.018	0.028 ± 0.005	0.02 ± 0.004	0.035 ± 0.036
4801	0.106 ± 0.003	0.15 ± 0.047	0.173 ± 0.021	0.103 ± 0.031	0.083 ± 0.011	0.102 ± 0.032	0.157 ± 0.035	0.089 ± 0.02	0.147 ± 0.031	0.108 ± 0.071	0.122 ± 0.061	0.182 ± 0.04	0.127 ± 0.032	0.161 ± 0.049	0.189 ± 0.075	0.194 ± 0.002	0.148 ± 0.05	0.213 ± 0.107
4802	0.072 ± 0.051	0.076 ± 0.04	0.106 ± 0.042	0.068 ± 0.015	0.043 ± 0.002	0.066 ± 0.021	0.069 ± 0.002	0.05 ± 0.03	0.085 ± 0.021	0.054 ± 0.027	0.073 ± 0.025	0.071 ± 0.007	0.073 ± 0.005	0.076 ± 0.007	0.074 ± 0.023	0.106 ± 0.004	0.102 ± 0.03	0.097 ± 0.073
4805	0.092 ± 0.077	0.095 ± 0.018	0.124 ± 0.032	0.075 ± 0.018	0.05 ± 0.011	0.063 ± 0.023	0.082 ± 0.017	0.054 ± 0.017	0.081 ± 0.009	0.073 ± 0.008	0.063 ± 0.021	0.081 ± 0.024	0.049 ± 0.003	0.079 ± 0.003	0.087 ± 0.04	0.089 ± 0.018	0.09 ± 0.026	0.083 ± 0.048
4806	0.125 ± 0.076	0.125 ± 0.056	0.167 ± 0.083	0.064 ± 0.021	0.072 ± 0.005	0.074 ± 0.017	0.116 ± 0.042	0.072 ± 0.03	0.108 ± 0.035	0.119 ± 0.016	0.088 ± 0.046	0.122 ± 0.04	0.1 ± 0.009	0.127 ± 0.031	0.143 ± 0.054	0.195 ± 0.057	0.129 ± 0.031	0.226 ± 0.057
5003	0.193 ± 0.022	0.246 ± 0.061	0.202 ± 0.063	0.181 ± 0.048	0.262 ± 0.003	0.182 ± 0.066	0.215 ± 0.083	0.199 ± 0.056	0.214 ± 0.06	0.219 ± 0.05	0.159 ± 0.114	0.226 ± 0.083	0.197 ± 0.05	0.247 ± 0.07	0.279 ± 0.049	0.222 ± 0.007	0.264 ± 0.081	0.246 ± 0.126
5101	0.259 ± 0.041	0.319 ± 0.029	0.193 ± 0.04	0.281 ± 0.018	0.181 ± 0.014	0.283 ± 0.072	0.209 ± 0.007	0.258 ± 0.039	0.285 ± 0.091	0.29 ± 0.058	0.276 ± 0.102	0.305 ± 0.118	0.195 ± 0.026	0.306 ± 0.097	0.192 ± 0.019	0.359 ± 0.07	0.154 ± 0.014	0.447 ± 0.028
5103	0.322 ± 0.018	0.39 ± 0.051	0.371 ± 0.061	0.341 ± 0.123	0.322 ± 0.047	0.346 ± 0.163	0.331 ± 0.021	0.274 ± 0.064	0.443 ± 0.121	0.301 ± 0.075	0.421 ± 0.048	0.294 ± 0.032	0.364 ± 0.054	0.251 ± 0.062	0.236 ± 0.086	0.288 ± 0.015	0.257 ± 0.086	0.35 ± 0.143
5104	0.148	0.169 ± 0.028	0.228 ± 0.064	0.147 ± 0.005	0.236 ± 0.028	0.109 ± 0.021	0.154 ± 0.006	0.195 ± 0.005	0.195 ± 0.047	0.122 ± 0.054	0.119 ± 0.081	0.179 ± 0.019	0.14 ± 0.051	0.108 ± 0.056	0.086 ± 0.047	0.136 ± 0.086	0.138 ± 0.014	0.16 ± 0.018
5105	0.352 ± 0.056	0.243 ± 0.034	0.346 ± 0.042	0.298 ± 0.126	0.362 ± 0.011	0.267 ± 0.113	0.345 ± 0.068	0.293 ± 0.016	0.393 ± 0.154	0.311 ± 0.049	0.285 ± 0.149	0.272 ± 0.067	0.394 ± 0.114	0.271 ± 0.063	0.329 ± 0.072	0.364 ± 0.138	0.309 ± 0.115	0.342 ± 0.08
5201	0.043 ± 0.015	0.049 ± 0.016	0.054 ± 0.007	0.037 ± 0.003	0.041	0.033 ± 0.016	0.054 ± 0.007	0.048 ± 0.001	0.078 ± 0.025	0.053 ± 0.012	0.038 ± 0.013	0.04 ± 0.015	0.042 ± 0.013	0.049 ± 0.001	0.063 ± 0.015	0.081 ± 0.022	0.076 ± 0.03	0.092 ± 0.032
5203	0.252 ± 0.134	0.344 ± 0.034	0.408 ± 0.116	0.225 ± 0.112	0.451 ± 0.049	0.262 ± 0.05	0.361 ± 0.023	0.338 ± 0.07	0.259 ± 0.052	0.248 ± 0.035	0.358 ± 0.032	0.244 ± 0.095	0.363 ± 0.109	0.304 ± 0.059	0.395 ± 0.134	0.187 ± 0.047	0.337 ± 0.036	0.293 ± 0.078
5207	0.042 ± 0.014	0.053 ± 0.021	0.053 ± 0.025	0.023 ± 0.019	0.058 ± 0.018	0.033 ± 0.02	0.061 ± 0.026	0.057 ± 0.045	0.039 ± 0.011	0.075 ± 0.022	0.02 ± 0.016	0.029 ± 0.011	0.053 ± 0.003	0.031 ± 0.008	0.048 ± 0.025	0.052 ± 0.042	0.035 ± 0.02	0.066 ± 0.028
5210	0.12 ± 0.015	0.085 ± 0.016	0.096 ± 0.028	0.068 ± 0.027	0.108 ± 0.022	0.112 ± 0.07	0.11 ± 0.013	0.102 ± 0.013	0.069 ± 0.04	0.108 ± 0.052	0.074 ± 0.07	0.111 ± 0.017	0.116 ± 0.066	0.101 ± 0.024	0.107 ± 0.079	0.131 ± 0.063	0.091 ± 0.043	0.115 ± 0.043
5303	0.131 ± 0.056	0.118 ± 0.035	0.176 ± 0.024	0.098 ± 0.011	0.096 ± 0.006	0.07 ± 0.012	0.116 ± 0.016	0.108 ± 0.021	0.156 ± 0.041	0.115 ± 0.031	0.123 ± 0.032	0.112 ± 0.019	0.087 ± 0.011	0.098 ± 0.049	0.1 ± 0.02	0.171 ± 0.018	0.132 ± 0.036	0.196 ± 0.051
5304	1.441 ± 0.297	1.877 ± 0.1	1.45 ± 0.061	1.201 ± 0.28	1.914 ± 0.009	1.24 ± 0.519	2.287 ± 0.186	1.411 ± 0.011	1.4 ± 0.61	1.744 ± 0.271	1.058 ± 0.47	1.464 ± 0.159	1.535 ± 0.421	1.696 ± 0.27	1.812 ± 0.158	2.436 ± 0.888	1.64 ± 0.721	2.084 ± 0.922

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
4503		0.16	0.44	-	0.08	0.71	-	-	-	-	-	-	-	-	-	-
4505		0.08	0.70	-	0.36	0.07	↗	-	-	-	-	-	-	-	-	-
4508		-0.17	0.41	-	0.29	0.16	-	-	-	-	-	-	-	-	-	-
4704	Phosphoglucomutase, cytoplasmic EC=5.4.2.2	0.26	0.20	-	0.62	0.0009	↗↗↗	-	-	-	-	-	-	-	-	-
4708	Succinate DH [ubi] flavoprotein / NADP-dep. malic enzyme / ATP synthase sub. Alpha	-0.47	0.017	↘↘	-0.49	0.01	↘↘	-	-	-	-	-	-	-	-	-
4801	ATP-dependent Clp protease ATP-binding	0.24	0.24	-	0.53	0.01	↗↗	-	-	-	-	-	-	-	-	-
4802		0.14	0.496	-	0.34	0.10	↗	-	-	-	-	-	-	-	-	-
4805		-0.13	0.55	-	0.10	0.62	-	-	-	-	-	-	-	-	-	-
4806	ATP-dependent Clp protease ATP-binding / Cyanate hydratase	0.00	0.99	-	0.73	< 0.0001	↗↗↗	-	-	-	-	-	-	-	-	-
5003		0.30	0.15	-	0.20	0.33	-	-	-	-	-	-	-	-	-	-
5101	Triosephosphate isomerase : TIM EC=5.3.1.1	-0.30	0.144	-	0.54	0.005	↗↗	-	-	-	-	-	-	-	-	NM >>
5103		-0.35	0.085	↘	-0.18	0.39	-	-	-	-	-	-	-	-	-	-
5104	ND	-0.48	0.014	↘↘	-0.03	0.88	-	-	-	M >	-	-	-	-	-	-
5105		-0.14	0.49	-	0.33	0.11	-	-	-	-	-	-	-	-	-	-
5201	ND	0.37	0.069	↗	0.68	0.0002	↗↗↗	-	-	-	-	-	-	-	-	-
5203		0.08	0.72	-	-0.12	0.57	-	-	-	-	-	-	-	-	-	-
5207		-0.20	0.33	-	0.23	0.27	-	-	-	-	-	-	-	-	-	-
5210		-0.10	0.63	-	0.33	0.11	-	-	-	-	-	-	-	-	-	-
5303	Fructose-1,6-bisphosphatase, cytosolic EC=3.1.3.11	-0.25	0.23	-	0.63	0.0007	↗↗↗	-	-	-	-	-	-	-	-	-
5304	Fructose-bisphosphate aldolase, chloroplastic EC=4.1.2.13	0.03	0.88	-	0.45	0.02	↗↗	-	-	-	M >	-	-	-	-	-

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 5401 to 6107



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

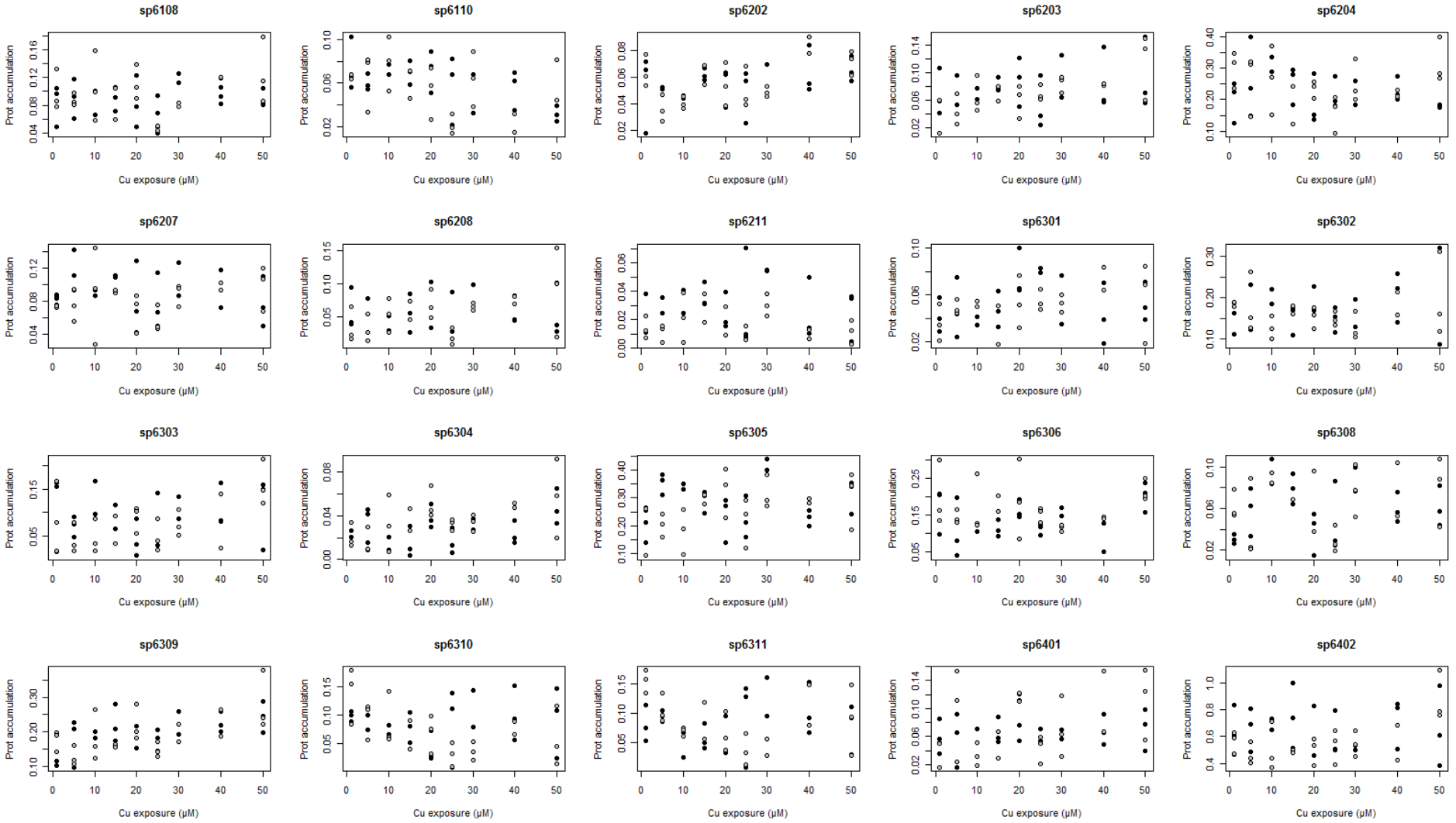
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
5401	0.125 ± 0.046	0.188 ± 0.054	0.162 ± 0.024	0.181 ± 0.062	0.113 ± 0.022	0.175 ± 0.016	0.131 ± 0.003	0.173 ± 0.01	0.147 ± 0.052	0.218 ± 0.006	0.163 ± 0.021	0.137 ± 0.004	0.133 ± 0.049	0.142 ± 0.018	0.17 ± 0.039	0.245 ± 0.087	0.158 ± 0.029	0.203 ± 0.033
5404	0.086 ± 0.023	0.066 ± 0.044	0.07 ± 0.045	0.062 ± 0.011	0.049 ± 0.008	0.05 ± 0.009	0.04 ± 0.019	0.06 ± 0.001	0.049 ± 0.003	0.065 ± 0.019	0.057 ± 0.016	0.086 ± 0.05	0.051 ± 0.004	0.062 ± 0.013	0.053 ± 0.028	0.091 ± 0.067	0.057 ± 0.037	0.09 ± 0.021
5412	0.348 ± 0.086	0.715 ± 0.243	0.752 ± 0.317	0.511 ± 0.074	0.701 ± 0.237	0.623 ± 0.181	0.654 ± 0.298	0.584 ± 0.174	0.603 ± 0.268	0.692 ± 0.134	0.691 ± 0.03	0.725 ± 0.103	0.765 ± 0.079	0.76 ± 0.041	0.927 ± 0.237	0.574 ± 0.13	0.855 ± 0.222	1.282 ± 0.368
5413	0.128 ± 0.077	0.231 ± 0.138	0.11 ± 0.013	0.134 ± 0.08	0.098 ± 0.035	0.233 ± 0.11	0.093 ± 0.025	0.168 ± 0.06	0.076 ± 0.025	0.132 ± 0.053	0.1 ± 0.054	0.182 ± 0.152	0.143 ± 0.043	0.176 ± 0.034	0.172 ± 0.054	0.11 ± 0.074	0.149 ± 0.09	0.331 ± 0.027
5501	0.036 ± 0.011	0.03 ± 0.01	0.026 ± 0.018	0.023 ± 0.015	0.011 ± 0.008	0.026 ± 0.01	0.018 ± 0.006	0.018 ± 0.014	0.02 ± 0.014	0.045 ± 0.011	0.029 ± 0.012	0.04 ± 0.008	0.027 ± 0.004	0.021 ± 0.005	0.018 ± 0.01	0.036 ± 0.022	0.023 ± 0.008	0.038 ± 0.025
5503	0.058 ± 0.02	0.054 ± 0.005	0.043 ± 0.01	0.051 ± 0.025	0.038 ± 0.022	0.075 ± 0.017	0.07 ± 0.015	0.073 ± 0.015	0.052 ± 0.02	0.069 ± 0.006	0.057 ± 0.011	0.086 ± 0.016	0.077 ± 0.003	0.072 ± 0.034	0.069 ± 0.01	0.077 ± 0.032	0.068 ± 0.025	0.102 ± 0.018
5507	0.025 ± 0.005	0.029 ± 0.003	0.033 ± 0.012	0.022 ± 0.015	0.029 ± 0.02	0.025 ± 0.015	0.052 ± 0.01	0.032	0.025 ± 0.013	0.049 ± 0.025	0.022 ± 0.007	0.032 ± 0.011	0.028 ± 0.001	0.04 ± 0.008	0.023 ± 0.012	0.053 ± 0.041	0.042 ± 0.017	0.033 ± 0.027
5508	0.066 ± 0.01	0.051 ± 0.02	0.058 ± 0.012	0.05 ± 0.009	0.048 ± 0.009	0.059 ± 0.022	0.065 ± 0.002	0.058 ± 0.02	0.079 ± 0.022	0.067 ± 0.036	0.046 ± 0.003	0.072 ± 0.027	0.083 ± 0.018	0.079 ± 0.01	0.073 ± 0.019	0.108 ± 0.021	0.086 ± 0.052	0.128 ± 0.067
5707	0.054 ± 0.018	0.05 ± 0.039	0.043 ± 0.036	0.046 ± 0.018	0.036 ± 0.002	0.039 ± 0.004	0.041 ± 0.016	0.036 ± 0.015	0.058 ± 0.017	0.044 ± 0.031	0.075 ± 0.026	0.056 ± 0.014	0.057 ± 0.006	0.067 ± 0.036	0.04 ± 0.017	0.029 ± 0.01	0.046 ± 0.011	0.083 ± 0.031
5708	0.055 ± 0.012	0.069 ± 0.023	0.055 ± 0.012	0.064 ± 0.019	0.063 ± 0.006	0.068 ± 0.014	0.063 ± 0.012	0.054 ± 0.001	0.087 ± 0.029	0.077 ± 0.007	0.08 ± 0.013	0.075 ± 0.028	0.124 ± 0.029	0.092 ± 0.017	0.085 ± 0.016	0.063 ± 0.018	0.081 ± 0.019	0.099 ± 0.04
5801	0.043 ± 0.021	0.053 ± 0.025	0.067 ± 0.022	0.042 ± 0.011	0.032 ± 0.001	0.033 ± 0.006	0.038 ± 0.022	0.048 ± 0.025	0.048 ± 0.022	0.05 ± 0.022	0.052 ± 0.015	0.04 ± 0.007	0.037 ± 0.027	0.055 ± 0.017	0.057 ± 0.029	0.06 ± 0.033	0.056 ± 0.018	0.083 ± 0.014
5802	0.444 ± 0.271	0.42 ± 0.094	0.423 ± 0.225	0.322 ± 0.046	0.265 ± 0.036	0.379 ± 0.187	0.364 ± 0.033	0.326 ± 0.099	0.372 ± 0.148	0.435 ± 0.116	0.372 ± 0.17	0.464 ± 0.126	0.29 ± 0.021	0.443 ± 0.161	0.365 ± 0.043	0.498 ± 0.075	0.467 ± 0.252	0.603 ± 0.203
5806	0.489 ± 0.216	0.87 ± 0.506	0.75 ± 0.164	0.632 ± 0.244	0.687 ± 0.197	0.564 ± 0.218	0.37 ± 0.067	0.95 ± 0.24	0.854 ± 0.344	0.81 ± 0.13	0.873 ± 0.478	0.662 ± 0.337	0.693 ± 0.03	0.785 ± 0.052	0.641 ± 0.031	0.729 ± 0.003	0.623 ± 0.02	0.87 ± 0.07
5807	0.352 ± 0.079	0.492 ± 0.253	0.594 ± 0.245	0.414 ± 0.206	0.554 ± 0.077	0.434 ± 0.206	0.612 ± 0.254	0.463 ± 0.134	0.523 ± 0.259	0.577 ± 0.098	0.541 ± 0.28	0.545 ± 0.21	0.391 ± 0.023	0.516 ± 0.175	0.496 ± 0.156	0.49 ± 0.116	0.434 ± 0.104	0.853 ± 0.021
5808	0.013 ± 0.01	0.023 ± 0.011	0.028 ± 0.025	0.016 ± 0.012	0.02 ± 0.011	0.013 ± 0.008	0.023 ± 0.022	0.023 ± 0.013	0.016 ± 0.009	0.023 ± 0.01	0.033 ± 0.005	0.011 ± 0.001	0.044 ± 0.01	0.022 ± 0.015	0.047 ± 0.013	0.011 ± 0.003	0.032 ± 0.019	0.035 ± 0.025
6001	2.618 ± 0.501	1.114 ± 0.596	2.229 ± 0.233	2.087 ± 0.939	0.814 ± 0.456	1.095 ± 0.287	1.406 ± 0.289	2.516 ± 0.373	1.535 ± 0.65	1.497 ± 0.612	1.244 ± 0.346	1.826 ± 0.883	1.773 ± 0.981	1.399 ± 0.28	1.177 ± 0.289	1.541 ± 1.011	1.862 ± 0.954	1.254 ± 0.807
6101	0.176 ± 0.055	0.25 ± 0.045	0.435 ± 0.055	0.328 ± 0.047	0.335 ± 0.038	0.273 ± 0.056	0.227 ± 0.019	0.395 ± 0.067	0.321 ± 0.091	0.266 ± 0.088	0.249 ± 0.032	0.316 ± 0.082	0.324 ± 0.072	0.271 ± 0.029	0.314 ± 0.221	0.448 ± 0.26	0.332 ± 0.078	0.469 ± 0.192
6103	2.947 ± 0.267	2.65 ± 1.103	2.559 ± 0.911	2.509 ± 0.403	2.407 ± 0.249	2.226 ± 0.371	2.668 ± 0.805	2.073 ± 0.254	1.809 ± 0.828	2.529 ± 0.507	1.877 ± 0.743	1.753 ± 0.447	2.58 ± 0.377	1.719 ± 0.495	2.609 ± 1.775	1.53 ± 0.788	1.838 ± 0.541	1.095 ± 0.474
6106	2.055 ± 0.504	1.721 ± 0.322	1.6 ± 0.642	1.724 ± 0.462	2.163 ± 0.54	1.31 ± 0.649	2 ± 0.229	1.907 ± 0.496	1.395 ± 0.52	1.763 ± 0.521	1.209 ± 0.538	1.257 ± 0.272	1.966 ± 0.442	1.5 ± 0.16	1.436 ± 0.748	1.029 ± 0.37	1.19 ± 0.102	0.815 ± 0.157
6107	0.194 ± 0.058	0.224 ± 0.06	0.239 ± 0.057	0.188 ± 0.041	0.175 ± 0.02	0.251 ± 0.096	0.229 ± 0.074	0.183 ± 0.016	0.256 ± 0.068	0.237 ± 0.011	0.205 ± 0.012	0.179 ± 0.056	0.206 ± 0.01	0.182 ± 0.028	0.231 ± 0.065	0.34 ± 0.019	0.245 ± 0.028	0.345 ± 0.205

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
5401		0.30	0.145	-	0.15	0.48	-	-	-	-	-	-	-	-	-	-
5404		-0.23	0.28	-	0.36	0.07	↗	-	-	-	-	-	-	-	-	-
5412	Elongation factor Tu / Phosphoglycerate kinase	0.50	0.011	↗↗	0.58	0.002	↗↗↗	-	-	-	-	-	-	-	-	-
5413		0.33	0.10	-	0.21	0.31	-	-	-	-	-	-	-	-	-	-
5501		-0.14	0.49	-	0.25	0.22	-	-	-	-	-	-	-	-	-	-
5503	Eukaryotic initiation factor 4A	0.40	0.051	↗	0.57	0.00	↗↗↗	-	-	-	-	-	-	-	-	-
5507		0.07	0.75	-	0.33	0.11	-	-	-	-	-	-	-	-	-	-
5508	Eukaryotic initiation factor 4A	0.33	0.10	-	0.68	0.0002	↗↗↗↗	-	-	-	-	-	-	-	-	-
5707		0.01	0.97	-	0.34	0.10	↗	-	-	-	-	-	-	-	-	-
5708	Phosphoglucomutase, cytoplasmic	0.51	0.010	↗↗↗	0.41	0.04	↗↗	-	-	-	-	-	-	-	-	-
5801	ATP-dependent Clp protease ATP-binding / Cyanate hydratase	0.13	0.55	-	0.52	0.008	↗↗↗	-	-	-	-	-	-	-	-	-
5802	Transketolase, chloroplastic EC=2.2.1.1	0.03	0.87	-	0.51	0.01	↗↗↗	-	-	-	-	-	-	-	-	-
5806		0.09	0.66	-	0.13	0.55	-	-	-	-	NM >	-	-	-	-	-
5807	Transketolase, chloroplastic / ATP synthase sub. a, chloro.	-0.09	0.67	-	0.52	0.01	↗↗↗	-	-	-	-	-	-	-	-	NM >
5808	Heat shock 70 kDa protein 10, mitochondrial	0.45	0.023	↗↗	0.22	0.29	-	-	-	-	-	-	M >>	-	M >>	-
6001		-0.28	0.18	-	-0.09	0.68	-	-	-	-	-	-	-	-	-	-
6101	ND	0.12	0.57	-	0.47	0.018	↗↗	-	-	-	-	-	-	-	-	-
6103	20 kDa chaperonin, chloroplastic / Chlorophyll a-b binding protein 8	-0.26	0.21	-	-0.68	0.0002	↘↘↘↘	-	-	-	-	-	-	-	-	-
6106	Chlorophyll a-b binding protein 1B-21, chloroplastic	-0.43	0.034	↘↘	-0.57	0.00	↘↘↘	-	-	-	-	-	-	-	-	-
6107	Triosephosphate isomerase EC=5.3.1.1	0.20	0.35	-	0.41	0.04	↗↗	-	-	-	-	-	-	-	-	-

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 6108 to 6402



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

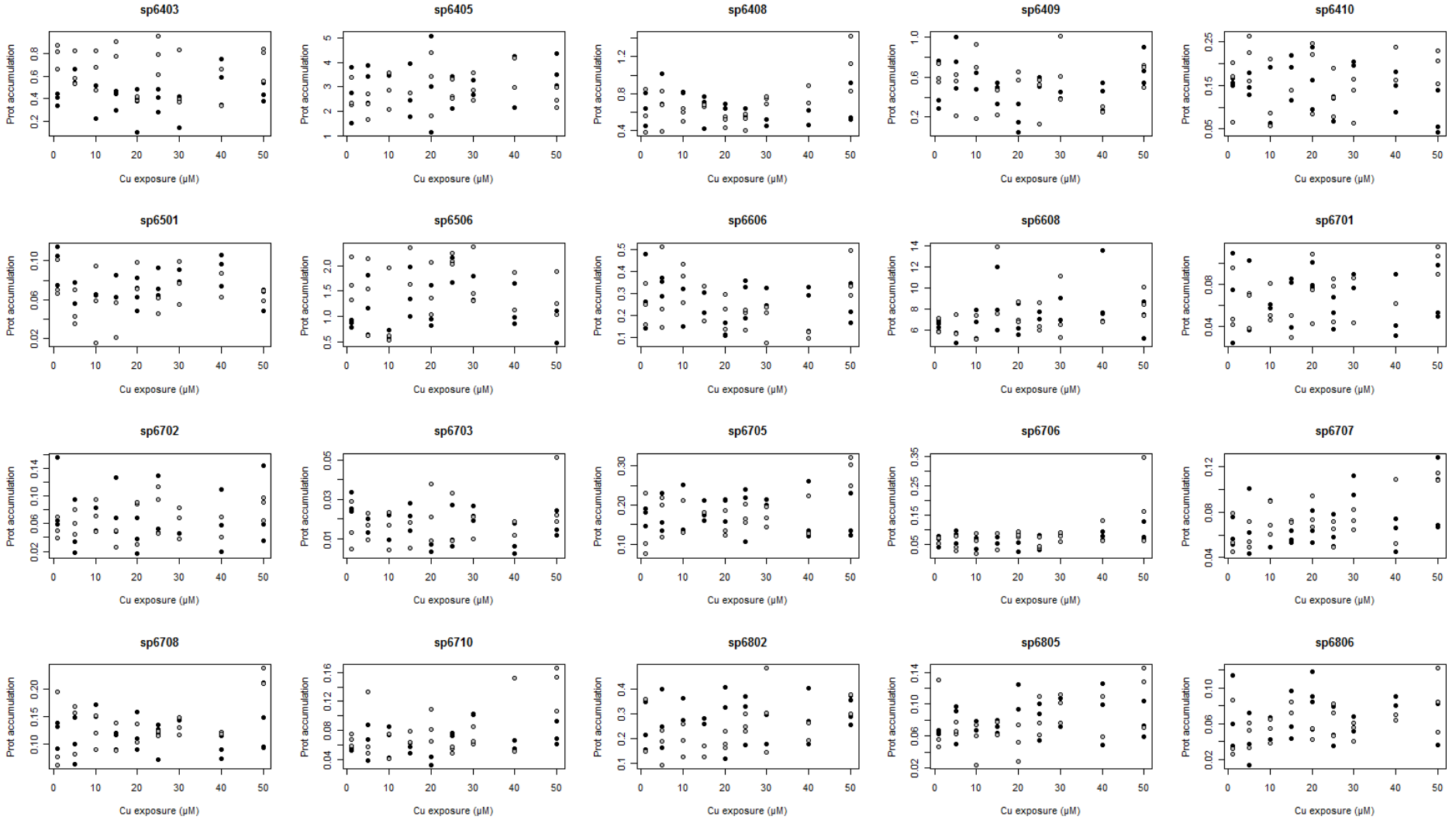
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
6108	0.083 ± 0.03	0.099 ± 0.029	0.091 ± 0.028	0.088 ± 0.009	0.083 ± 0.024	0.105 ± 0.051	0.09 ± 0.017	0.082 ± 0.032	0.084 ± 0.037	0.112 ± 0.025	0.068 ± 0.027	0.048 ± 0.004	0.12 ± 0.009	0.082 ± 0.003	0.094 ± 0.012	0.12	0.09 ± 0.013	0.127 ± 0.047
6110	0.074 ± 0.024	0.065 ± 0.002	0.06 ± 0.007	0.064 ± 0.027	0.073 ± 0.007	0.079 ± 0.025	0.07 ± 0.011	0.059 ± 0.018	0.072 ± 0.019	0.053 ± 0.024	0.057 ± 0.031	0.022 ± 0.009	0.05 ± 0.025	0.064 ± 0.025	0.056 ± 0.018	0.023 ± 0.012	0.032 ± 0.007	0.069 ± 0.021
6202	0.052 ± 0.029	0.064 ± 0.012	0.052 ± 0.001	0.036 ± 0.01	0.045 ± 0.001	0.04 ± 0.004	0.061 ± 0.005	0.061 ± 0.01	0.054 ± 0.015	0.054 ± 0.016	0.048 ± 0.02	0.05 ± 0.016	0.058 ± 0.017	0.049 ± 0.004	0.063 ± 0.018	0.084 ± 0.009	0.066 ± 0.009	0.071 ± 0.009
6203	0.068 ± 0.034	0.044 ± 0.028	0.081 ± 0.025	0.044 ± 0.023	0.069 ± 0.011	0.065 ± 0.027	0.082 ± 0.009	0.069 ± 0.016	0.088 ± 0.036	0.06 ± 0.024	0.052 ± 0.038	0.07 ± 0.011	0.094 ± 0.043	0.084 ± 0.012	0.084 ± 0.045	0.082 ± 0.001	0.093 ± 0.052	0.114 ± 0.048
6204	0.2 ± 0.066	0.299 ± 0.057	0.261 ± 0.128	0.259 ± 0.098	0.312 ± 0.033	0.263 ± 0.109	0.251 ± 0.06	0.182 ± 0.084	0.191 ± 0.081	0.233 ± 0.027	0.217 ± 0.049	0.16 ± 0.059	0.221 ± 0.054	0.252 ± 0.067	0.23 ± 0.038	0.221 ± 0.015	0.181 ± 0.006	0.317 ± 0.072
6207	0.085 ± 0.002	0.074 ± 0.002	0.115 ± 0.025	0.074 ± 0.02	0.09 ± 0.005	0.089 ± 0.059	0.103 ± 0.011	0.091 ± 0.003	0.079 ± 0.044	0.068 ± 0.024	0.077 ± 0.034	0.057 ± 0.016	0.106 ± 0.028	0.088 ± 0.013	0.087 ± 0.026	0.097 ± 0.006	0.077 ± 0.03	0.098 ± 0.027
6208	0.059 ± 0.031	0.035 ± 0.026	0.053 ± 0.025	0.032 ± 0.02	0.041 ± 0.015	0.053 ± 0.026	0.056 ± 0.029	0.06 ± 0.019	0.062 ± 0.036	0.068 ± 0.022	0.048 ± 0.034	0.02 ± 0.013	0.079 ± 0.027	0.066 ± 0.006	0.058 ± 0.021	0.075 ± 0.008	0.056 ± 0.04	0.092 ± 0.067
6211	0.02 ± 0.015	0.014 ± 0.008	0.025 ± 0.011	0.011 ± 0.006	0.033 ± 0.011	0.021 ± 0.018	0.036 ± 0.009	0.028 ± 0.014	0.024 ± 0.013	0.022 ± 0.012	0.029 ± 0.036	0.01 ± 0.005	0.055	0.03 ± 0.008	0.025 ± 0.022	0.01 ± 0.004	0.025 ± 0.018	0.011 ± 0.008
6301	0.042 ± 0.015	0.036 ± 0.016	0.048 ± 0.026	0.049 ± 0.007	0.038 ± 0.005	0.053 ± 0.003	0.047 ± 0.015	0.035 ± 0.024	0.077 ± 0.02	0.054 ± 0.022	0.076 ± 0.009	0.055 ± 0.009	0.056 ± 0.03	0.053 ± 0.007	0.043 ± 0.026	0.074 ± 0.014	0.053 ± 0.017	0.057 ± 0.035
6302	0.15 ± 0.035	0.185 ± 0.007	0.159 ± 0.063	0.18 ± 0.072	0.203 ± 0.026	0.127 ± 0.029	0.151 ± 0.036	0.171 ± 0.014	0.189 ± 0.032	0.153 ± 0.026	0.149 ± 0.031	0.148 ± 0.017	0.162 ± 0.048	0.128 ± 0.034	0.207 ± 0.06	0.187 ± 0.04	0.175 ± 0.127	0.197 ± 0.102
6303	0.112 ± 0.082	0.088 ± 0.074	0.072 ± 0.022	0.044 ± 0.033	0.132 ± 0.049	0.047 ± 0.036	0.073 ± 0.041	0.064 ± 0.042	0.042 ± 0.04	0.089 ± 0.029	0.071 ± 0.061	0.049 ± 0.034	0.111 ± 0.032	0.076 ± 0.028	0.109 ± 0.047	0.082 ± 0.081	0.11 ± 0.077	0.16 ± 0.048
6304	0.027 ± 0.007	0.021 ± 0.011	0.035 ± 0.017	0.016 ± 0.012	0.015 ± 0.008	0.032 ± 0.026	0.015 ± 0.014	0.037 ± 0.014	0.039 ± 0.011	0.051 ± 0.015	0.016 ± 0.012	0.033 ± 0.005	0.032 ± 0.007	0.034 ± 0.008	0.024 ± 0.011	0.049 ± 0.003	0.048 ± 0.016	0.057 ± 0.036
6305	0.203 ± 0.058	0.206 ± 0.1	0.354 ± 0.038	0.203 ± 0.042	0.341 ± 0.014	0.18 ± 0.082	0.292 ± 0.04	0.294 ± 0.024	0.233 ± 0.083	0.327 ± 0.089	0.226 ± 0.076	0.218 ± 0.09	0.421 ± 0.027	0.316 ± 0.061	0.228 ± 0.028	0.29 ± 0.011	0.313 ± 0.063	0.306 ± 0.105
6306	0.17 ± 0.062	0.198 ± 0.087	0.105 ± 0.083	0.144 ± 0.018	0.114 ± 0.014	0.17 ± 0.079	0.112 ± 0.023	0.18 ± 0.03	0.162 ± 0.025	0.19 ± 0.108	0.112 ± 0.015	0.152 ± 0.021	0.158 ± 0.014	0.115 ± 0.009	0.106 ± 0.048	0.142 ± 0.004	0.202 ± 0.041	0.215 ± 0.03
6308	0.03 ± 0.004	0.062 ± 0.014	0.058 ± 0.023	0.044 ± 0.039	0.096 ± 0.017	0.089 ± 0.007	0.079 ± 0.015	0.069	0.038 ± 0.021	0.057 ± 0.033	0.046 ± 0.034	0.03 ± 0.013	0.088 ± 0.016	0.077 ± 0.026	0.06 ± 0.014	0.078 ± 0.036	0.061 ± 0.02	0.079 ± 0.033
6309	0.137 ± 0.049	0.176 ± 0.03	0.177 ± 0.07	0.129 ± 0.029	0.191 ± 0.012	0.182 ± 0.074	0.221 ± 0.056	0.159 ± 0.007	0.174 ± 0.037	0.221 ± 0.051	0.177 ± 0.032	0.147 ± 0.022	0.226 ± 0.046	0.205 ± 0.03	0.227 ± 0.03	0.225 ± 0.056	0.243 ± 0.045	0.28 ± 0.085
6310	0.098 ± 0.009	0.139 ± 0.049	0.095 ± 0.019	0.094 ± 0.033	0.075 ± 0.011	0.087 ± 0.048	0.079 ± 0.027	0.065 ± 0.035	0.042 ± 0.027	0.069 ± 0.034	0.086 ± 0.069	0.031 ± 0.021	0.111 ± 0.045	0.036 ± 0.017	0.101 ± 0.048	0.077 ± 0.016	0.093 ± 0.062	0.058 ± 0.052
6311	0.081 ± 0.031	0.155 ± 0.019	0.095 ± 0.009	0.106 ± 0.025	0.045 ± 0.03	0.069 ± 0.007	0.058 ± 0.022	0.087 ± 0.044	0.075 ± 0.036	0.066 ± 0.034	0.092 ± 0.073	0.037 ± 0.027	0.128 ± 0.046	0.037 ± 0.016	0.104 ± 0.045	0.114 ± 0.049	0.078 ± 0.043	0.09 ± 0.06
6401	0.059 ± 0.025	0.039 ± 0.02	0.058 ± 0.039	0.096 ± 0.067	0.061 ± 0.014	0.034 ± 0.016	0.066 ± 0.019	0.048 ± 0.027	0.08 ± 0.029	0.118 ± 0.006	0.059 ± 0.01	0.044 ± 0.02	0.063 ± 0.009	0.071 ± 0.043	0.069 ± 0.022	0.111 ± 0.061	0.072 ± 0.03	0.112 ± 0.051
6402	0.636 ± 0.188	0.565 ± 0.082	0.663 ± 0.163	0.468 ± 0.083	0.692 ± 0.063	0.508 ± 0.182	0.753 ± 0.245	0.489 ± 0.014	0.607 ± 0.195	0.501 ± 0.104	0.6 ± 0.169	0.535 ± 0.13	0.518 ± 0.023	0.546 ± 0.096	0.719 ± 0.187	0.553 ± 0.185	0.659 ± 0.304	0.882 ± 0.186

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
6108		0.12	0.57	-	0.21	0.32	-	-	-	-	-	-	-	-	-	-
6110	Ras-related protein Rab7	-0.55	0.004	↘↘↘	-0.22	0.30	-	-	-	-	-	-	-	-	-	-
6202	ND	0.32	0.12	-	0.49	0.01	↗↗	-	-	-	-	-	-	-	-	-
6203	Thioredoxin H-type 4	0.18	0.40	-	0.68	0.0002	↗↗↗↗	-	-	-	-	-	-	-	-	-
6204		-0.25	0.23	-	0.03	0.90	-	-	-	-	-	-	-	-	-	-
6207		-0.23	0.26	-	0.25	0.23	-	-	-	-	-	-	-	-	-	-
6208	Thioredoxin H-type 4	0.07	0.76	-	0.48	0.01	↗↗	-	-	-	-	-	-	-	-	-
6211		0.06	0.77	-	-0.08	0.70	-	-	-	-	-	-	-	-	-	-
6301		0.13	0.55	-	0.39	0.05	↗	-	-	-	-	-	-	-	-	-
6302		0.16	0.43	-	0.09	0.66	-	-	-	-	-	-	-	-	-	-
6303	Leaf Ferredoxin--NADP reductase, chloro. EC=1.18.1.2	0.11	0.62	-	0.46	0.02	↗↗	-	-	-	-	-	-	-	-	-
6304	ND	0.28	0.173	-	0.55	0.005	↗↗↗	-	-	-	-	-	-	-	-	-
6305	FBP aldolase / Triosephosphate isomerase	0.04	0.83	-	0.44	0.03	↗↗	-	-	-	-	-	-	-	-	-
6306		0.22	0.28	-	0.03	0.90	-	-	-	-	-	-	-	-	-	-
6308		0.12	0.56	-	0.22	0.30	-	-	-	-	-	-	-	-	-	-
6309	Cysteine synthase EC=2.5.1.47	0.54	0.006	↗↗↗	0.58	0.002	↗↗↗	-	-	-	-	-	-	-	-	-
6310	ND	0.08	0.69	-	-0.47	0.02	↘↘	-	-	-	-	-	-	-	-	-
6311		0.17	0.43	-	-0.28	0.17	-	-	-	-	-	-	-	M >	-	-
6401		0.16	0.44	-	0.39	0.056	↗	-	-	-	-	-	-	-	-	-
6402	Actin	-0.01	0.96	-	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 6403 to 6806



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

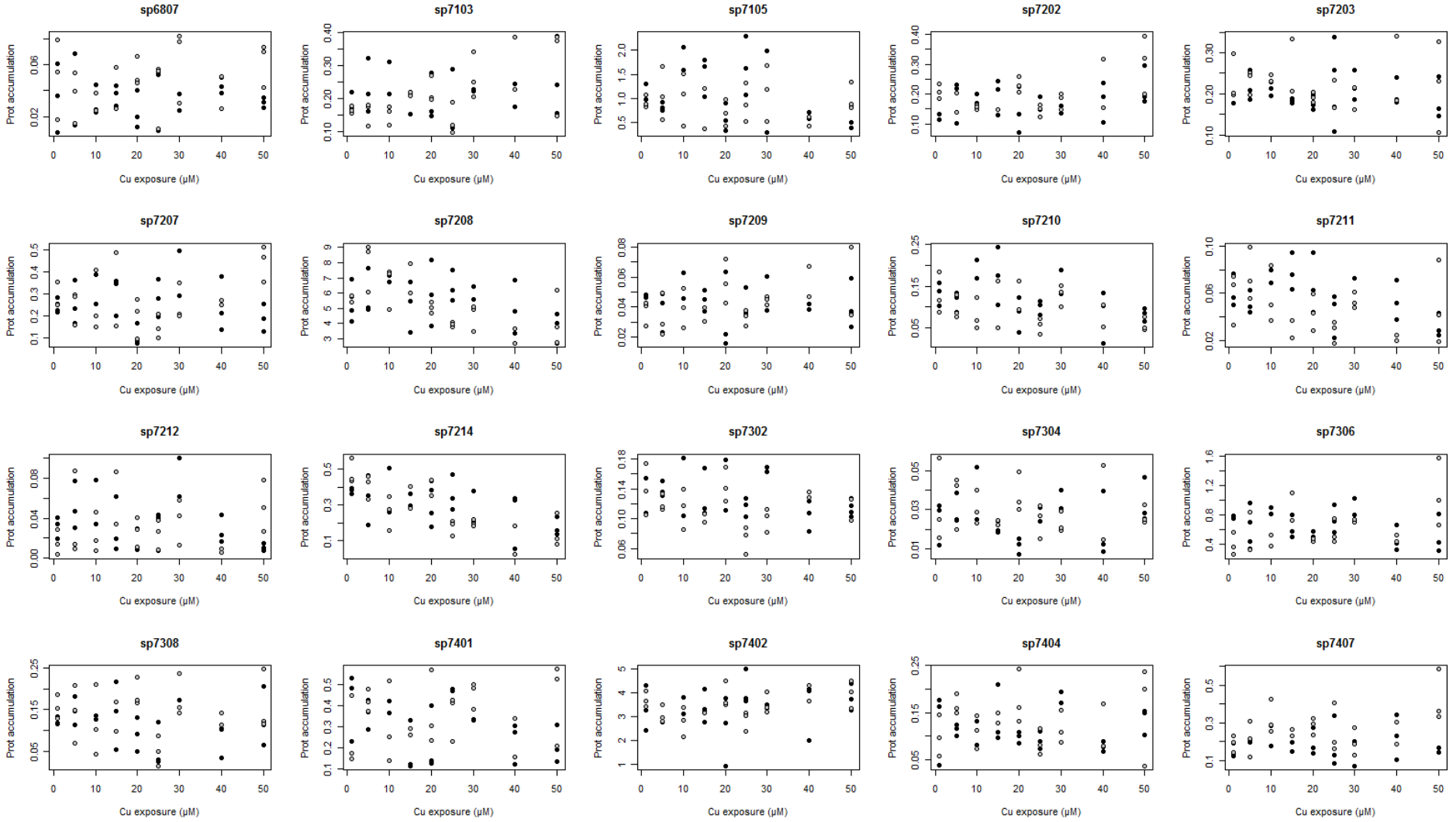
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
6403	0.396 ± 0.055	0.785 ± 0.107	0.582 ± 0.073	0.647 ± 0.157	0.37 ± 0.207	0.66 ± 0.175	0.403 ± 0.093	0.841 ± 0.094	0.323 ± 0.196	0.406 ± 0.014	0.394 ± 0.103	0.787 ± 0.173	0.284 ± 0.193	0.532 ± 0.26	0.562 ± 0.204	0.503 ± 0.228	0.452 ± 0.083	0.736 ± 0.154
6405	2.694 ± 1.128	2.663 ± 0.642	3.21 ± 0.785	2.236 ± 0.527	3.175 ± 0.429	2.828 ± 0.751	2.72 ± 1.118	2.767 ± 0.006	3.086 ± 1.977	3.216 ± 1.326	2.708 ± 0.674	2.809 ± 0.444	2.978 ± 0.438	2.971 ± 0.576	3.542 ± 1.2	3.57 ± 0.841	3.635 ± 0.678	2.529 ± 0.426
6408	0.636 ± 0.175	0.599 ± 0.233	0.802 ± 0.186	0.632 ± 0.222	0.813 ± 0.002	0.581 ± 0.071	0.633 ± 0.181	0.669 ± 0.011	0.627 ± 0.068	0.502 ± 0.064	0.578 ± 0.06	0.506 ± 0.089	0.492 ± 0.05	0.739 ± 0.042	0.591 ± 0.121	0.793 ± 0.132	0.661 ± 0.224	1.126 ± 0.299
6409	0.472 ± 0.255	0.629 ± 0.096	0.751 ± 0.26	0.465 ± 0.224	0.56 ± 0.116	0.607 ± 0.384	0.46 ± 0.11	0.343 ± 0.175	0.177 ± 0.144	0.627 ± 0.044	0.547 ± 0.047	0.409 ± 0.244	0.421 ± 0.044	0.667 ± 0.324	0.426 ± 0.142	0.279 ± 0.042	0.705 ± 0.183	0.638 ± 0.123
6410	0.157 ± 0.009	0.145 ± 0.072	0.151 ± 0.026	0.216 ± 0.053	0.127 ± 0.09	0.118 ± 0.082	0.175 ± 0.053	0.139 ± 0.001	0.165 ± 0.071	0.184 ± 0.087	0.104 ± 0.031	0.129 ± 0.056	0.199 ± 0.006	0.123 ± 0.053	0.139 ± 0.047	0.2 ± 0.053	0.079 ± 0.052	0.197 ± 0.039
6501	0.098 ± 0.021	0.079 ± 0.019	0.068 ± 0.011	0.049 ± 0.019	0.065	0.056 ± 0.04	0.068 ± 0.015	0.039 ± 0.025	0.064 ± 0.017	0.08 ± 0.016	0.076 ± 0.015	0.056 ± 0.009	0.085 ± 0.009	0.077 ± 0.022	0.092 ± 0.016	0.075 ± 0.017	0.059 ± 0.011	0.066 ± 0.006
6506	0.863 ± 0.066	1.704 ± 0.436	1.199 ± 0.59	1.427 ± 0.761	0.654 ± 0.095	1.036 ± 0.802	1.437 ± 0.491	1.997 ± 0.513	1.128 ± 0.42	1.492 ± 0.524	1.947 ± 0.252	2.114 ± 0.117	1.564 ± 0.337	1.711 ± 0.579	1.16 ± 0.435	1.502 ± 0.528	0.874 ± 0.347	1.392 ± 0.44
6606	0.296 ± 0.172	0.253 ± 0.095	0.339 ± 0.044	0.297 ± 0.193	0.236 ± 0.124	0.359 ± 0.09	0.284 ± 0.062	0.255 ± 0.111	0.129 ± 0.033	0.221 ± 0.08	0.293 ± 0.093	0.19 ± 0.049	0.286 ± 0.056	0.174 ± 0.087	0.25 ± 0.106	0.112 ± 0.021	0.244 ± 0.092	0.374 ± 0.107
6608	6.625 ± 0.299	6.308 ± 0.736	6.014 ± 1.341	6.28 ± 1.054	7.37 ± 0.774	5.944 ± 1.295	8.65 ± 3.054	10.732 ± 4.465	6.813 ± 1.536	7.502 ± 1.043	7.273 ± 0.396	7.025 ± 1.385	7.992 ± 1.447	7.7 ± 3.052	9.607 ± 3.439	6.868 ± 0.059	7.168 ± 1.777	8.648 ± 1.379
6701	0.069 ± 0.043	0.061 ± 0.03	0.059 ± 0.038	0.059 ± 0.018	0.059 ± 0.002	0.059 ± 0.019	0.068 ± 0.026	0.04 ± 0.015	0.085 ± 0.013	0.075 ± 0.033	0.052 ± 0.015	0.069 ± 0.022	0.083 ± 0.009	0.057 ± 0.025	0.054 ± 0.031	0.062	0.067 ± 0.027	0.104 ± 0.013
6702	0.093 ± 0.054	0.053 ± 0.015	0.049 ± 0.041	0.061 ± 0.018	0.066 ± 0.023	0.071 ± 0.023	0.082 ± 0.04	0.038 ± 0.017	0.041 ± 0.026	0.07 ± 0.034	0.076 ± 0.046	0.085 ± 0.035	0.057 ± 0.016	0.063 ± 0.023	0.062 ± 0.045	0.055 ± 0.021	0.08 ± 0.057	0.084 ± 0.018
6703	0.027 ± 0.005	0.015 ± 0.012	0.019 ± 0.005	0.016 ± 0.007	0.016 ± 0.009	0.015 ± 0.01	0.021 ± 0.007	0.012 ± 0.009	0.006 ± 0.003	0.023 ± 0.015	0.014 ± 0.011	0.025 ± 0.014	0.023 ± 0.005	0.018 ± 0.007	0.009 ± 0.008	0.015 ± 0.005	0.017 ± 0.007	0.03 ± 0.018
6705	0.173 ± 0.024	0.136 ± 0.083	0.173 ± 0.051	0.178 ± 0.054	0.194 ± 0.082	0.185 ± 0.047	0.183 ± 0.027	0.18 ± 0.002	0.194 ± 0.032	0.147 ± 0.034	0.187 ± 0.071	0.174 ± 0.025	0.207 ± 0.011	0.169 ± 0.026	0.173 ± 0.077	0.176 ± 0.068	0.162 ± 0.058	0.291 ± 0.037
6706	0.063 ± 0.02	0.066 ± 0.013	0.078 ± 0.022	0.049 ± 0.026	0.053 ± 0.027	0.056 ± 0.035	0.067 ± 0.011	0.06 ± 0.041	0.057 ± 0.032	0.082 ± 0.009	0.053 ± 0.025	0.052 ± 0.019	0.082 ± 0.003	0.077 ± 0.015	0.079 ± 0.015	0.098 ± 0.048	0.091 ± 0.033	0.19 ± 0.144
6707	0.061 ± 0.013	0.059 ± 0.018	0.069 ± 0.029	0.058 ± 0.012	0.07 ± 0.029	0.073 ± 0.015	0.058 ± 0.005	0.072 ± 0.001	0.066 ± 0.014	0.078 ± 0.014	0.062 ± 0.014	0.062 ± 0.011	0.103 ± 0.012	0.073 ± 0.009	0.062 ± 0.015	0.081 ± 0.04	0.088 ± 0.035	0.11 ± 0.004
6708	0.12 ± 0.025	0.11 ± 0.073	0.103 ± 0.043	0.135 ± 0.047	0.16 ± 0.015	0.12 ± 0.031	0.108 ± 0.016	0.112 ± 0.036	0.119 ± 0.035	0.125 ± 0.02	0.111 ± 0.034	0.118 ± 0.004	0.145 ± 0.003	0.131 ± 0.016	0.092 ± 0.021	0.119 ± 0.003	0.111 ± 0.031	0.219 ± 0.016
6710	0.055 ± 0.003	0.067 ± 0.008	0.065 ± 0.025	0.08 ± 0.047	0.064 ± 0.03	0.063 ± 0.019	0.056 ± 0.007	0.071 ± 0.011	0.047 ± 0.016	0.085 ± 0.022	0.069 ± 0.01	0.054 ± 0.004	0.102 ± 0.001	0.071 ± 0.013	0.058 ± 0.007	0.102 ± 0.071	0.074 ± 0.017	0.142 ± 0.031
6802	0.24 ± 0.098	0.219 ± 0.121	0.27 ± 0.121	0.171 ± 0.071	0.317 ± 0.063	0.192 ± 0.066	0.273 ± 0.013	0.146 ± 0.032	0.283 ± 0.149	0.188 ± 0.035	0.291 ± 0.105	0.259 ± 0.037	0.238 ± 0.084	0.31 ± 0.171	0.282 ± 0.113	0.229 ± 0.05	0.299 ± 0.05	0.351 ± 0.043
6805	0.065 ± 0.002	0.078 ± 0.046	0.08 ± 0.026	0.069 ± 0.008	0.073 ± 0.008	0.053 ± 0.026	0.071 ± 0.008	0.07 ± 0.011	0.098 ± 0.025	0.052 ± 0.023	0.081 ± 0.023	0.083 ± 0.025	0.09 ± 0.025	0.097 ± 0.019	0.091 ± 0.039	0.085 ± 0.036	0.079 ± 0.023	0.115 ± 0.039
6806	0.07 ± 0.04	0.048 ± 0.033	0.041 ± 0.029	0.049 ± 0.014	0.054 ± 0.017	0.053 ± 0.014	0.066 ± 0.028	0.078 ± 0.008	0.098 ± 0.018	0.05 ± 0.007	0.054 ± 0.022	0.067 ± 0.018	0.059 ± 0.011	0.052 ± 0.01	0.08 ± 0.01	0.067 ± 0.004	0.052 ± 0.026	0.086 ± 0.036

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
6403		0.06	0.78	-	-0.13	0.54	-	NM >	-	-	NM >	-	-	-	-	-
6405		0.24	0.24	-	0.17	0.42	-	-	-	-	-	-	-	-	-	-
6408	Chloroplast inner envelope protein / Actin / Phosphoglycerate kinase	-0.27	0.19	-	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
6409		0.03	0.880	-	-0.02	0.94	-	-	-	-	-	NM >	-	-	-	-
6410		-0.34	0.10	-	0.13	0.526	-	-	-	-	-	-	-	-	-	-
6501		-0.13	0.53	-	0.13	0.53	-	-	-	-	-	-	-	-	-	-
6506		0.07	0.75	-	0.02	0.93	-	-	-	-	-	-	-	-	-	-
6606		-0.18	0.40	-	-0.07	0.73	-	-	-	-	-	-	-	-	-	-
6608		0.28	0.18	-	0.29	0.16	-	-	-	-	-	-	-	-	-	-
6701	Vacuolar proton-ATPase subunit A / Phosphoglucomutase	-0.03	0.90	-	0.44	0.029	↗↗	-	-	-	-	-	-	-	-	-
6702		-0.01	0.97	-	0.27	0.19	-	-	-	-	-	-	-	-	-	-
6703		-0.34	0.099	↘	0.34	0.10	-	-	-	-	-	-	-	-	-	-
6705	V-type proton ATPase catalytic subunit A (Fragment)	-0.06	0.78	-	0.53	0.006	↗↗↗	-	-	-	-	-	-	-	-	-
6706	Chaperonin CPN60-2, mitochondrial : HSP60-2	0.32	0.116	-	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-
6707	Phosphoglycerate mutase EC=5.4.2.12	0.29	0.15	-	0.65	0.0004	↗↗↗↗	-	-	-	-	-	-	-	-	-
6708	V-type proton ATPase catalytic subunit A (Fragment)	-0.16	0.46	-	0.52	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
6710	Phosphoglycerate mutase EC=5.4.2.12	0.28	0.17	-	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
6802	Transketolase, chloroplastic	0.11	0.61	-	0.52	0.01	↗↗↗	-	-	-	M >	-	-	-	-	-
6805	Transketolase, chloroplastic	0.23	0.26	-	0.47	0.018	↗↗	-	-	-	-	-	-	-	-	-
6806	ATP-dependent zinc metalloprotease FTSH 1 / 70 kDa peptidyl-prolyl isomerase	0.05	0.83	-	0.46	0.02	↗↗	-	-	-	-	-	-	-	-	-

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 6807 to 7407



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

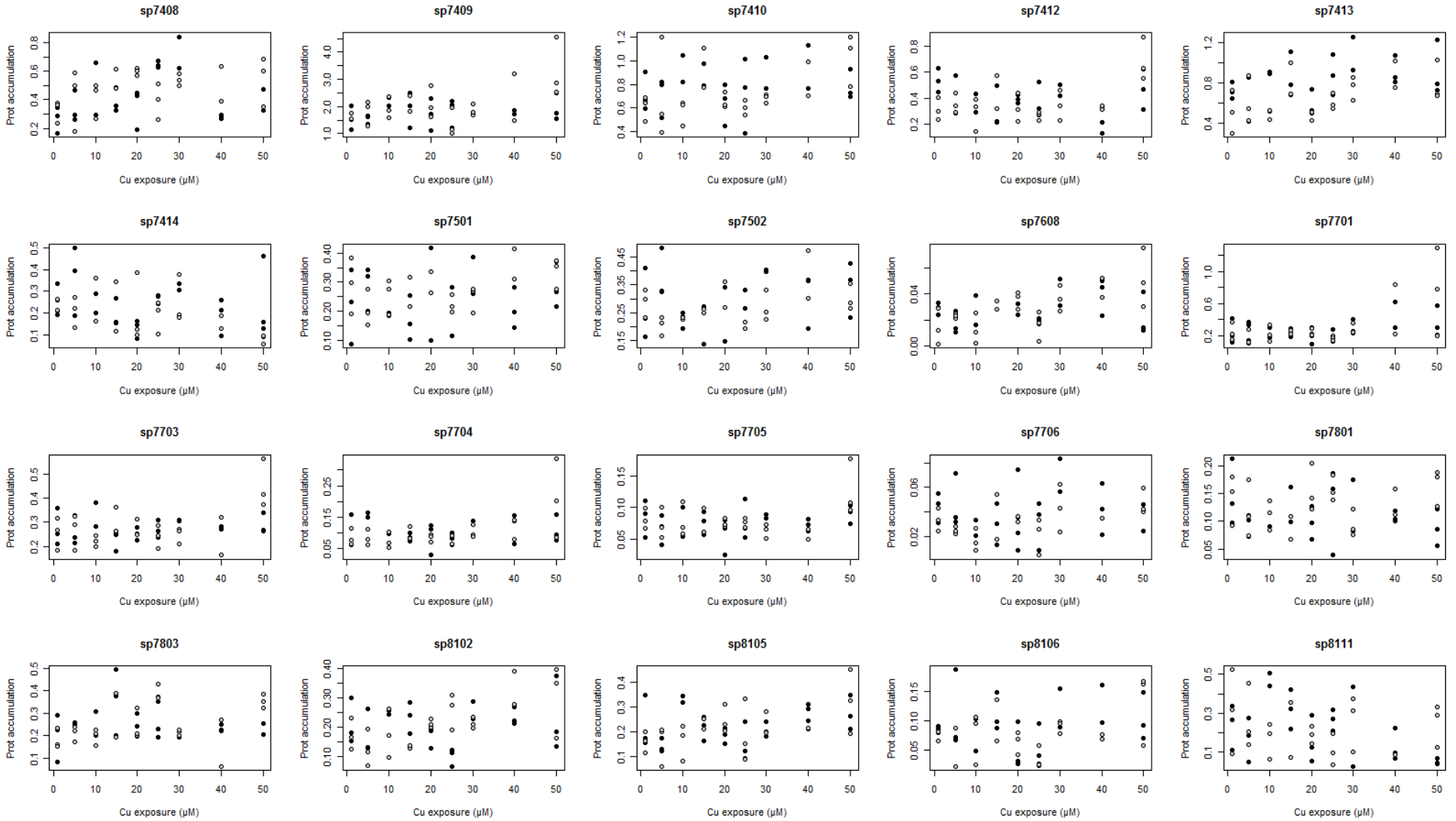
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
6807	0.035 ± 0.026	0.05 ± 0.031	0.041 ± 0.039	0.036 ± 0.02	0.034 ± 0.015	0.03 ± 0.007	0.037 ± 0.008	0.042 ± 0.022	0.024 ± 0.014	0.053 ± 0.011	0.039 ± 0.026	0.041 ± 0.026	0.031 ± 0.009	0.063 ± 0.029	0.044 ± 0.006	0.039 ± 0.017	0.031 ± 0.004	0.062 ± 0.017
7103	0.186 ± 0.03	0.165 ± 0.012	0.232 ± 0.082	0.157 ± 0.036	0.262 ± 0.067	0.153 ± 0.029	0.192 ± 0.035	0.214 ± 0.007	0.195 ± 0.072	0.224 ± 0.04	0.172 ± 0.101	0.135 ± 0.047	0.225 ± 0.004	0.265 ± 0.07	0.216 ± 0.037	0.307 ± 0.111	0.262 ± 0.118	0.304 ± 0.135
7105	1.081 ± 0.181	0.915 ± 0.129	0.822 ± 0.091	1.081 ± 0.558	1.828 ± 0.35	1.006 ± 0.554	1.497 ± 0.41	0.779 ± 0.61	0.586 ± 0.297	0.691 ± 0.287	1.665 ± 0.619	0.897 ± 0.4	1.133 ± 1.203	1.13 ± 0.587	0.625 ± 0.063	0.508 ± 0.131	0.575 ± 0.25	1.005 ± 0.291
7202	0.127 ± 0.011	0.208 ± 0.025	0.184 ± 0.071	0.182 ± 0.038	0.185 ± 0.021	0.157 ± 0.007	0.196 ± 0.059	0.149 ± 0.001	0.143 ± 0.077	0.232 ± 0.027	0.169 ± 0.022	0.147 ± 0.02	0.148 ± 0.015	0.178 ± 0.028	0.178 ± 0.067	0.235 ± 0.115	0.221 ± 0.066	0.305 ± 0.099
7203	0.184 ± 0.011	0.233 ± 0.057	0.217 ± 0.036	0.231 ± 0.029	0.204 ± 0.013	0.235 ± 0.009	0.183 ± 0.006	0.27 ± 0.091	0.177 ± 0.018	0.191 ± 0.012	0.235 ± 0.117	0.189 ± 0.037	0.223 ± 0.051	0.197 ± 0.03	0.202 ± 0.033	0.262 ± 0.112	0.184 ± 0.05	0.221 ± 0.111
7207	0.241 ± 0.038	0.285 ± 0.058	0.254 ± 0.098	0.248 ± 0.077	0.32 ± 0.094	0.253 ± 0.134	0.301 ± 0.089	0.321 ± 0.233	0.11 ± 0.049	0.198 ± 0.093	0.282 ± 0.085	0.151 ± 0.053	0.393 ± 0.145	0.252 ± 0.083	0.243 ± 0.121	0.259 ± 0.015	0.191 ± 0.061	0.443 ± 0.081
7208	5.306 ± 1.414	5.655 ± 0.21	5.878 ± 1.535	7.941 ± 1.631	6.967 ± 0.298	6.547 ± 1.401	5.202 ± 1.65	6.963 ± 1.362	5.964 ± 2.15	5.056 ± 0.361	6.412 ± 0.987	3.962 ± 0.152	6.002 ± 0.587	4.504 ± 0.881	5.003 ± 1.769	3.199 ± 0.675	3.79 ± 1.002	4.217 ± 1.759
7209	0.047 ± 0.001	0.037 ± 0.008	0.038 ± 0.014	0.033 ± 0.014	0.055 ± 0.012	0.039 ± 0.013	0.045 ± 0.007	0.035 ± 0.006	0.034 ± 0.026	0.057 ± 0.015	0.042 ± 0.01	0.033 ± 0.005	0.049 ± 0.016	0.044 ± 0.003	0.041 ± 0.002	0.057 ± 0.014	0.041 ± 0.016	0.05 ± 0.026
7210	0.133 ± 0.028	0.13 ± 0.05	0.115 ± 0.023	0.098 ± 0.028	0.191 ± 0.031	0.08 ± 0.038	0.175 ± 0.069	0.107 ± 0.079	0.084 ± 0.042	0.117 ± 0.038	0.099 ± 0.017	0.055 ± 0.019	0.16 ± 0.041	0.13 ± 0.026	0.084 ± 0.063	0.079 ± 0.038	0.083 ± 0.015	0.058 ± 0.017
7211	0.061 ± 0.014	0.058 ± 0.022	0.052 ± 0.009	0.075 ± 0.022	0.074 ± 0.008	0.057 ± 0.024	0.078 ± 0.015	0.03 ± 0.01	0.067 ± 0.025	0.044 ± 0.016	0.043 ± 0.018	0.028 ± 0.009	0.067 ± 0.008	0.054 ± 0.007	0.053 ± 0.017	0.022 ± 0.004	0.032 ± 0.01	0.05 ± 0.035
7212	0.031 ± 0.011	0.015 ± 0.013	0.051 ± 0.024	0.037 ± 0.044	0.056 ± 0.031	0.024 ± 0.02	0.03 ± 0.028	0.06 ± 0.037	0.016 ± 0.012	0.027 ± 0.015	0.03 ± 0.02	0.024 ± 0.015	0.081 ± 0.027	0.038 ± 0.023	0.028 ± 0.014	0.007 ± 0.002	0.011 ± 0.004	0.052 ± 0.026
7214	0.377 ± 0.015	0.48 ± 0.069	0.333 ± 0.138	0.406 ± 0.068	0.383 ± 0.172	0.259 ± 0.095	0.353 ± 0.053	0.342 ± 0.083	0.273 ± 0.102	0.407 ± 0.05	0.358 ± 0.099	0.177 ± 0.044	0.292 ± 0.122	0.198 ± 0.017	0.24 ± 0.157	0.104 ± 0.114	0.177 ± 0.05	0.15 ± 0.094
7302	0.133 ± 0.023	0.139 ± 0.035	0.139 ± 0.01	0.12 ± 0.012	0.143 ± 0.054	0.114 ± 0.028	0.13 ± 0.033	0.101 ± 0.007	0.157 ± 0.039	0.145 ± 0.023	0.116 ± 0.013	0.073 ± 0.019	0.166 ± 0.004	0.099 ± 0.016	0.105 ± 0.021	0.132 ± 0.005	0.109 ± 0.008	0.117 ± 0.017
7304	0.025 ± 0.011	0.032 ± 0.021	0.029 ± 0.008	0.036 ± 0.014	0.038 ± 0.019	0.031 ± 0.009	0.021 ± 0.003	0.023 ± 0.001	0.012 ± 0.004	0.038 ± 0.01	0.027 ± 0.003	0.025 ± 0.009	0.036 ± 0.007	0.023 ± 0.005	0.02 ± 0.017	0.034 ± 0.027	0.033 ± 0.012	0.027 ± 0.005
7306	0.762 ± 0.022	0.398 ± 0.154	0.703 ± 0.261	0.503 ± 0.293	0.856 ± 0.058	0.472 ± 0.087	0.63 ± 0.157	0.911 ± 0.27	0.516 ± 0.059	0.467 ± 0.024	0.741 ± 0.188	0.564 ± 0.163	0.912 ± 0.161	0.715 ± 0.02	0.471 ± 0.172	0.481 ± 0.058	0.517 ± 0.261	1.075 ± 0.457
7308	0.123 ± 0.01	0.156 ± 0.029	0.147 ± 0.034	0.142 ± 0.07	0.131 ± 0.006	0.119 ± 0.084	0.138 ± 0.081	0.134 ± 0.048	0.092 ± 0.04	0.189 ± 0.033	0.06 ± 0.053	0.052 ± 0.036	0.157 ± 0.022	0.178 ± 0.051	0.082 ± 0.039	0.128 ± 0.019	0.128 ± 0.072	0.161 ± 0.074
7401	0.413 ± 0.161	0.255 ± 0.165	0.36 ± 0.071	0.422 ± 0.052	0.391 ± 0.042	0.3 ± 0.194	0.188 ± 0.123	0.276 ± 0.021	0.22 ± 0.154	0.37 ± 0.176	0.391 ± 0.142	0.355 ± 0.108	0.332 ± 0.003	0.454 ± 0.062	0.233 ± 0.099	0.245 ± 0.129	0.212 ± 0.088	0.433 ± 0.197
7402	3.34 ± 0.939	3.722 ± 0.335	3.264 ± 0.412	3.079 ± 0.371	3.459 ± 0.488	2.803 ± 0.623	3.411 ± 0.71	3.179 ± 0.015	2.478 ± 1.43	3.857 ± 0.561	4.13 ± 0.739	2.86 ± 0.42	3.434 ± 0.112	3.54 ± 0.446	3.414 ± 1.24	3.983 ± 0.458	3.792 ± 0.566	3.969 ± 0.579
7404	0.126 ± 0.077	0.1 ± 0.044	0.114 ± 0.012	0.166 ± 0.021	0.107 ± 0.035	0.11 ± 0.035	0.138 ± 0.062	0.138 ± 0.016	0.098 ± 0.012	0.178 ± 0.057	0.091 ± 0.019	0.087 ± 0.028	0.183 ± 0.016	0.116 ± 0.035	0.078 ± 0.011	0.124 ± 0.062	0.135 ± 0.028	0.158 ± 0.107
7407	0.152 ± 0.034	0.189 ± 0.045	0.205 ± 0.007	0.214 ± 0.094	0.233 ± 0.076	0.323 ± 0.09	0.18 ± 0.028	0.247 ± 0.025	0.194 ± 0.071	0.285 ± 0.045	0.183 ± 0.136	0.257 ± 0.132	0.137 ± 0.092	0.196 ± 0.074	0.225 ± 0.12	0.246 ± 0.08	0.152 ± 0.015	0.426 ± 0.138

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
6807		-0.01	0.96	-	0.29	0.16	-	-	-	-	-	-	-	-	-	-
7103	Triosephosphate isomerase : TIM EC=5.3.1.1	0.16	0.43	-	0.62	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
7105		-0.32	0.116	-	-0.07	0.73	-	-	-	-	-	-	-	-	-	-
7202	Cysteine synthase, chloroplastic/chromoplastic	0.28	0.18	-	0.47	0.017	↗↗	-	-	-	-	-	-	-	-	-
7203		0.00	1.00	-	-0.08	0.72	-	-	-	-	-	-	-	-	-	-
7207		-0.11	0.60	-	0.30	0.14	-	-	-	-	-	-	-	-	-	-
7208	Oxygen-evolving enhancer protein 1, chloroplastic : OEE1	-0.34	0.095	↘	-0.65	0.0005	↘↘↘↘	-	-	-	-	-	-	-	-	-
7209		-0.10	0.65	-	0.38	0.06	↗	-	-	-	-	-	-	-	-	-
7210	ND	-0.40	0.048	↘↘	-0.33	0.10	-	-	-	-	-	-	-	-	-	-
7211	ND	-0.43	0.033	↘↘	-0.34	0.10	-	-	-	-	M >	-	-	-	-	-
7212		-0.27	0.19	-	0.17	0.42	-	-	-	-	-	-	-	-	-	NM >
7214	Chlorophyll a-b binding protein 8, chloroplastic	-0.53	0.006	↘↘↘	-0.75	< 0.0001	↘↘↘↘	-	-	-	-	-	-	-	-	-
7302		-0.34	0.096	↘	-0.16	0.45	-	-	-	-	-	-	-	-	-	-
7304		0.07	0.75	-	-0.17	0.42	-	-	-	-	-	NM >	-	-	-	-
7306	Sedoheptulose-1,7-bisphosphatase, chloroplastic EC=3.1.3.37	-0.38	0.062	↘	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
7308		-0.17	0.41	-	0.04	0.85	-	-	-	-	-	-	-	-	-	-
7401		-0.39	0.055	↘	0.21	0.32	-	-	-	-	-	-	-	-	-	-
7402		0.17	0.41	-	0.38	0.06	↗	-	-	-	-	-	-	-	-	-
7404		-0.02	0.94	-	0.09	0.67	-	-	-	-	-	-	-	-	-	-
7407	Phosphoribulokinase / Adenosine kinase	-0.05	0.81	-	0.42	0.04	↗↗	-	-	-	-	-	-	-	-	NM >

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 7408 to 8111



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

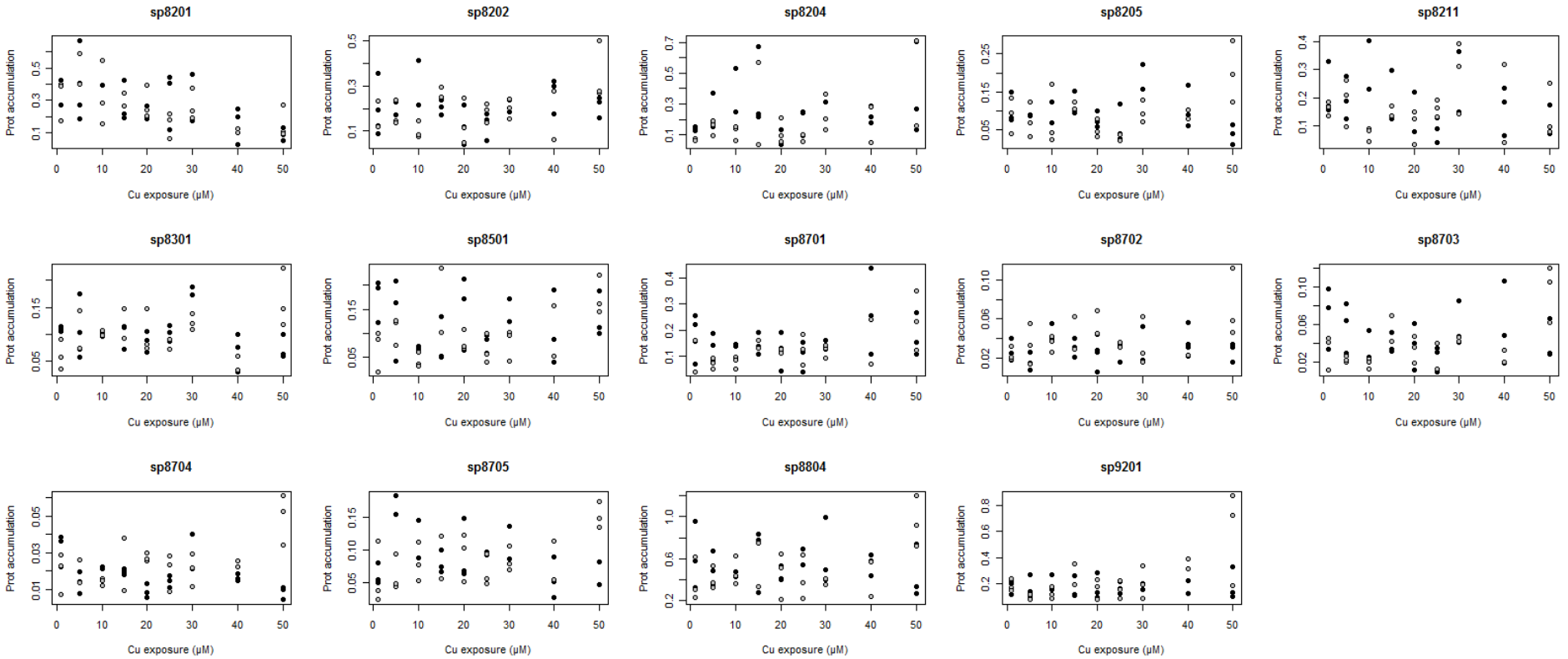
SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
7408	0.267 ± 0.092	0.324 ± 0.078	0.34 ± 0.109	0.42 ± 0.215	0.477 ± 0.255	0.41 ± 0.124	0.389 ± 0.084	0.548 ± 0.093	0.356 ± 0.142	0.597 ± 0.028	0.646 ± 0.021	0.391 ± 0.124	0.731 ± 0.155	0.538 ± 0.04	0.282 ± 0.015	0.51 ± 0.172	0.375 ± 0.084	0.543 ± 0.173
7409	1.58 ± 0.436	1.617 ± 0.119	1.534 ± 0.159	1.812 ± 0.469	2.173 ± 0.227	1.942 ± 0.399	1.912 ± 0.637	2.104 ± 0.405	1.711 ± 0.605	2.12 ± 0.591	1.829 ± 0.523	1.373 ± 0.506	1.736 ± 0.031	1.849 ± 0.212	1.762 ± 0.071	2.344 ± 1.195	1.933 ± 0.5	3.325 ± 1.087
7410	0.722 ± 0.165	0.605 ± 0.102	0.71 ± 0.166	0.715 ± 0.427	0.933 ± 0.16	0.572 ± 0.105	0.843 ± 0.111	0.938 ± 0.237	0.642 ± 0.177	0.657 ± 0.07	0.725 ± 0.315	0.604 ± 0.062	0.894 ± 0.187	0.681 ± 0.036	0.864 ± 0.228	0.844 ± 0.204	0.784 ± 0.128	1.027 ± 0.219
7412	0.535 ± 0.09	0.313 ± 0.086	0.398 ± 0.153	0.356 ± 0.078	0.362 ± 0.101	0.291 ± 0.13	0.31 ± 0.163	0.447 ± 0.178	0.391 ± 0.033	0.322 ± 0.111	0.371 ± 0.138	0.265 ± 0.035	0.46 ± 0.058	0.343 ± 0.116	0.226 ± 0.104	0.326 ± 0.024	0.47 ± 0.157	0.685 ± 0.168
7413	0.719 ± 0.082	0.512 ± 0.212	0.711 ± 0.257	0.614 ± 0.232	0.901 ± 0.014	0.495 ± 0.048	0.857 ± 0.221	0.844 ± 0.217	0.59 ± 0.128	0.484 ± 0.053	0.875 ± 0.199	0.606 ± 0.079	1.089 ± 0.233	0.753 ± 0.112	0.91 ± 0.139	0.889 ± 0.186	0.911 ± 0.272	0.795 ± 0.198
7414	0.262 ± 0.073	0.228 ± 0.031	0.361 ± 0.159	0.207 ± 0.071	0.244 ± 0.065	0.227 ± 0.115	0.193 ± 0.064	0.231 ± 0.162	0.128 ± 0.043	0.201 ± 0.16	0.265 ± 0.022	0.187 ± 0.075	0.321 ± 0.02	0.25 ± 0.112	0.188 ± 0.086	0.158 ± 0.04	0.248 ± 0.185	0.079 ± 0.021
7501	0.222 ± 0.128	0.291 ± 0.095	0.287 ± 0.077	0.209 ± 0.063	0.19 ± 0.008	0.257 ± 0.061	0.173 ± 0.076	0.267 ± 0.072	0.26 ± 0.224	0.301 ± 0.052	0.219 ± 0.089	0.223 ± 0.031	0.323 ± 0.089	0.248 ± 0.045	0.208 ± 0.069	0.362 ± 0.073	0.285 ± 0.078	0.334 ± 0.052
7502	0.268 ± 0.126	0.288 ± 0.05	0.378 ± 0.09	0.205 ± 0.033	0.223 ± 0.04	0.232 ± 0.006	0.227 ± 0.078	0.256 ± 0.011	0.245 ± 0.136	0.315 ± 0.063	0.288 ± 0.039	0.21 ± 0.013	0.401 ± 0.004	0.271 ± 0.054	0.308 ± 0.1	0.387 ± 0.119	0.342 ± 0.099	0.303 ± 0.047
7608	0.029 ± 0.005	0.014 ± 0.014	0.017 ± 0.009	0.023 ± 0.002	0.028 ± 0.016	0.013 ± 0.012	0.032 ± 0.004	0.032 ± 0.005	0.028 ± 0.004	0.036 ± 0.006	0.02 ± 0.002	0.016 ± 0.011	0.041 ± 0.015	0.037 ± 0.01	0.039 ± 0.014	0.045 ± 0.01	0.023 ± 0.016	0.052 ± 0.023
7701	0.242 ± 0.158	0.243 ± 0.113	0.283 ± 0.122	0.166 ± 0.096	0.235 ± 0.089	0.224 ± 0.099	0.245 ± 0.05	0.243 ± 0.035	0.206 ± 0.106	0.232 ± 0.047	0.225 ± 0.088	0.169 ± 0.024	0.321 ± 0.122	0.283 ± 0.066	0.381 ± 0.212	0.526 ± 0.436	0.359 ± 0.189	0.759 ± 0.549
7703	0.275 ± 0.076	0.256 ± 0.067	0.26 ± 0.061	0.266 ± 0.074	0.333 ± 0.07	0.221 ± 0.022	0.228 ± 0.04	0.313 ± 0.068	0.251 ± 0.026	0.27 ± 0.036	0.27 ± 0.036	0.241 ± 0.049	0.308 ± 0.003	0.249 ± 0.032	0.277 ± 0.007	0.243 ± 0.111	0.291 ± 0.043	0.45 ± 0.098
7704	0.099 ± 0.052	0.085 ± 0.026	0.125 ± 0.055	0.085 ± 0.025	0.1 ± 0.005	0.075 ± 0.026	0.086 ± 0.013	0.103 ± 0.027	0.088 ± 0.051	0.085 ± 0.013	0.083 ± 0.017	0.072 ± 0.016	0.132 ± 0.008	0.103 ± 0.02	0.12 ± 0.048	0.111 ± 0.043	0.111 ± 0.043	0.207 ± 0.125
7705	0.085 ± 0.03	0.082 ± 0.016	0.066 ± 0.024	0.074 ± 0.025	0.077 ± 0.033	0.079 ± 0.027	0.076 ± 0.018	0.08 ± 0.026	0.057 ± 0.029	0.075 ± 0.007	0.078 ± 0.032	0.077 ± 0.007	0.086 ± 0.004	0.063 ± 0.011	0.072 ± 0.009	0.057 ± 0.011	0.09 ± 0.015	0.127 ± 0.044
7706	0.044 ± 0.012	0.033 ± 0.009	0.046 ± 0.022	0.024 ± 0.003	0.027 ± 0.009	0.016 ± 0.009	0.03 ± 0.017	0.036 ± 0.026	0.035 ± 0.035	0.035 ± 0.003	0.031 ± 0.02	0.021 ± 0.015	0.07 ± 0.019	0.043 ± 0.019	0.042 ± 0.021	0.035	0.038 ± 0.012	0.047 ± 0.011
7801	0.147 ± 0.06	0.142 ± 0.045	0.095 ± 0.02	0.12 ± 0.052	0.088 ± 0.004	0.113 ± 0.027	0.11 ± 0.048	0.089 ± 0.03	0.098 ± 0.03	0.157 ± 0.043	0.128 ± 0.078	0.158 ± 0.023	0.129 ± 0.065	0.095 ± 0.025	0.108 ± 0.009	0.135 ± 0.033	0.088 ± 0.033	0.166 ± 0.033
7803	0.2 ± 0.108	0.183 ± 0.044	0.251 ± 0.009	0.211 ± 0.034	0.255 ± 0.075	0.198 ± 0.035	0.357 ± 0.146	0.291 ± 0.139	0.247 ± 0.052	0.245 ± 0.07	0.259 ± 0.082	0.392 ± 0.034	0.209 ± 0.024	0.216 ± 0.012	0.233 ± 0.016	0.167 ± 0.146	0.261 ± 0.058	0.353 ± 0.033
8102	0.211 ± 0.079	0.174 ± 0.054	0.173 ± 0.076	0.125 ± 0.064	0.248 ± 0.009	0.177 ± 0.081	0.233 ± 0.054	0.133 ± 0.008	0.17 ± 0.038	0.211 ± 0.014	0.1 ± 0.029	0.258 ± 0.061	0.256 ± 0.041	0.213 ± 0.019	0.234 ± 0.029	0.332 ± 0.079	0.231 ± 0.127	0.302 ± 0.123
8105	0.227 ± 0.106	0.16 ± 0.043	0.143 ± 0.028	0.157 ± 0.082	0.331 ± 0.019	0.163 ± 0.073	0.217 ± 0.05	0.232 ± 0.03	0.187 ± 0.031	0.248 ± 0.056	0.152 ± 0.078	0.192 ± 0.127	0.212 ± 0.04	0.227 ± 0.047	0.284 ± 0.034	0.213 ± 0.003	0.274 ± 0.069	0.323 ± 0.129
8106	0.085 ± 0.006	0.07 ± 0.009	0.109 ± 0.068	0.065 ± 0.038	0.076 ± 0.039	0.075 ± 0.045	0.112 ± 0.033	0.101 ± 0.051	0.051 ± 0.041	0.063 ± 0.02	0.053 ± 0.037	0.035 ± 0.02	0.122 ± 0.048	0.091 ± 0.011	0.109 ± 0.048	0.072 ± 0.005	0.103 ± 0.041	0.13 ± 0.063
8111	0.237 ± 0.116	0.311 ± 0.216	0.17 ± 0.114	0.267 ± 0.167	0.474 ± 0.046	0.168 ± 0.092	0.32 ± 0.1	0.213 ± 0.2	0.156 ± 0.122	0.188 ± 0.045	0.266 ± 0.055	0.109 ± 0.08	0.231 ± 0.288	0.262 ± 0.143	0.13 ± 0.084	0.09 ± 0.003	0.051 ± 0.014	0.25 ± 0.11

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50 µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
7408		0.14	0.51	-	0.39	0.05	↗	-	-	-	-	-	-	-	-	-
7409	Glutamine synthetase / OEE 1 / RuBisCO activase A / Phosphoglycerate kinase	0.16	0.44	-	0.53	0.007	↗↗	-	-	-	-	-	-	-	-	-
7410	Phosphoribulokinase, chloroplastic EC=2.7.1.19	0.13	0.52	-	0.46	0.021	↗↗	-	-	-	-	-	-	-	-	-
7412	Glutamine synthetase leaf isozyme, chloroplastic EC=6.3.1.2	-0.16	0.44	-	0.50	0.011	↗↗	-	-	-	-	-	-	-	-	-
7413	Phosphoribulokinase, chloroplastic EC=2.7.1.19	0.35	0.087	↗	0.51	0.010	↗↗	-	-	M >	-	-	-	-	-	-
7414		-0.16	0.45	-	-0.39	0.05	↘	-	-	-	-	-	-	-	-	-
7501		0.13	0.55	-	0.39	0.06	↗	-	-	-	-	-	-	-	-	-
7502	Ribulose bisphosphate carboxylase/oxygenase activase A, chloro.	0.20	0.347	-	0.41	0.05	↗↗	-	-	-	-	-	-	-	-	-
7608	Tubulin alpha	0.16	0.43	-	0.67	0.0002	↗↗↗	-	-	-	-	-	-	-	-	-
7701	60 kDa chaperonin subunit beta	0.34	0.098	↗	0.58	0.002	↗↗	-	-	-	-	-	-	-	-	-
7703	ATP-dependent zinc metalloprotease FTSH 2, chloroplastic	0.14	0.52	-	0.48	0.014	↗↗	-	-	-	-	-	-	-	-	-
7704	60 kDa chaperonin subunit beta	0.11	0.62	-	0.55	0.005	↗↗	-	-	-	-	-	-	-	-	-
7705		0.15	0.49	-	0.30	0.15	-	-	-	-	-	-	-	-	-	-
7706	RuBisCO large subunit-binding protein subunit beta, chloro.	0.04	0.86	-	0.44	0.03	↗↗	-	-	-	-	-	-	-	-	-
7801		-0.16	0.46	-	0.23	0.28	-	-	-	-	-	-	-	-	-	-
7803		0.01	0.97	-	0.40	0.05	↗	-	-	-	-	-	-	-	-	-
8102	Thioredoxin peroxidase EC=1.11.1.15 / 2-Cys peroxiredoxin BAS1	0.13	0.55	-	0.67	0.0003	↗↗↗	-	-	-	-	-	NM >	-	-	-
8105	Thioredoxin peroxidase EC=1.11.1.15 / 2-Cys peroxiredoxin BAS1	0.29	0.16	-	0.53	0.01	↗↗	-	-	-	-	-	-	-	-	-
8106		0.11	0.61	-	0.35	0.09	↗	-	-	-	-	-	-	-	-	-
8111	ND	-0.45	0.025	↘↘	-0.17	0.42	-	-	-	M >	-	-	-	-	-	NM >>

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Spots 8201 to 9201



Spots variation (Prot accumulation) in response to Cu exposure for M (black points) and NM (white points) populations

SSP	mM1	mNM1	mM5	mNM5	mM10	mNM10	mM15	mNM15	mM20	mNM20	mM25	mNM25	mM30	mNM30	mM40	mNM40	mM50	mNM50
8201	0.324 ± 0.088	0.32 ± 0.128	0.376 ± 0.258	0.467 ± 0.107	0.396 ± 0.001	0.33 ± 0.198	0.277 ± 0.129	0.305 ± 0.058	0.218 ± 0.044	0.282 ± 0.102	0.324 ± 0.178	0.155 ± 0.078	0.317 ± 0.203	0.268 ± 0.098	0.159 ± 0.115	0.113 ± 0.015	0.092 ± 0.039	0.157 ± 0.1
8202	0.214 ± 0.135	0.159 ± 0.065	0.2 ± 0.038	0.173 ± 0.056	0.313 ± 0.141	0.101 ± 0.038	0.205 ± 0.034	0.272 ± 0.03	0.126 ± 0.088	0.137 ± 0.101	0.129 ± 0.061	0.183 ± 0.043	0.211 ± 0.037	0.199 ± 0.045	0.267 ± 0.078	0.169 ± 0.152	0.212 ± 0.045	0.349 ± 0.132
8204	0.141 ± 0.011	0.071 ± 0.009	0.233 ± 0.12	0.151 ± 0.051	0.391 ± 0.198	0.119 ± 0.049	0.374 ± 0.258	0.305 ± 0.377	0.076 ± 0.051	0.123 ± 0.08	0.196 ± 0.089	0.07 ± 0.025	0.225 ± 0.124	0.235 ± 0.119	0.229 ± 0.055	0.165 ± 0.162	0.186 ± 0.074	0.525 ± 0.318
8205	0.103 ± 0.041	0.09 ± 0.047	0.09 ± 0.002	0.076 ± 0.046	0.097 ± 0.038	0.08 ± 0.079	0.115 ± 0.032	0.115 ± 0.014	0.077 ± 0.022	0.053 ± 0.024	0.057 ± 0.053	0.034 ± 0.009	0.19 ± 0.045	0.097 ± 0.029	0.106 ± 0.055	0.091 ± 0.016	0.039 ± 0.025	0.202 ± 0.081
8211	0.219 ± 0.095	0.163 ± 0.024	0.196 ± 0.075	0.19 ± 0.084	0.317 ± 0.123	0.074 ± 0.024	0.185 ± 0.097	0.154 ± 0.024	0.112 ± 0.096	0.104 ± 0.061	0.087 ± 0.044	0.163 ± 0.029	0.257 ± 0.151	0.282 ± 0.127	0.162 ± 0.086	0.181 ± 0.196	0.109 ± 0.059	0.143 ± 0.094
8301	0.11 ± 0.004	0.061 ± 0.027	0.112 ± 0.059	0.096 ± 0.041	0.102 ± 0.001	0.1 ± 0.006	0.099 ± 0.024	0.12 ± 0.039	0.087 ± 0.02	0.101 ± 0.04	0.102 ± 0.015	0.084 ± 0.01	0.18 ± 0.01	0.122 ± 0.015	0.068 ± 0.035	0.047 ± 0.018	0.074 ± 0.023	0.163 ± 0.054
8501	0.174 ± 0.045	0.069 ± 0.044	0.139 ± 0.086	0.108 ± 0.028	0.07 ± 0.004	0.043 ± 0.015	0.079 ± 0.048	0.169 ± 0.095	0.15 ± 0.077	0.083 ± 0.022	0.081 ± 0.019	0.066 ± 0.031	0.148 ± 0.034	0.08 ± 0.033	0.106 ± 0.077	0.105 ± 0.075	0.134 ± 0.047	0.176 ± 0.04
8701	0.182 ± 0.099	0.12 ± 0.069	0.139 ± 0.054	0.074 ± 0.02	0.141 ± 0.005	0.079 ± 0.023	0.148 ± 0.042	0.145 ± 0.022	0.123 ± 0.074	0.121 ± 0.008	0.103 ± 0.057	0.127 ± 0.058	0.145 ± 0.026	0.123 ± 0.026	0.268 ± 0.164	0.156 ± 0.118	0.175 ± 0.082	0.236 ± 0.114
8702	0.028 ± 0.011	0.024 ± 0.007	0.016 ± 0.009	0.034 ± 0.021	0.047 ± 0.011	0.035 ± 0.008	0.031 ± 0.01	0.046 ± 0.023	0.02 ± 0.012	0.053 ± 0.014	0.027 ± 0.01	0.033 ± 0.002	0.035 ± 0.024	0.034 ± 0.025	0.04 ± 0.014	0.022 ± 0.001	0.027 ± 0.009	0.072 ± 0.035
8703	0.07 ± 0.033	0.033 ± 0.019	0.059 ± 0.027	0.023 ± 0.003	0.039 ± 0.021	0.018 ± 0.005	0.039 ± 0.011	0.056 ± 0.02	0.038 ± 0.025	0.034 ± 0.014	0.025 ± 0.014	0.031 ± 0.016	0.067 ± 0.027	0.043 ± 0.003	0.057 ± 0.045	0.026 ± 0.009	0.041 ± 0.022	0.096 ± 0.03
8704	0.032 ± 0.009	0.019 ± 0.011	0.016 ± 0.007	0.018 ± 0.007	0.022 ± 0.001	0.014 ± 0.002	0.02 ± 0.002	0.024 ± 0.02	0.009 ± 0.004	0.027 ± 0.002	0.014 ± 0.003	0.02 ± 0.01	0.031 ± 0.014	0.021 ± 0.009	0.016 ± 0.002	0.024 ± 0.002	0.008 ± 0.004	0.049 ± 0.014
8705	0.061 ± 0.017	0.058 ± 0.048	0.128 ± 0.073	0.062 ± 0.028	0.117 ± 0.041	0.081 ± 0.03	0.08 ± 0.017	0.088 ± 0.047	0.093 ± 0.049	0.092 ± 0.037	0.094 ± 0.003	0.066 ± 0.024	0.111 ± 0.036	0.084 ± 0.019	0.056 ± 0.032	0.084 ± 0.041	0.059 ± 0.021	0.153 ± 0.02
8804	0.621 ± 0.316	0.385 ± 0.203	0.501 ± 0.165	0.411 ± 0.111	0.45 ± 0.033	0.475 ± 0.133	0.63 ± 0.303	0.539 ± 0.292	0.451 ± 0.074	0.456 ± 0.224	0.623 ± 0.08	0.41 ± 0.213	0.743 ± 0.356	0.387 ± 0.031	0.555 ± 0.103	0.406 ± 0.232	0.446 ± 0.254	0.947 ± 0.242
9201	0.18 ± 0.057	0.188 ± 0.049	0.168 ± 0.091	0.107 ± 0.024	0.213 ± 0.078	0.129 ± 0.045	0.166 ± 0.084	0.275 ± 0.112	0.17 ± 0.101	0.163 ± 0.079	0.169 ± 0.043	0.154 ± 0.069	0.18 ± 0.035	0.207 ± 0.127	0.191 ± 0.054	0.351 ± 0.053	0.19 ± 0.121	0.594 ± 0.36

Mean values (± sd, n = 2 or 3) for both population (M and NM) at each Cu exposure (1, 5, 10, 15, 20, 25, 30, 40, 50µM Cu).

Sp	ID	rM	pval		rNM	pval		ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
8201	Oxygen-evolving enhancer protein 1, chloroplastic : OEE1	-0.54	0.005	↘↘↘	-0.60	0.001	↘↘↘	-	-	-	-	-	-	-	-	-
8202	Putative ribose-5-phosphate isomerase / Light harvesting chlorophyll a/b binding protein	0.02	0.91	-	0.49	0.014	↗↗	-	-	-	-	-	-	-	-	-
8204	Chlorophyll a-b binding protein 2 / Ribulose-phosphate 3-epimerase	-0.09	0.68	-	0.52	0.008	↗↗↗	M >	-	-	-	-	-	-	-	-
8205	14-3-3-like protein A	-0.19	0.36	-	0.44	0.030	↗↗	-	-	-	-	-	-	-	-	NM >
8211		-0.33	0.10	-	0.12	0.57	-	-	-	M >	-	-	-	-	-	-
8301		-0.27	0.19	-	0.39	0.054	↗	-	-	-	-	-	-	-	-	-
8501	Glutamine synthetase, chloroplastic EC=6.3.1.2	-0.09	0.67	-	0.41	0.044	↗↗	-	-	-	-	-	-	-	-	-
8701	60 kDa chaperonin subunit alpha	0.22	0.30	-	0.57	0.003	↗↗↗	-	-	-	-	-	-	-	-	-
8702		0.16	0.44	-	0.39	0.05	↗	-	-	-	-	-	-	-	-	-
8703	RuBisCO large subunit-binding protein subunit alpha, chloro.	-0.14	0.51	-	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
8704	Nucleoredoxin EC=1.8.1.8	-0.44	0.026	↘↘	0.60	0.001	↗↗↗	-	-	-	-	NM >	-	-	-	NM >>
8705	Protein disulfide isomerase : PDI EC=5.3.4.1	-0.31	0.13	-	0.57	0.003	↗↗↗	-	-	-	-	-	-	-	-	NM >
8804	Heat shock 70 kDa protein 7, chloroplastic	-0.07	0.74	-	0.48	0.015	↗↗	-	-	-	-	-	-	-	-	-
9201	Cp31BHv	0.06	0.78	-	0.64	0.0006	↗↗↗↗	-	-	-	-	-	-	-	-	-

Sp: spots number; ID: results of protein identification (ND = non determined); rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; ratio (1-50): comparative ratio between populations at each Cu exposure, =: no difference; M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Annex 20 - Correlation with Cu in M and NM leaves

Down -regulated in M, up-regulated in NM (2 spots)

SSP	ID	rM	pval		rNM	pval		Ratio 1	Ratio 5	Ratio 10	Ratio 15	Ratio 20	Ratio 25	Ratio 30	Ratio 40	Ratio 50
7306	exc.	-0.38	0.062	↘	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
8704	exc.	-0.44	0.026	↘↘	0.60	0.001	↗↗↗	-	-	-	-	NM >	-	-	-	NM >>

Up-regulated spots (14 spots)

SSP	ID	rM	pval		rNM	pval		Ratio 1	Ratio 5	Ratio 10	Ratio 15	Ratio 20	Ratio 25	Ratio 30	Ratio 40	Ratio 50
6309	exc.	0.54	0.006	↗↗↗	0.58	0.00	↗↗↗	-	-	-	-	-	-	-	-	-
2801	exc.	0.45	0.025	↗↗	0.43	0.03	↗↗	-	-	-	-	-	-	-	-	-
3503	exc.	0.50	0.010	↗↗	0.49	0.01	↗↗	-	-	-	-	-	-	-	-	-
2704	exc.	0.38	0.062	↗	0.34	0.09	↗	-	-	-	M >	-	-	-	-	-
3404		0.34	0.092	↗	0.36	0.08	↗	-	-	-	-	-	-	-	-	-
5708	exc.	0.51	0.010	↗↗↗	0.41	0.04	↗↗	-	-	-	-	-	-	-	-	-
2703	exc.	0.39	0.055	↗	0.40	0.05	↗↗	-	-	-	-	-	-	-	-	-
4401	exc.	0.36	0.077	↗	0.45	0.02	↗↗	-	-	-	-	-	-	-	-	-
2809	exc.	0.37	0.065	↗	0.55	0.00	↗↗↗	-	-	-	-	-	-	-	-	-
5503	exc.	0.40	0.051	↗	0.57	0.00	↗↗↗	-	-	-	-	-	-	-	-	-
7413	exc.	0.35	0.087	↗	0.51	0.010	↗↗↗	-	-	M >	-	-	-	-	-	-
7701	exc.	0.34	0.098	↗	0.58	0.002	↗↗↗	-	-	-	-	-	-	-	-	-
5201	exc.	0.37	0.069	↗	0.68	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
5412	exc.	0.50	0.011	↗↗	0.58	0.002	↗↗↗	-	-	-	-	-	-	-	-	-

Down-regulated spots (10 spots)

SSP	ID	rM	pval		rNM	pval		Ratio 1	Ratio 5	Ratio 10	Ratio 15	Ratio 20	Ratio 25	Ratio 30	Ratio 40	Ratio 50
3104	exc.	-0.65	< 0.001	↘↘↘↘	-0.75	< 0.001	↘↘↘↘	-	-	-	-	-	-	-	-	-
8201	exc.	-0.54	0.005	↘↘↘	-0.60	0.00	↘↘↘	-	-	-	-	-	-	-	-	-
4708	exc.	-0.47	0.017	↘↘	-0.49	0.01	↘↘	-	-	-	-	-	-	-	-	-
4203		-0.36	0.077	↘	-0.36	0.07	↘	-	-	-	-	-	-	-	-	-
2104	exc.	-0.51	0.009	↘↘↘	-0.46	0.03	↘↘	-	-	-	-	-	-	-	-	-
7208	exc.	-0.34	0.095	↘	-0.65	< 0.001	↘↘↘↘	-	-	-	-	-	-	-	-	-
3102	exc.	-0.47	0.019	↘↘	-0.54	0.01	↘↘↘	-	-	-	-	-	-	-	-	-
6106	exc.	-0.43	0.034	↘↘	-0.57	0.00	↘↘↘	-	-	-	-	-	-	-	-	-
4107	exc.	-0.56	0.004	↘↘↘	-0.75	< 0.001	↘↘↘↘	NM >	-	-	-	-	-	-	-	-
7214	exc.	-0.53	0.006	↘↘↘	-0.75	< 0.001	↘↘↘↘	-	-	-	-	-	-	-	-	-

Annex 21 - Correlation with Cu only in M leaves

Up-regulated spots in M (4 spots)

SSP	ID	rM	pval		rNM	pval		Ratio 1	Ratio 5	Ratio 10	Ratio 15	Ratio 20	Ratio 25	Ratio 30	Ratio 40	Ratio 50
1305	exc.	0.46	0.021	↗↗	-0.12	0.58	-	-	-	-	-	-	-	-	-	-
5808	exc.	0.45	0.023	↗↗	0.22	0.29	-	-	-	-	-	-	M >>	-	M >>	-
1506		0.36	0.078	↗	0.13	0.54	-	-	-	-	-	-	-	-	-	-
3507		0.34	0.098	↗	-0.02	0.94	-	-	-	-	-	-	-	-	-	-

Down-regulated spots in M (15 spots)

SSP	ID	rM	pval		rNM	pval		Ratio 1	Ratio 5	Ratio 10	Ratio 15	Ratio 20	Ratio 25	Ratio 30	Ratio 40	Ratio 50
2308		-0.34	0.098	↘	0.17	0.42	-	-	-	-	-	-	-	-	-	-
4408		-0.39	0.057	↘	0.10	0.63	-	-	-	-	-	-	-	-	-	-
5103		-0.35	0.085	↘	-0.18	0.39	-	-	-	-	-	-	-	-	-	-
6703		-0.34	0.099	↘	0.34	0.10	-	-	-	-	-	-	-	-	-	-
7302		-0.34	0.096	↘	-0.16	0.45	-	-	-	-	-	-	-	-	-	-
7401		-0.39	0.055	↘	0.21	0.32	-	-	-	-	-	-	-	-	-	-
2106	exc.	-0.41	0.040	↘↘	-0.28	0.18	-	-	-	-	-	-	-	-	-	-
3707	exc.	-0.46	0.022	↘↘	-0.27	0.19	-	-	-	NM >	-	-	-	-	-	-
4105	exc.	-0.40	0.045	↘↘	0.16	0.45	-	-	-	-	-	-	-	-	-	-
5104	exc.	-0.48	0.014	↘↘	-0.03	0.88	-	-	-	M >	-	-	-	-	-	-
7210	exc.	-0.40	0.048	↘↘	-0.33	0.10	-	-	-	-	-	-	-	-	-	-
7211	exc.	-0.43	0.033	↘↘	-0.34	0.10	-	-	-	-	M >	-	-	-	-	-
8111	exc.	-0.45	0.025	↘↘	-0.17	0.42	-	-	-	M >	-	-	-	-	-	NM >>
4308	exc.	-0.55	0.005	↘↘↘	-0.11	0.59	-	-	-	-	-	-	-	-	-	-
6110	exc.	-0.55	0.004	↘↘↘	-0.22	0.30	-	-	-	-	-	-	-	-	-	-

Annex 22 - Correlation with Cu only in NM leaves

Up-regulated spots in NM (80 spots)

SSP	ID	rM	pval	rNM	pval		Ratio 1	Ratio 5	Ratio 10	Ratio 15	Ratio 20	Ratio 25	Ratio 30	Ratio 40	Ratio 50
2303	exc.	-0.04	0.84	0.69	< 0.001	↗↗↗↗	-	-	-	-	-	NM >	NM >>	-	-
4704	exc.	0.26	0.20	0.62	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
4806	exc.	0.00	0.99	0.73	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
5303	exc.	-0.25	0.23	0.63	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
5508	exc.	0.33	0.10	0.68	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
6203	exc.	0.18	0.40	0.68	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
6707	exc.	0.29	0.15	0.65	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
7103	exc.	0.16	0.43	0.62	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
7608	exc.	0.16	0.43	0.67	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
8102	exc.	0.13	0.55	0.67	< 0.001	↗↗↗↗	-	-	-	-	-	NM >	-	-	-
9201	exc.	0.06	0.78	0.64	< 0.001	↗↗↗↗	-	-	-	-	-	-	-	-	-
2402	exc.	0.13	0.53	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
2806	exc.	0.33	0.112	0.60	0.001	↗↗↗	-	-	-	-	-	-	-	NM >	-
3202	exc.	0.00	0.98	0.56	0.004	↗↗↗	-	-	M >	-	-	-	-	NM >>	-
4407	exc.	0.14	0.49	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-
4801	exc.	0.24	0.24	0.53	0.01	↗↗↗	-	-	-	-	-	-	-	-	-
5101	exc.	-0.30	0.144	0.54	0.005	↗↗↗	-	-	-	-	-	-	-	-	NM >>
5801	exc.	0.13	0.55	0.52	0.008	↗↗↗	-	-	-	-	-	-	-	-	-
5802	exc.	0.03	0.87	0.51	0.01	↗↗↗	-	-	-	-	-	-	-	-	-
5807	exc.	-0.09	0.67	0.52	0.01	↗↗↗	-	-	-	-	-	-	-	-	NM >
6304	exc.	0.28	0.173	0.55	0.00	↗↗↗	-	-	-	-	-	-	-	-	-
6402	exc.	-0.01	0.96	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-
6408	exc.	-0.27	0.19	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
6705	exc.	-0.06	0.78	0.53	0.006	↗↗↗	-	-	-	-	-	-	-	-	-
6706	exc.	0.32	0.116	0.55	0.004	↗↗↗	-	-	-	-	-	-	-	-	-
6708	exc.	-0.16	0.46	0.52	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
6710	exc.	0.28	0.17	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
6802	exc.	0.11	0.61	0.52	0.01	↗↗↗	-	-	-	M >	-	-	-	-	-
7409	exc.	0.16	0.44	0.53	0.007	↗↗↗	-	-	-	-	-	-	-	-	-
7704	exc.	0.11	0.62	0.55	0.005	↗↗↗	-	-	-	-	-	-	-	-	-
8105	exc.	0.29	0.16	0.53	0.01	↗↗↗	-	-	-	-	-	-	-	-	-
8204	exc.	-0.09	0.68	0.52	0.008	↗↗↗	M >	-	-	-	-	-	-	-	-
8701	exc.	0.22	0.30	0.57	0.00	↗↗↗	-	-	-	-	-	-	-	-	-
8703	exc.	-0.14	0.51	0.61	0.001	↗↗↗	-	-	-	-	-	-	-	-	-
8705	exc.	-0.31	0.13	0.57	0.003	↗↗↗	-	-	-	-	-	-	-	-	NM >
1104	exc.	0.15	0.48	0.41	0.04	↗↗	-	-	-	-	-	-	-	-	-
1107	exc.	0.06	0.772	0.45	0.03	↗↗	M >>	M >>	M >>	M >>	M >>	-	-	M >>	M >>
3301	exc.	-0.03	0.87	0.44	0.026	↗↗	-	-	-	-	-	-	-	-	-

4501	exc.	0.27	0.20	0.45	0.02	↗↗	-	-	-	-	-	-	-	-	-
5304	exc.	0.03	0.88	0.45	0.02	↗↗	-	-	-	M >	-	-	-	-	-
6101	exc.	0.12	0.570	0.47	0.018	↗↗	-	-	-	-	-	-	-	-	-
6107	exc.	0.20	0.35	0.41	0.04	↗↗	-	-	-	-	-	-	-	-	-
6202	exc.	0.32	0.12	0.49	0.01	↗↗	-	-	-	-	-	-	-	-	-
6208	exc.	0.07	0.76	0.48	0.01	↗↗	-	-	-	-	-	-	-	-	-
6303	exc.	0.11	0.62	0.46	0.02	↗↗	-	-	-	-	-	-	-	-	-
6305	exc.	0.04	0.83	0.44	0.03	↗↗	-	-	-	-	-	-	-	-	-
6701	exc.	-0.03	0.90	0.44	0.029	↗↗	-	-	-	-	-	-	-	-	-
6805	exc.	0.23	0.26	0.47	0.018	↗↗	-	-	-	-	-	-	-	-	-
6806	exc.	0.05	0.83	0.46	0.02	↗↗	-	-	-	-	-	-	-	-	-
7202	exc.	0.28	0.18	0.47	0.017	↗↗	-	-	-	-	-	-	-	-	-
7407	exc.	-0.05	0.81	0.42	0.04	↗↗	-	-	-	-	-	-	-	-	NM >
7410	exc.	0.13	0.52	0.46	0.021	↗↗	-	-	-	-	-	-	-	-	-
7412	exc.	-0.16	0.44	0.50	0.011	↗↗	-	-	-	-	-	-	-	-	-
7502	exc.	0.20	0.347	0.41	0.05	↗↗	-	-	-	-	-	-	-	-	-
7703	exc.	0.14	0.52	0.48	0.014	↗↗	-	-	-	-	-	-	-	-	-
7706	exc.	0.04	0.86	0.44	0.03	↗↗	-	-	-	-	-	-	-	-	-
8202	exc.	0.02	0.91	0.49	0.014	↗↗	-	-	-	-	-	-	-	-	-
8205	exc.	-0.19	0.36	0.44	0.030	↗↗	-	-	-	-	-	-	-	-	NM >
8501	exc.	-0.09	0.67	0.41	0.044	↗↗	-	-	-	-	-	-	-	-	-
8804	exc.	-0.07	0.74	0.48	0.015	↗↗	-	-	-	-	-	-	-	-	-
2102		-0.20	0.33	0.35	0.08	↗	-	-	-	-	-	-	-	-	-
2205		0.00	0.98	0.38	0.06	↗	-	-	-	-	-	-	-	-	-
2508		-0.02	0.92	0.35	0.091	↗	-	-	-	-	-	-	-	-	-
3103		0.01	0.95	0.34	0.10	↗	-	-	-	-	-	-	-	-	-
3709		-0.06	0.772	0.34	0.092	↗	-	-	-	-	-	-	-	-	-
4405		0.20	0.35	0.36	0.077	↗	-	-	-	-	-	-	-	-	-
4505		0.08	0.70	0.36	0.07	↗	-	-	-	-	-	-	-	-	-
4802		0.14	0.496	0.34	0.10	↗	-	-	-	-	-	-	-	-	-
5404		-0.23	0.28	0.36	0.07	↗	-	-	-	-	-	-	-	-	-
5707		0.01	0.97	0.34	0.10	↗	-	-	-	-	-	-	-	-	-
6301		0.13	0.55	0.39	0.05	↗	-	-	-	-	-	-	-	-	-
6401		0.16	0.44	0.39	0.056	↗	-	-	-	-	-	-	-	-	-
7209		-0.10	0.65	0.38	0.06	↗	-	-	-	-	-	-	-	-	-
7402		0.17	0.41	0.38	0.06	↗	-	-	-	-	-	-	-	-	-
7408		0.14	0.51	0.39	0.05	↗	-	-	-	-	-	-	-	-	-
7501		0.13	0.55	0.39	0.06	↗	-	-	-	-	-	-	-	-	-
7803		0.01	0.97	0.40	0.05	↗	-	-	-	-	-	-	-	-	-
8106		0.11	0.61	0.35	0.09	↗	-	-	-	-	-	-	-	-	-
8301		-0.27	0.19	0.39	0.054	↗	-	-	-	-	-	-	-	-	-
8702		0.16	0.44	0.39	0.05	↗	-	-	-	-	-	-	-	-	-

Down-regulated spots in NM (11 spots)

SSP	ID	rM	pval	rNM	pval		Ratio 1	Ratio 5	Ratio 10	Ratio 15	Ratio 20	Ratio 25	Ratio 30	Ratio 40	Ratio 50
1101	exc.	-0.05	0.80	-0.70	< 0.001	↘↘↘↘	-	-	-	-	-	-	-	-	-
6103	exc.	-0.26	0.21	-0.68	< 0.001	↘↘↘↘	-	-	-	-	-	-	-	-	-
2312	exc.	0.13	0.53	-0.53	0.01	↘↘↘	-	-	-	-	-	-	-	-	-
2707	exc.	-0.22	0.30	-0.59	0.002	↘↘↘	-	-	-	-	-	-	-	-	M >>
1501	exc.	0.30	0.14	-0.47	0.02	↘↘	-	-	NM >>	-	-	-	-	-	-
2103	exc.	0.03	0.89	-0.43	0.034	↘↘	-	-	-	-	-	-	-	-	-
2105	exc.	-0.07	0.75	-0.42	0.04	↘↘	-	-	-	-	-	-	-	-	-
6310	exc.	0.08	0.69	-0.47	0.02	↘↘	-	-	-	-	-	-	-	-	-
1201		0.27	0.20	-0.34	0.10	↘	-	-	-	-	-	-	-	-	-
2808	exc.	-0.29	0.16	-0.36	0.08	↘	-	-	NM >>	-	-	-	-	-	-
7414		-0.16	0.45	-0.39	0.05	↘	-	-	-	-	-	-	-	-	-

Annex 23 - Over-expressed spots in leaves

SSP	ID	rM		rNM		Pop	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
1107	exc.	0.06	-	0.45	↗↗	M	M >>	M >>	M >>	M >>	M >>	-	-	M >>	M >>
1111		-0.11	-	-0.14	-	M	-	-	-	-	-	-	M >	-	-
1803	exc.	0.11	-	-0.15	-	M	-	-	-	-	M >	-	-	-	M >
2704	exc.	0.38	↗	0.34	↗	M	-	-	-	M >	-	-	-	-	-
2707	exc.	-0.22	-	-0.59	↘↘↘	M	-	-	-	-	-	-	-	-	M >>
3315		-0.09	-	0.05	-	M	-	-	-	-	-	-	-	M >	-
4104		0.10	-	0.08	-	M	-	-	-	-	-	-	-	M >	-
4414	exc.	0.33	-	NA		M	M >>	M >>	M >>	M >>	M >>	M >>	M >>	M >>	M >>
5104	exc.	-0.48	↘↘	-0.03	-	M	-	-	M >	-	-	-	-	-	-
5304	exc.	0.03	-	0.45	↗↗	M	-	-	-	M >	-	-	-	-	-
5808	exc.	0.45	↗↗	0.22	-	M	-	-	-	-	-	M >>	-	M >>	-
6311		0.17	-	-0.28	-	M	-	-	-	-	-	-	M >	-	-
6802	exc.	0.11	-	0.52	↗↗↗	M	-	-	-	M >	-	-	-	-	-
7211	exc.	-0.43	↘↘	-0.34	-	M	-	-	-	M >	-	-	-	-	-
7413	exc.	0.35	↗	0.51	↗↗↗	M	-	-	M >	-	-	-	-	-	-
8204	exc.	-0.09	-	0.52	↗↗↗	M	M >	-	-	-	-	-	-	-	-
8211		-0.33	-	0.12	-	M	-	-	M >	-	-	-	-	-	-
1501	exc.	0.30	-	-0.47	↘↘	NM	-	-	NM >>	-	-	-	-	-	-
2303	exc.	-0.04	-	0.69	↗↗↗↗	NM	-	-	-	-	-	NM >	NM >>	-	-
2507		0.11	-	0.13	-	NM	-	-	NM >	-	-	-	-	-	-
2806	exc.	0.33	-	0.60	↗↗↗	NM	-	-	-	-	-	-	-	NM >	-
2808	exc.	-0.29	-	-0.36	↘	NM	-	-	NM >>	-	-	-	-	-	-
3205		0.25	-	0.22	-	NM	-	-	NM >	-	-	-	-	-	-
3707	exc.	-0.46	↘↘	-0.27	-	NM	-	-	NM >	-	-	-	-	-	-
4107	exc.	-0.56	↘↘↘	-0.75	↘↘↘↘	NM	NM >	-	-	-	-	-	-	-	-
5101	exc.	-0.30	-	0.54	↗↗↗	NM	-	-	-	-	-	-	-	-	NM >>
5806		0.09	-	0.13	-	NM	-	-	-	NM >	-	-	-	-	-
5807	exc.	-0.09	-	0.52	↗↗↗	NM	-	-	-	-	-	-	-	-	NM >
6403		0.06	-	-0.13	-	NM	NM >	-	-	NM >	-	-	-	-	-
6409		0.03	-	-0.02	-	NM	-	-	-	-	NM >	-	-	-	-
7212		-0.27	-	0.17	-	NM	-	-	-	-	-	-	-	-	NM >
7304		0.07	-	-0.17	-	NM	-	-	-	-	NM >	-	-	-	-
7407	exc.	-0.05	-	0.42	↗↗	NM	-	-	-	-	-	-	-	-	NM >
8102	exc.	0.13	-	0.67	↗↗↗↗	NM	-	-	-	-	-	NM >	-	-	-
8205	exc.	-0.19	-	0.44	↗↗	NM	-	-	-	-	-	-	-	-	NM >
8704	exc.	-0.44	↘↘	0.60	↗↗↗	NM	-	-	-	-	NM >	-	-	-	NM >>
8705	exc.	-0.31	-	0.57	↗↗↗	NM	-	-	-	-	-	-	-	-	NM >
1804	exc.	0.33	-	0.25	-	M NM	-	-	-	M >>	-	-	-	NM >	-
3202	exc.	0.00	-	0.56	↗↗↗	M NM	-	-	M >	-	-	-	-	NM >>	-
8111	exc.	-0.45	↘↘	-0.17	-	M NM	-	-	M >	-	-	-	-	-	NM >>

Annex 24 - Over-expressed leaf spots correlated with Cu

Sp: spots number; ID Exc.: excised; rM/rNM: r coefficient of Pearson's correlation for population M or NM, p-val: 1 < - < 0.1 < ↗ < 0.05 < ↗↗ < 0.1 < ↗↗↗ < 0.001< ↗↗↗↗; Ratio (1-50): ratio between populations at each Cu exposure, -: no difference; Pop: M/NM indicated the population with higher mean; >/>>: ratio of x1.5/x2.

Sp	ID	rM	rNM	Pop	ratio 1	ratio 5	ratio 10	ratio 15	ratio 20	ratio 25	ratio 30	ratio 40	ratio 50
2704	exc.	0.38 ↗	0.34 ↗	M	-	-	-	M >	-	-	-	-	-
5104	exc.	-0.48 ↘↘	-0.03 -	M	-	-	M >	-	-	-	-	-	-
5808	exc.	0.45 ↗↗	0.22 -	M	-	-	-	-	-	M >>	-	M >>	-
7211	exc.	-0.43 ↘↘	-0.34 -	M	-	-	-	M >	-	-	-	-	-
7413	exc.	0.35 ↗	0.51 ↗↗↗	M	-	-	M >	-	-	-	-	-	-
3707	exc.	-0.46 ↘↘	-0.27 -	NM	-	-	NM >	-	-	-	-	-	-
4107	exc.	-0.56 ↘↘↘	-0.75 ↘↘↘↘	NM	NM >	-	-	-	-	-	-	-	-
8704	exc.	-0.44 ↘↘	0.60 ↗↗↗	NM	-	-	-	-	NM >	-	-	-	NM >>
8111	exc.	-0.45 ↘↘	-0.17 -	M NM	-	-	M >	-	-	-	-	-	NM >>
1107	exc.	0.06 -	0.45 ↗↗	M	M >>	M >>	M >>	M >>	M >>	-	-	M >>	M >>
2707	exc.	-0.22 -	-0.59 ↘↘↘	M	-	-	-	-	-	-	-	-	M >>
5304	exc.	0.03 -	0.45 ↗↗	M	-	-	-	M >	-	-	-	-	-
6802	exc.	0.11 -	0.52 ↗↗↗	M	-	-	-	M >	-	-	-	-	-
8204	exc.	-0.09 -	0.52 ↗↗↗	M	M >	-	-	-	-	-	-	-	-
1501	exc.	0.30 -	-0.47 ↘↘	NM	-	-	NM >>	-	-	-	-	-	-
2303	exc.	-0.04 -	0.69 ↗↗↗↗	NM	-	-	-	-	-	NM >	NM >>	-	-
2806	exc.	0.33 -	0.60 ↗↗↗	NM	-	-	-	-	-	-	-	NM >	-
2808	exc.	-0.29 -	-0.36 ↘	NM	-	-	NM >>	-	-	-	-	-	-
5101	exc.	-0.30 -	0.54 ↗↗↗	NM	-	-	-	-	-	-	-	-	NM >>
5807	exc.	-0.09 -	0.52 ↗↗↗	NM	-	-	-	-	-	-	-	-	NM >
7407	exc.	-0.05 -	0.42 ↗↗	NM	-	-	-	-	-	-	-	-	NM >
8102	exc.	0.13 -	0.67 ↗↗↗↗	NM	-	-	-	-	-	NM >	-	-	-
8205	exc.	-0.19 -	0.44 ↗↗	NM	-	-	-	-	-	-	-	-	NM >
8705	exc.	-0.31 -	0.57 ↗↗↗	NM	-	-	-	-	-	-	-	-	NM >
3202	exc.	0.00 -	0.56 ↗↗↗	M NM	-	-	M >	-	-	-	-	NM >>	-

Annex 25 - Leaf spots not influenced by treatments

Sp	rM	pval	rNM	pval
1105	0.13	0.54	0.02	0.93
1106	-0.26	0.22	0.00	0.99
1203	0.21	0.31	0.12	0.58
1205	0.08	0.72	0.06	0.79
1304	0.20	0.35	-0.31	0.13
1401	0.20	0.35	-0.16	0.45
1802	0.10	0.63	-0.29	0.15
2101	0.20	0.33	0.29	0.16
2204	-0.27	0.20	0.31	0.13
2206	0.08	0.70	-0.22	0.29
2211	-0.10	0.63	0.17	0.42
2301	-0.10	0.62	0.27	0.19
2309	-0.30	0.15	0.11	0.60
2903	-0.03	0.89	-0.10	0.64
3105	0.08	0.72	-0.29	0.16
3201	-0.10	0.64	0.23	0.28
3303	-0.05	0.83	0.23	0.28
3309	0.13	0.53	0.21	0.30
3406	-0.02	0.94	0.28	0.17
3613	-0.30	0.14	0.06	0.77
3704	-0.10	0.64	-0.15	0.47
3802	-0.09	0.67	-0.02	0.93
3805	-0.06	0.79	-0.27	0.19
4001	0.25	0.22	0.05	0.82
4103	-0.15	0.49	0.22	0.29
4303	0.30	0.145	0.17	0.41
4404	-0.06	0.793	-0.06	0.76
4413	0.10	0.63	0.15	0.49
4503	0.16	0.44	0.08	0.71
4508	-0.17	0.41	0.29	0.16
4805	-0.13	0.55	0.10	0.62

Sp	rM	pval	rNM	pval
5105	-0.14	0.49	0.33	0.11
5203	0.08	0.72	-0.12	0.57
5207	-0.20	0.33	0.23	0.27
5210	-0.10	0.63	0.33	0.11
5401	0.30	0.145	0.15	0.48
5413	0.33	0.10	0.21	0.31
5501	-0.14	0.49	0.25	0.22
5507	0.07	0.75	0.33	0.11
6001	-0.28	0.18	-0.09	0.68
6108	0.12	0.57	0.21	0.32
6204	-0.25	0.23	0.03	0.90
6207	-0.23	0.26	0.25	0.23
6211	0.06	0.77	-0.08	0.70
6302	0.16	0.43	0.09	0.66
6306	0.22	0.28	0.03	0.90
6308	0.12	0.56	0.22	0.30
6405	0.24	0.24	0.17	0.42
6410	-0.34	0.10	0.13	0.526
6501	-0.13	0.53	0.13	0.53
6506	0.07	0.75	0.02	0.93
6606	-0.18	0.40	-0.07	0.73
6608	0.28	0.18	0.29	0.16
6702	-0.01	0.97	0.27	0.19
6807	-0.01	0.96	0.29	0.16
7105	-0.32	0.116	-0.07	0.73
7203	0.00	1.00	-0.08	0.72
7207	-0.11	0.60	0.30	0.14
7308	-0.17	0.41	0.04	0.85
7404	-0.02	0.94	0.09	0.67
7705	0.15	0.49	0.30	0.15
7801	-0.16	0.46	0.23	0.28

5003	0.30	0.15	0.20	0.33

Annex 26 - Identification details for the 70 leaf spots with a single protein identity

Sp: spot number; Dtb: consulted database, V: viridiplantae of Uniprot and A: Agrostis spp. EST database; ID: Protein identity; Uniprot: Uniprot Accession; gb Access: Genbank Accession; e-value: e-value of the blastx on NCBI; Cov: % of coverage between experimental and database sequences; (nb): number of peptides matched between both sequences; peptids: list of matched peptides.

Sp	Db	ID	gb / e-val	Uniprot	Cov (nb)	MW	pI	Peptides
1101	A	Oxygen-evolving enhancer protein 2, chloroplastic	DV853316_3 / 4e-123	M7YV65	41.31 (9)	33.91	9.25	TDSEGGFESDAVATANVLESSAPVVDGK YEDNFDATSNLSVVINPTTK HQLITATVADGK YGEAANVFGK QYYSITVLTR TADGDEGGKHQLITATVADGK EFPGQVLR TITEYGSPEQFLSEVGFLLGQQSYGGK EREFPGQVLR
		Oxygen-evolving enhancer protein 2, chloroplastic	DV853283_3 / 8e-118	M7YV65	29.04 (6)	32.82	8.82	xEDNFDATSNLSVVINPTTK HQLITATVADGK QYYSITVLTR EREFPGHVLRxEDNFDATSNLSVVINPTTK TADGDEGGKHQLITATVADGK TITEYGSPEQFLSEVGFLLGQQSxGGK
	V	Oxygen-evolving enhancer protein 2, chloroplastic		Q00434	31.78 (8)	27.25	8.70	TDSEGGFESDAVATANVLESSAPVVDGK HQLITATVADGK FVENAAGSFSVA QYYSITVLTR TADGDEGGKHQLITATVADGK KFVENAAGSFSVA EFPGQVLR EREFPGQVLR
1104	A	50S ribosomal protein L10, chloroplastic	DY543708_6 / 5e-42	M8BNG8	12.77 (2)	15.29	9.01	VEETNDFIGAVFEGK EERVEETNDFIGAVFEGK
1803	A	Polyphenol oxidase	GR279139_4 / 3e-22	Q6PLR1	32.89 (4)	17.12	5.05	ILGDLVSDYVNPETK NNNLYNmYR AFYEQTPK AFmDLNIGPANQTDLLR
		Polyphenol oxidase	DV854107_3 / 4e-34	Q6PLR0	12.17 (2)	29.64	9.41	TLESDEEVLVVDmK ITINDVVDLNNLGYTYEK

1804	A	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	DV856495_2 / 1e-121	M7ZHT1	25.78 (6)	36.28	8.98	EGVVYGAGIGPGVYDIHSPR IPSKEEIADR SEHAFYLDWAVHSFR YAEVKPALTNmVEAAK EVEDLEAGGIQVIQIDEAALR KYAEVKPALTNmVEAAK
		5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	GR278720_5 / 3e-86	M7ZHT1	21.82 (2)	17.78	9.07	DEAYFAANAAALASR LNLPILPTTTIGSFPQTVELR
		5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	GR280925_5 / 9e-135	M7YTL8	27.51 (3)	21.40	5.57	GmLTGPVTILNWSFVR SEHAFYLDWAVHSFR EVEDLEAGGIQVIQIDEAALR
	V	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase		P93263	12.68 (7)	84.77	6.28	YLFAGVVDGR GmLTGPVTILNWSFVR DEAFFSANAAALASR LNLPILPTTTIGSFPQTVELR VLEVNALAK YGAGIGPGVYDIHSPR FALESFWDGK
2103	A	Ribulose-1,5-bisphosphate carboxylase small subunit EC=4.1.1.39	GR279297_6 / 1e-74	Q9SDY8	52.69 (9)	19.34	8.44	LPmFGcTDASQVIK KFETLSYLPPLSEEALLK QVQcVSFIAFKPPGcEESGK IIGFDNIR FETLSYLPPLSEEALLK EHGSTPGYYDGR QIDFLIR KEYPDAYVR EYPDAYVR
	V	Ribulose bisphosphate carboxylase small chain (Fragment)		P13951	24.39 (2)	9.60	6.51	IIGFDNNR EHGSTPGYYDGR
2105	V	Nucleoside diphosphate kinase 2, chloroplastic		P47923	11.3 (3)	25.60	8.40	GLVGEIISR KLIGATDPLQAEPGTIR LIGATDPLQAEPGTIR
2106	A	Ribulose-1,5-bisphosphate carboxylase small subunit	GR279297_6 / 1e-74	Q9SDY8	48.5 (7)	19.34	8.44	QVQcVSFIAFKPPGcEESGK LPmFGcTDASQVIK KFETLSYLPPLSEEALLK IIGFDNIR EHGSTPGYYDGR FETLSYLPPLSEEALLK WVPcLEFSK
	V	Ribulose bisphosphate carboxylase small chain clone 512 (Fragment)		P07398	25.66 (3)	13.05	6.06	QVQcVSFIAFKPPGcEESGK QVQcVSFIAFKPPGcEESGKA EYPDAYVR

2303	A	Bark storage protein A	DV857196_1 / 8e-131	M8CRB0	17.89 (4)	34.61	7.17	YGDGKENELPLEAAGDYTR GcSANVYLDNAR ENELPLEAAGDYTR YYALAAQLEGmELPAcLDATTcLPR
		Glutelin type-A 1	DV856120_3 / 2e-105	M7Z0L4	7.64 (2)	33.19	9.76	VVVLNTVNLPLVK EVGLGADLVR
2312	A	Putative L-ascorbate peroxidase, chloroplastic	DV855736_2 / 6e-101	M8BMC6	21.32 (6)	37.01	8.82	GGPLSFADLIQIAAQQALK TLYSAYGSSGQWGFFDK VPQWGSASVQEIK FIAVGLGPR DKFIAVGLGPR DDAQEPDPEGR
2402	A	Fructose-bisphosphate aldolase EC=4.1.2.13	DV858099_2 / 1e-104	I1GXE4	29.69 (6)	34.29	10.36	VAAEVIAEYTVAALR VLLEGTLLKPNmVTPGSDSPK YAGAAAGGDAAASESLYVSGYK ENVADAQATFLAR KENVADAQATFLAR TVPPAVPGVVFLSGGQSEEEATK
	V	Fructose-bisphosphate aldolase, cytoplasmic isozyme 1		P46256	5.88 (2)	38.42	6.79	GILAADESTGTIGK YADELIK
2707	A	Polyphenol oxidase	GR279139_4 / 3e-22	Q6PLR1	32.89 (4)	17.12	5.05	ILGDLVSDYVNPETK NNNLYNmYR AFmDLNIGPANQTDLLR AFYEQTPK
		Polyphenol oxidase	DV854107_3 / 4e-34	Q6PLR1	8.37 (2)	29.64	9.41	TLESDEEVLVVDmK GLAPLVPR
2801	A	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	GR278720_5 / 3e-86	M7ZHT1	40.61 (4)	17.78	9.07	DEAYFAANAAALASR VLEVNALAK KLNLPILPTTTIGSFPQTVELR LVVSTScSLmHTAVDLVNETK
		5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	DV856495_1 / 1e-121	M7ZHT1	11.18 (3)	36.07	9.73	AxPPRPmKGmLTGPVTILNWSFVR GmLTGPVTILNWSFVR FETcYQIALAIK
		5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	DV856495_2 / 1e-121	M7ZHT1	21.12 (4)	36.28	8.98	EGVVYGAGIGPGVYDIHSPR IPSKEEIADR EVEDLEAGGIQVIQIDEAALR KYAEVKPALTNmVEAAK
	V	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase		P93263	12.94 (7)	84.77	6.28	GmLTGPVTILNWSFVR DEAFFSANAAALASR FALESFWDGK YLFAGVVDGR VLEVNALAK LQEELDIDVLVHGEPER KLNLPILPTTTIGSFPQTVELR

		5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase		Q42662	18.98 (10)	84.54	6.51	GmLTGPVTILNWSFVR FALESFWDGK FETcYQIALAIK YLFAGVVDGR LQEELDIDVLVHGEPER KLNLPILPTTTIGSFPQTVELR WFDTNYHFIVPELGPDVK IVEVNALAK EVIAELK ALGVDTVPVLVGPVSYLILSKPAK
2806	A	Cobalamin-independent methionine synthase	DV856495_1 / 9e-126	A6XMY7	7.45 (2)	36.07	9.73	GmLTGPVTILNWSFVR AxPPRPMKGmLTGPVTILNWSFVR
		5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	GR278720_5 / 3e-86	M7ZHT1	21.82 (2)	17.78	9.07	DEAYFAANAAALASR LVVSTScSLmHTAVDLVNETK
		Cobalamin-independent methionine synthase	DV854375_1 / 3e-95	A6XMY7	10.07 (2)	32.90	8.51	EGVVYGAGIGPGVYDIHSPR IPSKEEIADR
	V	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase		P93263	10.85 (5)	84.77	6.28	YLFAGVVDGR GmLTGPVTILNWSFVR DEAFFSANAAALASR ALGVDTVPVLVGPVSYLLLSK LNLPILPTTTIGSFPQTVELR
2808	A	Polyphenol oxidase	DV854107_3 / 4e-34	Q6PLR0	39.54 (8)	29.64	9.41	KTLESDEEVLVVDmK VDPSDNAYFDVLVNVAEGEVLDR LPPAGFPIVLGDGK WLNTSFVFYDEK TLESDEEVLVVDmK ITINDVVDLNNLGYTYEK YLGNFAQVPHGSmK GLAPLVPR
		Polyphenol oxidase	GR279139_4 / 3e-22	Q6PLR1	32.89 (4)	17.12	5.05	ILGDLVSDYVNPETK AFmDLNIGPANQTDLLR NNNLYNmYR AFmDLNIGPANQTDLLRDDcTAEEK
2809	A	GTP-binding protein TypA	DV864812_1 / 2e-78	G3K3T1	20.22 (3)	31.08	9.28	DQGSLVAFEGGSTTSYAcINAQER GILFVKPGQDVYK GQIVGIHQRPGDLALNVcK

3104	A	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic EC=1.10.9.1	DV853200_2 / 7e-141	Q7X9A6	40.58 (8)	30.01	8.48	GPAPLSLALVHADVDDGK GDPTYLVVESDK LGNDIIAADWLNTHGPNDR VVFVPWVETDFR DKLGNDIIAADWLNTHGPNDR TLATYGVNAVcTHLGcVVPWNAAENK FLcPcHGSQYNNQGK TGEEPWWK
	V	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic		Q7X9A6	28.83 (5)	23.71	8.18	GPAPLSLALVHADVDDGK GDPTYLVVESDK VVFVPWVETDFR FLcPcHGSQYNNQGK TLAQGLK
3503	A	Isocitrate dehydrogenase [NADP] EC=1.1.1.42	DV867425_1 / 8e-119	M7YI34	24.91 (6)	29.40	8.40	YEAAGIWYEHR LEEAcVGTVESGK DLALLVHGSSK TIEAEAAHGTVTR LIDDmVAYALK LLDFTQK
	V	Isocitrate dehydrogenase [NADP], chloroplastic (Fragment)		Q40345	20.32 (8)	48.35	6.55	TIEAEAAHGTVTR VANPIVEmDGDEmTR YEAAGIWYEHR LIFPFVELDIK NILNGTVFR LIDDmVAYALK SEGGYVWAcK HAFGDQYR
3707	V	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1, mitochondrial		O82663	7.89 (4)	69.61	6.29	SSQTILATGGYGR ImQNNAAVFR GSDWLGDQDAIQYmcR AFGGQSLDFGK
4105	A	Ribulose-phosphate 3-epimerase EC=5.1.3.1	DV856160_1 / 3e-142	I1H9A1	25.55 (5)	35.10	8.09	VIEAGANALVAGSAVFGAK GVNPWIEVDGGVSPK DYAEAISGIK AVELAGcDWIHVDVmDGR AGADIVSVHcEQTATIHLHR
	V	Ribulose-phosphate 3-epimerase, chloroplastic		Q43157	18.6 (3)	30.35	8.06	VIEAGANALVAGSAVFGAK SDIIVSPSILSANFAK AVELAGcDWIHVDVmDGR
4501	A	Apyrase EC=3.6.1.5	DV858912_5 / 5e-24	B9U140	6.69 (2)	36.51	9.19	YAVIFDAGSTATR VHVFSFDKK

4704	A	Phosphoglucomutase, cytoplasmic EC=5.4.2.2	GR280735_5 / 2e-80	Q9SNX2	42.64 (5)	14.37	5.03	YDYENVDAEAAK ESSDALSPLVDVALK IYIEQYEK LSGTGSVGATIR YLFGDGSR
	V	Phosphoglucomutase, cytoplasmic		Q9SNX2	20.65 (10)	62.63	5.54	YNmGNGGPAPESVTDK YDYENVDAEAAK LSGTGSVGATIR ESSDALSPLVDVALK SmPTSAALDVVAK GATIVVSGDGR IYIEQYEK FFEVPTGWK YLFGDGSR EDFGGGHPDPNLTYAK
		Phosphoglucomutase, cytoplasmic 2		P93805	23.33 (10)	63.00	5.71	YNmGNGGPAPESVTDK LSGTGSVGATIR ATGAFILTASHNPGGPTEDFGIK SmPTSAALDVVAK SSSNVEPPEFGAAADGDADR GATIVVSGDGR DAVQIITK FFEVPTGWK YLFGDGSR EDFGGGHPDPNLTYAK
		Probable phosphoglucomutase, cytoplasmic 1		O49299	15.27 (7)	63.13	6.30	ATGAFILTASHNPGGPTEDFGIK SmPTSAALDVVAK GATLVVSGDGR LYIEQYEK FFEVPTGWK EDFGGGHPDPNLTYAK SIFDFEAIR

4801	A	ATP-dependent Clp protease ATP-binding subunit clpA-like CD4A protein, chloroplastic	GR277864_5 / 0	M8C5W2	51.77 (11)	31.29	6.98	NTLLImTSNVGSSVIEK TLASYYFGSEEAmIR LDmSEFmER IGFDLESDEK AHPDVFNmmLQILEDGR RPYSVVLFDEIEK IGFDLESDEKDSSYGR LIGSPPGYVGYTEGGQLTEAVR NPNRPIASFIFAGPTGVGK VIGQDEAVK AQITALIDK
		ATP-dependent Clp protease ATP-binding subunit clpA-like CD4A protein, chloroplastic	DV853298_2 / 2e-105	M8C5W2	21.5 (6)	33.36	9.66	NTLLImTSNVGSSVIEK EIADImLQEVFNR LDEmIVFR IGFDLESDEK IGFDLESDEKDSSYGR EINLQVTEK
	V	Chaperone protein ClpC2, chloroplastic		Q2QVG9	32.75 (24)	101.95	7.06	NTLLImTSNVGSSVIEK mIGETTEAVGAGVGGGSSGNK VImLAQEEAR GELQcIGATTLDEYR NNPcLIGEPGVGK VITLDmGLLVAGTK AIDLIDEAGSR GNGFVAVEIPFTPR TAIAEGLAQR EGDSAIVDVDSEGK VLELSLEEAR LDmSEFmER mPTLEEYGTNLTK LDEmIVFR LSYQYISDR HAQVPEEAR AHPDVFNmmLQILEDGR AQITALIDK LIGSPPGYVGYTEGGQLTEAVR NPNRPIASFIFAGPTGVGK LLEDSLAEK VIGQDEAVK QLGHNYIGSEHLLLGLLR GELQcIGATTLDEYRK
		Chaperone protein ClpC1, chloroplastic		Q7F9I1	25.38 (19)	101.74	6.51	NTLLImTSNVGSSVIEK VImLAQEEAR GELQcIGATTLDEYR NNPcLIGEPGVGK VITLDmGLLVAGTK AIDLIDEAGSR

					TAIAEGLAQR EGDSAIVDVDSEGK VLELSLEEAR LDmSEFmER mPTLEEYGTNLTK LDEmIVFR AHPDVFNmmLQILEDGR AQITAIIDK LIGSPPGYVGYTEGGQLTEAVR LLEDSLAEK QLGHNYIGSEHLLLGLLR GELQcIGATTLDEYRK EIADImLKEVFDR
ATP-dependent Clp protease ATP-binding subunit clpA homolog CD4A, chloroplastic	P31541	22.03 (16)	102.49	6.64	NTLLImTSNVGSSVIEK VImLAQEEAR GELQcIGATTLDEYR NNPcLIGEPGVGK SLATYYFGSEEAmIR VITLDmGLLVAGTK AIDLIDEAGSR TAIAEGLAQR VLELSLEEAR LDmSEFmER mPTLEEYGTNLTK AHPDVFNmmLQILEDGR LIGSPPGYVGYTEGGQLTEAVR VIGQDEAVK QLGHNYIGSEHLLLGLLR GELQcIGATTLDEYRK
Chaperone protein ClpB4, mitochondrial	Q8VYJ7	2.39 (2)	108.59	6.98	TAIAEGLAQR RPYSVVLFDEIEK
Chaperone protein ClpD1, chloroplastic	Q6H795	2.67 (3)	101.82	7.17	AIDLIDEAGSR QLPDKAIDLIDEAGSR LDmSEYMER

5101	A	Triosephosphate isomerase EC=5.3.1.1	GR278906_4 / 8e-103	E0X6V4	73.51 (11)	19.70	7.09	GGAFTGEVSAEmLANLGVPWVILGHSER EAGSTmDVVAAQTK ALLGESNEFVGDK ASLRPEIQVAAQNcWVK VIAcVGETLEQR cNGTTEQVEK VAYALAQGLK RALLGESNEFVGDK KGGAFTGEVSAEmLANLGVPWVILGHSER ITATNVEVVVSPPYVFLPTVK TFFVGGNWK
		Triosephosphate isomerase	DV857222_3 / 3e-95	I1HC04	32.45 (8)	32.69	9.51	VATPAQAQEVHANLR EAGSTmDVVAAQTK TNVSPEVAETTR ALLGESNEFVGDK VIAcVGETLEQR ITDWTNVVIAYEPVWAIGTGK VAYALAQGLK RALLGESNEFVGDK
	V	Triosephosphate isomerase, cytosolic		P12863	24.11 (5)	27.01	5.68	EAGSTmDVVAAQTK ALLGESNEFVGDK VIAcVGETLEQR RALLGESNEFVGDK IKDWSNVVVAYEPVWAIGTGK
		Triosephosphate isomerase, cytosolic		P34937	26.09 (5)	26.72	5.47	GGAFTGEVSAEmLANLGVPWVILGHSER VATPAQAQEVHANLR VIAcVGETLEQR VAYALAQGLK KGGAFTGEVSAEmLANLGVPWVILGHSER
		Triosephosphate isomerase, cytosolic		P48495	24.02 (5)	27.12	5.71	ALLGESNEFVGDK VIAcVGETLEER RALLGESNEFVGDK VKDWTNVVVAYEPVWAIGTGK ESGSTmDVVAAQTK

5303	A	Fructose-1,6-bisphosphatase, cytosolic	DV862215_3 / 5e-85	D8L9K9	38.96 (8)	26.49	9.85	SPIFLGSYDDVEEIK TLLYGGIFLYPADKK YIGSmVADVHR TLLYGGIFLYPADK VmYEVFPmSFLmEQAGGQSFTGK SLDLIPTK ALYAEEAK IYSVNEGNAK
	V	Fructose-1,6-bisphosphatase, cytosolic		P46267	13.57 (4)	37.13	5.48	SPIFLGSYDDVEEIK TLLYGGIFLYPADKK TLLYGGIFLYPADK LIGLAGDTNIQGEEQK
		Fructose-1,6-bisphosphatase, cytosolic		A2WXB2	17.7 (6)	37.01	5.77	GDLTILLSHIVLGcK YIGSmVADVHR TDLmTITR FVASAVNK YVLNEQSR IYSVNEGNAK
		Fructose-1,6-bisphosphatase, cytosolic		P14766	16.42 (5)	37.17	5.76	YIGSmVADVHR TDLmTITR LIGLAGETNIQGEEQK cKFPTDGSSPK IYSVNEGNAK
5304	A	Fructose-bisphosphate aldolase EC=4.1.2.13	GR278946_1 / 4e-104	F2D6R8	35.87 (5)	19.55	8.60	GLVPLTGSNDESWcQGLDGLASR IVDILVEQGIVPGIK GILAmDESNATcGK LDSIGLENTEANR RLDSIGLENTEANR
		Fructose-bisphosphate aldolase EC=4.1.2.13	DV858706_2 / 5e-130	F2D6R8	29.36 (5)	26.14	7.28	AAQEALLLR ANSLAQLGK YTSDGEAAEAK ATPEQVADYTLK YAAISQDNGLVPIVEPEILLDGEHGIER
	V	Fructose-bisphosphate aldolase, chloroplastic		Q40677	22.68 (8)	41.98	6.80	GILAmDESNATcGK ANSLAQLGK YTSDGEAAEAK LASIGLENTEANR TVVSIPNGPSELAVK EAAYYQQGAR ALQNTcLK EAAWGLAR

5503	V	Eukaryotic initiation factor 4A		P41378	35.02 (12)	46.90	5.48	GLDVIQQAQSGTGK mFVLDEADEmLSR ILASGVHVVVGTPGR GVAINFVTR DQIYDIFQLLPGK FYNVVIEELPANVADLL RDELTLEGIK VLITTDLLAR ELAQQIEK FGRKGVAINFVTR DHTVSATHGDmDQNTR GIYAYGFEKPSAIQQR
5508	A	Eukaryotic initiation factor 4A-1	DV856378_2 / 6e-91	M8A8P0	15.41 (4)	33.39	8.57	KGVAINFVTR GIDVQQVSLVINYDLPTQPENYLHR GVAINFVTR VLITTDLLAR
	V	Eukaryotic initiation factor 4A		P41378	43.48 (18)	46.90	5.48	mFVLDEADEmLSR GLDVIQQAQSGTGK ILASGVHVVVGTPGR DQIYDIFQLLPGK KGVAINFVTR RDELTLEGIK GVAINFVTR FYNVVIEELPANVADLL VLITTDLLAR FGRKGVAINFVTR DELTLEGIK ELAQQIEK GIYAYGFEKPSAIQQR DHTVSATHGDmDQNTR KVDWLTDK ALGDYLGVK mLFDIQK VHAcVGGTSVR

5708	A	Phosphoglucomutase, cytoplasmic EC=5.4.2.2	GR280735_5 / 2e-80	Q9SNX2	36.43 (4)	14.37	5.03	ESSDALSPLVDVALK YDYENVDAEAAK LSGTGSVGATIR IYIEQYEK
	V	Phosphoglucomutase, cytoplasmic		Q9SNX2	22.38 (10)	62.63	5.54	YNmGNGGPAPESVTDK ESSDALSPLVDVALK FFGNLmDAGmcSVcGEESFGTGSDHIR YDYENVDAEAAK SmPTSAALDVVAK LSGTGSVGATIR GATIVVSGDGR IYIEQYEK ELmANLVK YLFGDGSR
		Phosphoglucomutase, cytoplasmic 1		P93804	15.09 (7)	63.06	5.72	YNmGNGGPAPESVTDK SmPTSAALDVVAK LSGTGSVGATIR GATIVVSGDGR DAVQIITK YLFGDGSR SSSNVEPPEFGAAADGDADR
5802	A	Transketolase, chloroplastic	DV863383_1 / 3e-56	N1QRK9	21.91 (3)	19.80	9.41	ISIEAGSTLGWQK EYGITAEAVVAAAK FGASAPAGIIYK
	V	Transketolase, chloroplastic		Q7SIC9	10.07 (7)	72.95	5.72	ISIEAGSTLGWQK VTTTIGFGSPNK FLAIDAVEK RPSILALSR ESVLPAAVTAR NPYWFNR mFGDFQK
5808	V	Heat shock 70 kDa protein 9, mitochondrial		Q8GUM2	7.62 (5)	73.03	5.62	EVDEVLLVGGmTR ETAEAYLGK mKETAEAYLGK GVNPDEAVAmGAAIQGGILR HLNITLTR
		Heat shock 70 kDa protein 10, mitochondrial		Q9LDZ0	8.94 (5)	72.95	5.78	EVDEVLLVGGmTR ETAEAYLGK SQVFSTAADNQTQVGIR mKETAEAYLGK GVNPDEAVAmGAALQGGILR
6106	A	Chlorophyll a-b binding protein 1B-21, chloroplastic	DY543567_5 / 2e-118	Q9SDM1	9.06 (3)	29.43	8.68	YPGGAFDPLGFSK FKESEIYHcR KYPGGAFDPLGFSK
	V	Chlorophyll a-b binding protein 1B-21, chloroplastic		Q9SDM1	9.8 (2)	26.45	6.20	KYPGGAFDPLGFSK FKESEIYHcR

6107	A	Triosephosphate isomerase EC=5.3.1.1	DV857222_3 / 3e-95	I1HC04	32.12 (7)	32.69	9.51	VATPAQAQEVHANLR ALLGESNEFVGDK VIAcVGETLEQR EAGSTmDVVAAQTK TNVSPEVAETTR VAYALAQGLK ITDWTNVVIAYEPVWAIGTGK
		Triosephosphate isomerase EC=5.3.1.1	GR278906_4 / 9e-103	E0X6V4	67.03 (8)	19.70	7.09	ALLGESNEFVGDK VIAcVGETLEQR EAGSTmDVVAAQTK ASLRPEIQVAAQNcWVK VAYALAQGLK GGAFTGEVSAEmLANLGVPWVILGHSER ITATNVEVVVSPPYVFLPTVK TFFVGGNWK
	V	Triosephosphate isomerase, cytosolic		P12863	15.42 (3)	27.01	5.68	ALLGESNEFVGDK VIAcVGETLEQR EAGSTmDVVAAQTK
		Triosephosphate isomerase, cytosolic		P34937	25.69 (4)	26.72	5.47	VATPAQAQEVHANLR VIAcVGETLEQR VAYALAQGLK GGAFTGEVSAEmLANLGVPWVILGHSER
		Triosephosphate isomerase, cytosolic		P48495	18.11 (3)	27.12	5.71	ALLGESNEFVGDK VIAcVGETLEER VKDWTNVVVAYEPVWAIGTGK
6110	V	Ras-related protein Rab7		P31022	15.53 (3)	23.03	5.08	FQSLGVAFYR VIILGDSGVGK GNIPYFETSAK
6203	A	Thioredoxin H-type 4	DV865481_2 / 3e-85	M8CV70	23.5 (5)	26.23	8.00	DmEVVEVPTFLFIR mNGDENDAcmEFLR ADVEALmK TmADTAVFAR GELIGEILR
	V	Thioredoxin-like protein CDSP32, chloroplastic		Q84NN4	11.63 (3)	32.14	6.73	LVVVEFAASHSVNSSR GELIGEILR IYPcmVELSR
		Thioredoxin-like protein CDSP32, chloroplastic		Q9SGS4	10.6 (3)	33.66	8.46	LIVLDVGLK GELIGEILR DmNVIEVPTFLFIR

6208	A	Thioredoxin H-type 4	DV865481_2 / 3e-85	M8CV70	30.77 (7)	26.23	8.00	DmEVVEVPTFLFIR GELIGEILR mNGDENDAcmEFLR LLVLDVGLK ADVEALmK ADVEALmKENSGEDGK TmADTAVFAR
	V	Thioredoxin-like protein CDSP32, chloroplastic		Q84NN4	16.28 (4)	32.14	6.73	LVVVEFAASHSVNSSR GELIGEILR mNGDENDScMEFLR IYPcmVELSR
		Thioredoxin-like protein CDSP32, chloroplastic		Q9SGS4	10.6 (3)	33.66	8.46	GELIGEILR LIVLDVGLK DmNVIEVPTFLFIR
6303	A	Ferredoxin--NADP reductase, leaf isozyme, chloroplastic	DV855685_1 / 2e-137	M8B795	34.23 (12)	38.18	7.55	GIDDImVDLAAK LVYTNDAGEVVK DPNATIImLGTGTGIAPFR GVcSNFLcDLK DNTYVYmcGLK mFFEEHEDYK mVEIGGDNFR DGIVWSDYK mAEYKEELWEmLK RLVYTNDAGEVVK mAEYKEELWEmLKK mYIQTR
		Ferredoxin--NADP reductase, leaf isozyme, chloroplastic	DV855672_3 / 4e-123	M8B795	31.82 (9)	34.91	8.69	GIDDImVDLAAK DPNATIImLGTGTGIAPFR GVcSNFLcDLK DNTYVYmcGLK mFFEEHEDYK mVEIGGDNFR DGIVWSDYK YTNDAGEVVK mYIQTR
		Ferredoxin--NADP reductase, leaf isozyme, chloroplastic	DV852798_3 / 3e-174	N1R101	10.89 (3)	34.36	8.47	LYSIASSALGDFGDSK DNTYVYmcGLK mYIQTR
	V	Ferredoxin--NADP reductase, leaf isozyme, chloroplastic		P10933	12.78 (5)	40.17	8.40	LYSIASSAIGDFGDSK LVYTNDAGEVVK LDFAVSR KAEQWNVEVY RLVYTNDAGEVVK
		Ferredoxin--NADP reductase, leaf isozyme 1, chloroplastic		Q9FKW6	14.44 (4)	40.30	8.13	LYSIASSAIGDFGDSK DPNATIImLGTGTGIAPFR mFFEEHEDYK LDFAVSR

		Ferredoxin--NADP reductase, chloroplastic (Fragments)		P84210	51.35 (2)	3.86	4.44	GIDDImVDLAAK LDFAVSR
		Ferredoxin--NADP reductase, chloroplastic		P41343	7.67 (3)	41.04	8.38	DNTYVYmcGLK LDFAVSR KAEQWNVEVY
6309	A	Cysteine synthase EC=2.5.1.47	GR282134_5 / 2e-64	I1HC84	62.07 (6)	15.08	5.12	VDIFIGGIGTGGTISGSGR QLALQEGLmVGISSGAAAAAAIK LIVVIFPSFGER IQGIGAGFVPR NLDSDVLNEVIEISSDEAIETAK GKVDIFIGGIGTGGTISGSGR
		Cysteine synthase	DY543696_4 / 7e-50	F2D8H2	30.08 (5)	29.39	8.81	QLALQEGLmVGISSGAAAAAAIK YLSSVLFQSIR LIVVIFPSFGER IQGIGAGFVPR NLDSDVLNEVIEISSDEAIETAK
		Cysteine synthase	DV858932_2 / 2e-70	M8CF13	11.52 (2)	28.75	8.94	IDGLISGIGTGGTITGTGR LFVVVFPSFGER
	V	Cysteine synthase		O81154	14.15 (4)	34.32	6.62	IGYSmITDAEEK LIVVIFPSFGER LESmEPcSSVK YLSSVLFETVR
		Cysteine synthase		P38076	20.92 (5)	34.09	5.57	IGYSmITDAEEK LFVVVFPSFGER DVTELIGNTPLVYLNK TPNSYILQQFENAANPK LESmEPcSSVK
		Cysteine synthase, mitochondrial		Q43725	7.91 (2)	45.79	8.18	QLALKEGLmVGISSGAAAAAAIK LEImEPccSVK
6402	A	Actin-3	DV857524_2 / 1e-154	M8AIA9	37.72 (9)	36.52	5.71	LAYVALDYEQELESAK SYELPDGQVITIGAER GEYDESGPAIVHR TTGIVLDSGDGVSHTVPIYEGYALPHAILR DLYGNIVLSGGSTmFPGIADR EITALAPSSmK GYSFTTTAER KDLYGNIVLSGGSTmFPGIADR DLTDcLmK
	V	Actin		Q05214	59.68 (17)	41.71	5.71	SYELPDGQVITIGAER IVLSGGSTmFPGIADR AGFAGDDAPR DAYVGDEAQSK VAPEEHPVLLTEAPLNPK IWHHTFYNELR YPIEHGIVSNWDDmEK DAYVGDEAQSKR TTGIVLDSGDGVSHTVPIYEGYALPHAILR

					GYSFTTTAER AVFPSIVGRPR EITALAPSSmK HTGVMVGmGQK RGILTLK AEYDESGPSIVHR cDVDIR cPEVLFQPSmIGmEAAGIHETTYNSImK
Actin-3	A2XNS1	29.71 (12)	41.68	5.49	SYELPDGQVITIGAER AGFAGDDAPR DAYVGDEAQSK IWHHTFYNELR DAYVGDEAQSKR GYSFTTTAER AVFPSIVGRPR EITALAPSSmK HTGVMVGmGQK DLTDcLmK RGILTLK cDVDIR
Actin-7	P0C542	28.99 (11)	41.59	5.39	SYELPDGQVITIGAER AGFAGDDAPR LAYVALDYEQELDTAR DAYVGDEAQSK IWHHTFYNELR DAYVGDEAQSKR GYSFTTTAER AVFPSIVGRPR HTGVMVGmGQK RGILTLK cDVDIR
Actin-65 (Fragment)	P93585	34.42 (11)	37.24	5.97	AGFAGDDAPR DAYVGDEAQSK IWHHTFYNELR YPIEHGIVSNWDDmEK DAYVGDEAQSKR GYSFTTTAER AVFPSIVGRPR EITALAPSSmK VVPEEHPVLLTEAPLNPK HTGVMVGmGQK cDVDIR
Actin	O65316	31.03 (12)	41.56	5.48	AGFAGDDAPR DAYVGDEAQSK VAPEEHPVLLTEAPLNPK IWHHTFYNELR DAYVGDEAQSKR DLYGNIVLSGGSTmxPGIADR

								GYSFTTTAER AVFPSIVGRPR EITALAPSSmK KDLYGNIVLSGGSTMxPGIADR RGILTLK cDVDIR
		Actin-2		Q96292	27.06 (9)	41.85	5.58	AGFAGDDAPR DAYVGDEAQSK IWHHTFYNELR DAYVGDEAQSKR TTGIVLDSGDGVSHTVPIYEGFSLPHAILR EITALAPSSmK NYELPDGQVITIGAER RGILTLK cDVDIR
		Actin-1		P02582	21.07 (7)	41.59	5.39	AGFAGDDAPR LAYVALDYEQELETAK AVFPSIVGRPR VSPEDHPVLLTEAPLNPK HTGVMVGmGQK RGILTLK cDVDIR
6705	A	Vacuolar proton-ATPase subunit A	FE527958_5 / 1e-116	Q1W681	23.69 (6)	37.59	8.10	FEDPAEGEDVLVAK NIIHFNTLANQAVER EDYLAQNAFTPYDK LYDDLTTGFR YATALEGFYDK EDDLNEIVQLVGK
	V	V-type proton ATPase catalytic subunit A (Fragment)		Q40002	27.07 (13)	64.06	5.55	TTLVANTSNmPVAAR FEDPAEGEDVLVAK LAEmPADSGYPAYLASR LAADTPLLTGQR DmGYNVSmmADSTSR TVISQALSK EDYLAQNAFTPYDK EDDLNEIVQLVGK YSNSDTVVYVGcGER DQLWEFQPNK mGDLFYR LASFYER VQcLGSPDR
		V-type proton ATPase catalytic subunit A		P09469	21.99 (11)	68.79	5.45	TTLVANTSNmPVAAR LAADTPLLTGQR DmGYNVSmmADSTSR VSGPVVVADGmGGAAmYELVR TVISQALSK EDYLAQNAFTPYDK EDDLNEIVQLVGK

								YSNSDTVVYVGcGER SGDVYIPR LASFYER ESEYGYVR
6706	V	Chaperonin CPN60-2, mitochondrial		Q05046	19.3 (10)	61.09	6.64	GISmAVDSVVTNLK IGGASEAEVGEK IGVQIIQNALK GYISPYFITNQK DDTVILDGAGDKK NVVIEQSYGAPK SVAAGmNAmDLR VTDALNATK APGFGENR AGIIDPLK
		Chaperonin CPN60-1, mitochondrial		Q05045	18.61 (10)	61.02	5.77	GISmAVDSVVTNLK IGGASEAEVGEK IGVQIIQNALK GYISPYFITNQK DDTVILDGAGDKK GEYVDmVK VTDALNATK APGFGENR SVASGMNAmDLR AGIIDPLK
		Chaperonin CPN60-2, mitochondrial		Q43298	16.67 (9)	60.90	5.85	IGGASEAEVGEK IGVQIIQNALK DDTVILDGAGDKK cELEDPLILIHDK SVAAGmNAmDLR GVEELADAVK VTDALNATK APGFGENR AGIIDPLK
6707	A	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	GR277914_4 / 7e-50	M7YLI9	28.32 (3)	18.94	6.51	LVDAALESGK AHGTAVGLPSDDDmGNSEVGHNALGAGR IWEDEGFNYIK
		Phosphoglycerate mutase	DV862103_5 / 2e-46	S5TM29	8.87 (3)	31.73	9.04	TSGEYLVK TFAcSETVK SGYFDETK
	V	2,3-bisphosphoglycerate-independent phosphoglycerate mutase		P30792	13.77 (6)	60.58	5.53	GWDAQVLGEAPYK YAGmLQYDGELK AHGTAVGLPSDDDmGNSEVGHNALGAGR TFAcSETVK TSGEYLVK mYVTmDR

		2,3-bisphosphoglycerate-independent phosphoglycerate mutase		Q42908	8.94 (5)	61.15	5.62	GWDAQVLGEAPYK YAGmLQYDGELK YENDWSVVK TFAcSETVK mYVTmDR
		2,3-bisphosphoglycerate-independent phosphoglycerate mutase (Fragment)		O24246	7.58 (4)	53.36	5.58	YAGmLQYDGELK LDQLLLLVK TFAcSETVK mYVTmDR
6708	A	Vacuolar proton-ATPase subunit A	FE527958_5 / 1e-116	Q1W681	35.38 (11)	37.59	8.10	FEDPAEGEDVLVAK YATALEGFYDKFDSDFIDmR DALGEGDKITLETAK EDYLAQNAFTPYDK LYDDLTTGFR NIIHFNTLANQAVER YATALEGFYDK EDDLNEIVQLVGK FDSDFIDmR NLEDEAR mGDLFYR
	V	V-type proton ATPase catalytic subunit A (Fragment)		Q40002	33.79 (15)	64.06	5.55	TTLVANTSNmPVAAR FEDPAEGEDVLVAK LAEmPADSGYPAYLASR LAADTPLLTGQR DmGYNVSmmADSTSR EDYLAQNAFTPYDK YSNSDTVVYVGcGER VGHDSLIGEIIR mGDLFYR TVISQALSK EDDLNEIVQLVGK ISYIAPAGQYSLQDTVLELEFQGIK GVSVPALDKDQLWEFQPNK VQcLGSPDR DQLWEFQPNK
		V-type proton ATPase catalytic subunit A (Fragment)		P49087	21.39 (10)	61.91	6.20	TTLVANTSNmPVAAR LAADTPLLTGQR DmGYNVSmmADSTSR EDYLAQNAFTPYDK SGDVYIPR LYDDLTTGFR NIIHFNTLANQAVER TVISQALSK EDDLNEIVQLVGK VKcLGSPDR
6710	A	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	GR277914_4 / 7e-50	M7YLI9	28.32 (3)	18.94	6.51	LVDAALESGK IWEDEGFNYIK AHGTAVGLPSDDDmGNSEVGHNALGAGR

		2,3-bisphosphoglycerate-independent phosphoglycerate mutase	DV867908_1 / 3e-41	M7YLI9	42.58 (2)	16.68	9.60	SGSIQILTSHTLQPVPVAIGGPGLHPGVK SDINTPGLANVAATVmNLHGFLAPDDYETT LIEVADK
	V	2,3-bisphosphoglycerate-independent phosphoglycerate mutase		P30792	15.56 (8)	60.58	5.53	GWDAQVLGEAPYK YAGmLQYDGELK AHGTAVGLPSDDDmGNSEVGHNALGAGR FKSALEAVK RGWDAQVLGEAPYK mYVTmDR TSGEYLVK TFAcSETVK
		2,3-bisphosphoglycerate-independent phosphoglycerate mutase		Q42908	9.12 (6)	61.15	5.62	GWDAQVLGEAPYK YAGmLQYDGELK YENDWSVVK RGWDAQVLGEAPYK mYVTmDR TFAcSETVK
		2,3-bisphosphoglycerate-independent phosphoglycerate mutase (Fragment)		O24246	12.3 (5)	53.36	5.58	YAGmLQYDGELK mYVTmDR LDQLLLLVK TFAcSETVK VNLPNSDMVGHTSSIEATVVAcK
		2,3-bisphosphoglycerate-independent phosphoglycerate mutase		P35493	5.4 (4)	60.78	5.82	GWDAQVLGEAPYK RGWDAQVLGEAPYK FKSAVEAIK mYVTmDR
6802	A	Transketolase, chloroplastic	DV863383_1 / 2e-56	M8APV9	28.09 (4)	19.80	9.41	EYGITAEAVVAAAK ISIEAGSTLGWQK ESVLPAAVTAR FGASAPAGIIYK
	V	Transketolase, chloroplastic		Q7SIC9	9.78 (7)	72.95	5.72	FLAIDAVEK ESVLPAAVTAR VTTTIGFGSPNK ISIEAGSTLGWQK NPYWFNR mFGDFQK FAEYEKK
6805	A	Transketolase, chloroplastic	DV863383_1 / 2e-56	M8APV9	28.09 (4)	19.80	9.41	ISIEAGSTLGWQK FGASAPAGIIYK EYGITAEAVVAAAK ESVLPAAVTAR
	V	Transketolase, chloroplastic		Q7SIC9	4.44 (3)	72.95	5.72	ESVLPAAVTAR FAEYEKK VTTTIGFGSPNK
7103	A	Triosephosphate isomerase EC=5.3.1.1	DV853744_1 / 4e-133	M7Z1M4	37.95 (11)	36.69	8.27	IIYGGSVNAANSAELAK KEDIDGFLVGGASLK HVIGEDDQFIGK

								VHxLIALRVSAQNTWIGK TNVSADVASAVR VmAcIGELLEER VASPEQAQEVHAAVR GPDFATIcNSVTSK EDIDGFLVGGASLK AAYALSQNLK RHVIGEDDQFIGK
	V	Triosephosphate isomerase, chloroplastic		P46225	44.97 (12)	31.61	6.37	IEVSAQNTWIGK KEDIDGFLVGGASLK VmAcIGELLEER IIYGGSVNAANcAELAK VASPEQAQEVHAAVR GPDFATIcNSVTSK EDIDGFLVGGASLK TNVSADVASTVR AAYALSQNLK FFVGGNWK HVIGEDDEFIGK TFDVcFK
		Triosephosphate isomerase, chloroplastic		Q9M4S8	11.46 (4)	33.51	7.80	HVIGEDDQFIGK EEDIDGFLVGGASLK FFVGGNWK RHVIGEDDQFIGK
7202	A	Cysteine synthase, chloroplastic/chromoplastic	GR282134_5 / 2e-63	M8AZ01	60.69 (5)	15.08	5.12	VDIFIGGIGTGGTISGSGR NLDSDVLNEVIEISSDEAIETAK QLALQEGLmVGISSGAAAAAAAIK IQGIGAGFVPR LIVVIFPSFGER
		Cysteine synthase, chloroplastic/chromoplastic	DY543696_4 / 2e-49	M8AZ01	30.08 (5)	29.39	8.81	NLDSDVLNEVIEISSDEAIETAK QLALQEGLmVGISSGAAAAAAAIK IQGIGAGFVPR LIVVIFPSFGER YLSSVLFQSIR
	V	Cysteine synthase		O81154	7.08 (2)	34.32	6.62	LIVVIFPSFGER YLSSVLFETVR
		Cysteine synthase, mitochondrial		Q43725	7.91 (2)	45.79	8.18	QLALKEGLmVGISSGAAAAAAAIK LEImEPccSVK
7208	A	Oxygen-evolving enhancer protein 1, chloroplastic	DV859364_2 / 3e-169	M8AE10	61.9 (19)	33.87	8.60	NASSSTGNITLSVTK DGIDYAAVTVQLPGGER FEEKDGIDYAAVTVQLPGGER GGSTGYDNAVALPAGGR QLVATGKPESFSGPFLVPSYR SNPDTGEVIGVFESVQPSDTDLGAK GGSTGYDNAVALPAGGRGDEEELAK LTYTLDEmEGPLEVSSDGTLK GTGTANQcPTIDGGVDTFPFK

								FcLEPTSFTVK GDEEELAK KFcLEPTSFTVK GGSTGYDNAVALPAGGRGDEEELAKENVK GDEEELAKENVK GSSFLDPK VPFLFTVK AEGIQKNEPPAFQK VPFLFTVKQLVATGKPESFSGPFLVPSYR NEPPAFQK
		Oxygen-evolving enhancer protein 1, chloroplastic	DV855155_2 / 5e-80	M8AE10	56.54 (10)	20.45	8.46	NASSSTGNITLSVTK GGSTGYDNAVALPAGGR QLVATGKPESFSGPFLVPSYR SNPDTGEVIGVFESVQPSDTDLGAK GGSTGYDNAVALPAGGRGDEEELAK GDEEELAK GGSTGYDNAVALPAGGRGDEEELAKENVK GDEEELAKENVK GSSFLDPK ARGPFLFTVKQLVATGKPESFSGPFLVPSYR
		Oxygen-evolving enhancer protein 1, chloroplastic	DV853571_2 / 1e-71	M8AE10	43.09 (6)	20.32	9.76	NASSSTGNITLSVTK SNPDTGEVIGVFESVQPSDTDLGAK GDEEELAK GDEEELAKENVK GSSFLDPK QVVATGKPESFSGPFLVPSYR
	V	Oxygen-evolving enhancer protein 1, chloroplastic		P27665	28.62 (12)	34.72	8.56	NASSSTGNITLSVTK IQGVWYAQLESN DGIDYAAVTVQLPGGER FEEKDGIDYAAVTVQLPGGER LTFDEIQSK RLTFDEIQSK GDEEELAKENVK KFcLEPTSFTVK FcLEPTSFTVK GDEEELAK TLKFEEKDGIDYAAVTVQLPGGER GSSFLDPK
		Oxygen-evolving enhancer protein 1-1, chloroplastic		P23321	12.35 (4)	35.12	5.66	GGSTGYDNAVALPAGGR VPFLFTVK GGSTGYDNAVALPAGGRGDEEELVK GSSFLDPK
		Oxygen-evolving enhancer protein 1, chloroplastic		P12853	9.28 (2)	30.50	8.16	AGSYKLENFcIEPTSFTVK GSSFLDPK
7214	A	Chlorophyll a-b binding protein 8, chloroplastic	DV856057_1 / 1e-123	M8A6M9	24.74 (5)	32.16	9.07	TAmmGVVGmIAPEALGK YLGGSGDPAYPGGPIFNPLGFGTK WLAYGEIFNGR

								LQDWYNPGSmGK QYFLGLEK
7306	A	Sedoheptulose-1,7-bisphosphatase, chloroplastic EC=3.1.3.37	DV854814_1 / 3e-127	P46285	27.24 (7)	33.48	9.83	LTGVTGGDQVAAAMGIYGPR YTGGmVPDVNQIIVK GIFTNVTSPTAK FEETLYGSSR VITVLDER ATFDNPDYDK LVNYYVK
		Sedoheptulose-1,7-bisphosphatase, chloroplastic EC=3.1.3.37	DV859601_4 / 2e-94	P46285	31.4 (7)	26.92	9.48	LLFEVAPLGFLIEK YTGGmVPDVNQIIVK GIFTNVTSPTAK FEETLYGSSR VITVLDER ATFDNPDYDK LVNYYVK
	V	Sedoheptulose-1,7-bisphosphatase, chloroplastic		P46285	30.28 (9)	42.03	6.43	LTGVTGGDQVAAAMGIYGPR LLFEALEYSHVcK GIFTNVTSPTAK YTGGmVPDVNQIIVK LLFEVAPLGFLIEK LLIcmGEAmR FEETLYGSSR DcPGTHEFLLLDEGK ATFDNPDYDK
7410	A	Phosphoribulokinase EC=2.7.1.19	GR279308_6 / 1e-151	F2DD69	50.34 (9)	33.30	6.32	FYGEVTQQmLK KPDFDAYIDPQK DLYQQIIAER LDELIYVESHLSNLSTK FSYGPDTYFGQEVSVLEmDGQFDR FFNPVYLFDEGSTINWIPcGR QYADAVIEVLPTQLIPDDNEGK HADFPGSNNGTGLFQTIVGLK VGAPAEAAK
	V	Phosphoribulokinase, chloroplastic		P26302	36.63 (10)	45.11	6.05	FYGEVTQQmLK ANDFDLmYEQVK IRDLYEQIIAER DLYEQIIAER LDELIYVESHLSNLSTK FSYGPDTYFGQEVSVLEmDGQFDR FFNPVYLFDEGSTINWIPcGR HADFPGSNNGTGLFQTIVGLK QYADAVIEVLPTQLIPDDNEGK GVTALDPK
		Phosphoribulokinase, chloroplastic		P27774	16.62 (6)	44.09	6.46	FYGEVTQQmLK ANDFDLmYEQVK KPDFDAYIDPQK

								LDELIYVESHLSNLSTK LTSVFGGAAEPPR RLTSVFGGAAEPPR
7412	A	Glutamine synthetase EC=6.3.1.2	GR278149_5 / 5e-105	I1J2T4	22.33 (3)	22.64	7.17	HDLHISEYGEGNER LTGLHETASISDFSWGVANR AmREDGGFEVIK
		Glutamine synthetase EC=6.3.1.2	GR279277_3 / 3e-52	B4FT28	39.36 (2)	10.41	7.18	TISKPVEDPSELPK GGNNIIVVcDTYTPQGEPIPTNK
	V	Glutamine synthetase leaf isozyme, chloroplastic		P13564	11.52 (3)	47.06	5.29	IIAEYIWVGGSGIDLR TISKPVEDPSELPK LTGLHETASISDFSWGVANR
		Glutamine synthetase, chloroplastic		P25462	10.87 (3)	45.99	6.87	TISKPVEDPSELPK GGNNVLVIcDTYTPQGEPLPTNK AAQIFSDPK
		Glutamine synthetase, chloroplastic		P14655	5.37 (2)	46.61	6.34	TISKPVEDPSELPK EDGGFEVIK
7413	A	Phosphoribulokinase EC=2.7.1.19	DV866058_6 / 5e-122	F2DD69	44.4 (8)	30.45	7.11	FYGEVTQQmLK LDELIYVESHLSNLSTK KPDFDAYIDPQK QYADAVIEVLPTQLIPDDNEGK FSYGPDTYFGQEVSVLEmDGQFDR DLYEQIIAER IRDLYEQIIAER HADFPGSNNGTGLFQTIVGLK
	V	Phosphoribulokinase, chloroplastic		P26302	31.93 (9)	45.11	6.05	FYGEVTQQmLK LDELIYVESHLSNLSTK ANDFDLmYEQVK QYADAVIEVLPTQLIPDDNEGK DLYEQIIAER FSYGPDTYFGQEVSVLEmDGQFDR HADFPGSNNGTGLFQTIVGLK KLTcSYPGIK IRDLYEQIIAER
		Phosphoribulokinase, chloroplastic		P27774	18.89 (6)	44.09	6.46	FYGEVTQQmLK LDELIYVESHLSNLSTK LTSVFGGAAEPPR ANDFDLmYEQVK KPDFDAYIDPQK KLTcSYPGIK
7502	A	Ribulose bisphosphate carboxylase/oxygenase activase A, chloroplastic	DV855440_2 / 0	Q40073	44.44 (10)	35.11	6.24	IVDTFPGQSIDFFGALR mcALFINDLDAGAGR VPIIVTGNDFSTLYAPLIR GIFQTDNVSDESVVK LLEYGHmLVQEQDNVK VQLADTYmSQAALGDANQDAmK WVTATGIENIGK VYDDEVR

								GAQQGTLPVPEGcTDR KWVTATGIENIGK
		Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic	DV859387_6 / 4e-157	P93431	12.98 (3)	37.15	7.72	IPLILGIWGGK mGINPImmSAGELESGNAGEPAK SFQcELVFAK
	V	Ribulose bisphosphate carboxylase/oxygenase activase A, chloroplastic		Q40073	30.17 (10)	51.04	7.94	IVDTFPGQSIDFFGALR IPLILGIWGGK mGINPImmSAGELESGNAGEPAK GIFQTDNVSDESVVK LLEYGHmLVQEQDNVK VQLADTYmSQAALGDANQDAmK SFQcELVFAK VYDDEVR DGPVTFEQPK NFmTLPNIK
		Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic		O98997	22.78 (7)	47.87	7.78	LVDTFPGQSIDFFGALR mcALFINDLDAGAGR mGINPImmSAGELESGNAGEPAK VPIIVTGNDFSTLYAPLIR SFQcELVFAK VYDDEVR NFmTLPNIK
7608	A	Tubulin alpha-1 chain	DV858436_1 / 4e-150	O22347	50.3 (11)	36.99	5.30	AFVHWYVGEGmEEGEFSEAR AVcmISNSTSVVEVFSR IHFmLSSYAPVISAEK LVSQVISSLTASLR DVNAAVATIK TIQFVDWcPTGFK FDGALNVDVNEFQTNLVPYPR cGINYQPPSVVPGGDLAK AYHEQLSVAEITNSAFEPSSmmAK FDLmYAK EDLAALEK
		Alpha-tubulin 2	GR281625_5 / 1e-89	Q8H6M0	67.94 (5)	14.69	5.43	IHFmLSSYAPVISAEK LVSQVISSLTASLR FDGALNVDVNEFQTNLVPYPR AYHEQLSVAEITNSAFEPSSmmAK SLDIERPTYTNLNR
	V	Tubulin alpha-3 chain		O22349	30 (11)	49.58	5.06	AIFVDLEPTVIDEVR IHFmLSSYAPVISAEK DVNAAVATIK cGINYQPPSVVPGGDLAK EDAANNFAR EDLAALEK EIVDLcLDR AFVHWYVGEGmEEGEFSEAR SLDIERPTYTNLNR

								YmAccLmYR FDLmYAK
		Tubulin alpha chain		Q9FT36	33.48 (11)	49.61	5.01	AIFVDLEPTVIDEVR IHFmLSSYAPVISAEK LVSQVISSLTASLR TIQFVDWcPTGFK EDAANNFAR EDLAALEK EIVDLcLDR AFVHWYVGEGmEEGEFSEAR AYHEQLSVAEITNSAFEPSSmmAK SLDIERPTYTNLNR YmAccLmYR
		Tubulin alpha-3 chain		P22275	33.78 (12)	49.53	5.24	AVcmISNSTSVVEVFSR AIFVDLEPTVIDEVR DVNAAVATIK LVSQVISSLTASLR TIQFVDWcPTGFK EDAANNFAR FDGALNVDVNEFQTNLVPYPR EDLAALEK EIVDLcLDR AFVHWYVGEGmEEGEFSEAR YmAccLmYR FDLmYAK
		Tubulin alpha-3 chain		Q56WH1	24.89 (9)	49.62	5.10	AVFVDLEPTVIDEVR EDAANNFAR EDLAALEK EIVDLcLDR AFVHWYVGEGmEEGEFSEAR FDGAINVDITEFQTNLVPYPR SLDIERPTYTNLNR YmAccLmYR FDLmYAK
7701	A	60 kDa chaperonin subunit beta, chloroplastic	GR278090_5 / 1e-114	Q43831	58.29 (12)	21.07	5.24	LAGGVAVIQVGAQTETELK AAVEEGIVVGGGcTLLR ESTTIVGDGSTQEEVTK VEDALNATK NLIENAEQDYEK LRVEDALNATK LAGGVAVIQVGAQTETELKEK NAGVNGSVVTEK ESTTIVGDGSTQEEVTKR DEVGLSxDK NLIENAEQDYEKEK VDAIKDTLDNDEQK
		60 kDa chaperonin subunit beta, chloroplastic	DV857419_2 / 9e-74	M7ZYP1	32.66 (6)	33.16	9.20	TFLTSDVVVVEIK FGYNAATGQYEDLmAAGIIDPTK

								ccLEHAASVAK NAGVNGSVVTEK MLxTQPRLPVEEGIVVGGGcTLLR VDAIKDTLDNDEQK
	V	60 kDa chaperonin subunit beta, chloroplastic (Fragment)		Q43831	50.7 (23)	53.38	4.94	GYISPYFVTDSEK TQYLDDIAILTGGTVIR LAGGVAVIQVGAQTETELK TFLTSDVVVVEIK EVELEDPVENIGAK AAVEEGIVVGGGcTLLR VIAAGANPVQITR KTQYLDDIAILTGGTVIR DLINVLEEAIR TNDLAGDGTTTSVVLAQGLIAEGVK FGYNAATGQYEDLmAAGIIDPTK ESTTIVGDGSTQEEVTK VEDALNATK ccLEHAASVAK KGVVTLEEGR LRVEDALNATK GVVTLEEGR LAGGVAVIQVGAQTETELKEK NAGVNGSVVTEK ESTTIVGDGSTQEEVTKR LLLVDKK mTTEYENcK ALcYPLK
		Chaperonin 60 subunit beta 2, chloroplastic		Q9LJE4	19.8 (10)	63.30	5.73	GYISPYFVTDSEK EVELEDPVENIGAK AAVEEGIVVGGGcTLLR FGYNAATGKYEDLmAAGIIDPTK VEDALNATK ccLEHAASVAK LADLVGVTLGPK LRVEDALNATK IVNDGVTVAR LLLVDKK
7703	A	ATP-dependent zinc metalloprotease FTSH 2, chloroplastic	DV853223_2 / 3e-151	Q655S1	38.03 (7)	33.53	6.43	QLSDQAYEIALQQIR AAEEIIFGEPEVTTGAAGDLQQITGLAK IVAGmEGTVmTDGK GLTWFIPmDDPTLISR SLVAYHEVGHAVcGTLTPGHDPVQK LALDIDSAIK IVEVLLEK
	V	ATP-dependent zinc metalloprotease FTSH 2, chloroplastic		Q655S1	36.09 (16)	72.49	5.72	FQmEPNTGVTFDDVAGVDEAK VQLPGLSQELLQK AAEEIIFGEPEVTTGAAGDLQQITGLAK IVAGmEGTVmTDGK

								QDFmEVVEFLK ENAPcIVFVDEIDAVGR QVSVDVPDVR TPGFSGADLANLLNEAAILAGR IVEVLLEK GLTWFIPmDDPTLISR GVLLVGPPGTGK ETLSGDEFR VHGSNKKFDTDVSLEVIAmR ADILDSALLRPGR KVDLFENGTIAIVEAISPELGNR FLEYLDK
		ATP-dependent zinc metalloprotease FTSH 6, chloroplastic		Q67WJ2	3.94 (2)	72.58	6.44	TGVTFDDVAGVDEAK GVLLVGPPGTGK
7704	A	60 kDa chaperonin subunit beta, chloroplastic	DV854853_1 / 4e-121	Q43831	48.06 (12)	30.94	8.44	LAGGVAVIQVGAQTETELK FGYNAATGQYEDLmAAGIIDPTK AAVEEGIVVGGGcTLLR TFLTSDVVVVEIK NLIENAEQDYEK LRVEDALNATK NAGVNGSVVTEK NLIENAEQDYEKEK VEDALNATK LAGGVAVIQVGAQTETELKEK VDAIKDTLDNDEQK ccLEHAASVAK
		60 kDa chaperonin subunit beta, chloroplastic	GR278090_5 / 1e-114	Q43831	58.29 (12)	21.07	5.24	LAGGVAVIQVGAQTETELK AAVEEGIVVGGGcTLLR NLIENAEQDYEK LRVEDALNATK NAGVNGSVVTEK NLIENAEQDYEKEK ESTTIVGDGSTQEEVTKR VEDALNATK ESTTIVGDGSTQEEVTK LAGGVAVIQVGAQTETELKEK VDAIKDTLDNDEQK DEVGLSxDK
	V	60 kDa chaperonin subunit beta, chloroplastic (Fragment)		Q43831	49.1 (22)	53.38	4.94	TNDLAGDGTTTSVVLAQGLIAEGVK VIAAGANPVQITR LAGGVAVIQVGAQTETELK FGYNAATGQYEDLmAAGIIDPTK KTQYLDDIAILTGGTVIR TQYLDDIAILTGGTVIR AAVEEGIVVGGGcTLLR DLINVLEEAIR TFLTSDVVVVEIK

								LRVEDALNATK GYISPYFVTDSEK NAGVNGSVVTEK KGVVTLEEGR GVVTLEEGR EVELEDPVENIGAK ESTTIVGDGSTQEEVTKR VEDALNATK ESTTIVGDGSTQEEVTK LAGGVAVIQVGAQTETELKEK ccLEHAASVAK mTTEYENcK LLLVDK
		Chaperonin 60 subunit beta 2, chloroplastic		Q9LJE4	19.63 (10)	63.30	5.73	FGYNAATGKYEDLmAAGIIDPTK AAVEEGIVVGGGcTLLR LADLVGVTLGPK LRVEDALNATK GYISPYFVTDSEK IVNDGVTVAR EVELEDPVENIGAK VEDALNATK ccLEHAASVAK LLLVDK
7706	A	RuBisCO large subunit-binding protein subunit beta, chloroplastic	GR278090_5 / 1e-114	Q43831	18.59 (3)	21.07	5.24	LAGGVAVIQVGAQTETELK ESTTIVGDGSTQEEVTKR ESTTIVGDGSTQEEVTK
	V	RuBisCO large subunit-binding protein subunit beta, chloroplastic (Fragment)		Q43831	38.28 (15)	53.38	4.94	VIAAGANPVQITR FGYNAATGQYEDLmAAGIIDPTK DLINVLEEAIR KGVVTLEEGR ESTTIVGDGSTQEEVTK LAGGVAVIQVGAQTETELK TQYLDDIAILTGGTVIR EVELEDPVENIGAK GVVTLEEGR VEDALNATK TNDLAGDGTTTSVVLAQGLIAEGVK ESTTIVGDGSTQEEVTKR mTTEYENcK ccLEHAASVAK NAGVNGSVVTEK
		RuBisCO large subunit-binding protein subunit beta, chloroplastic		P21241	13.44 (6)	62.43	6.93	LADLVGVTLGPK FGYNAATGKYEDLmAAGIIDPTK IVNDGVTVAR EVELEDPVENIGAK VEDALNATK ccLEHAASVAK

8102	A	Thioredoxin peroxidase	DV856996_5 / 5e-129	O81480	25.78 (5)	34.98	9.31	SFGVLIADQGIALR INTEILGVSVDSVFSHLAWVQTER APDFAAEAVFDQEFINVK AANDLPLVGNK EGVIQHSTINNLGIGR
		Thioredoxin peroxidase	DV865047_4 / 1e-101	O81480	34.84 (4)	24.64	7.14	TLQALQYVQENPDEVcPAGWKPGEK INTEILGVSVDSVFSHLAWVQTER EGVIQHSTINNLGIGR VcPTEITAFSDR
	V	2-Cys peroxiredoxin BAS1, chloroplastic (Fragment)		P80602	35.24 (5)	23.31	5.99	INTEILGVSVDSVFSHLAWVQTER APDFAAEAVFDQEFINVK EGVIQHSTINNLGIGR YPLVSDVTK GLFIIDK
		2-Cys peroxiredoxin BAS1-like, chloroplastic		Q9C5R8	17.58 (3)	29.76	5.74	TLQALQYVQENPDEVcPAGWKPGEK EGVIQHSTINNLGIGR GLFIIDK
8105	A	Thioredoxin peroxidase	DV865047_4 / 1e-101	O81480	48.42 (7)	24.64	7.14	TLQALQYVQENPDEVcPAGWKPGEK SFGVLIEDQGIALR YPLVSDVTK SVDETLR EGVIQHSTINNLGIGR VcPTEITAFSDR INTEILGVSVDSVFSHLAWVQTER
		Thioredoxin peroxidase	DV856996_5 / 5e-129	O81480	26.4 (6)	34.98	9.31	AANDLPLVGNK APDFAAEAVFDQEFINVK YPLVSDVTK SVDETLR EGVIQHSTINNLGIGR INTEILGVSVDSVFSHLAWVQTER
	V	2-Cys peroxiredoxin BAS1, chloroplastic (Fragment)		P80602	30 (6)	23.31	5.99	APDFAAEAVFDQEFINVK YPLVSDVTK SVDETLR EGVIQHSTINNLGIGR GLFIIDK EYFAAI
		2-Cys peroxiredoxin BAS1-like, chloroplastic		Q9C5R8	17.58 (3)	29.76	5.74	TLQALQYVQENPDEVcPAGWKPGEK EGVIQHSTINNLGIGR GLFIIDK
		2-Cys peroxiredoxin BAS1, chloroplastic		Q6ER94	8.43 (3)	28.08	6.00	GLFIIDK YPLISDVTK EYFAAI
8201	A	Oxygen-evolving enhancer protein 1, chloroplastic	DV859364_2 / 3e-169	M8AE10	53.65 (11)	33.87	8.60	NASSSTGNITLSVTK GGSTGYDNAVALPAGGRGDEEELAK DGIDYAAVTVQLPGGER SNPDTGEVIGVFESVQPSDTDLGAK QLVATGKPESFSGPFLVPSYR

								GGSTGYDNAVALPAGGR GTGTANQcPTIDGGVDTFPFK KFcLEPTSFTVK FEEKDGIDYAAVTVQLPGGER LTYTLDEmEGPLEVSSDGTLK VPFLFTVK
	V	Oxygen-evolving enhancer protein 1, chloroplastic		P27665	21.54 (8)	34.72	8.56	NASSSTGNITLSVTK DGIDYAAVTVQLPGGER IQGVWYAQLESN FcLEPTSFTVK RLTFDEIQSK LTFDEIQSK KFcLEPTSFTVK FEEKDGIDYAAVTVQLPGGER
		Oxygen-evolving enhancer protein 1-2, chloroplastic		Q9S841	13.9 (3)	35.00	6.16	GGSTGYDNAVALPAGGR VPFLFTVK FKEEDGIDYAAVTVQLPGGER
8205	A	14-3-3-like protein A	DV853825_1 / 1e-145	P29305	28.35 (8)	36.60	8.12	SAQDIALADLPTTHPIR KEAAENTLVAYK EAAENTLVAYK GNEAYVASIK DSTLImQLLR QAFDEAIAELDSLGEESYK LLDSHLVPSATAAESK TRIETELSK
		14-3-3-like protein A	GR281480_5 / 2e-45	P29305	30 (4)	14.71	8.32	TADVGELTVEER YEEMVEFMEK GNEAYVASIK LAEQAERYEEmVEFmEK
	V	14-3-3-like protein A		P29305	49.62 (12)	29.33	4.88	SAQDIALADLPTTHPIR TADVGELTVEER KEAAENTLVAYK EAAENTLVAYK YEEMVEFMEK GNEAYVASIK DSTLImQLLR TRIETELSK IISSIEQK QAFDEAIAELDSLGEESYK LLDSHLVPSATAAESK LAEQAERYEEmVEFmEK
8501	A	Glutamine synthetase EC=6.3.1.2	GR279277_3 / 8e-52	P25462	39.36 (2)	10.41	7.18	GGNNIIVVcDTYTPQGEPIPTNK TISKPVEDPSELPK
		Glutamine synthetase EC=6.3.1.2	GR278149_5 / 5e-105	I1J2T4	10.68 (2)	22.64	7.17	AILNLSLR HDLHISEYGEGNER
	V	Glutamine synthetase, chloroplastic		P25462	9.46 (3)	45.99	6.87	AAQIFSDPK AILNLSLR

								GGNNVLVIcDTYTPQGEPLPTNK
8701	A	60 kDa chaperonin subunit alpha, chloroplastic	DV859255_1 / 1e-75	P08823	18.63 (4)	33.97	7.39	YENLIESGVLDPAK VGAATETELEDR ALLAPASLIANNAGVEGEVVIEK LGADIIQK
		60 kDa chaperonin subunit alpha, chloroplastic	DV855990_3 / 8e-114	P08823	11.38 (3)	31.57	6.80	VGAATETELEDR ELSETDSIYDSEK LGADIIQK
		60 kDa chaperonin subunit alpha, chloroplastic	DV860259_6 / 2e-25	P08823	33.74 (3)	17.87	4.86	YENLIESGVLDPAK ESEWEmGYNAmTDK cALQNAASVAGmVLTTQAIIVEKPKPK
	V	60 kDa chaperonin subunit alpha, chloroplastic (Fragment)		P08823	42.54 (18)	57.49	4.91	TNDSAGDGTTTAcVLAR LANAVGVTLGPR YENLIESGVIDPAK LGILSVTSGANPVSLK AIELANPmENAGAALIR EIIPLLEQTTQLR GYISPQFVTNLEK ELSETDSIYDSEK VVNDGVTIAR ESEWEmGYNAmTDK GIINVAAIK AVASISAGNDELIGAmIADAIDK LGADIIQK SIVEFENAR EIAFDQK AALQAGVEK ETIEDHDER DLGLLVENATVDQLGTAR
		60 kDa chaperonin subunit alpha, chloroplastic (Fragment)		P08824	9.09 (4)	52.35	4.87	VGAATETELEDR LGLLSVTSGANPVSIK GILNVAAIK LGADILQK
8703	A	RuBisCO large subunit-binding protein subunit alpha, chloroplastic : CPN-60 alpha	DV855990_3 / 9e-114	P08823	14.48 (4)	31.57	6.80	LGADIIQK ELSETDSIYDSEK VGAATETELEDR LSGGVAVIK
	V	RuBisCO large subunit-binding protein subunit alpha, chloroplastic (Fragment)		P08823	28.55 (13)	57.49	4.91	TNDSAGDGTTTAcVLAR AIELANPmENAGAALIR LGADIIQK EIIPLLEQTTQLR GIINVAAIK LGILSVTSGANPVSLK ELSETDSIYDSEK LANAVGVTLGPR GYISPQFVTNLEK LSGGVAVIK

								VVNDGVTIAR AALQAGVEK SIVEFENAR
8704	A	Nucleoredoxin	DV853833_1 / 2e-96	N1R275	21.24 (5)	34.21	6.61	GQDAAEAAPAGYVcEGDVcR APIAVHGADAFPFTEDR NSDFEIVFVSSDR GIPSLVAIGPDGK EKGQDAAEAAPAGYVcEGDVcR
		Nucleoredoxin	GR280877_2 / 2e-97	N1R275	15.34 (2)	18.09	4.94	GIPHLVILDAK mPWLAVPFSDSEGR
8705	A	Protein disulfide isomerase EC=5.3.4.1	EV519572_1 / 4e-135	Q9FEG4	54.42 (11)	24.08	5.36	DFDVSALESFIEASSTPK SAYYGAAEEFK APEDAASIEDGK YEIQGFPTLK LFKPFDELVVDSK EAEGIVDYLKK SAYYGAAEEFKDK SEYEFGHTLHANHLPR EAEGIVDYLK VVTFDKNPDNHPYLLK FFQGDSSK
		Protein disulfide isomerase	DV854185_1 / 3e-66	Q6JAC4	19.44 (5)	31.75	8.51	TADEIVDYIK NVLIEFYAPWcGHcK mVSYDGGR LAPILDEAAATLQSEEDVVIAK TADEIVDYIKK
	V	Protein disulfide-isomerase		P52589	19.81 (9)	56.50	5.10	TADEIVDYIK NVLIEFYAPWcGHcK LFKPFDELVVDSK TADEIVDYIKK SEPIPEANNEPVK KSEPIPEANNEPVK LAPILDEAAATLQSEEDVVIAK GDAAVERPLVR VVTFDKNPDNHPYLLK
		Protein disulfide-isomerase		P52588	10.72 (4)	57.06	5.41	NVLIEFYAPWcGHcK YEIQGFPTIK FLIGDIEASQGAFQYFGLK GDAAVERPLVR
		Protein disulfide-isomerase		P29828	5.47 (2)	57.05	5.10	NVLIEFYAPWcGHcK VVVGQTLEDVVFK
		Protein disulfide-isomerase		Q43116	5.62 (2)	55.53	5.08	NVLLEFYAPWcGHcK SEPIPEVNNEPVK
8804	A	70 kDa heat shock protein	DV857735_5 / 2e-167	C7ENF7	21.79 (6)	33.51	9.42	IINEPTAASLAYGFEK QFAAEEISAQVLR IAGLEVLR MAEVDDEAK

								LDcPAIGK AVVTVPAYFNDSQR
		Heat shock protein 70 kDa	DV860338_6 / 2e-93	H6UG34	21.35 (5)	29.02	6.81	KQDITITGASTLPK FDIDANGILSVAAVDK NQADSVVYQTEK QDITITGASTLPK mVEEADKFAQEDKEK
		70 kDa heat shock protein	GR279194_1 / 6e-113	D3YE92	25.71 (4)	18.81	4.82	FEELcSDLIDR LSVSNLDEVILVGGSTR NDEGIDLLK TPVNNALK
	V	Heat shock 70 kDa protein 7, chloroplastic		Q9LTX9	9.47 (8)	76.95	5.30	KQDITITGASTLPK QFAAEEISAQVLR FEELcSDLLDR NQADSVVYQTEK GKFEELcSDLLDR IAGLEVLR QDITITGASTLPK LDcPAIGK
		Stromal 70 kDa heat shock-related protein, chloroplastic (Fragment)		Q08080	11.85 (6)	64.86	4.97	IINEPTAASLAYGFEK QFAAEEISAQVLR NQADSVVYQTEK IAGLEVLR AVVTVPAYFNDSQR LEcPAIGK
9201	A	Cp31BHv	DV853271_2 / 4e-118	O81988	30.03 (8)	34.13	4.61	GFGFVTmSTVEEADKAIETFNR GFGFVTmSTVEEADK LVQLFSQHGEVLNATVVYDR AYVGNLPWQAEDSR GFGFVTmASK EDLDSAISALDGEELDGRPLR QFASAFRAYVGNLPWQAEDSR AIETFNR
		Cp31BHv	DV862991_3 / 1e-59	O81988	24.88 (3)	22.87	9.45	GFGFVTmASK EDLESAISALDGEELDGRPLR LVQLFSAHGEVLNATVVYDR

Annex 27 - Identification details for the 23 leaf spots with multiple identifications

Sp: spot number; Dtb: consulted database, V: viridiplantae of Uniprot and A: Agrostis spp. EST database; ID: Protein identity; Uniprot: Uniprot Accession; gb Access: Genbank Accession; e-value: e-value of the blastx on NCBI; Cov: % of coverage between experimental and database sequences; (nb): number of peptides matched between both sequences; peptids: list of matched peptides.

Sp	Db	ID	gb / e-val	Uniprot	Cov (nb)	MW	pI	Peptides
1305	A	Cysteine synthase EC=2.5.1.48	DV855923_3 / 7e-165	I1IVG1	23.17 (5)	34.11	8.31	AFGAQLVLTDPAK GYELVLTmPSYTSLER ATQLYEDHPSAFmLQQFENPANVK mAQQLAVK LIVTIHPSAGER
		Cysteine synthase	DV853264_2 / 3e-115	M7Z105	15.07 (4)	31.82	8.03	FmLQQFENPANVK YLSSALFEGLR mAQQLAVK LIVTIHPSAGER
	V	Malate dehydrogenase 1, mitochondrial		Q9ZP06	9.09 (2)	35.78	8.35	VAILGAAGGIGQPLALLmK TQDGGTEVVEAK
2703	V	ATP synthase subunit alpha, chloroplastic		A1EA05	45.35 (19)	55.43	6.48	GQNVIcVYVAIGQR HTLIIYDDLSK IIGLGEImSGELVEFAEGTR IAQIPVSEAYLGR GYLDSLEIEQVNK TAVATDTILNQK GEIIASESR TFTEQAEILLK VVQVGDGIAR EAIQEQLER EAYPGDVFYLHSR LIESAAPSIISR DTKPQFQEIISSSK VGIENIGR GIALNLESK SVYEPLQTGLIAIDSmIPIGR QSQANPLPVEEQIATIYTGTR ERHTLIIYDDLSK ELIIGDR
		Ribulose bisphosphate carboxylase large chain		A8Y9H8	4.4 (2)	52.79	6.48	LTYYTPEYETK ASVGFQAGVK
		RuBisCO large subunit-binding protein subunit beta, chloroplastic (Fragment)		Q43831	6.41 (2)	53.38	4.94	LAGGVAVIQVGAQTETELK TFLTSDVVVVEIK

2704	A	Fructose-bisphosphate aldolase EC=4.1.2.13	DV859690_2 / 9e-28	M8BHV4	73.08 (2)	5.51	4.64	GLVPLVGSNDESWcQGLDGLASR IVDILVEQGIVPGIK
	V	ATP synthase subunit alpha, chloroplastic		A1EA05	43.76 (18)	55.43	6.48	GYLDSLEIEQVNK TAVATDTILNQK HTLIIYDDLSK IAQIPVSEAYLGR GQNVIcVYVAIGQR EAIQEQLER TFTEQAEILLK QSQANPLPVEEQIATIYTGTR EAYPGDVFYLHSR GEIIASESR SVYEPLQTGLIAIDSmIPIGR KVGIENIGR VVQVGDGIAR LIESAAPSIISR DTKPQFQEIISSSK VGIENIGR GIALNLESK IIGLGEImSGELVEFAEGTR
		ATP synthase subunit alpha, chloroplastic		Q6ENH7	29.98 (13)	55.63	6.25	TAVATDTILNQK HTLIIYDDLSK IAQIPVSEAYLGR EAIQEQLER EAYPGDVFYLHSR SVYEPLQTGLIAIDSmIPIGR KVGIENIGR VVQVGDGIAR DTKPQFQEIISSSK VGIENIGR GIALNLESK TFTEEAEILLK IIGLGEImSGELVEFAEGTR
		Ketol-acid reductoisomerase, chloroplastic		Q65XK0	4.5 (2)	62.34	6.43	GVAFmVDNcSTTAR VSLAGHEEYIVR
3301	A	ATP synthase subunit gamma	DV868568_3 / 2e-87	M8BFL3	33.62 (6)	25.80	8.84	GEIcDVNGIcVDASEDELFK mSAmSSATDNAIDLR ALQESLASELAAR SDPIIQTLLPmSPK NLSmVYNR VELVYSK
		Malate dehydrogenase EC=1.1.1.37	DV855137_2 / 4e-105	F2D4W6	7.17 (2)	32.55	8.62	LNVQVSDVK mDATAQELSEEK
	V	ATP synthase subunit gamma, chloroplastic		P0C1M0	12.53 (5)	39.77	8.19	VALVVLTGER ALQESLASELAAR SDPIIQTLLPmSPK

								KGNAYFQR GNAYFQR
		Malate dehydrogenase, cytoplasmic		O24047	7.53 (2)	35.48	6.43	LNVQVSDVK VLVVANPANTNALILK
		Malate dehydrogenase, cytoplasmic		Q7XDC8	8.43 (2)	35.55	6.09	VLVVANPANTNALILK mDATAQELSEEK
4107	A	Ferritin	DV855748_2 / 3e-135	B6UZ79	23.86 (7)	34.93	6.79	GDALYAmELALALEK ISEYVSQLR GELSLVPQGK EVLSGVmFQPFEELK cNDPQLSDFVESEFLQEQVDAIK EVLSGVmFQPFEELKGELSLVPQGK cNDPQLSDFVESEFLQEQVDAIKK
		ferritin	DV853035_2 / 4e-45	Q945F6	39.24 (2)	9.17	6.79	GDALYAmELALALKK LQSIVTPLTEFDHAEK
		chlorophyll a-b binding protein	GR279311_6 / 2e-116	B6T1H1	30.23 (3)	18.46	4.70	WAmLGALGcVFPEILAK IYPGGSFDPLGLADDPDTAAELK VGGGPLGEGLDK
4308	A	Fructose-bisphosphate aldolase EC=4.1.2.13	DV855046_3 / 3e-179	M8BHV4	47.84 (11)	35.58	8.47	GLVPLVGSNDESWcQGLDGLASR ATPEEVASYTLK ANSLAQLGK TWGGRPENVAAAQEALLLR YTSDGEAAAAK EAAYYQQGAR TVVSIPNGPSELAVK VWAETFYYmALNNVmFEGILLKPSmVTPGAEcK TFEVAQK ALQNTcLK EAAWGLAR
		Fructose-bisphosphate aldolase	GR278812_5 / 2e-97	M8BHV4	41.85 (7)	19.72	9.03	GILAmDESNATcGK LASIGLENTEANR GLVPLVGSNDESWcQGLDGLASR RLASIGLENTEANR IVDILVKQGIVPGIK ASAYADELVK KIVDILVKQGIVPGIK
		Fructose-bisphosphate aldolase	GR278946_1 / 4e-104	F2D6R8	35.87 (5)	19.55	8.60	KIVDILVEQGIVPGIK GILAmDESNATcGK IVDILVEQGIVPGIK GLVPLTGSNDESWcQGLDGLASR LDSIGLENTEANR
		Putative oxidoreductase	DV855669_3 / 5e-163	M8C8T0	32.61 (6)	35.73	9.44	GVPLAVNQVNYSLIYR AAcDELGVTLIAYSPIAQGVLSGK NAGQAmDFAGALGWSLTADEVEELR AVGVSNYNEK

								FAALPWR NPTQVSLNWLTcQGNVVPIPGAK
	V	Fructose-bisphosphate aldolase, chloroplastic		Q40677	21.91 (9)	41.98	6.80	GILAmDESNATcGK LASIGLENTEANR ANSLAQLGK RLASIGLENTEANR EAAYYQQGAR TVVSIPNGPSELAVK EAAWGLAR ALQNTcLK TFEVAQK
		Probable fructose-bisphosphate aldolase 1, chloroplastic		Q9SJU4	12.78 (6)	42.90	6.58	LASIGLENTEANR ANSLAQLGK ATPEQVASYTLK RLASIGLENTEANR EAAWGLAR ALQNTcLK
		Uncaracterized oxidoreductase At1g06690, chloroplastic		Q94A68	6.1 (2)	41.47	8.82	GIPLASNQVNYSLIYR FAALPWR
4401	A	Glyceraldehyde-3-phosphate dehydrogenase B, chloroplastic	DV856385_2 / 9e-140	M7ZNG9	53.67 (11)	32.66	8.05	VPTPNVSVVDLVINTVK VVAWYDNEWGYSQR VVDLAHLVASK AAALNIVPTSTGAAK AADGPLNGILAVcDEPLVSVDFR VLDEEFGIVK TGSGDPLEDYcK cSDVSTTIDASLTmVmGDDmVK AVSLVLPQLK TGITADDVNAAFR GTMTTTHSYTGDQR
		Glyceraldehyde-3-phosphate dehydrogenase B, chloroplastic	GR278640_1 / 3e-127	M7ZNG9	44.5 (8)	22.70	6.54	VPTPNVSVVDLVINTVK AAALNIVPTSTGAAK VLDEEFGIVK cSDVSTTIDASLTmVmGDDmVK AVSLVLPQLK VIITAPAK KVIITAPAK GTMTTTHSYTGDQR
	V	Glyceraldehyde-3-phosphate dehydrogenase GAPB, chloroplastic		P25857	34 (13)	47.63	6.80	VVAWYDNEWGYSQR VVDLAHLVASK IVDNETISVDGK AAALNIVPTSTGAAK VLDEEFGIVK AVSLVLPQLK VIITAPAK VAINGFGR

								YDSmLGTFK LLDASHR LIKVVSNRDPLK cSDVSTTIDSSLTMVmGDDmVK GTMTTTHSYTGDQR
		Phosphoglycerate kinase, cytosolic		P12783	8.23 (2)	42.10	5.86	GVKLLLPTDVVVADK LASVADLYVNDAFGTAHR
4407	A	Glyceraldehyde-3-phosphate dehydrogenase B, chloroplastic	DV856385_2 / 8E-140	M7ZNG9	38.67 (9)	32.66	8.05	VPTPNVSVVDLVINTVK VVDLAHLVASK VVAWYDNEWGYSQR VLDEEFGIVK AAALNIVPTSTGAAK TGITADDVNAAFR TGSGDPLEDYcK GTmTTTHSYTGDQR AVSLVLPQLK
		Aspartate aminotransferase EC=2.6.1.1	DV867720_2 / 2e-103	M7YWZ4	24.48 (5)	26.95	9.44	IGAINVIcSAPEVADR ISLAGLNLAK LYDSLSAK IVANVVGDPTmFGEWKEEmAQmAGR IVANVVGDPTmFGEWK
	V	Glyceraldehyde-3-phosphate dehydrogenase GAPB, chloroplastic		P25857	18.12 (7)	47.63	6.80	IVDNETISVDGK VVDLAHLVASK VVAWYDNEWGYSQR VLDEEFGIVK AAALNIVPTSTGAAK AVSLVLPQLK YDSmLGTFK
		Aspartate aminotransferase, chloroplastic		P46248	8.39 (4)	49.80	8.15	EYLPIEGLAAFNK LNLGVGAYR IADVIQEK NLGLYAER
		Glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic (Fragment)		Q8VXQ9	6.05 (2)	33.55	7.15	VLDEKFGIVK KVLITAPAK
4414	A	Fructose-bisphosphate aldolase EC=4.1.2.13	DV858099_2 / 9e-105	I1GXE4	22.5 (5)	34.29	10.36	KENVADAQATFLAR VAAEVIAEYTVAALR YAGAAAGGDAAASESLYVSGYK ENVADAQATFLAR VLLEGTLLKPNmVTPGSDSPK
		Ribulose-1,5-bisphosphate carboxylase small subunit EC=4.1.1.39	GR279297_6 / 1e-74	Q9SDY8	23.35 (4)	19.34	8.44	LPmFGcTDASQVIK QIDFLIR KFETLSYLPPLSEEALLK FETLSYLPPLSEEALLK
	V	Fructose-bisphosphate aldolase, cytoplasmic isozyme 1		P46256	5.88 (2)	38.42	6.79	GILAADESTGTIGK YADELIK

		Fructose-bisphosphate aldolase, cytoplasmic isozyme		P08440	5.92 (2)	38.58	7.61	GILAADESTGTIGK YYEAGAR
4708	A	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	DV862115_2 / 5e-72	M8A968	11.32 (2)	25.15	7.72	TQETLEEGcELISK ImQNNAAVFR
		succinate dehydrogenase flavoprotein subunit,mitochondrial	GR280547_4 / 1e-80	B6U124	25.95 (3)	13.99	8.02	LGANSLLDIVVFGR ImQNNAAVFR VAEISKPGDK
		NADP-dependent malic enzyme, chloroplastic EC=1.1.1.40	DV860156_6 / 1e-28	P43279	16.15 (2)	15.11	9.61	AYELGLATR YAEScmYTPIYR
	V	Succinate dehydrogenase [ubiquinone] flavoprotein subunit 1, mitochondrial		O82663	16.4 (7)	69.61	6.29	AFGGQSLDFGK SSQTILATGGYGR ImQNNAAVFR LPGISETAAIFAGVDVTK AYFSATSAHTcTGDGNAmVAR LGANSLLDIVVFGR AAIGLSEHGFNTAcITK
		ATP synthase subunit alpha, chloroplastic		A1EA05	4.16 (2)	55.43	6.48	LIESAAPSIISR GEIIASESR
4806	A	ATP-dependent Clp protease ATP-binding subunit clpA-like CD4B protein, chloroplastic	DV856880_1 / 3e-117	M7Y8C6	23.93 (7)	34.40	8.92	NTLLImTSNVGSSVIEK EGDSAIVDVDADGK IGFDLESDEKDTSYNR LLEDSLAEK LDEmIVFR KIGFDLESDEKDTSYNR SLVTEELK
		ATP-dependent Clp protease ATP-binding subunit clpA-like CD4A protein, chloroplastic	GR279426_5 / 1e-108	M8C5W2	32.66 (4)	22.15	8.87	NTLLImTSNVGSSVIEK LDmSEFmER AHPDVFNmmLQILEDGR LIGSPPGYVGYTEGGQLTEAVR
		Cyanate hydratase EC=4.2.1.104	DV857698_4 / 2e-92	B6TTW1	13.6 (3)	37.62	11.02	AIDLIDEAGSR VPEPTVDESIQILR YTDEALVAAAQLSYQYISDR
		ATP-dependent Clp protease ATP-binding subunit clpA-like protein CD4B, chloroplastic	GR279038_6 / 1e-71	M7Z383	35.77 (3)	15.02	11.28	VImLAQEEAR GSGFVAVEIPFTPR LGHNFVGTEQILLGLIGEGTGIAAK
		ATP-dependent Clp protease ATP-binding subunit clpA-like CD4B protein, chloroplastic	DV853298_2 / 2e-105	M8AGK1	11.6 (3)	33.36	9.66	NTLLImTSNVGSSVIEK LDEmIVFR EINLQVTEK
	V	Chaperone protein ClpC1, chloroplastic		Q7F9I1	39.32 (28)	101.74	6.51	NTLLImTSNVGSSVIEK mVGESTEAVGAGVGGGSSGQK AIDLIDEAGSR GELQcIGATTLDEYR NNPcLIGEPGVGK

					NPNRPIASFIFSGPTGVGK VImLAQEEAR VPEPTVDETIQILR GSGFVAVEIPFTPR LGHNFVGTEQILLGLIGEGTGIAAK VITLDmGLLVAGTK IIGQDEAVK VLELSLEEAR VLESLGADPNNIR LDmSEFmER RPYTVVLFDEIEK mPTLEEYGTNLTK TAIAEGLAQR LLEDSLAEK AHPDVFNmmLQILEDGR LDEmIVFR GELQcIGATTLDEYRK LIGSPPGYVGYTEGGQLTEAVR HAQLPDEAK HIEKDPALER SLVTEELK AQITAIIDK QLGHNYIGSEHLLLGLLR
Chaperone protein ClpC2, chloroplastic	Q2QVG9	30.14 (22)	101.95	7.06	NTLLImTSNVGSSVIEK AIDLIDEAGSR GELQcIGATTLDEYR NNPcLIGEPGVGK VImLAQEEAR LGHNFVGTEQILLGLIGEGTGIAAK VITLDmGLLVAGTK VLELSLEEAR LDmSEFmER RPYTVVLFDEIEK mPTLEEYGTNLTK TAIAEGLAQR LLEDSLAEK LSYQYISDR AHPDVFNmmLQILEDGR LDEmIVFR GELQcIGATTLDEYRK LIGSPPGYVGYTEGGQLTEAVR HIEKDPALER AQITALIDK VPEPTVDETIEILR QLGHNYIGSEHLLLGLLR
ATP-dependent Clp protease ATP-binding subunit clpA homolog, chloroplastic (Fragment)	P46523	23.23 (16)	97.26	6.18	NTLLImTSNVGSSVIEK GELQcIGATTLDEYR NNPcLIGEPGVGK

								VImLAQEEAR GSGFVAVEIPFTPR LGHNFVGTEQILLGLIGEGTGIAAK VLELSLEEAR LDmSEFmER RPYTVVLFDEIEK mPTLEEYGTNLTK TAIAEGLAQR AHPDVFNmmLQILEDGR GELQcIGATTLDEYRK HIEKDPALER SLVTQELK QLGHNYIGSEHLLLGLLR
		Chaperone protein ClpD1, chloroplastic		Q6H795	1.71 (2)	101.82	7.17	AIDLIDEAGSR QLPDKAIDLIDEAGSR
5412	A	Elongation factor Tu	DY543537_4 / 2e-134	N1R5E7	32.18 (7)	29.21	8.75	TmDDAIAGDNVGLLLR MVVELIQPVAcEQGmR VGDPVDLVGIR TTDVTGNVTNImNDK SATVTGVEmFQK FEAVVYVLK TTDVTGNVTNImNDKDEEAK
		Elongation factor Tu	DV859340_3 / 3e-134	N1R5E7	27.27 (7)	34.51	9.04	TmDDAIAGDNVGLLLR MVVELIQPVAcEQGmR VGDPVDLVGIR NATVTGVEmFQK TTDVTGNVTNImNDK FEAVVYVLK TTDVTGNVTNImNDKDEEAK
		Phosphoglycerate kinase EC=2.7.2.3	DV858247_3 / 2e-115	I1HI26	10.4 (2)	31.74	7.31	GVSLLLPSDVVIADK cDILLLGGGmIFTFYK
	V	Elongation factor Tu, chloroplastic		O24310	16.19 (7)	53.02	7.12	KYDEIDAAPEER GITINTATVEYETETR QDQVDDEELLELVELEVR VGDVVDLVGLR YDEIDAAPEER HYAHVDcPGHADYVK EHILLAK
		Elongation factor TuA, chloroplastic		Q40450	15.27 (6)	51.92	6.81	KYDEIDAAPEER NmITGAAQmDGAILVcSGADGPmPQTK YDEIDAAPEER HYAHVDcPGHADYVK STTVTGVEMFQK EHILLAK
		Elongation factor Tu, chloroplastic		P50371	7.84 (4)	45.29	6.14	KYDEIDAAPEER QVGVPSIVVFLNK

								YDEIDAAPEER EHILLAK
		Elongation factor Tu, chloroplastic		Q9TKZ5	14.39 (4)	44.78	5.54	QDQVDDEELLELVELEVR DTDKSFLMAVEDVFSITGR HYAHVDcPGHADYVK EHILLAK
5801	A	ATP-dependent Clp protease ATP-binding subunit clpA-like CD4B protein, chloroplastic	DV856880_1 / 3e-117	M7Y8C6	21.31 (7)	34.40	8.92	NTLLImTSNVGSSVIEK LDEmIVFR IGFDLESDEKDTSYNR EGDSAIVDVDADGK IGFDLESDEK LLEDSLAEK KIGFDLESDEKDTSYNR
		ATP-dependent Clp protease ATP-binding subunit clpA-like CD4A protein, chloroplastic	GR277864_5 / 0	M8C5W2	32.98 (7)	31.29	6.98	NTLLImTSNVGSSVIEK IGFDLESDEK VIGQDEAVK LIGSPPGYVGYTEGGQLTEAVR LDmSEFmER AQITALIDK AHPDVFNmmLQILEDGR
		Cyanate hydratase EC=4.2.1.104	DV857698_4 / 2e-92	B6TTW1	13.6 (3)	37.62	11.02	AIDLIDEAGSR VPEPTVDESIQILR YTDEALVAAAQLSYQYISDR
		ATP-dependent Clp protease ATP-binding subunit clpA-like protein CD4B, chloroplastic	GR279038_6 / 1e-71	M7Z383	35.77 (3)	15.02	11.28	GSGFVAVEIPFTPR VImLAQEEAR LGHNFVGTEQILLGLIGEGTGIAAK
	V	Chaperone protein ClpC1, chloroplastic		Q7F9I1	35.19 (26)	101.74	6.51	mVGESTEAVGAGVGGGSSGQK NTLLImTSNVGSSVIEK AIDLIDEAGSR VITLDmGLLVAGTK GSGFVAVEIPFTPR VLELSLEEAR VImLAQEEAR LGHNFVGTEQILLGLIGEGTGIAAK NNPcLIGEPGVGK LDEmIVFR HAQLPDEAK HIEKDPALER TAIAEGLAQR mPTLEEYGTNLTK IIGQDEAVK RPYTVVLFDEIEK GELQcIGATTLDEYR VLESLGADPNNIR LDmSEFmER LLEDSLAEK

								LRHAQLPDEAK AHPDVFNmmLQILEDGR AQITAIIDK NPNRPIASFIFSGPTGVGK GELQcIGATTLDEYRK LIGSPPGYVGYTEGGQLTEAVR
		Chaperone protein ClpC2, chloroplastic		Q2QVG9	27.64 (21)	101.95	7.06	NTLLImTSNVGSSVIEK AIDLIDEAGSR VITLDmGLLVAGTK VLELSLEEAR VImLAQEEAR LGHNFVGTEQILLGLIGEGTGIAAK NNPcLIGEPGVGK LDEmIVFR LSYQYISDR HIEKDPALER TAIAEGLAQR mPTLEEYGTNLTK RPYTVVLFDEIEK GELQcIGATTLDEYR VIGQDEAVK LDmSEFmER LLEDSLAEK AHPDVFNmmLQILEDGR AQITALIDK GELQcIGATTLDEYRK LIGSPPGYVGYTEGGQLTEAVR
		Chaperone protein ClpD1, chloroplastic		Q6H795	2.67 (3)	101.82	7.17	AIDLIDEAGSR LDmSEYMER QLPDKAIDLIDEAGSR
5807	A	Transketolase, chloroplastic	DV863383_1 / 3e-56	N1QRK9	28.09 (4)	19.80	9.41	ISIEAGSTLGWQK EYGITAEAVVAAAK FGASAPAGIIYK ESVLPAAVTAR
	V	Transketolase, chloroplastic		Q7SIC9	10.81 (7)	72.95	5.72	VTTTIGFGSPNK ISIEAGSTLGWQK FEALGWHTIWVK ESVLPAAVTAR FLAIDAVEK RPSILALSR FAEYEKK
		ATP synthase subunit alpha, chloroplastic		A1EA05	4.95 (2)	55.43	6.48	GQNVIcVYVAIGQR TFTEQAEILLK
6103	A	20 kDa chaperonin, chloroplastic	DV858714_1 / 4e-81	M8AVR4	35.36 (10)	37.99	9.07	VAETSDTTAGGLILSESTK EDDIIGILETDDVK QPLSVSAGSTVLYSK

								VEVSIPTGSQVIYSK HLImKEDDIIGILETDDVK YAGTEVEYNNAK EKPSIGTVVAVGPGALDEEGKR GTDGTNYIVLK KVEVSIPTGSQVIYSK EDDIIGILETDDVKDmKPLNDR
		Chlorophyll a-b binding protein 8, chloroplastic	DV856057_1 / 1e-123	M8A6M9	25.09 (6)	32.16	9.07	TAmMGVVGmIAPEALGK WLAYGEIFNGR RLQDWYNPGSmGK QYFLGLEK YLGGSGDPAYPGGPIFNPLGFGTK LQDWYNPGSmGK
		Chlorophyll a-b binding protein 8, chloroplastic	DV856707_3 / 5e-122	M8A6M9	23.41 (6)	33.07	9.61	TAmMGVVGmIAPEALGK RLQDWYNPGSmGK QYFLGLEK YLGGSGDPAYPGGPIFNPLGFGTK mARSSTARTAmMGVVGmIAPEALGK LQDWYNPGSmGK
		Chlorophyll a-b binding protein 8, chloroplastic	DV857962_3 / 4e-46	M8A6M9	16.16 (3)	24.32	9.51	QYFLGLEK YLGGSGDPAYPGGPIFNPLGFGTK APmGKQYFLGLEK
6305	A	Fructose-bisphosphate aldolase EC=4.1.2.13	GR278946_1 / 4e-95	M8BHV4	36.41 (6)	19.55	8.60	RLDSIGLENTEANR KIVDILVEQGIVPGIK IVDILVEQGIVPGIK GILAmDESNATcGK GLVPLTGSNDESWcQGLDGLASR LDSIGLENTEANR
		Fructose-bisphosphate aldolase	DV855628_5 / 4e-171	M8BHV4	27.36 (8)	34.08	5.78	YTSDGEAAEAK ATPEQVADYTLK AAQEALLLR EAAYYQQGAR TVVSIPNGPSELAVK ANSLAQLGK ALQNTcLK TWGGRPENVK
		Fructose-bisphosphate aldolase	GR278311_1 / 1e-109	M8BHV4	35.48 (5)	19.68	8.24	KIVDILVEQGIVPGIK IVDILVEQGIVPGIK GILAmDESNATcGK LASIGLENTEANR GLVPLVGSNDESWcQGLDGLASR
		Fructose-bisphosphate aldolase, chloroplastic	GR277910_5 / 5e-143	M7Z4Y9	21.89 (5)	29.12	5.12	TWGGRPENVAAAQEALLLR TVVSIPNGPSELAVK ANSLAQLGK ALQNTcLK YYQQGAR

		Triosephosphate isomerase EC=5.3.1.1	DV853744_1 / 4e-133	M7Z1M4	9.64 (2)	36.69	8.27	IIYGGSVNAANSAELAK KEDIDGFLVGGASLK
	V	Fructose-bisphosphate aldolase, chloroplastic		Q40677	22.42 (8)	41.98	6.80	GILAmDESNATcGK LASIGLENTEANR YTSDGEAAEAK EAAYYQQGAR TVVSIPNGPSELAVK ANSLAQLGK ALQNTcLK TFEVAQK
		Fructose-bisphosphate aldolase 2, chloroplastic		Q01517	10.03 (4)	37.80	5.59	RLDSIGLENTEANR GILAmDESNATcGK LDSIGLENTEANR TFEVAQK
6408	A	putative chloroplast inner envelope protein	DV853317_5 / 2e-141	A8R7E5	47.06 (10)	32.56	5.54	NLIQENISSALSILK LFDEVAADmFR ALGLDDVDAANmHmVVGR GLDIGTLIEVR HLFGITDYQIDIAmR SELcDLYASFVYSVLPPGHEDLK YGVSTQDAAFK AALELAVVAAAAAAGYTLGTR GNEVEAIIK SNPGSTSIPK
		putative chloroplast inner envelope protein	DV856061_2 / 2e-134	A8R7E5	38.81 (8)	32.08	9.01	NLIQENISSALSILK EAEAIIEGVTSNVK LFDEVAADmFR GLDIGTLIEVR HLFGITDYQIDIAmR GLGPVSLGGDFDHDR ILYAAYATEVLSDGSLDDEK SNPGSTSIPK
		Actin-3	DV857524_2 / 1e-154	M8AIA9	28.74 (6)	36.52	5.71	SYELPDGQVITIGAER LAYVALDYEQELESAK GEYDESGPAIVHR TTGIVLDSGDGVSHTVPIYEGYALPHAILR EITALAPSSmK GYSFTTTAER
		Actin	GR281989_5 / 2e-91	B9VJF4	30.88 (3)	15.33	4.96	LAYVALDYEQELETAR SYEmPDGQVITIGSER GYSLTTTAER
	V	Actin-66 (Fragment)		P81228	39.58 (9)	37.17	5.82	SYELPDGQVITIGAER AGFAGDDAPR DAYVGDEAQSK YPIEHGIVSNWDDmEK VAPEEHPVLLTEAPLNPK

					TTGIVLDSGDGVSHTVPIYEGYALPHAILR GYSFTTTAER EITALAPSSmK IWHHTFYNELR
Actin-97	P30171	35.28 (9)	41.62	5.49	SYELPDGQVITIGAER AGFAGDDAPR DAYVGDEAQSK GYSFTTSAER YPIEHGIVSNWDDmEK VAPEEHPVLLTEAPLNPK TTGIVLDSGDGVSHTVPIYEGYALPHAILR EITALAPSSmK IWHHTFYNELR
Actin-1	P53504	32.36 (8)	41.84	5.69	SYELPDGQVITIAADR AGFAGDDAPR DAYVGDEAQSK YPIEHGIVSNWDDmEK VAPEEHPVLLTEAPLNPK TTGIVLDSGDGVSHTVPIYEGYALPHAILR GYSFTTTAER IWHHTFYNELR
Actin-54 (Fragment)	P93373	32.15 (8)	37.46	5.99	NYELPDGQVITIGAER LAYVALDYEQELETAR AGFAGDDAPR DAYVGDEAQSK VAPEEHPVLLTEAPLNPK EITALAPSSmK IWHHTFYNELR YPIEHGIASNWDDmEK
Actin-7	P0C542	20.21 (6)	41.59	5.39	SYELPDGQVITIGAER AGFAGDDAPR DAYVGDEAQSK VAPEEHPVLLTEAPmNPK GYSFTTTAER IWHHTFYNELR
Actin (Fragment)	P53491	22.38 (5)	39.48	6.55	AGFAGDDAPR VAPEEHPVLLTEAPmNPK TTGIVLDSGDGVSHTVPIYEGYALPHAILR GYSFTTTAER IWHHTFYNELR
Actin-1	P02582	14.13 (4)	41.59	5.39	LAYVALDYEQELETAK AGFAGDDAPR SYEmPDGQVITIGSER DAYVGDEAQAK
Phosphoglycerate kinase, chloroplastic	P12782	6.25 (2)	49.81	7.03	ELDYLDGAVSNPK GVTTIIGGGDSVAAVEK

		Phosphoglycerate kinase, chloroplastic		Q42961	6.65 (2)	50.15	8.38	GVTTIIGGGDSVAAVEK GVSLLLPSDVVIADK
6701	A	Vacuolar proton-ATPase subunit A	FE527958_5 / 1e-116	Q1W681	10.77 (3)	37.59	8.10	LYDDLTTGFR FEDPAEGEDVLVAK YATALEGFYDK
		Phosphoglucomutase, cytoplasmic EC=5.4.2.2	GR280735_5 / 2e-80	Q9SNX2	36.43 (4)	14.37	5.03	LSGTGSVGATIR IYIEQYEK YDYENVDAEAAK ESSDALSPLVDVALK
	V	Phosphoglucomutase, cytoplasmic		Q9SNX2	14.97 (7)	62.63	5.54	LSGTGSVGATIR IYIEQYEK YDYENVDAEAAK SmPTSAALDVVAK YNmGNGGPAPESVTDK GATIVVSGDGR ESSDALSPLVDVALK
		V-type proton ATPase catalytic subunit A (Fragment)		Q40002	23.28 (10)	64.06	5.55	TTLVANTSNmPVAAR FEDPAEGEDVLVAK DmGYNVSmmADSTSR LAEmPADSGYPAYLASR LAADTPLLTGQR EASIYTGITIAEYFR YSNSDTVVYVGcGER VGHDSLIGEIIR EFTmLHTWPVR VQcLGSPDR
		V-type proton ATPase catalytic subunit A		P09469	16.21 (7)	68.79	5.45	TTLVANTSNmPVAAR VSGPVVVADGmGGAAmYELVR DmGYNVSmmADSTSR LAADTPLLTGQR EASIYTGITIAEYFR YSNSDTVVYVGcGER ESEYGYVR
6806	A	ATP-dependent zinc metalloprotease FTSH 1, chloroplastic	DV855902_3 / 3e-174	M8ADT2	40.44 (8)	35.26	6.99	VAEEVIFGTNNVTTGASSDFmQVSR SYLENQmAVALGGR GQAGGLTFFAPSEER TPGFTGADLQNLMNEAAILAAR LVAYHEAGHALVGALmPEYDPVAK EISKDEISDALER LAQLLIEK LESGLYSR
	V	ATP-dependent zinc metalloprotease FTSH 1, chloroplastic		Q39102	23.46 (12)	76.71	5.83	AQGGPGGGPGGLGGPmDFGR APcIVFIDEIDAVGR SYLENQmAVALGGR LAQLLIEK TPGFTGADLQNLmNEAAILAAR

								LELQEVVDFLK LVAYHEAGHALVGALmPEYDPVAK GQAGGLTFFAPSEER EISKDEISDALER GcLLVGPPGTGK LESGLYSR DVDFDK
		70 kDa peptidyl-prolyl isomerase		Q43207	10.2 (5)	62.02	5.40	TDEEAVIEGLDR ITcNLNNAAcK ExEGYERPNEGAVVTVK LQDGTVFLK LGQGQVIK
7407	A	Phosphoribulokinase EC=2.7.1.19	DV866058_6 / 5e-122	F2DD69	44.03 (8)	30.45	7.11	FYGEVTQQmLK KPDFDAYIDPQK LDELIYVESHLSNLSTK FFNPVYLFDEGSTINWIPcGR DLYEQIIAER IRDLYEQIIAER HADFPGSNNGTGLFQTIVGLK FSYGPDTYFGQEVSVLEmDGQFDR
		Adenosine kinase	DV866906_3 / 5e-65	Q8L5P6	23.03 (2)	19.17	9.01	IAVITQGADPVVVAEDGK LVDTNGAGDAFVGGFLSQLVQGK
	V	Phosphoribulokinase, chloroplastic		P27774	18.64 (6)	44.09	6.46	FYGEVTQQmLK ANDFDLmYEQVK KPDFDAYIDPQK LTSVFGGAAEPPR LDELIYVESHLSNLSTK LTcSYPGIK
		Phosphoribulokinase, chloroplastic		P26302	27.48 (9)	45.11	6.05	FYGEVTQQmLK ANDFDLmYEQVK LDELIYVESHLSNLSTK FFNPVYLFDEGSTINWIPcGR DLYEQIIAER LTcSYPGIK GVTALDPK IRDLYEQIIAER HADFPGSNNGTGLFQTIVGLK
7409	A	Glutamine synthetase EC=6.3.1.2	DV858937_3 / 7e-138	I1J2T4	19.75 (10)	35.89	7.93	RLTGLHETASISDFSWGVANR LTGLHETASISDFSWGVANR HDLHISEYGEGNER EDGGFEVIKK AILNLSLR AILNLSLRHDLHISEYGEGNER AmREDGGFEVIK KAILNLSLR AmREDGGFEVIKK

							DISDAHYK
	Glutamine synthetase	GR278149_5 / 5e-105	I1J2T4	35.92 (10)	22.64	7.17	RLTGLHETASISDFSWGVANR LTGLHETASISDFSWGVANR HDLHISEYGEGNER EDGGFEVIKK AILNLSLR AILNLSLRHDLHISEYGEGNER AmREDGGFEVIK KAILNLSLR AmREDGGFEVIKK QVGPSVGIDAGDHIWASR
	glutamine synthetase, chloroplastic EC=6.3.1.2	GR279277_3 / 9e-52	P25462	40.43 (2)	10.41	7.18	GGNNIIVVcDTYTPQGEPIPTNKR TISKPVEDPSELPK
	Oxygen-evolving enhancer protein 1, chloroplastic	DV855937_1 / 4e-158	M8AE10	30.94 (5)	33.40	9.26	NASSSTGNITLSVTK DGIDYAAVTVQLPGGER GGSTGYDNAVALPAGGR SNPDTGEVIGVFESVQPSDTDLGAK LTYTLDEmEGPLEVSSDGTLK
	Ribulose bisphosphate carboxylase/oxygenase activase A, chloroplastic	DV855440_2 / 0	M7ZAC1	21.9 (4)	35.11	6.24	mcALFINDLDAGAGR GIFQTDNVSDESVVK VQLADTYmSQAALGDANQDAmK IVDTFPGQSIDFFGALR
	Phosphoglycerate kinase EC=2.7.2.3	DV858247_3 / 2e-115	I1HI26	16.44 (3)	31.74	7.31	KGVTTIIGGGDSVAAVEK GVSLLLPSDVVIADK cDILLLGGGmIFTFYK
	Ribulose bisphosphate carboxylase/oxygenase activase A, chloroplastic	DV856736_3 / 6e-98	M7ZAC1	21.99 (3)	30.91	8.94	VQLADTYmSQAALGDANQDAmK IVDTFPGQSIDFFGALR RPVLVSARGISQTDNVSDESVVK
	Ribulose bisphosphate carboxylase/oxygenase activase B, chloroplastic	FD933088_1 / 0	M8AZL6	11.3 (2)	39.51	8.92	LVDTFPGQSIDFFGALR mGINPImmSAGELESGNAGEPAK
V	Glutamine synthetase leaf isozyme, chloroplastic		P13564	13.82 (6)	47.06	5.29	RLTGLHETASISDFSWGVANR IIAEYIWVGGSGIDLR LTGLHETASISDFSWGVANR TISKPVEDPSELPK AILNLSLR KAILNLSLR
	Glutamine synthetase, chloroplastic		P25462	20.57 (6)	45.99	6.87	AAQIFSDPK TISKPVEDPSELPK AILNLSLR GGNNVLVIcDTYTPQGEPLPTNK GGNNVLVIcDTYTPQGEPLPTNKR ITEQAGVVLTLDPKPIQGDWNGAGcHTNYSTK
	Glutamine synthetase, chloroplastic		P14655	19.16 (7)	46.61	6.34	EDGGFEVIKK TISKPVEDPSELPK

								AILNLSLR MEQLLNmDTTPFTDK SMREDGGFEVIK KAILNLSLR ITEQAGVVLTLDPKPIQGDWNGAGcHTNYSTK
		Phosphoglycerate kinase, chloroplastic		P12782	9.79 (4)	49.81	7.03	ELDYLDGAVSNPK GVTTIIGGGDSVAAVEK KGVTTIIGGGDSVAAVEK cDILLLGGGmIFTFYK
		Ribulose bisphosphate carboxylase/oxygenase activase A, chloroplastic		Q40073	18.75 (5)	51.04	7.94	GIFQTDNVSDESVVK VQLADTYmSQAALGDANQDAmK IVDTFPGQSIDFFGALR mGINPImmSAGELESGNAGEPAK DGPVTFEQPK
		Glutamine synthetase leaf isozyme, chloroplastic		Q9XQ94	14.49 (6)	47.09	6.73	EDGGFEVIKK AILNLSLR AAEIFSNPK ITEQAGVVLTLDPKPIEGDWNGAGcHTNYSTK SMREDGGFEVIK KAILNLSLR
		Phosphoglycerate kinase, chloroplastic		Q42961	12.89 (4)	50.15	8.38	GVTTIIGGGDSVAAVEK GVSLLLPSDVVIADK FYKEEEKNEPEFAK cDILLLGGGmIFTFYK
		Oxygen-evolving enhancer protein 1, chloroplastic		P27665	9.85 (2)	34.72	8.56	NASSSTGNITLSVTK DGIDYAAVTVQLPGGER
		Oxygen-evolving enhancer protein 1, chloroplastic (Fragments)		P84989	34 (2)	10.66	5.49	GGSTGYDNAVALPAGGR DGIDYAAVTVQLPGGER
		Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic		O98997	12.53 (3)	47.87	7.78	mcALFINDLDAGAGR LVDTFPGQSIDFFGALR mGINPImmSAGELESGNAGEPAK
		Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic		P10896	6.96 (2)	51.95	6.15	mGINPImmSAGELESGNAGEPAK SFQcELVMAKMGINPImmSAGELESGNAGEPAK
8202	A	Putative ribose-5-phosphate isomerase	DV859524_6 / 5e-121	M8CHY5	28.95 (4)	24.83	6.09	LQGLFNEEGVEAK LNEDGKPYVTDNSNYIVDLYFK LVTGLGGSGLAmPVEVVQFcWK FVVVVDETK
		Light harvesting chlorophyll a/b binding protein3	GR279604_2 / 1e-61	K7V1F9	26.04 (2)	10.27	5.00	WAmLGALGcVFPELLAR ELEVIHSR
8204	A	Light harvesting chlorophyll a/b-binding protein Lhcb1	DV858561_2 / 1e-150	D6RSA1	18.59 (4)	33.18	6.84	LAmFSmFGFFVQAIVTGK AKPSASGSPWYGSDR WAmLGALGcVFPELLAR FGEAVWFK

	Light harvesting chlorophyll a/b-binding protein Lhcb1	DY543483_4 / 8e-102	H6BDG5	14.98 (3)	24.16	6.73	LAmFSmFGFFVQAIVTGK FGEAVWFK VFPELLAR
	Light harvesting chlorophyll a/b-binding protein Lhcb1	DV854994_2 / 1e-60	H6BDG5	10 (2)	32.69	9.28	LAmFSmFGFFVQAIVTGK KVVLmGAVEGYR
V	Chlorophyll a-b binding protein 2, chloroplastic		P09755	19.92 (5)	27.10	6.20	LAmFSmFGFFVQAIVTGK WAmLGALGcVFPELLAR FGEAVWFK ELEVIHSRWAmLGALGcVFPELLAR ELEVIHSR
	Ribulose-phosphate 3-epimerase, chloroplastic		Q43157	12.28 (2)	30.35	8.06	VIEAGANALVAGSAVFGAK SDIIVSPSILSANFAK

Annex 28 - Identification details for the 3 leaf spots with uncharacterized identity

Sp: spot number; Dtb: consulted database, V: viridiplantae of Uniprot and A: Agrostis spp. EST database; ID: Protein identity; Uniprot: Uniprot Accession; gb Access: Genbank Accession; e-value: e-value of the blastx on NCBI; Cov: % of coverage between experimental and database sequences; (nb): number of peptides matched between both sequences; peptids: list of matched peptides.

Sp	Db	ID	gb / e-val	Uniprot	Cov (nb)	MW	pI	Peptides
1501	A	Uncharacterized protein	DV853256_3 / 5e-55	I1HH93	13.73 (2)	34.01	6.81	QGAPEDAPEDAPQAEESK DGTANVEEEKEEEDKEmTLDEFEK
5201	A	hypothetical protein	DV852843_1 / 2e-124	C5YJV9	36.99 (8)	32.36	9.14	GLVDANQVLAYFAVSK LIWISAFmLVGAR NDDLDGVLEATPK SEVSSLIAELASAAGAER LQNGGLTcK HPGATVGVVEK NGWFYSLSEK ALAEGKPDPcSLHTAWLK
		Predicted protein	DV866774_2 / 3e-50	B9GLQ4	32.95 (4)	19.28	8.53	SEVSSLIAELASAAGAER GLLFDEGIEER NGWFYSISEK ALGEGKPDPcPLHTAWLK
6202	A	Putative uncharacterized protein Sb07g009470	DV852843_1 / 2e-124	C5YJV9	30.82 (7)	32.36	9.14	GLVDANQVLAYFAVSK LIWISAFmLVGAR NDDLDGVLEATPK SEVSSLIAELASAAGAER GLSFDEGIEER LQNGGLTcK NGWFYSLSEK

Thanks for reading …

That's all for the "Pingu" team !