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Intraspecific variability in embolism resistance and functional trade-offs in sunflower (*Helianthus annuus* L.) and Maritime pine (*Pinus pinaster* Ait.)

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Title: Intraspecific variability in embolism resistance and functional trade-offs in sunflower (*Helianthus annuus* L.) and Maritime pine (*Pinus pinaster* Ait.)

Summary

As a consequence of current global climate change, forest and crop ecosystems are decreasing in productivity worldwide and are under increased threat of widespread drought-induced mortality due to hydraulic failure caused by xylem embolism. The range of embolism resistance across species has been intensively studied but little attention has been paid to intraspecific variability. This thesis aimed therefore at improving our understanding of the intraspecific variation in embolism resistance in one crop species (*Helianthus annuus* L.) and one conifer species (*Pinus pinaster*). In the first place, vulnerability to embolism was determined across four sunflower accessions with significant variations in P_{50} (pressure inducing 50% loss of hydraulic conductivity) ranging between -2.67 and -3.22 MPa. A trade-off between hydraulic safety and growth was observed but not between hydraulic safety and efficiency. Only few anatomical traits, such as vessel density and vessel lumen area, were found related to embolism resistance. Secondly, we phenotyped 477 *P. pinaster* full-sib F2 genotypes corresponding to a three-generation inbred pedigree obtained by the self-pollination of an inter "Corsica × Landes" provenance hybrid, for embolism resistance and wood density. The variability for P_{50} within species was remarkably low (CV_P=5.6%). We also found a lack of trade-off between hydraulic safety and wood density. The phenotypic variance explained by QTLs (PEV) detected for P_{50} accounted for 23.6% of the phenotypic variation but no QTL collocated between P_{50} and wood density, suggesting that embolism resistant genotypes in this family are not dependent on denser wood. A multi-trait analysis of leaf and xylem functioning was performed through seasonal measurements of photosystem II fluorescence and stem hydraulic traits on a full-sib family of an inter "Morocco × Landes" provenance hybrid of *P. pinaster*; it revealed that with decreasing water potential, the efficiency of both photosystem II and stomatal conductance decreased which resulted in rapid decrease in assimilation rate. Xylem resistance to embolism was found having no relationship with leaf functioning traits. The absence of trade-off between these traits makes it possible to consider selection for both efficient and resistant genotypes.

Key words: embolism, drought, intraspecific variability, xylem, vessel anatomy, QTL, wood density

Titre: Variabilité intraspécifique de la résistance à l'embolie et trade-offs fonctionnels chez le tournesol (*Helianthus annuus* L.) et le pin maritime (*Pinus pinaster* Ait.)

Résumé

Plusieurs études ont montré que la productivité des forêts et des cultures décroît sur l'ensemble de la planète en réponse au changement climatique, et que les risques de mortalité liée à la sécheresse s'accroissent. Ces mortalités massives ont été observées dans tous les écosystèmes forestiers et seraient dues à un dysfonctionnement hydraulique des plantes, causé par de l'embolie xylémiène. Le spectre interspécifique de résistance à l'embolie a été largement étudiée, mais peu d'attention a été accordée à la variabilité intra-spécifique de ce trait. C'est pourquoi, l'objectif de ce travail de thèse était d'améliorer notre compréhension de la variabilité intra-spécifique de la résistance à l'embolie chez une espèce cultivée (*Helianthus annuus* L.) et chez un conifère (*Pinus pinaster*). Nous avons déterminé la vulnérabilité à l'embolie de quatre variétés de tournesol, présentant des différences significatives de P_{50} (pression induisant 50% de perte de conductivité hydraulique), allant de -2.67 à -3.22 MPa. Un compromis entre sécurité hydraulique et croissance a été observé mais pas entre la sécurité hydraulique et la capacité de transport de l'eau du xylème. Seuls quelques caractères anatomiques, tels que la densité des vaisseaux et la surface du lumen, présentent une corrélation avec la résistance à l'embolie. Dans un second temps, nous avons phénotypé pour la résistance à l'embolie et la densité du bois 477 génotypes de *P. pinaster,* issus d'une F2 de pleins-frères correspondant à la $3^{\text{ème}}$ génération consanguine obtenue par auto-fécondation d'un hybride inter-provenance "Corse × Landes. La variabilité de la P_{50} au sein de l'espèce était extrêmement faible (CV_P=5.6%). D'autre part, nous avons mis en évidence l'absence de compromis entre sécurité hydraulique et densité du bois. La part de variance du caractère P_{50} expliquée par QTLs (PEV) correspondait à 23.6% de la variance phénotypique totale mais aucun QTL commun n'a pu être détecté entre P_{50} et densité du bois, suggérant qu'au sein de cette famille, la plus forte résistance à l'embolie de certains génotypes n'est pas liée à un bois plus dense. Une analyse multi-caractère du fonctionnement de la feuille et du xylème a été réalisée via l'étude de la dynamique saisonnière de la fluorescence du photosystème II et des traits hydrauliques de la tige, sur une famille de pleins-frères d'un hybride inter-provenance "Maroc × Landes" de *P. pinaster* ; en réponse à une diminution du potentiel hydrique, l'efficience du photosystème II et la conductance stomatique ont tous deux diminué, induisant une décroissance rapide du taux d'assimilation. La résistance à l'embolie du xylème n'a pas montré de relation significative avec les traits foliaires. L'absence de compromis entre ces caractères permet donc d'envisager la sélection génétique de génotypes à la fois efficients et résistants.

Mots-Clefs: embolie, sécheresse, variabilité intraspécifique, xylème, anatomie des vaisseaux, QTL, densité du bois

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Introduction

Introduction

Drought-induced plant mortality and crop productivity decline

Ecosystems across the globe are facing continuous threats by anthropogenic climate change, resulting natural populations to adapt, migrate or extinct (Walther et al., 2002). Shifting precipitation patterns, rising temperatures, heat waves combined with severe and frequent drought spells, triggered by climate change during the last few decades, may increase plant mortality (Allen et al., 2010; Bentz et al., 2010; Elderd et al., 2010; O' Grady and Mitchell, 2015;). The impacts of this changing climate have already been well recognized in ecosystems across the world, ranging from coral-reef dieback due to ocean acidification and temperature rise (Hoegh-Guldberg et al., 2007), extreme climatic events affecting many terrestrial ecosystems (Allen et al., 2010; Zhao and Running, 2010) to severely impacting human population globally, through crop failure, storm damage, extensive flooding and causing serious health issues by the spread of infectious fatal diseases (Patz et al., 2005). Existing climate models predict further decreases in soil moisture contents in the top 10 cm layer (Dai, 2013) and widespread increases in the intensity and duration of periodic water stress having severe consequences on plant growth and distribution worldwide during the 21st century (Van Mantgem et al., 2009; Anderegg et al., 2012; Engelbrecht, 2012; IPCC, 2014; McDowell et al., 2015).

According to FAO (2006) 30% of the world's land surface is under forests, including woodlands and savannas. Innumerable tangible and intangible benefits, around the globe, are derived from forests, ranging from the provision of essential goods and services such as timber (Payn *et al.*, 2015) and watershed protection/conservation to recreational, aesthetic, and spiritual amity. Understanding the mechanism underlying as well as predicting the consequences of these climatic changes in ecosystems and forecasting their impacts on forests and other vegetation types, is therefore, of particular importance and emerging as one of the striking challenges for scientists focusing on global change impacts (Boisvenue and Running, 2006; Bonan, 2008). The common implicated causal factor of forest die-offs and tree mortality in recent decades in southern Europe (Breda et al., 2006; Bigler et al., 2006), temperate forests of Western North America (van Mantgem et al., 2009), boreal forests of Canada (Peng et al., 2011) and extensive death of several tree species across different forest types over an area of about 10 million hectares (Raffa et al., 2008) is elevated temperatures and/or water stress. This indicates that the forests at a global scale across all ecoregions are under greater risk and respond increasingly to drought and ongoing warming and drying (Allen et al., 2010; 2015).

In the face of climate change, similar to forest research, studies on predicting responses to non-lethal water stress levels in crops have also been focused for better understanding of breeding programs and water management (Blum, 2005; Tardieu, 2012) in order to obtain varieties with improved drought tolerance Xoconostle-Cásarez et al., 2010). A pronounced decline in crop net primary productivity (NPP) was observed in 2003 throughout Europe following strong heatwaves (northern Italy, France) and drought (Ukraine, Romania). For instance, the greatest drop in maize NPP (-36%) was found in the Italian Po valley, where extremely high temperatures prevailed (Ciais et al., 2005). A continental scale analysis for 1901–2012 conducted by Trnka et al. (2016) also highlighted that barley and wheat yields were affected by climate variability in Central and Western Europe. The yield decline was more pronounced in Central Europe than in Western Europe due to comparatively drier conditions and variation in annual temperatures to a greater range. Pinke and Lövei, (2017) recently showed that increasing temperatures over the 1981-2010 period have negatively impacted the yields of barley, wheat, maize and rye in Hungary. Specifically, a 1°C rise in temperature reduced the yield of all four cereals by 9.6 to 14.8% in this Eastern European country that is situated on the western border of the Eurasian Steppe Belt. Overall, these studies confirm the high vulnerability of European agricultural regions to ongoing climate warming. Similarly, 32 to 39% of maize, rice, wheat and soybean yields are vulnerable to climate variability worldwide (Ray et al. 2015), which translates into large fluctuations in global crop production.

Direct and indirect effects of climate change (Wheeler and von Braun, 2013) associated with soil degradation/erosion (Pimentel et al., 1995), heatwave and drought (Ciais et al., 2005) and shrinking freshwater reserves (Tester & Langridge, 2010; Trnka et al., 2011) along with some other socio-economic changes such as decreasing fertilization in Europe (Peltonen-Sainio and Jauhiainen, 2014) are the main divers of the recent decreases in crop productivity recorded in some regions. This causes serious concerns about global food security (Godfray et al., 2010) due to increase in per capita food consumption, which is predicted to further increase by ca. 70% by 2050 (FAO, 2009; Mansharamani, 2015) by rapidly growing human population (Mitchell, 2008). Many regions worldwide are recognized being affected from more frequent, prolonged, and severe drought episodes (Stocker et al., 2013). The subsequent impact of transformation in climatic regimes on the biosphere is a long-term process, small data sets, based on reginal observations, allow only limited conclusions (IPCC, 2013). Hence, in-depth analyses of regional patterns of climate change would be required to complement usefully in global analyses for better understanding of geographical variation of all these factors and to improve our knowledge of plants' response to drought and other abiotic stresses associated with climate change. It is, indeed, also required for improved modelling of future plant distributions, to predict areas highly vulnerable for plant mortality and to plan ahead by selection of species and/or varieties to assist in breeding programs for agriculture as well as forestry sector.

Water transport in vascular plants (The cohesion-tension theory)

The ability of plants to transport water through xylem is one of the most important aspects in plant-water relations. Plant growth depends on a number of morphological and physiological traits as well as on environmental factors like precipitation, temperature and nutrient availability (Wright and Westoby 1999). Efficiency of nutrient uptake (Cornelissen et al. 1997) and water status (Major and Johnsen 1999; Hsiao and Xu 2000) are important physiological factors which are known to influence plant growth. During the process of photosynthesis 200 to 400 molecules of water are needed to fixate one molecule of CO_2. Generally about 90% of the water absorbed from soil is transmitted to the atmosphere during this process (Zimmermann, 1983; Kramer and Boyer, 1995; Cruziat et al., 2001; Chavarria and Pessoa Dos Santos, 2012).Trees are the unique organisms capable of transporting huge amounts of water from the soil to the leaves at the top of the crown covering long distances of more than 100 meters (Ryan et al., 2006). Water moves through the entire plant body and evaporates into the atmosphere reaching the surface of the leaf through microscopic pores called stomata, which also allow the entrance of carbonic gas required essentially for photosynthesis. The phenomenon is called transpiration. To replace the water lost by transpiration, continuous flow of water is necessary to insure the growth and survival of plants.

The cohesion-tension theory is generally accepted mechanism for long-distance passive water-transport (Dixon and Joly, 1894; Sperry et al., 1996; Wei et al., 1999; Tyree and Zimmermann, 2002; Angeles et al., 2004). Evaporation of water from surface of leaves, a process called transpiration, is the driving force for water transport. Water molecules stick together due to cohesive forces (hydrogen bonding) and are gradually pulled up in the form of water-column through the entire plant by the surface-tension of each air/sap meniscus in the leaves creating a water-potential gradient which becomes more negative following the soil-plant water continuum. A water potential is the potential energy of water per unit volume relative to pure water in a reference condition which is 0 MPa. Plant water potentials are negative (from 0 to -10 MPa), whereas water potential in the atmosphere is even more negative. This difference in water potentials creates a pressure gradient that drives the evaporation of water from leaves into the atmosphere (Zimmermann, 1983). Water in a plant is in a metastable thermodynamic state and remains in liquid phase even under negative pressure due to the substantial cohesive forces among the water molecules. Therefore, according to the cohesion-tension theory, as water evaporates via stomata, it decreases the leaf water potential, enabling water to move from stems to leaves thus lowering the water potential in stems and consequently causing water movement from roots to stems and from soil to roots.

Cavitation and embolism formation in Plants

The efficient water transport system enables plants to extract water from dry soils, and move it to long distances above the ground against the gravity pull, it depends entirely on maintaining an intact vascular network filled with water essentially in a metastable liquid state and hence, under a permanent risk of breakdown of column which disrupts the water flow (Tyree and Sperry, 1989). Indeed, during drought, the pressure becomes more negative and tension in the plant increases which means that air-bubbles can be drawn into conductive xylem elements from neighboring air-filled spaces or cells, according to the “air-seeding hypothesis” (Cochard et al., 2009), leading to partial blockage of water flow through the conduits, reducing the ability of plants to transport water from soil to leaf. This phenomenon, also called cavitation, has been described repeatedly over the last decades but clear understanding is still to be achieved (Schenk et al., 2015). Trees can protect themselves from this situation by reducing transpiration through closing their stomata and prevent the spread of embolism. However, during long and/or intense drought spells, stomatal control is not sufficient to regulate the gradient pressure and tension may reach such a highly negative value that the small initial gas-bubble spreads to fill the entire cell, which is then air-filled, or embolized, and therefore no longer remain functional, thereby reducing the plant’s overall capacity to transport water to its aerial organs (Sperry and Tyree, 1988; Cochard et al., 2006). The number of cavitation events increases with increasing evaporative demand at the leaf level which results in lowering (more negative) the soil water-potential, reducing the hydraulic conductance drastically (Sperry and Sullivan, 1992). Hydraulic failure is one of the major causes of plant mortality due to xylem embolism under severe drought events (Choat et al., 2012). A conclusive evidence of a direct relation between plant death and dramatic failure of water transport has been highlighted in conifers (Brodribb and Cochard, 2009; Brodribb et al., 2010) as well as in angiosperms (Barigah et al., 2013; Urli et al., 2013) demonstrating that the species tolerance to water stress and their ecological distribution depends, at least partly, on the efficiency of their hydraulic system and their ability to withstand embolism (Maherali et al., 2004; Cochard et al., 2008; Anderegg et al., 2015). In a recent meta-analysis on mortality rates across 475 species from 33 studies around the globe, it was found that species-specific mortality rates in a given drought were associated with plant hydraulic traits. It showed that across all species, including angiosperms and gymnosperms, P_{50} is the trait that better explained mortality in trees, supporting the hypothesis that hydraulic traits hold key mechanisms determining tree death and highlight that physiological traits can improve prediction of tree mortality during climatic extremes (Anderegg et al., 2016).

Resistance to embolism formation

Air leakage from embolized to functional conduits is the main reason of xylem cavitation due to the presence of tiny inter-conduit openings called pits (Crombie et al., 1985; Cochard et al., 1992; Jarbeau et al., 1995; Tyree and Zimmermann, 2002). It was thought that in both angiosperms and gymnosperms, when the pressure in the xylem becomes negative enough, capillary rupture of an air-sap meniscus in a pit pair (air-seeding) results in xylem cavitation, enabling air to spread from a non-functional (air-filled, embolized) conduit to an adjacent functional (sap filled) conduit (Cochard et al., 2009; Delzon et al., 2010). Porosity of the xylem conduit wall would therefore influence the resistance to cavitation. Thus, variations in xylem anatomical traits among species are related to cavitation resistance in angiosperms (Sperry and Hacke, 2004; Jansen et al., 2009; Lens et al., 2011) and conifers (Hacke et al., 2004; Domec et al., 2006; Delzon et al., 2010; Pittermann et al., 2010, Bouche et al. 2014). In angiosperms, a porous membrane of micro fibrils is located inside the pit chamber in inter-vessel pits. Embolism may spread by capillary rupture of an air-sap meniscus depending on the size of the largest pores in the pit membranes (Christman et al., 2009). However, it has been suggested by some authors that embolism formation might not be caused by immediate expansion of an air bubble as described by the air-seeding hypothesis due to the presence of nanobubbles in the sap of angiosperms (Schenk et al., 2015). Instead, these authors suggest that due to gas supersaturation of xylem sap or decreasing pressure, stable nanobubbles might become unstable and could lead to embolism formation. However, the presence of nanobubbles in conifer sap is not confirmed and so far, the air-seeding hypothesis related to xylem anatomy is still privileged. In bordered pit pairs of conifers, pit membranes are composed of two distinct regions: a torus located in the center serving as a valve to isolate the air-filled tracheids from the rest of the system (Bailey, 1916) and a porous margo region which surrounds the torus acting as a barrier to air bubbles and prevents the spread of embolism throughout the xylem. In a well-watered condition, the torus-margo structure is in a neutral position within the pit chamber and water flows through the large pores of the margo. Whereas under water stress conditions, the negative pressure in the xylem deflects the torus against the pit aperture. This valve effect creates a seal that prevents the spread of air into the xylem depending on the width of the torus relative to the pit aperture (Bailey, 1913; Hacke et al., 2004; Domec et al., 2006; Delzon et al., 2010). This seems to confer increased resistance to drought-induced cavitation in Gymnosperms, which are generally more resistant than Angiosperms (Maherali et al., 2004; Choat et al., 2012). Two hypotheses have been proposed for the spread of cavitation in this sophisticated “airtight” bordered pit: i) when the torus and the inner wall of the pit membrane are not completely sealed, air bubbles pass through pores at the edge of the torus (seal capillary seeding hypothesis, Cochard et al., 2009; Delzon et al., 2010; Pittermann et al., 2010) and ii) the torus structure is not fully airtight enabling the air bubble pass through tiny pores (torus capillary seeding hypothesis, Jansen et al., 2012). However, the later hypothesis was not confirmed by Bouche et al. (2014) who proposed that embolism was most likely to occur by seal capillary-seeding by overlapping the torus on pit aperture, whereas torus capillary-seeding could provide an additional mechanism in Pinaceae.

Assessment of xylem vulnerability to drought induced embolism

Cavitation is a fundamental phenomenon of plant-water relations and its multidimensional links with xylem anatomy and ecophysiological implications are important aspect to understand. Indeed, xylem vulnerability to cavitation is one of the major issue driving forest productivity and drought resistance in plants (Anderegg et al. 2012, Choat et al. 2012). With the increased understanding of plant water-transport, a variety of techniques have been developed to induce and measure the extent of cavitation in order to estimate xylem vulnerability to drought induced cavitation (VCs, Cochard et al., 2013).

Embolism induction:

Bench dehydration method is the most simple and natural way of inducing embolism slowly in a whole intact plant or in cut branches by letting them dehydrate *in situ* or on a bench under ambient light conditions. Xylem pressure is assessed with a pressure chamber on non-transpiring covered leaves or with stem psychrometers (Tyree et al., 1992; Bréda et al., 1993).

Air pressurization method induces cavitation by forcing the air into a xylem segment inserted into a standard Scholander pressure chamber or a double ended pressure chamber, which allows the increase of air pressure, while the water flow through the sample is also measured at the same time (Crombie et al., 1985b; Sperry and Tyree, 1990; Cochard et al., 1992; Choat et al., 2005).

Centrifugation method exposes xylem to highly negative pressure by applying centrifugal forces (Pockman et al., 1995). The principle is to reduce xylem pressure in the middle part of a short branch segment by spinning it in a centrifuge.

Embolism Measurement:

1. ***Acoustic emissions*** (AEs), from audible “clicks” to ultrasound emissions, produced during drought stress are recorded automatically by a sensor clamped to a branch, leaf or trunk, even under field conditions, while the plant is subjected to natural or induced water stress (Tyree et al., 1984; Tyree and Sperry, 1989a).

2. ***Observations of xylem water content*** can be made with different methodologies to make direct or indirect observations of xylem water content such as magnetic resonance imaging technology (Holbrook et al., 2001) or cryo-scanning electron microscopy (Canny, 1997; Cochard et al., 2004). Another appropriate method for visualizing water or air in xylem conduits with relatively high spatial resolution is the high-resolution X-ray computed tomography (Fromm et al., 2001; Brodersen et al., 2012; Torres-Ruiz et al., 2015). However, this method is yet not being used for the routine study of xylem embolism in plants.

3. ***Hydraulic detection of embolism*** involve directly monitoring decrease in water-flow as stress is imposed to a sample to induce negative pressure in the xylem analogous to that experienced by the plant during drought. Decrease in xylem hydraulic efficiency (specific conductance, k_S) caused by cavitation events is measured. The degree of embolism in a xylem segment can be quantified as the percentage loss of hydraulic conductance (PLC), which can be determined gravimetrically (Sperry et al. 1988) or with a Xylem apparatus. The principle of these techniques is to measure the relative decrease in the efficiency of xylem to transport water caused by the presence of air bubbles which considerably increase the resistance to water flow through the conduits.

Cavitron technique is the *in situ* flow centrifuge technique described by Cochard, (2002a) and Cochard et al. (2005) that enables the construction of a vulnerability curve in a very short time of less than half an hour. Samples are placed in a custom-built rotor with both ends in reservoirs filled with water. Small holes are made in the reservoirs at two distances from the rotation axis in order to generate a pressure gradient (ΔP, MPa) across the sample and to induce water flow through the sample. The hydraulic conductivity can then be determined directly during centrifugation and an entire vulnerability curve is constructed, thus allowing large scale investigations of interspecific, intraspecific, genetic and phenotypic variability of cavitation resistance in Gymnosperm as well as in Angiosperms (Delzon et al., 2010; Lamy et al., 2011; Wortemann et al., 2011).

By visualizing the percent loss of xylem conductance (PLC) as a function of xylem pressure (MPa), one obtains a vulnerability curve (VC, generally a sigmoid), from which the cavitation resistance of a sample is estimated, notably from the inflexion point, i.e., the pressure at which 50% of total xylem hydraulic conductance is lost (P_{50}, in Mega Pascal), and the slope of the curve (S, in % MPa^{-1}). A sigmoid function (Pammenter and Vander Willigen 1998) is fitted to the VC using the following equation:

$$PLC = \frac{100}{\left[1 + exp\left(\frac{S}{25} \times (\Psi - P_{50})\right)\right]}$$

where P_{50} (MPa) is the xylem pressure inducing 50% loss of conductance and S (% MPa^{-1}) is the slope of the vulnerability curve at the inflexion point (Figure 1).

Although there is general agreement for the use of centrifuge technique to generate the vulnerability curves for short conduits bearing species (e.g. conifers), the application of both the static and *in the situ* flow centrifuge methods for long vessel bearing species remains a subject of debate, as they are potentially prone to artefact (Torres-Ruiz et al., 2014, 2017; Hacke et al., 2015, Choat et al., 2016). The high losses of hydraulic conductivity (K_s) induced by the centrifuge technique at relatively mild xylem pressures for

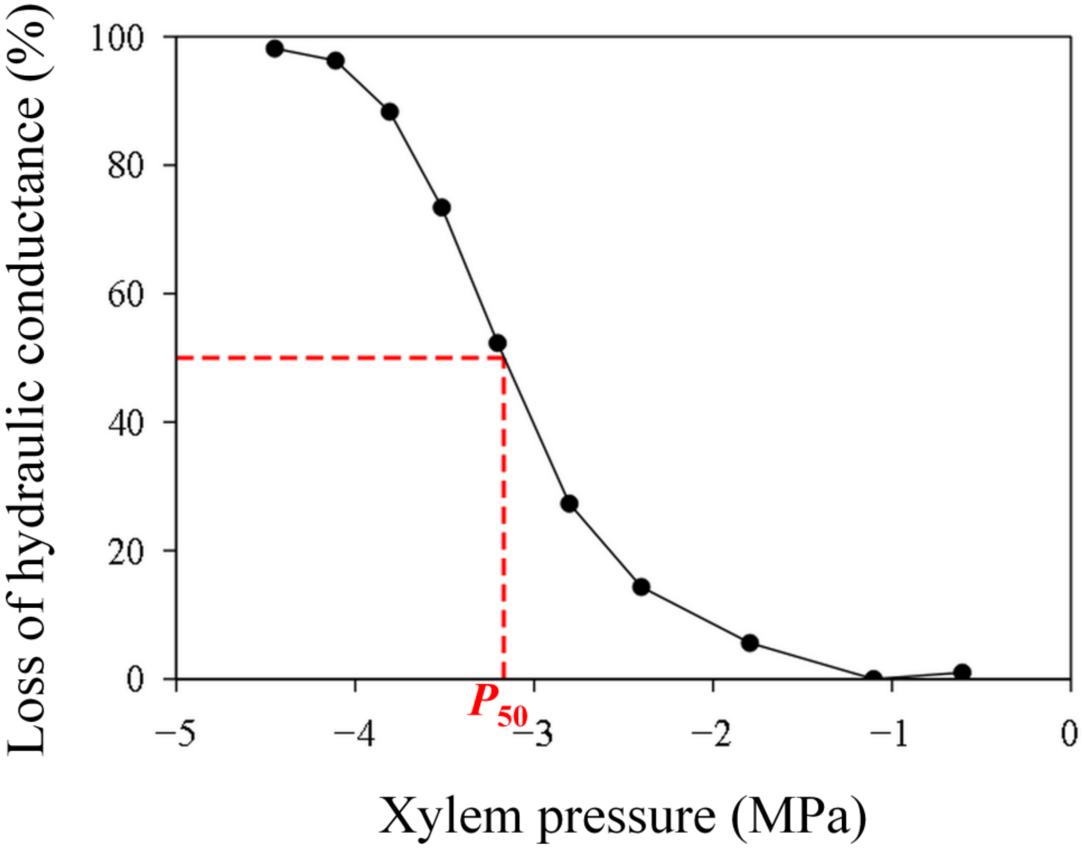

Figure: 1 Example of *Pinus pinaster* vulnerability curve carried out with the cavitron technique showing the percentage loss of xylem conductance as a function of xylem pressure (MPa). The main hydraulic parameter P_{50} (dashed lines) fitted using the Pammenter and Vander Willingen model (1998).

species with long vessels results in the production of exponential vulnerability curves (the so-called 'r-shaped' curves). It is considered that these curves are methodological artefact resulting due to the presence of open vessels, i.e. vessels having no end within the stem segment (Choat et al., 2010; Cochard et al., 2013; Wheeler et al., 2013; Torres-Ruiz et al., 2014; Rockwell et al., 2014; Pivovaroff et al., 2016). However, these curves have been taken by some other authors to be a reliable estimate of embolism resistance in these species, i.e. the significant embolism within moderate ranges of water deficit (Sperry et al., 2012; Hacke et al., 2015). These contradictive views generate a confusion reaching conclusive results in the field of plant hydraulics and impedes the progress in understanding the role of hydraulics in plant function (Delzon & Cochard, 2014). A lot of work has been done to clarify whether there is an open-vessel artefact in using the standard centrifuge technique, but even working with the same plant species, contradictions in results are still there, For example, olive (*Olea europaea*) has been observed to produce contrasting vulnerability curves and resistances to embolism according to the methods being used for assessing of the degree of embolism. Although it has been observed with high-resolution computed tomography that olive stems are not embolized until a certain threshold in xylem pressure is reached (approximately -3.0 MPa) and that vulnerability curves are sigmoidal (s-shaped curve) for this species (Torres- Ruiz et al., 2014), significant decreases in K_s at relatively high xylem water potentials have been reported by (Hacke et al., 2015), suggesting high embolism vulnerability with r-shaped curve in this species. In a recent study Torres-Ruiz et al. (2017) demonstrated the unreliability of the standard centrifuge technique for the measurement of resistance to embolism in xylem samples with some proportion of open vessels, regardless of the rotor design used. They observed significant decrease in hydraulic conductance at a less negative xylem pressure than the minimum pressure the plant material had experienced previously, indicating that embolism resistance in long vessels bearing speciss, is not as low as previously suggested.

Interspecific variability in embolism resistance

During water stress, hydraulic failure in trees has been reported to be one of the primary causes of forest decline (Anderegg et al., 2015, 2016). The negative pressure inside the water conducting cells get intensified due to drought, making the liquid xylem sap more vulnerable to air-entry and hence embolized (Lens et al., 2013). High levels of embolisms may ultimately lead to desiccation and plant death (Barigah et al., 2013; Urli et al., 2013). Plant resistance to embolism is, therefore, considered a key-parameter in determining the drought tolerance of plants and is estimated on the basis of so-called vulnerability curves (VCs), from which the P_{50} (i.e. the xylem pressure causing 50% loss of hydraulic conductivity) can be estimated (Cochard et al., 2013). P_{50} values are, therefore, good proxies for drought stress tolerance in plants which varies widely among species (Maherali et al., 2004) and have been reported for hundreds of angiosperms and gymnosperms (Delzon et al., 2010; Choat et al., 2012), with vulnerable species rapidly reducing hydraulic conductance at moderate negative pressure (-2.0 to -0.5 MPa), whereas some species can resist high xylem negative pressure (< -19 MPa) before embolism starts to occur (Larter et al., 2015). This shows a huge range of P_{50} at interspecific level. However, a global convergence in tree vulnerability to drought across ecosystems was found in a study comparing the severity of drought experienced by trees (i.e. the lowest water-potentials observed seasonally) to levels of drought inducing death by hydraulic failure (Choat et al., 2012), indicating that wet and dry forest are equally prone to lethal levels of drought due to limited hydraulic safety margin. Martin-StPaul et al. (2017) reported the existence of an absolute limit by which stomata close to avoid rapid death under drought events. They showed that the water potential triggering stomatal closure and the xylem pressure causing embolism formation were equal for only a small number of species, and the difference between these two traits increased continuously with increasing embolism resistance, indicating the absence of functional coordination between stomatal closure and the onset of embolism formation.

Previous work on hydraulic traits highlights considerable variation in resistance to cavitation among coniferous species from contrasting environments, which seems to be linked to their ecology, with species from xeric regions being more resistant than those from mesic areas (Brodribb and Hill, 1999; Maherali et al., 2004; Choat et al., 2005, 2012; Vinya et al., 2013). It shows that the evolution of cavitation resistance in conifers enables them to adapt in arid environmental conditions. However, little is known about the relationship between cavitation resistance and xylem anatomy in conifers. It could be suggested that the variability of cavitation resistance and species distribution may be explained on the basis of structure of bordered pits.

In recent studies it has been revealed that cavitation events occur at air–sap interfaces within pores between embolized and functional conduits (Choat et al., 2008; Lens et al., 2013). A valve-like structure called torus, present in bordered pits of conifers, is deflected to block the pit aperture, protecting functional tracheids from air entry (Bailey, 1916). Though it provides conifers with increased protection from embolism compared to angiosperms (Maherali et al., 2004; Pittermann et al., 2005; Choat et al., 2012), embolism still occurs due to incomplete sealing of the pit aperture by the torus (Bouche et al., 2014). Embolism resistance is required to balance (xylem safety) with optimizing rates of water transport (xylem efficiency) to the photosynthetic organs of the plants (Tyree & Zimmermann, 2002). In competitive settings, the xylem safety gains are obvious (i.e. increased survival during drought), whereas increased carbon allocation to leaves relative to sapwood area is allowed by xylem efficiency, resulting in rapid growth and optimum photosynthetic rate (Santiago et al., 2004; Poorter et al., 2010). In drought-tolerant species, the efficiency of wider conductive elements to transport water but their weakness under the mechanical stress imposed by negative xylem pressure, creates an indirect link between safety and efficiency (Sperry et al., 2008). Pit morphology, notably the pit membrane, is influenced by thicker cell walls, thereby increasing xylem embolism resistance (Tyree & Sperry, 1989b; Li et al., 2016). Species ecology and phylogeny strongly determine the final compromise between safety and efficiency. For example, species growing in comparatively wetter environments are more likely to maximize efficiency over safety (Sperry et al., 2006; Choat et al., 2012). At a higher ecological and taxonomic scale, only a few or no evidence is available of a universal and strong relationship between safety and efficiency (Gleason et al., 2016).

In the context of evolutionary biogeography of conifers, pit structure in 15 species of Cupressaceae revealed the hydraulic tradeoffs associated with cavitation resistance at the pit level, and the way they vary within the structural components of the inter tracheid pit. Cavitation resistance was found strongly correlated with the ratio of the torus to pit aperture diameter but did not vary systematically with the porosity of margo. Moreover, it was found that pit conductivity was restricted by pit aperture in more drought-resistant taxa, whereas in species that are more vulnerable to cavitation, pit conductivity was more likely to be regulated by increased margo resistance (Pittermann et al., 2010). The morphological and physiological differences between modern-day members of the Cupressaceae brought by Cenozoic climatic change described by Pittermann et al. (2012) showed that drought-resistant crown clades - the Cupressoid and Callitroid clades - most likely evolved from drought-intolerant Mesozoic ancestors, and that the pattern was consistent with the shifts in post-Eocene paleoclimates. Analyses based on phylogenetic methods suggest that the ancestors of the Cupressaceae conifers were dependent upon humid habitats and the current drought-resistant physiology evolved along with increasing habitat aridity from the Oligocene onward, largely by their capacity to adapt to drought. It was evidenced that within the Cupressaceae, the evolution of drought-resistant xylem was coupled with increased carbon allocation in xylem conduit walls and reduced xylem transport efficiency, and at leaf level, with reduced photosynthetic capacity (Pittermann et al., 2012).

In a recent study based on a time-calibrated phylogeny, Larter et al. (2017) found a wide range in embolism resistance across the *Callitris* clade (P_{50}: -3.8 to 18.8 MPa) which was significantly correlated with water scarcity and tracheid diameter. It was further evidenced that the evolution of extreme xylem embolism resistance and diversification into xeric environments accompanied the radiation of this clade and was driven by increasing aridity of the Australian continent during the last 30 million years.

Intraspecific variability in embolism resistance

While the interspecific differences in functional traits have been investigated by a vast majority of research, variation at intraspecific level is less understood, particularly for hydraulic traits, and is important for understanding and modeling the impacts of climate change on vegetation. The degree of variation of intraspecific traits has major consequences for species sensitivity to climatic shifts and required migration rates to track these shifts. Hydraulic characteristics variability within a single species could have a major impact on population demographics and responses to extreme climatic changes, such as ability of a population to withstand a drought, as well as spatial patterns and thresholds in drought-induced mortality (Martınez-Vilalta et al., 2009; L. D. L. Anderegg et al., 2013).

Intraspecific studies showed no variability of cavitation resistance in branches among populations belonging to different environments, arguing that within a species, this trait is highly confined to the branch level (Sáenz-Romero et al., 2013; Anderegg et al., 2014; Lamy et al., 2014). A comparative study of vulnerability to cavitation between the different organs within a single plant, has been studied to understand drought resistance at the whole-plant level (Bouche et al., 2015). Stomatal conductance and photosynthesis essentially depend on efficient water transport from roots to the leaves. Thus, the way different plant organs cope with cavitation risks has an impact on plant growth and survival. The hydraulic segmentation hypothesis was first proposed by Zimmermann, (1983) suggesting that distal plant organs would be more subject to cavitation events because of the decline in water potential from proximal to distal organs. Later on, this hypothesis was slightly modified by Tyree and Ewers (1991) showing that distal tissues were more vulnerable to cavitation than proximal tissues to prevent cavitation events in the main stem axis. However, significant variability in resistance to cavitation has been obtained between intra-plant studies for a given species and organs (Sperry and Ikeda, 1997; Martínez-Vilalta et al., 2002;

Cochard et al., 2004; Domec et al., 2006; Dalla-Salda et al., 2009; Charra-Vaskou et al., 2012; McCulloh et al., 2014), which might be linked to the different methods applied to measure cavitation resistance across organs.

Cavitation resistance at intra-specific level shows a lack of scaling with environment even for species distributed very widely. For example, climatic aridity had no significant effect on P_{50} whereas other traits such as branch leaf-to-sapwood area ratio were plastic over the wide range of scots pine (*P. sylvestris*) (Martínez-Vilalta et al., 2009). Similarly, Lamy et al., (2011) found less genetic differentiation for cavitation resistance (Qst) than expected under genetic drift (neutral markers, Fst), while comparing populations from whole climatic range of maritime pine (*P. pinaster*), with the authors concluding that the trait is likely canalized, suggesting genetic constraints on cavitation resistance variability in this species.

Variation in xylem resistance to embolism (P_{50}) and xylem specific hydraulic conductivity (K_s) among *P. pinaster* populations and families in another study (Corcuera et al., 2011) were found to be mainly controlled by phenotypic plasticity, while wood density was found to be controlled genetically and was not under the influence of environment. Rosner *et al.* (2017) used wood density as a functional trait to highlight its variability within a conifer species, Norway spruce (*Picea abies*) and found that P_{50} of trunk wood of different cambial age from mature and young trees was strongly related to wood density.

There is scarcity of literature on genetic determinism of cavitation resistance, although studies on some species such as Arabidopsis (Tixier et al., 2013), and search for QTLs (Quantative Trait Loci – Lamy, 2012) are being pursued to follow in the future. As cavitation resistance is related to the xylem anatomy, especially at the inter-tracheid pit level, genetic architecture of cell and cell-wall development is probably linked to it, and is, therefore, likely under strong genetic regulation, consequently growth, water-transport efficiency and mechanical support may be affected by random changes. Same gene could be implicated in determining P_{50} and other traits (pleiotropy), and a gene cluster could also interact to establish the same phenotype (polygenic trait) - such complex genetic networks necessarily limit the impact of a single mutation on a single gene, therefore reducing the probability of variation on which natural (and artificial) selection can work. However, it is important to mention that recent results from a widespread angiosperm species, *Fagus sylvatica*, are opposite to this trend (Schuldt et al., 2015), showing within-species variation for P_{50} is higher in Angiosperms than in Gymnosperms (Anderegg, 2015).

Intraspecific variability in embolism resistance in crops

Vulnerability of woody species (Angiosperm and gymnosperm) to embolism have been investigated in many studies, but there are only a few studies focusing on crop xylem hydraulics (Sperry et al., 2003). Lens et al. (2016) recently studied hydraulic failure in 26 species of herbaceous angiosperms (mainly European grasses, Poaceae)) and found a wide range in P_{50} (the xylem pressure inducing a 50% loss of hydraulic conductivity) from -0.5 to -7.5 MPa, showing that they were generally more resistant to embolism than previously assumed. It was further revealed that embolism formation were not routinely experienced by herb as they had the ability to withstand high negative water potentials without embolism formation during drought stress, but the intraspecific variability of embolism resistance is yet to be investigated in herbs.

The limited number of studies have been performed on the relation between hydraulic failure and the agronomic performance of different crops such as sugarcane (Neufeld et al., 1992), maize (Tyree et al., 1986; Cochard, 2002b; Li et al., 2009), soybean (Sperry, 2000), rice (Stiller et al., 2003) and sunflower (Stiller and Sperry, 2002; Trifilo et al., 2003a, 2003b), but there is lack of information on intraspecific variability in embolism resistance and other hydraulic traits. In the face of enhanced drought risk and increasing drought-affected area globally with the rising global temperature associated with Climatic variability (IPCC, 2014), negative yield trends of important cereal crops, even in high-technology, high-yielding areas across the world, have been recorded (Kang et al., 2009; Ray et al., 2012; van Wart et al., 2013; Martín-Retortillo & Pinilla, 2015). Knowledge of the role of embolism formation in crops and its variability at intraspecific level is, thus essential, if we are to understand the susceptibility of the water transport pathway of crop plants to drought and to cope with the threats posed by changing climatic conditions, which may be crucial for selecting drought resistant, high yielding genotypes to meet the future food demands of increasing population.

Objectives of the thesis

The main objective of this thesis was to investigate the intraspecific variability in embolism resistance and other hydraulic traits in combination with xylem anatomy, and genetics. The work was categorized in two parts. The first part deals with intraspecific variability in hydraulic traits in sunflower, which is an important oilseed crop in both Europe and South Asia. In Pakistan, the local production of edible oil meets only 32% of the country's domestic requirement while the remaining 68% demand is met through import involving huge expenditure, which accounts for almost 10% of the country's total import bill (Hussain, 2000; Joyo et al., 2016). 75 to 87% area of Pakistan already lies in extremely arid to semi-arid zones and only less than 10% is in humid regions, which are vulnerable to drought under the current climate change scenario, showing a significant increasing trend in maximum annual temperature by 0.014 °C $year^{-1}$ and 0.018 °C $year^{-1}$ in extremely arid and humid regions, respectively (Haider and Adnan, 2014; Adnan et al., 2017). Recent studies show that the sunflower seed yield could reduce by up to 15% by the 2020s and may further decrease up to 25% by the 2050s (Nasim et al., 2016). Characterization of the hydraulic traits of sunflower is, therefore, of utmost important for agricultural scientists, agronomists and hydrologists to plan the growing and breeding programs of the crop in accordance with the regional climate so that varieties/accessions with improved drought tolerance may be selected. The second part aims at finding the intraspecific variability in hydraulic traits and their link with genetics and other physiological traits in Maritime pine, which is the biggest coniferous forest species in Europe and presents a high ecological and economic importance in the Western Mediterranean basin. In France this species covers 6% of the total forest land (88% of the private forest area; CNPPF-IDF, 2015), 5% of the standing volume and more than 26% of the annual wood production (IGN 2016). In Southwestern France, forest productivities are known to be more negatively affected than in Northern France, in response to increase in air and soil drought frequency and intensity because of a more pronounced shift in seasonal rainfall from summer to winter (Loustau et al., 2005). It is, therefore, important to investigate the genetic basis of hydraulic traits and the correlations between key traits that determines the survival of maritime pine during severe periods of drought if such characteristics are to be incorporated into breeding programs.

Part I: Intraspecific variability in embolism resistance in sunflower crop

The main goals of this study were to investigate the embolism resistance of sunflower (*Helianthus annuus* L.), its intraspecific variability among accessions, and its relationships with xylem anatomy. This provided new insights into our understanding of the mechanism of embolism resistance in sunflower and highlighted the potential trade-offs resulting from an increase in embolism resistance. To properly define a hydraulic strategy, it is essential to account both for the growth/drought resistance trade-off and the hydraulic safety/efficiency trade-off in xylem conduits.

Chapter 1: Intraspecific variation in embolism resistance and stem anatomy across four sunflower (*Helianthus annuus* L.) accessions. (*Accepted in Physiologia Plantarum*)

The major question to be answered was how much sunflower (*Helianthus annuus* L.) accessions, growing under the same environmental conditions, vary in their ability to resist drought-induced embolism? Four sunflower accessions were investigated to access the vulnerability to embolism using the *in situ* flow centrifuge method for the first time, using a 42 cm rotor to prevent open-vessel artifacts. The potential trade-off between growth traits and embolism resistance and the relationship between embolism resistance and xylem anatomical traits was also determined.

Part II: Intraspecific variability in embolism resistance and its relation with genetic architecture and photosynthetic traits in Maritime pine.

Maintaining the productivity under reduced water availability and survival under severe drought and/or heatwaves are two major challenges, regarding the tree adaptation to water deficit in the face of climate change. Maritime pine (*Pinus pinaster* Ait.) is known to have low intra-specific variability both between *in situ* natural populations and provenances growing in common gardens (Lamy et al. 2011, 2014) but provenance hybrids, that would have increased variability, have never been tested so far (see Plomion et al., 2016 for a review). In view of recent predictions of future climatic scenarios (IPCC, 2014), the results obtained will make it possible to estimate basic genetic parameters for embolism resistance and to evaluate breeding opportunities.

Chapter 2: Dissecting the genetic architecture of resistance in Maritime pine reveals that hydraulic safety is not linked to wood density. (*in preparation*)

Due to the inaccessibility of high throughput phenotyping techniques for large populations until recently, insights into the genetic architecture, i.e., the number of chromosomes, chromosomal location and effect of quantitative trait loci (QTL) on vulnerability to embolism and xylem hydraulic conductivity are still less known. In the context of improving our understanding and in continuation of work already done on the genetic determinisms and evolutionary history of embolism resistance in Maritime pine, this study aimed at finding the relationship between embolism related traits (hydraulic safety) and wood density related traits by dissecting the genetic architecture of this species. To improve our understanding of the maintenance and evolution of genetic variation in the wild, recent studies showed that evolutionary forces have shaped phenotypic variability for these traits towards a low variability between natural populations. We used hybrids (F2 genotypes) that display more variations as they are potential catalysts of phenotypic evolution and hence diversification both in plants and animals. Moreover, hybridization can be used for transgressive segregation to develop qualitatively or quantitatively novel phenotypes in addition to get specific adaptive traits through lateral gene transfer and introgression.

Chapter 3: Intraspecific variability of the physiological response to water stress in a full-sib Maritime pine family. (*in preparation*)

The objective of this study was to investigate the intra-specific variability within a Maritime pine tree family in response to water availability by performing a multi-trait analyses of leaf and xylem functioning through seasonal measurements of photosystem II fluorescence, gas exchange and stem hydraulic properties. This will be the first step towards selecting the genotypes having the ability to sustain changes in water availability by resisting to drought, while maintaining the same growth rates during pre and post water stress phases.

Part I: Intraspecific variability in embolism resistance in sunflower crop

Chapter 1: Intraspecific variation in embolism resistance and stem anatomy across four sunflower (*Helianthus annuus* L.) accessions. (*Accepted in Physiologia Plantarum*)

Intraspecific variation in embolism resistance and stem anatomy across four sunflower (*Helianthus annuus* L.) accessions

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Abstract

Drought-induced xylem embolism is a key process closely related to plant mortality during extreme drought events. However, this process has been little investigated in crop species to date, despite the observed decline of crop productivity under extreme drought conditions. Interspecific variation in hydraulic traits has frequently been reported, but less is known about intraspecific variation in crops. We assessed the intraspecific variability of embolism resistance in four sunflower (*Helianthus annuus* L) accessions grown in well-watered conditions. Vulnerability to embolism was determined by the *in situ* flow centrifuge method (cavitron), and possible trade-offs between xylem safety, xylem efficiency and growth were assessed. The relationship between stem anatomy and hydraulic traits was also investigated. Mean P_{50} was -3 MPa, but considerable variation was observed between accessions, with values ranging between -2.67 and -3.22 MPa. Embolism resistance was negatively related to growth and positively related to xylem-specific hydraulic conductivity. There is, therefore, a trade-off between hydraulic safety and growth but not between hydraulic safety and efficiency. Finally, we found that a few anatomical traits, such as vessel density and the area of the vessel lumen relative to that of the secondary xylem, were related to embolism resistance, whereas stem tissue lignification was not. Further investigations are now required to investigate the link between the observed variability of embolism resistance and yield, to facilitate the identification of breeding strategies to improve yields in an increasingly arid world.

Abbreviations

PLC, percentage loss of hydraulic conductance; VCs, vulnerability curves.

Introduction

Crop productivity and biodiversity are currently undergoing major transformations due to climate change, with increases in global temperature and atmospheric CO_2 concentration and changes in land use (Vitousek et al. 1997, Parmesan and Yohe 2003). Global surface temperature increased by about 0.8°C from 1861 to 2005, and further increases are predicted for the future (IPCC 2007, Dai 2013). The predicted increases in temperature and the prevalence of extreme climatic events (Sterl et al. 2008, Wigley 2009) have been accompanied by increases in precipitation during the winter and fall and decreases in precipitation during the summer, resulting in an intensification of the summer drought period in Western Europe (Beniston et al. 2007, van Oldenborgh et al. 2009). These changes will undoubtedly have a major impact on crop production (Boyer 1982, Hussain et al. 2012), with adverse effects on all stages of plant growth and development (Jensen and Mogenson 1984), particularly for summer crops, such as maize, soybean, sugar beet and sunflower. Ciais *et al*. (2005) reported a pronounced decrease in net primary productivity (NPP) for maize (*Zea mays* L.) in agricultural regions of Europe affected by the 2003 heatwave and drought, with a record decrease of 36% in Italy.

World food security is mostly under threat from drought (Somerville and Briscoe 2001, Farooq et al. 2009), which affects crop plants immediately after their germination, resulting in poor seedling establishment (Harris et al. 2002, Kaya et al. 2006) and, ultimately, low yields (Jaleel et al. 2007), due to low rates of absorption of photosynthetically active radiation, poor radiation-use efficiency, and a low harvest index (Earl and Davis 2003). Crop sensitivity to water deficit varies with phenological stage and is, thus, crop-dependent. For example, water deficit has been shown to affect vegetative growth in soybean, flowering and boll formation in cotton and the vegetative and yield formation stages of sunflower and sugar beet (Kirda 2002). Critically low levels of vegetative growth and poor plant development due to water deficit have also been reported in rice (Tripathy et al. 2000, Manickavelu et al. 2006). Many studies have demonstrated effects of drought on crop phenology and gas exchanges, but little is known about the breakdown of the water transport due to embolism under drought conditions, even though this process has been shown to be the major cause of drought-related death in perennial plants (Urli et al. 2013, Anderegg et al. 2015).

The water transport system of plants consists of a complicated network of xylem conduits through which water moves under negative pressure (tension), due to the pull of transpiration at the leaves, transmitted via a continuous column of water down to the roots, where the resulting decrease in pressure leads to the absorption of water from the soil (Tyree & Zimmermann 2002, Wheeler & Stroock 2008, Trifilo et al. 2014). Water columns in plants must consist entirely of liquid and be free of air bubbles, despite being under negative pressure. This metastable liquid state is prone to cavitation, a phase change from liquid water to water vapor, resulting in the formation of gas bubbles (air embolism) that prevent water from flowing through xylem conduits, thereby reducing the hydraulic conductivity of the xylem from the soil to the foliage, where water is required for the maintenance of optimal cell hydration levels (Tyree and Sperry 1989, Salleo et al. 2000, Cochard 2006). During prolonged and severe droughts, the tension of the xylem sap increases the probability of embolism formation in the xylem, reaching potentially lethal levels causing desiccation and mortality (Davis et al. 2002, Brodribb and Cochard 2009, Hoffmann et al. 2011, Choat 2013, Urli et al. 2013).

Water stress-induced xylem embolism is one of the major causes of plant mortality during extreme drought events (Anderegg et al. 2015). Many studies have focused on the vulnerability of woody species to embolism, but there have been far fewer investigations of crop xylem hydraulics (Sperry et al. 2003). Nevertheless, the limited number of studies performed to date on sunflower (Stiller and Sperry 2002), rice (Stiller et al. 2003), maize (Tyree et al. 1986, Cochard 2002a, Li et al. 2009), sugarcane (Neufeld et al. 1992) and soybean (Sperry 2000) have indicated that hydraulic failure also has a major effect on crops, within the physiological range of xylem pressure. Knowledge of the role of embolism formation in crops is thus essential if we are to understand the susceptibility of the water transport pathway of plants to drought.

There have been few assessments of vulnerability to embolism in herbaceous plants (Lens et al. 2016). Lens et al. (2016) showed that herbaceous plants are generally more resistant to embolism than previously thought and that they do not routinely experience xylem embolism. Major differences in embolism resistance between herbaceous species have been reported, based on estimates from vulnerability curves (VCs), on which P_{50} — the xylem pressure inducing a 50% loss of hydraulic conductivity — ranged from -0.5 to -7.5 MPa, but nothing is known about the intraspecific variability of embolism resistance.

We focus here on sunflower (*Helianthus annuus*) a major high-yield oilseed crop (Lawal et al. 2011, Bera et al. 2014) cultivated over a broad geographical area with diverse environmental conditions worldwide (Liu and Baird 2003, Lopez-Valdez et al. 2011). Sunflower is generally considered to be a drought-resistant crop (Unger 1990, Connor and Hall 1997). However, it has been reported to be affected by extreme and frequent periods of water stress in southern Europe, where it is cultivated in low-rainfall areas in which the soil is shallow and irrigation facilities are not available (Casadebaig et al. 2008). Water deficit affecting the vegetative and flowering stages strongly has been reported to cause a 29% decrease in yield (Velue and Palanisami 2001). The susceptibility of sunflower-producing areas to drought may increase considerably in the near future, if the predicted climate changes occur (Dufresne et al. 2006).

The objective of this study was to investigate the variability of embolism resistance between sunflower (*Helianthus annuus*) accessions grown in the same environmental conditions. We assessed vulnerability to embolism in four sunflower accessions by the *in situ* flow centrifuge method, using a 42 cm rotor to prevent open-vessel artifacts (Cochard et al. 2013, Pivovaroff et al. 2016). We then investigated (i) the potential trade-off between growth traits and embolism resistance and (ii) the link between embolism resistance and xylem anatomical traits.

Materials and methods

Plant material and experimental design

The study was performed on four sunflower (*Helianthus annuus*) accessions: a rustic accession capable of growing on shallow soil (Melody), two early-sown accessions (ES_Biba and ES_Ethic) and one late-sown accession (LG_5660). Seeds for all four accessions were provided by free of charge by the *Centre Technique Interprofessionnel des Oléagineux Métropolitains* (CETIOM), France. Plants were grown in a randomized complete block design (RCBD) with four blocks, including six plants per accession per block, giving a total of 96 plants ((6*4)*4). Seeds were sown on March 14, 2014, in pots filled with Peltracom substrate containing peat, clay, plant fibers, vulcanic stones, sand and compost, with 14-16-18 kg m^{-3} NPK fertilizer. The pots were placed in a greenhouse under full light, in non-limiting growth conditions, at the University of Bordeaux, France. Three seeds were initially sown in each pot at the start of the experiment. After germination (about 10 to 12 days after sowing), when the seedlings had reached a height of about 10 to 15 cm, we removed two of the seedlings, leaving only one healthy seedling per pot. An automatic irrigation system fitted with an electronic water timer (Dual logic, CLABER, Italy) was used to irrigate the pots. It was set to irrigate all the pots simultaneously, for 10 minutes every 12 hours, to ensure that all the plants remained well-watered and free of water stress and embolism throughout the growing period.

Sample preparation for the assessment of embolism resistance

Plants were harvested about 12-15 weeks after sowing (i.e. between June 19 and July 9, 2014), when they had reached a mean height of 0.85 m. Plant height (H, m) and stem diameter (D mm) were measured with a tape measure and an electronic Vernier scale, respectively, and the plants were then cut for hydraulic measurements. Five to seven plants with intact flowers from the different accessions were selected at random each day and cut 1 cm above the soil at 8:00 am. All the leaves were removed from the plants just before cutting, to reduce loss of water due to transpiration. The plants were immediately wrapped in wet cotton cloth and placed in a plastic bag to minimize dehydration during their transport to the laboratory. They were then cut, under water, to a standard length of 42 cm, and both ends were trimmed with a fresh razor blade. On average, we were able to take measurements for 16 stems per accession by the end of the experiment.

Xylem vulnerability curves

Xylem vulnerability to embolism was assessed with the *in situ* flow-centrifuge technique (Cavitron), in which the percentage loss of hydraulic conductivity relative to xylem under negative pressure is determined (Cochard 2002b, Cochard et al. 2005). All measurements were performed at the high-throughput phenotyping platform for hydraulic traits (CaviPlace, University of Bordeaux, Talence, France). Centrifugal force was used to establish negative pressure in the xylem and to provoke water stress-induced cavitation, in a 42 cm-wide custom-built honeycomb aluminum rotor (DGmeca, Gradignan, France) mounted on a temperature-controlled high-speed centrifuge (J6-MI, Beckman Coulter, USA). The ends of the sample were placed in 25 mm OD polycarbonate centrifuge tubes (38 mL, Beckman Coulter, USA) with holes located 42 and 14 mm from the extremities for the upstream and downstream reservoirs, respectively. Samples were then secured in a slit across the center of the rotor, with the lid screwed down tightly to hold the sample in place. A solution of 10 mM KCl and 1 mM $CaCl_2$ in ultrapure deionized water was used as the reference ionic solution. The rotor was first spun at low xylem pressure (P_x = -0.8 MPa), corresponding to a rotation speed of 2087 rpm (revolutions per minute). The rotation speed of the centrifuge was then gradually increased by -0.3 or -0.5 MPa, to expose samples to lower xylem pressures. Rotor velocity was monitored with a 10 rpm-resolution electronic tachymeter (A2108-LSR 232, Compact Inst, Bolton, UK) and xylem pressure was adjusted to about ± 0.02 MPa. Hydraulic conductances (K_i, m^2 MPa^{-1} s^{-1}) were determined at every rotation by measuring the displacement speed of the air-water meniscus from the upstream to the downstream extremity of the sample, according to the equations of Wang et al. (2014). These measurements were performed with a calibrated CCD camera (Scout sca640, Basler, Germany) coupled to custom-written software (Cavisoft version 4.0, BIOGECO, University of Bordeaux). Hydraulic conductances were measured three times per rotation. The mean values were used to determine the percentage loss of hydraulic conductance (PLC) at each pressure, as follows:

$$PLC = 100\left(1 - \frac{K_i}{K_{max}}\right)$$

, where K_{max} is the maximum hydraulic conductance measured at low speed, i.e. at very high xylem pressure. Vulnerability curves (VCs), corresponding to the percentage loss of xylem conductance as a function of xylem pressure (MPa), were determined for each sample as follows (Pammenter and Vander Willigen 1998):

$$PLC = \frac{100}{\left[1+exp\left(\frac{s}{25}(\psi - P_{50})\right)\right]}$$

, where P_{50} (MPa) is the xylem pressure inducing a 50% loss of conductance and S (% MPa^{-1}) is the slope of the vulnerability curve at the inflexion point. The xylem-specific hydraulic conductivity (K_s, m^2 MPa^{-1} s^{-1}) was calculated by dividing the maximum hydraulic conductivity measured at low speed (K_{max}) by the xylem area of the sample. The xylem pressures at which 12 and 88% conductivity were lost (P_{12} and P_{88}, MPa, respectively) were calculated as follows:

$$P_{12} = 2/(s/25) + P_{50}$$

and

$$P_{88} = -2/(s/25) + P_{50}$$

The mean embolism vulnerability values were calculated from the data for 15 to 17 samples per accession.

Anatomical observations

Anatomical observations were carried out on the samples used for hydraulic measurements. Three stems per accession were selected at random. A 2.5-3.0 cm-long segment was cut from the central portion of each sample, and the 12 segments obtained in this way were stored in jars filled with 60% ethanol. The samples were then taken to the Naturalis Biodiversity Center (Leiden, the Netherlands) for sectioning. Three to four transverse stem sections, each about 20-25 µm thick, were cut from each sample with a sledge microtome (Reichert, Germany), for light microscopy (DM2500 microscope, Leica, Germany). The sections were prepared according to the standardized protocol described by Lens et al. (2005). Briefly, sections were treated with household bleach for 1 minute and rinsed at least three times with distilled water. They were then stained with safranin-Alcian blue (consisting of two parts 1% safranin in 50% ethanol and one part 1% Alcian blue in H_2O) for 15 seconds, and then subjected to dehydration by successive steps of at least one minute in 50%, 70% and 96% ethanol. Sections were finally treated with a 1:1 mixture of ethanol 96%-Histoclear before complete immersion in Histoclear. The sections were mounted in Euparal mounting medium and dried in an oven at 60°C for at least three weeks. The slides were then scanned with a Hamamatsu NANOZOOMER 2.0HT (Bordeaux Imaging Center, University of Bordeaux, France). Images of complete cross sections were taken at × 10 magnification and analyzed with Adobe Photoshop CS2 (Version 9.0, Adobe Systems Inc., San Jose, CA, USA) and ImageJ (Version 1.44p) software, using the particle analysis function.

For all subsequent calculations, the complete stem cross section with pith and bark was analyzed. The parameters measured included: total stem cross section area (A_{stem}, µm^2); total xylem area (A_{Tx}, µm^2); primary xylem area (A_{Px}, µm^2) and secondary xylem area (A_{Sx}, µm^2); pith area (A_{pith}, µm^2); proportion of pith area per unit stem area ($P_{p\ s}$); area of the cellular part of the stem (A_{cp}, µm^2) calculated by subtracting A_{pith} from A_{stem}; lignified area (A_{lig}, µm^2) calculated by adding total xylem area (A_{Tx}) and fiber cap area (A_{fcap}, µm^2); proportion of lignified area per stem area ($P_{lig\ s}$) obtained dividing A_{lig} by A_{stem}; proportion of lignified area relative to the cellular part of the stem ($P_{lig\ cp}$) calculated by dividing A_{lig} by A_{cp}; fiber cell wall area (A_{fcw}, µm^2) in the secondary xylem measured by subtracting fiber lumen area (A_{flumen}, µm^2) from fiber cell area (A_{fcell}, µm^2); proportion of cell wall per fiber cell ($P_{cw\ f}$) obtained by dividing (A_{fcw}) by (A_{fcell}); total fiber wall area in the lignified area ($A_{fcw\ in}\ A_{lig}$, µm^2) calculated by multiplying $P_{cw\ f}$ by A_{lig}; proportion of fiber wall in the lignified area per stem area ($P_{fcw\ in\ lig\ s}$) measured by dividing $A_{fcw\ in}\ A_{lig}$ by A_{stem}. We also calculated the following parameters for both primary and secondary xylem: vessel density

(VD); vessel lumen area (A_v); cumulative vessel lumen area (A_{cv}); relative vessel lumen area (A_{rv}) obtained by dividing cumulative vessel lumen area by the corresponding xylem area; thickness-to-span ratio of vessels (TD^{-1}) obtained by dividing double intervessel wall thickness (T_{vw}) by the maximum diameter of the vessel (D_{max}); equivalent circle diameter (D) and hydraulically weighted vessel diameter (D_h), respectively, calculated as $D = (4A/\pi)^{1/2}$ where A is vessel cross sectional surface area (µm^2), and $D_h = \sum D^5/\sum D^4$ (Scholz et al. 2013). A list of all the measured traits, their symbols and units is provided in Table 1.

Statistical analyses

The differences in hydraulic traits (P_{50}, P_{12}, P_{88}, S, K_s) between accessions were assessed by one-way analysis of variance (ANOVA). Correlations between variables were evaluated by calculating the Pearson correlation coefficient (r), and the relationship between variables was considered to be significant if $P = <0.05$. Statistical analyses of the data were performed with SAS software (version 9.4, SAS Institute, Cary, NC, USA).

Results

Differentiation between accessions

Stem diameter did not differ significantly between the four sunflower accessions ($F = 1.69$, $P = 0.1781$) but significant differences in height were observed: Melody and LG_5660 were significantly taller than ES_Ethic and ES_Biba (F = 20.96, $P = < 0.0001$; Table 2). The vulnerability curves of the four accessions followed a similar sigmoidal shape (Fig. 1). Embolism resistance (P_{50}) differed significantly between accessions ($F = 45.59$, $P = < 0.0001$), with LG_5660 and ES_Ethic the most vulnerable and the most resistant accession to embolism, respectively (Table 2, Fig. 2). ES_Ethic also differed significantly from the other accessions in terms of P_{12} ($F = 6.27$, $P = 0.0009$), whereas mean P_{88}, which differed significantly between accessions ($F = 33.95$, $P = < 0.0001$), was lowest for Melody and highest for LG_5660, respectively (Table 2). We also found significant differences in S ($F = 6.66$, $P = 0.0006$) and K_s ($F = 3.15$, $P = 0.0313$; Table 2) between accessions.

Correlation between hydraulic, growth and anatomical traits

The Pearson correlation analysis revealed several relationships between growth, hydraulic and anatomical traits. Height was positively correlated with P_{50} ($r = 0.42$; $P = 0.0027$; Table 3, Fig. 3A) and P_{12} ($r = 0.54$; $P = < 0.0001$), indicating that the vulnerability of the xylem to embolism increased with height. Height was negatively associated with S ($r = -0.35$; $P = 0.0128$), indicating that embolism occurred more rapidly in faster growing individuals. It was also negatively associated with xylem-specific hydraulic conductivity

(K_s) (r = -0.56; P = <0.0001; Fig. 3B), indicating a lower xylem efficiency in faster growing individuals. A negative correlation was also observed between P_{50} and K_s (r = -0.30; P = 0.0174; Fig. 3C), suggesting a lack of trade-off between xylem safety and efficiency. No correlation was found between stem diameter (D, mm) and hydraulic traits, except for K_s (r = -0.47; P = 0.0007; Table 3).

Hydraulic traits were significantly correlated with seven of the 34 anatomical traits measured (Tables 1 and S1). P_{12} and P_{50} were strongly and negatively correlated with VD_{Tx} and VD_{Sx} (Table 3, Fig. 4A, B), indicating that stems with a higher vessel density in the total xylem area and the secondary xylem area are more resistant to both the entry of air into the xylem and a substantial loss of conductance. P_{50} was also negatively correlated with A_{rvSx} (r = -0.60; P = 0.0485; Table 3, Fig. 4C), whereas P_{12} was negatively correlated with inter-vessel double-wall thickness in the primary xylem (T_{vwPx}, r = -0.60; P = 0.0490) and the thickness-to-span ratio of vessels in the primary xylem ($TD^{-1}{}_{Px}$, r = -0.63; P = 0.0385; Table 3). P_{50} was not related to the lignified area (A_{lig}) or to the proportion of lignified area per unit stem area ($P_{lig\ s}$; Table SA, Fig. 5). No correlation was detected between P_{88} and anatomical traits (Table 3 and S1). Xylem-specific hydraulic conductivity (K_s) was negatively correlated with A_{vPx} (r = -0.63; P = 0.0380) and D_{Px} (r = -0.64; P = 0.0319; Table 3) but this variable was not correlated with any other anatomical trait (Table S1).

Discussion

The mean P_{50} value of -2.99 ± 0.15 MPa found here is similar to that reported by Stiller and Sperry (2002) for well-watered *Helianthus annuus* (P_{50}, -3.0 ± 0.1 MPa). Sunflower is, thus, moderately vulnerable to embolism relative to other herbaceous and woody species, which have P_{50} values ranging from -0.5 to -7.5 MPa (Lens et al. 2016) and from -0.5 to -18.8 MPa (Delzon et al. 2010, Choat et al. 2012, Bouche et al. 2014, Larter et al. 2015), respectively. We found significant intraspecific differences in vulnerability to xylem embolism in sunflower, with the accessions at the two extremes of the scale differing in P_{50} by about 0.55 MPa. Vulnerability to embolism (P_{50}) was also positively related to growth and negatively related to xylem-specific hydraulic conductivity, highlighting a trade-off between embolism resistance and growth, but not between xylem safety and efficiency. Finally, we found that various anatomical traits, such as vessel density, were related to embolism resistance, whereas the degree of stem tissue lignification was not.

Intraspecific variability of embolism resistance

Despite the critical role of xylem embolism resistance in plant survival during drought events, only one previous study has reported variation in this trait (from -0.8 to -3MPa) in sunflower (Stiller and Sperry 2002). However, the authors used a single genotype subjected to drought and rewatering cycles. Our study is thus the first to investigate intraspecific variation in xylem embolism resistance in sunflower. We found a 0.55 MPa difference in P_{50} between the most resistant sunflower accession, the early-sown ES_Ethic, and the most vulnerable accession, the late-sown LG_5660. This finding highlights the possibility of selecting specific sunflower accessions on the basis of their greater resistance to xylem embolism, and therefore to drought, opening up opportunities for the development of new varieties better adapted to the drier environmental conditions of the future. Studies investigating the intraspecific variability of embolism resistance in other crops have yielded contrasting results. Neufeld et al. (1992) and Li et al. (2009) highlighted genetic differences in P_{50} in sugarcane clones (from -0.83 to -1.36 MPa) and maize hybrid stems (-1.56 to -1.78 MPa), respectively. By contrast, Cochard (2002b) and Stiller et al. (2003) found no such differences for maize hybrids, and for comparisons of upland and lowland rice varieties, respectively. Mixed results for the intraspecific variation of xylem embolism resistance have also been reported for woody plants. Moderate to low levels of intraspecific variation have been reported for P_{50} (-2.21 ± 0.19 to -2.97 ± 0.12 MPa) in poplar (*Populus* sp) demes (Hajek et al. 2014), whereas no significant differences in P_{50} were observed in European beech (*Fagus sylvatica* L.) populations (Hajek et al. 2016). Similarly diverse observations have been reported for conifers (Lamy et al. 2011, Sáenz-Romero et al. 2013, Lamy et al. 2014). These findings suggest that the resistance to embolism may often be linked to uniform evolutionary selection and canalization (Lamy et al. 2011, 2014).

Trade-off between growth traits and embolism resistance

It has often been suggested that increases in resistance to xylem embolism are achieved at the expense of slower plant growth, due to conflicts in the allocation of carbon to the construction of denser wood with thicker cell walls (Hacke et al. 2001) or the construction of foliar and axial tissues to increase canopy carbon gains and growth rate (Wikberg and Ögren, 2004, Ducrey et al. 2008). Our findings suggest that height is a key factor governing embolism resistance in sunflower accessions. We found that shorter plants had greater embolism resistance. Conflicting results have been published concerning the possible existence of such a trade-off between P_{50} and growth-related traits. Cochard et al. (2007) found a close relationship between xylem vulnerability and productivity in poplar and willow clones. However, Fichot et al. (2010), for instance, observed that embolism-resistant genotypes of poplar grew more rapidly than vulnerable genotypes. Similarly, Sterck et al. (2012) found that embolism resistance had a positive effect on branch growth in Scots pine, whereas Hajek et al. (2014) found no relationship between vulnerability to embolism and growth rate in poplar demes. Several recent studies have also failed to detect a trade-off between vulnerability to embolism and growth-related traits (Guet et al. 2015, Hajek et al. 2016). The relationship between embolism resistance and growth therefore remains a matter of debate.

Xylem-specific hydraulic conductivity (K_s) was negatively correlated with both height and stem diameter, consistent with a trade-off between hydraulic efficiency and growth. However, no direct effect of xylem specific hydraulic conductivity was observed on growth in Scots pine (Sterck et al. 2012). By contrast, Hajek et al. (2014) found a positive relationship between K_s and growth rate in poplar, suggesting that water conductance capacity is a useful growth-determining factor. Similarly, Schuldt et al. (2015) reported a significant positive relationship between K_s and growth in European beech, showing that fast-growing branches had a more efficient hydraulic system than slower growing branches. This conflicting results suggest that there is still a lack of consensus concerning possible trade-offs between K_s and growth traits.

Relationship between hydraulic traits and anatomy

Vessel density in the total xylem (VD_{Tx}) and in the secondary xylem (VD_{Sx}) strongly influenced both the point of air entry during embolism formation (P_{12}) and the xylem pressure inducing a 50% loss of conductance (P_{50}). These results indicate that embolism resistance in sunflower is increased by the production of more vessels per unit xylem area. This finding is also supported by the close relationship between P_{50} and vessel lumen area relative to secondary xylem area (A_{rvSx}). A similar relationship between vessel density (VD) and P_{12} was reported by Schuldt et al. (2015) for European beech; however, Hajek et al. (2014) found no close relationship between P_{50} and relative vessel lumen area in poplar.

We found no relationship between P_{50} and greater stem tissue lignification. The development of embolism-resistant stems does not therefore involve tissue lignification. Several studies have reported a link between greater embolism resistance and higher levels of lignification in herbaceous (Lens et al. 2013, Tixier et al. 2013, Lens et al. 2016) as (Awad et al. 2012) woody plants, but an increase in lignification is not always required to achieve higher levels of embolism resistance (Watkins et al. 2010, Pittermann et al. 2011).

Strong correlations between P_{12} and both intervessel double-wall thickness in primary xylem (T_{vwPx}) and the thickness-to-span ratio of vessels in the primary xylem (TD^{-1}_{Px}) suggest that these two traits are important for the onset of embolism formation. Greater wall thickness and thickness-to-span ratios result in a lower xylem air entry pressure (i.e. resistant accessions have thicker tracheid walls relative to lumen area). This association between increasing cavitation resistance and increasing thickness-to-span ratio has also been reported in conifers (Bouche *et al.* 2014; Hacke et al. 2001) and in *Acer* species (Chave et al. 2009, Lens et al. 2011). A higher thickness-to-span ratio is thought to strengthen the vessel walls against implosion, higher embolism resistance being associated with a lower negative sap pressure.

The negative relationship between K_s and P_{50} observed here shows that there is no trade-off between xylem-specific hydraulic conductivity and embolism resistance. Plants with higher embolism resistance also transport water more efficiently. This finding contrasts with that reported by Lens et al. (2011), who found that higher levels of embolism resistance were strongly associated with lower stem-specific (K_{Sa}) and xylem-specific (K_{Xa}) conductivities. Ideally, plants should be able to maintain both the efficient conductivity and safety of the hydraulic system. However, this is not the case in natural conditions, and little or no support for a safety-efficiency trade-off has been obtained across species (Gleason et al. 2015). A few studies have evaluated this trade-off at the intraspecific level, and found either no support for the existence of a trade-off (Martínez-Vilalta et al. 2009, Schuldt et al. 2015) or, as here, an association between greater conductivity and lower embolism resistance (Corcuera et al. 2011). The water-conducting efficiency of vessels depends on vessel diameter, with wider vessels more efficient than narrower ones (Sperry et al. 2006). Our finding that K_s is negatively correlated with vessel diameter in primary xylem (D_{Px}) and vessel lumen area in the primary xylem (A_{vPx}) is, therefore, surprising. It may reflect the limitation of water flow through vascular tissue mostly by the pit resistivity of the conduit end walls (about 56% according to Sperry et al. 2006). Indeed, increases in lumen conductivity are not necessarily associated with increases in total conduit conductivity. However, further studies are required to determine how pits can be efficient for water transport, leading to low values of pit resistivity, whilst also limiting air-seeding under high xylem tension. This contrasts with the findings of Lens et al. (2011) and Hajek et al. (2014), who found a positive relationship between K_s and relative vessel lumen area and vessel diameter, indicating that hydraulic conductivity was determined by vessel size. This view is supported by other studies carried out on woody species (Zwieniecki et al. 2001, Sperry et al. 2005, Sperry et al. 2008, Schuldt et al. 2015).

Conclusion

Our findings demonstrate the existence, in sunflower, of intraspecific variation in resistance to drought-induced xylem embolism. It may, therefore, be possible to select drought-resistant accessions/genotypes, which will be crucial for future farming, particularly in areas prone to drought. There was no trade-off between hydraulic efficiency (xylem-specific hydraulic conductivity) and xylem safety (embolism resistance), but we did find trade-offs between height and hydraulic safety and height and hydraulic efficiency. Future studies should investigate (i) the variability of embolism resistance across a wider range of accessions and (ii) the possible existence of a trade-off between embolism resistance and yield potential. Indeed, high yield potential, which is the main target of most crop breeding programs, may not be compatible with higher embolism resistance.

Author contributions

S.D. and H.B.A. designed the study. H.B.A. and G.C. performed the greenhouse experiment. G.C. and R.B. provided assistance for hydraulic measurements. S.D., F.L. and H.B.A. carried out the anatomical

observations. S.D. performed the statistical analyses. H.B.A. and L.J.L. wrote the first version of the manuscript, which was reviewed and revised by all the authors.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Correlations between all the hydraulic and anatomical traits studied.

Table S1. Non-significant ($P > 0.05$) correlations between anatomical and hydraulic traits. r values are Pearson correlation coefficients. See Table 1 for abbreviations.

Anatomical traits	Hydraulic traits									
	P_{12}		P_{50}		P_{88}		S		K_s	
	r	P	r	P	r	P	r	P	r	P
A_{stem} (μm^2)	0.43	0.1884	0.26	0.4453	-0.09	0.7936	-0.43	0.1855	-0.33	0.3152
A_{Tx} (μm^2)	0.51	0.1103	0.31	0.3513	-0.09	0.7768	-0.49	0.1199	-0.27	0.4213
A_{Px} (μm^2)	0.30	0.3638	0.16	0.6404	-0.09	0.7764	-0.26	0.4424	-0.23	0.4931
A_{Sx} (μm^2)	0.51	0.1080	0.31	0.3441	-0.09	0.7843	-0.50	0.1143	-0.26	0.4309
A_{pith} (μm^2)	0.41	0.2054	0.25	0.4435	-0.07	0.8323	-0.41	0.2083	-0.38	0.2515
$P_{p\ s}$	-0.01	0.9809	0.05	0.8691	0.09	0.7906	0.02	0.9420	-0.34	0.2964
A_{cp} (μm^2)	0.41	0.2045	0.22	0.4992	-0.11	0.7334	-0.43	0.1894	-0.21	0.5423
A_{lig} (μm^2)	0.52	0.1041	0.32	0.3305	-0.08	0.8006	-0.50	0.1162	-0.25	0.4481
$P_{lig\ s}$	0.29	0.3878	0.22	0.5156	0.01	0.9841	-0.22	0.5037	0.26	0.4340
$P_{lig\ cp}$	0.46	0.1580	0.49	0.1210	0.22	0.5027	-0.31	0.3578	-0.29	0.3914
A_{fcap} (μm^2)	0.37	0.2571	0.36	0.2723	0.12	0.7176	-0.31	0.3481	0.11	0.7517
A_{fcell} (μm^2)	0.46	0.1493	0.34	0.2977	-0.00	0.9990	-0.34	0.3008	-0.16	0.6383
A_{flumen} (μm^2)	0.47	0.1470	0.15	0.6613	-0.29	0.3901	-0.46	0.1488	0.00	0.9874
A_{fcw} (μm^2)	0.32	0.3343	0.39	0.2276	0.23	0.5005	-0.14	0.6763	-0.24	0.4723
$P_{cw\ f}$	-0.12	0.7331	0.19	0.5769	0.40	0.2217	0.25	0.4498	-0.17	0.6185
$A_{fcw\ in}\,A_{lig}$ (μm^2)	0.43	0.1854	0.42	0.1929	0.15	0.6574	-0.32	0.3349	-0.30	0.3649
$P_{fw\ in\ lig\ s}$	0.07	0.8452	0.27	0.4226	0.31	0.3383	0.08	0.8111	0.01	0.9679
VD_{Px} (n mm^{-2})	-0.57	0.0637	-0.49	0.1207	-0.09	0.7737	0.32	0.3434	0.02	0.9526
A_{vSx} (μm^2)	0.38	0.2477	0.31	0.3496	0.04	0.9019	-0.23	0.4902	-0.49	0.1186
A_{cvPx} (μm^2)	0.11	0.7380	-0.01	0.9719	-0.14	0.6793	-0.15	0.6519	-0.42	0.1931
A_{cvSx} (μm^2)	0.22	0.5000	-0.09	0.7900	-0.38	0.2508	-0.36	0.2741	-0.33	0.3143
A_{rvPx} (μm^2)	-0.13	0.7021	-0.21	0.5255	-0.17	0.6146	-0.01	0.9662	-0.56	0.0732
T_{vwSx} (μm)	-0.25	0.4556	-0.31	0.3458	-0.18	0.5851	-0.03	0.9202	-0.23	0.4892
$TD^{-1}{}_{Sx}$	-0.09	0.7845	-0.01	0.9732	0.08	0.8053	0.00	0.9903	-0.11	0.7509
D_{Sx} (μm)	0.42	0.1966	0.35	0.2809	0.06	0.8532	-0.25	0.4595	-0.48	0.1330
D_{hPx} (μm)	0.02	0.9493	-0.13	0.6960	-0.21	0.5212	-0.09	0.7986	-0.45	0.1666
D_{hSx} (μm)	0.10	0.7626	0.04	0.8980	-0.05	0.8891	-0.07	0.8451	-0.44	0.1759

593 **Table 1.** List of the traits studied, including their units and descriptions

Traits (units)	Description
Growth	
H (m)	Height
D (mm)	Stem diameter
Hydraulics	
P_{12} (MPa)	Xylem pressure inducing a 12% loss of hydraulic conductance
P_{50} (MPa)	Xylem pressure inducing a 50% loss of hydraulic conductance
P_{88} (MPa)	Xylem pressure inducing a 88% loss of hydraulic conductance
S (% MPa^{-1})	Slope of the vulnerability curve at the inflexion point
K_s (m^2 MPa^{-1} s^{-1})	Xylem specific hydraulic conductivity
Anatomy	
A_{stem} (µm^2)	Stem cross-section area
A_{Tx} (µm^2)	Total xylem area
A_{Px} (µm^2)	Primary xylem area
A_{Sx} (µm^2)	Secondary xylem area
A_{pith} (µm^2)	Pith area
A_{cp} (µm^2)	Area of the cellular part of stem
A_{lig} (µm^2)	Lignified area
A_{fcap} (µm^2)	Fiber cap area (sum of the areas of all fiber caps in the stem cross-section)
A_{fcell} (µm^2)	Fiber cell area
A_{flumen} (µm^2)	Fiber lumen area
A_{fcw} (µm^2)	Fiber cell wall area
$A_{fcw\ in}\,A_{lig}$ (µm^2)	Total fiber wall area in the lignified area
A_{vPx} (µm^2)	Vessel lumen area in the primary xylem
A_{vSx} (µm^2)	Vessel lumen area in the secondary xylem
A_{cvPx} (µm^2)	Cumulative vessel lumen area in the primary xylem
A_{cvSx} (µm^2)	Cumulative vessel lumen area in the secondary xylem
A_{rvPx}	Vessel lumen area relative to primary xylem area
A_{rvSx}	Vessel lumen area relative to secondary xylem area

$P_{p\ s}$	Pith area as a proportion of stem area
$P_{lig\ s}$	Lignified area as a proportion of stem area
$P_{lig\ cp}$	Lignified area as a proportion of the area of the cellular part of the stem
$P_{cw\ f}$	Cell wall area as a proportion of fiber cell area
$P_{fw\ in\ lig\ s}$	Fiber wall area in the lignified area as a proportion of stem area
VD_{Px} (n mm^{-2})	Vessel density in the primary xylem
VD_{Sx} (n mm^{-2})	Vessel density in the secondary xylem
VD_{Tx} (n mm^{-2})	Vessel density in the total xylem
T_{vwPx} (µm)	Inter-vessel double-wall thickness in the primary xylem
T_{vwSx} (µm)	Inter-vessel double-wall thickness in the secondary xylem
$TD^{-1}{}_{Px}$	Thickness-to-span ratio of vessels in the primary xylem
$TD^{-1}{}_{Sx}$	Thickness-to-span ratio of vessels in the secondary xylem
D_{Px} (µm)	Equivalent circle diameter of vessels in the primary xylem
D_{Sx} (µm)	Equivalent circle diameter of vessels in the secondary xylem
D_{hPx} (µm)	Hydraulically weighted vessel diameter in the primary xylem
D_{hSx} (µm)	Hydraulically weighted vessel diameter in the secondary xylem

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Table 2. Mean values (± SE) of traits related to growth and hydraulic properties for four sunflower accessions. Letters in bold indicate significant statistical differences between accessions ($P < 0.05$). Sampling sizes are indicated in brackets.

Traits	Sunflower accessions			
	Melody	LG_5660	ES_Ethic	ES_Biba
Growth				
H	0.95 ± 0.07**a** (16)	0.91 ± 0.07**a** (14)	0.78 ± 0.06**b** (20)	0.78 ± 0.11**b** (18)
D	8.75 ± 1.18**a** (16)	8.68 ± 1.24**a** (14)	8.02 ± 0.92**a** (20)	8.57 ± 1.12**a** (18)
Hydraulics				
P_{12}	-2.19 ± 0.23**a** (16)	-2.18 ± 0.27**a** (17)	-2.57 ± 0.33**b** (16)	-2.29 ± 0.31**a** (15)
P_{50}	-3.09 ± 0.12**b** (16)	-2.67 ± 0.16**a** (17)	-3.22 ± 0.13**c** (16)	-3.01 ± 0.18**b** (15)
P_{88}	-3.99 ± 0.27**c** (16)	-3.14 ± 0.15**a** (17)	-3.86 ± 0.27**bc** (16)	-3.73 ± 0.30**b** (15)
S	59.78 ± 17.88**c** (16)	120.48 ± 52.91**a** (17)	95.10 ± 48.49**ab** (16)	78.78 ± 30.78**bc** (15)
K_s	$1.6\times10^{-4} \pm 0.9\times10^{-4}$**ab** (16)	$1.4\times10^{-4} \pm 0.8\times10^{-4}$**b** (17)	$2.4\times10^{-4} \pm 0.9\times10^{-4}$**a** (16)	$1.7\times10^{-4} \pm 0.7\times10^{-4}$**ab** (15)

Table 3. Relationships between hydraulic, growth and anatomical traits. The values shown are the Pearson correlation coefficients. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. See Table 1 for trait descriptions.

Hydraulic traits	Growth traits		Anatomical traits						
	H	D	VD_{Tx}	VD_{Sx}	A_{rvSx}	T_{vwPx}	TD^{-1}_{Px}	A_{vPx}	D_{Px}
P_{12}	0.54***	0.15	-0.76**	-0.77**	-0.34	-0.60*	-0.63*	0.37	0.41
P_{50}	0.42**	0.08	-0.85***	-0.92***	-0.60*	-0.31	-0.43	0.21	0.27
P_{88}	0.09	-0.02	-0.41	-0.51	-0.51	0.21	0.06	-0.09	-0.06
S	-0.35*	-0.20	0.44	0.41	0.10	0.56	0.45	-0.31	-0.32
K_s	-0.56***	-0.47***	0.19	0.25	-0.18	-0.26	-0.09	-0.63*	-0.64*

Figure legends

Fig. 1. Vulnerability curves (VCs) for individuals of the four sunflower accessions studied, for which xylem embolism was induced by *in situ* flow centrifugation according to the Cavitron technique. $n = 16$, 17, 16 and 15 for Melody, LG_5660, ES_Ethic and ES_Biba, respectively. VCs are expressed as the percentage loss of hydraulic conductivity (PLC) as a function of xylem pressure.

Fig. 2. Mean vulnerability curves (VCs) (± SE) for each of the four sunflower accessions studied. $n = 16$, 17, 16 and 15 for Melody, LG_5660, ES_Ethic and ES_Biba, respectively. VCs were generated by the *in situ* flow centrifugation (Cavitron) technique and are expressed as the percentage loss of hydraulic conductivity (PLC) as a function of xylem pressure.

Fig. 3. Relationships between height (H, m), xylem embolism resistance (P_{50}, MPa) and xylem specific hydraulic conductivity (K_s, m^2 MPa^{-1} s^{-1}). $n = 49$, 63 and 49 for panels A, B and C, respectively.

Fig. 4. Relationship between vessel density in secondary xylem (VD_{Sx}; A), vessel density in total xylem (VD_{Tx}; B), vessel lumen area relative to secondary xylem area (A_{rvSx}; C) and xylem embolism resistance (P_{50}, MPa). $n = 11$.

Fig. 5. Transverse sections of (A, C) the most resistant (ES_Ethic) and (B, D) the most vulnerable (LG_5660) sunflower accessions. Plants were grown in pots filled with Peltracom substrate placed in a greenhouse under full light and non-limiting growing conditions. They were sectioned with a sledge microtome ((Reichert, Germany). (A, C) Sections cut from the middle of the plant stem. (B, D) Overview

of mature stems highlighting the similarity in lignified area (A_{lig}) and the difference in vessel density in the secondary xylem (VD_{Sx}) (black arrows).

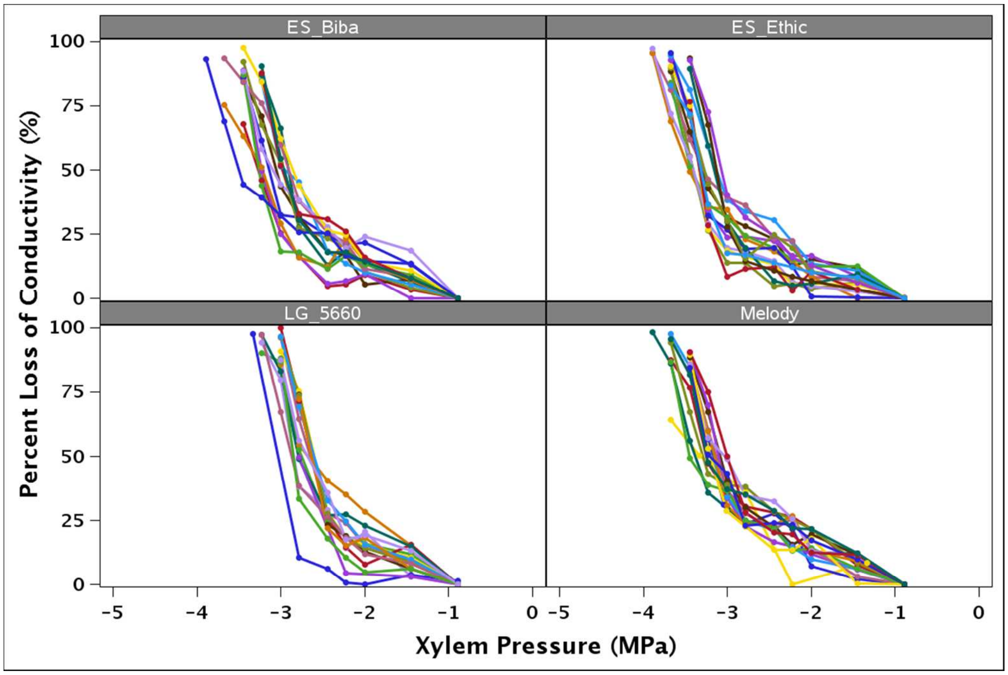

Fig. 1. Vulnerability curves (VCs) for individuals of the four sunflower accessions studied, for which xylem embolism was induced by in situ flow centrifugation according to the Cavitron technique. n = 16, 17, 16 and 15 for Melody, LG_5660, ES_Ethic and ES_Biba, respectively. VCs are expressed as the percentage loss of hydraulic conductivity (PLC) as a function of xylem pressure.

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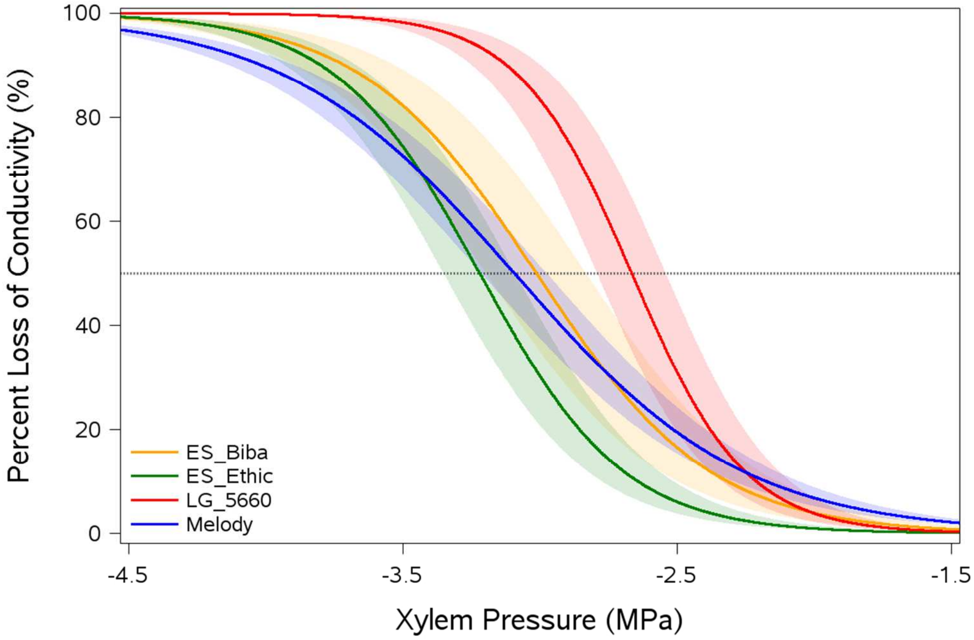

Fig. 2. Mean vulnerability curves (VCs) (± SE) for each of the four sunflower accessions studied. n = 16, 17, 16 and 15 for Melody, LG_5660, ES_Ethic and ES_Biba, respectively. VCs were generated by the in situ flow centrifugation (Cavitron) technique and are expressed as the percentage loss of hydraulic conductivity (PLC) as a function of xylem pressure.

317x211mm (96 x 96 DPI)

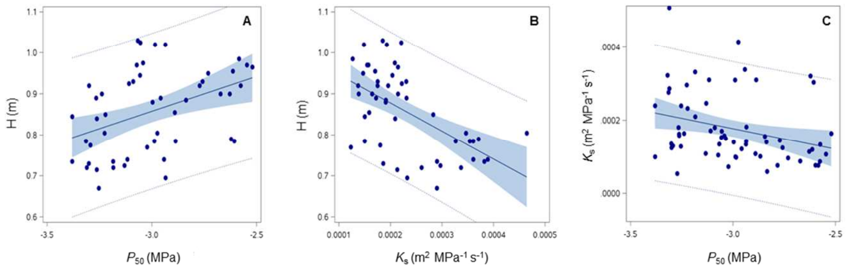

Fig. 3. Relationships between height (H, m), xylem embolism resistance (P50, MPa) and xylem specific hydraulic conductivity (Ks, m2 MPa-1 s-1). n = 49, 63 and 49 for panels A, B and C, respectively.

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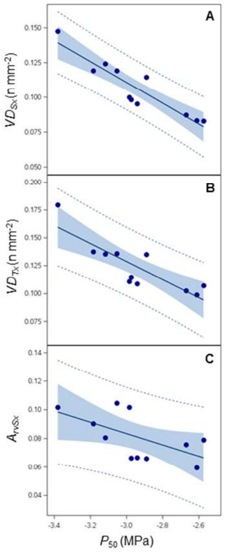

Fig. 4. Relationship between vessel density in secondary xylem (VDSx; A), vessel density in total xylem (VDTx; B), vessel lumen area relative to secondary xylem area (ArvSx; C) and xylem embolism resistance (P50, MPa). n = 11.

338x190mm (96 x 96 DPI)

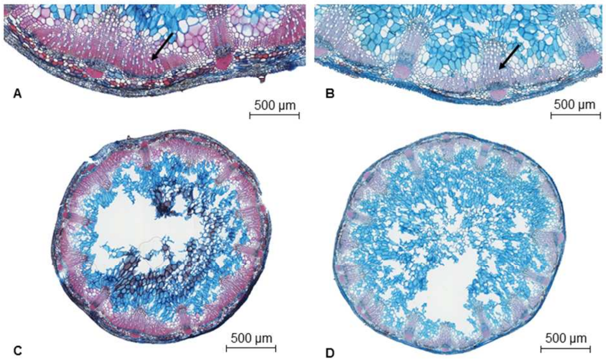

Fig. 5. Transverse sections of (A, C) the most resistant (ES_Ethic) and (B, D) the most vulnerable (LG_5660) sunflower accessions. Plants were grown in pots filled with Peltracom substrate placed in a greenhouse under full light and non-limiting growing conditions. They were sectioned with a sledge microtome ((Reichert, Germany). (A, C) Sections cut from the middle of the plant stem. (B, D) Overview of mature stems highlighting the similarity in lignified area (Alig) and the difference in vessel density in the secondary xylem (VDSx) (black arrows).

338x190mm (96 x 96 DPI)

Table: S1 Non-significant ($P > 0.05$) correlations between anatomical and hydraulic traits. *r* values are Pearson correlation coefficients. See Table 1 for abbreviations.

	Hydraulic traits									
Anatomical traits	P_{12}		P_{50}		P_{88}		S		K_s	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
A_{stem} (µm^2)	0.43	0.1884	0.26	0.4453	-0.09	0.7936	-0.43	0.1855	-0.33	0.3152
A_{Tx} (µm^2)	0.51	0.1103	0.31	0.3513	-0.09	0.7768	-0.49	0.1199	-0.27	0.4213
A_{Px} (µm^2)	0.30	0.3638	0.16	0.6404	-0.09	0.7764	-0.26	0.4424	-0.23	0.4931
A_{Sx} (µm^2)	0.51	0.1080	0.31	0.3441	-0.09	0.7843	-0.50	0.1143	-0.26	0.4309
A_{pith} (µm^2)	0.41	0.2054	0.25	0.4435	-0.07	0.8323	-0.41	0.2083	-0.38	0.2515
$P_{p\ s}$	-0.01	0.9809	0.05	0.8691	0.09	0.7906	0.02	0.9420	-0.34	0.2964
A_{cp} (µm^2)	0.41	0.2045	0.22	0.4992	-0.11	0.7334	-0.43	0.1894	-0.21	0.5423
A_{lig} (µm^2)	0.52	0.1041	0.32	0.3305	-0.08	0.8006	-0.50	0.1162	-0.25	0.4481
$P_{lig\ s}$	0.29	0.3878	0.22	0.5156	0.01	0.9841	-0.22	0.5037	0.26	0.4340
$P_{lig\ cp}$	0.46	0.1580	0.49	0.1210	0.22	0.5027	-0.31	0.3578	-0.29	0.3914
A_{fcap} (µm^2)	0.37	0.2571	0.36	0.2723	0.12	0.7176	-0.31	0.3481	0.11	0.7517
A_{fcell} (µm^2)	0.46	0.1493	0.34	0.2977	-0.00	0.9990	-0.34	0.3008	-0.16	0.6383
A_{flumen} (µm^2)	0.47	0.1470	0.15	0.6613	-0.29	0.3901	-0.46	0.1488	0.00	0.9874
A_{fcw} (µm^2)	0.32	0.3343	0.39	0.2276	0.23	0.5005	-0.14	0.6763	-0.24	0.4723
$P_{cw\ f}$	-0.12	0.7331	0.19	0.5769	0.40	0.2217	0.25	0.4498	-0.17	0.6185
$A_{fcw\ in}A_{lig}$ (µm^2)	0.43	0.1854	0.42	0.1929	0.15	0.6574	-0.32	0.3349	-0.30	0.3649
$P_{fw\ in\ lig\ s}$	0.07	0.8452	0.27	0.4226	0.31	0.3383	0.08	0.8111	0.01	0.9679
VD_{Px} (n mm^{-2})	-0.57	0.0637	-0.49	0.1207	-0.09	0.7737	0.32	0.3434	0.02	0.9526
A_{vSx} (µm^2)	0.38	0.2477	0.31	0.3496	0.04	0.9019	-0.23	0.4902	-0.49	0.1186
A_{cvPx} (µm^2)	0.11	0.7380	-0.01	0.9719	-0.14	0.6793	-0.15	0.6519	-0.42	0.1931
A_{cvSx} (µm^2)	0.22	0.5000	-0.09	0.7900	-0.38	0.2508	-0.36	0.2741	-0.33	0.3143
A_{rvPx} (µm^2)	-0.13	0.7021	-0.21	0.5255	-0.17	0.6146	-0.01	0.9662	-0.56	0.0732
T_{vwSx} (µm)	-0.25	0.4556	-0.31	0.3458	-0.18	0.5851	-0.03	0.9202	-0.23	0.4892
TD^{-1}_{Sx}	-0.09	0.7845	-0.01	0.9732	0.08	0.8053	0.00	0.9903	-0.11	0.7509
D_{Sx} (µm)	0.42	0.1966	0.35	0.2809	0.06	0.8532	-0.25	0.4595	-0.48	0.1330
D_{hPx} (µm)	0.02	0.9493	-0.13	0.6960	-0.21	0.5212	-0.09	0.7986	-0.45	0.1666
D_{hSx} (µm)	0.10	0.7626	0.04	0.8980	-0.05	0.8891	-0.07	0.8451	-0.44	0.1759

Part II: Intraspecific variability in embolism resistance and its relation with genetic architecture and photosynthetic traits in Maritime pine.

Chapter 2: Dissecting the genetic architecture of resistance in Maritime pine reveals that hydraulic safety is not linked to wood density. (*in preparation*)

Dissecting the genetic architecture of embolism resistance in maritime pine (*Pinus pinaster* Ait.) reveals that hydraulic safety is not linked to wood density. (*in preparation*)

Abstract

Hydraulic failure due to vascular embolism is likely the main cause of drought-induced forest mortality reported worldwide. The vulnerability to embolism has therefore been intensively investigated across species during the last decades, but we still know little about the intraspecific variability of this trait and its genetic determinism. To provide better insight about the genetic determinism of resistance to embolism, we dissected here its genetic architecture in concert with that of wood density in a large-size three-generation inbred pedigree of maritime pine (*Pinus pinaster* Ait.). We carried out the largest effort of phenotyping both hydraulic and wood density in 477 maritime pine genotypes. The variability of embolism resistance was remarkably low compared to that of wood density and radial growth. No significant correlations between embolism resistance and wood density were found. In addition, the comparison of quantitative trait loci (QTL) locations between embolism resistance and wood density related traits failed to detect co-locating genomic regions. The lack of phenotypic correlation and physical coincidence of QTLs in this family strongly suggest the absence of causality between these two traits in this species.

Introduction

Extreme droughts are predicted to be more intense, more frequent, and longer in the 21st Century (Meehl and Tebaldi 2004; Bahn et al., 2014; Lindner et al., 2014), placing forest systems in a more and more vulnerable situation. In the light of these events, the European heat wave of 2003 was a drastic demonstration of the extent of impacts we need to expect more often in the future (Ciais et al., 2005). Forest ecosystems have already been altered by climate change, leading to decreased growth (Piao et al., 2011; Zhao et al., 2010) and species distribution shift (Delzon et al., 2013), and to widespread mortality of trees (Allen et al., 2010; Michaelian et al., 2011). This global drought-induced forest mortality could be explained by the high vulnerability of trees to drought (Choat et al., 2012). With their long life-spans, trees cannot adapt rapidly to environmental changes, and most forest tree species will have to cope with these changes within a single generation (Lindner et al., 2010; Kremer et al., 2014). To better predict the resilience of forests to extreme droughts, a deeper understanding is needed about the key physiological traits underlying drought tolerance, their variations across and within species and their genetic determinisms (Plomion et al., 2016).

Xylem sap being transported under negative pressure in the vascular system of trees, during drought stress, sap pressures become excessively negative inside vessels and air embolism can form, responsible of hydraulic failure. Several studies demonstrated experimentally that the vulnerability to embolism is related to the survival of tree species in drought conditions (Brodribb and Cochard, 2009; Brodribb et al., 2010; Urli et al., 2013). In addition, the breakdown of the vascular system through embolism was found to be involved in multiple mass mortality events observed *in situ* under drought conditions (Anderegg et al., 2015, 2016). This mechanistic trait is therefore a crucial adaptive trait for trees to cope with severe drought (Larter et al., 2017). At the interspecific level, resistance to embolism has been described as highly variable across a wide range of species, species from xeric environments being more resistant to cavitation than species from mesic environments (Maherali et al., 2004; Cochard et al., 2008; Choat et al., 2012; Bouche et al., 2014). The incorporation of phylogenetic information also showed that embolism-related traits are under natural selection (Maherali et al., 2004; Pittermann et al., 2012; Larter et al., 2017). Conversely, little is known about the level and structure of intraspecific variability of embolism resistance, as well as its relationships with species autoecology. Based on common garden experiments (e.g. Lamy et al., 2011 for *Pinus pinaster* and Kavanagh et al., 1999 for *Douglas-fir ponderosa*) it was shown that intra-specific genetic variation in embolism resistance did not reveal significant differences between populations which leads Lamy et al. (2014) to suggest that this trait was under strong canalization, at least in *Pinus pinaster* where the large sample size of their study allowed such inference.

At the cellular level, vulnerability to cavitation has been shown to be intimately linked to the anatomy of xylem cells (Hacke et al., 2001), and in conifers, the mechanism of drought-induced cavitation largely depends on pit membrane properties (Delzon et al., 2010; Pittermann et al., 2010; Bouche et al., 2014). However, high throughput measurement of pit membrane anatomy is not feasible yet. At the interspecific level, Hacke et al. (2001) associated greater wall thickness per unit diameter in conifers (12 species) and angiosperms (36 species) with greater wood density and proposed to use wood density as good proxy of resistance for embolism. Delzon et al. (2010) studied 40 conifers and also reported a significant relationship between wood density and resistance to embolism but returns the density of the wood to a secondary role because all species that are in the higher density range are not all resistant to embolism. Such a relationship between wood density and embolism resistance was tested at the intraspecific level in maritime pine (Lamy et al., 2012) and in Douglas-fir (Dalla-Salda et al., 2009). Whereas Douglas-fir trees with higher wood density exhibited higher probability to survive drought than trees with a lower density, no relationships with embolism resistance was reported in maritime pine. By contrast, Rosner et al. (2017) found that trunk wood density was strongly related to resistance to embolism across cambial age in Norway spruce.

If we are beginning to better understand the genetics of embolism resistance (reviewed by Plomion et al., 2016 for *Pinus pinaster*), insights into the genetic architecture (number, chromosomal location and effect of quantitative trait loci, QTL) of vulnerability to embolism are still lacking, owing to the fact that high throughput phenotyping techniques of large populations (Liebhard et al., 2003) were inaccessible until recently. To our knowledge, a single study published by Lauri et al. (2011) failed to detect any QTL for embolism resistance in a *Malus domestica* full-sib mapping population of 123 trees.

In this context, the objectives of the present study were two-fold: i/ dissect the genetic architecture of embolism related traits (hydraulic safety) and wood density related traits, and (ii) test the relationship between wood density and xylem safety at the phenotypic and QTL level. To this end we phenotyped an already genotyped (Bartholomé et al., 2016) large-size three-generation inbred pedigree of *Pinus pinaster*. This species presents a high ecological and economic importance in the Western Mediterranean basin where it spans over 4M ha. The most recent climatic scenarios (IPCC, 2014) predict a strong modification of summer precipitation and temperature in this region by the end of this century. It is therefore important to know about the genetic architecture of traits that determines maritime pine survival during severe period of droughts if such characteristics are to be incorporated into breeding programs.

Material and methods

Plant material, genotyping and linkage map construction

The mapping population used in this study consisted of a three-generation inbred pedigree (F2) obtained by the self-pollination of an inter “Landes x Corsica” provenance hybrid (accession H12). Seeds were planted in a nursery (June 1998) and transplanted into the field (March 1999). Genotyping and phenotypic data were obtained for 477 F2 trees. The genotyping of this population is detailed in Bartholomé et al. (2016). A total of 248 SNP markers were mapped along the 12 linkage groups of the linkage map established for accession H12. Overall, 530 branches (i.e. assessment of some trees were replicated) were used to phenotype the F2s for cavitation resistance and wood density.

Phenotyping

Cavitation resistance related traits

For each F2, two branches fully exposed to the sun were collected in the early morning from November 2012 to April 2013. Forty cm branches of less than 1 cm diameter were chosen. All needles were immediately removed, and then the branches were labeled and placed in black bags, wrapped in wet paper towels. They were kept wet and cool (3°C) until measurement within three weeks after collection. Prior to embolism resistance measurement, branches were cut under water to a standard length of 27 cm, and bark was removed with a razor blade.

Xylem vulnerability to embolism was assessed with the CAVITRON, a centrifuge technique following the procedure described by Cochard (Cochard, 2002; Cochard et al., 2005) at the high-throughput phenotyping platform for hydraulic traits (CavitPlace, University of Bordeaux, Talence, France; http://sylvain-delzon.com/?page_id=536). Centrifugal force was used to establish negative pressure in the xylem and to provoke water stress-induced embolism, using a custom-built honeycomb rotor (Precis 2000, Bordeaux, France) mounted on a high-speed centrifuge (Sorvall RC5, USA). This technique enables measurement of the hydraulic conductance of a branch under negative pressure. Xylem pressure (P) was first set to a reference pressure (-0.5 MPa) and the maximal hydraulic conductivity (K) was determined by measuring the flux of a reference ionic solution (10 mM KCl and 1 mM $CaCl_2$ in deionized water) through the sample. The centrifugation speed was then set to a higher value for 3 min to expose the sample at a more negative pressure. Conductances were measured four times for each step, and the average was used to compute the percent loss of xylem conductance (PLC in %). The procedure was repeated for at least eight pressure steps with a -0.5 MPa step increment until PLC reached at least 90%. Rotor velocity was monitored with a 10 rpm resolution electronic tachymeter and xylem pressure was adjusted to about -0.02 MPa. We used Cavi_soft software (version2.0, BIOGECO, University of Bordeaux) for conductance measurements.

The percent loss of xylem conductivity as a function of xylem pressure (MPa) represents the sample's vulnerability curve (VC). A sigmoid function (Pammenter and van der Willigen, 1998) was fitted to the VC from each sample using the following equation:

$$PLC = \frac{100}{\left[1 + \exp\left(\frac{S}{25} x \left(P - P_{50}\right)\right)\right]}$$

where P_{50} (MPa) is the xylem pressure inducing 50% loss of hydraulic conductivity and S (% MPa $^{-1}$) is the slope of the vulnerability curve at the inflexion point. The xylem pressure inducing 12 and 88% of PLC (P_{12}, P_{88}, MPa) was calculated from the equation of Domec and Gartner (2001):

$$P_{12} = P_{50} + \frac{50}{S} ; P_{88} = P_{50} - \frac{50}{S}$$

Finally, the traits listed in Table 1 were available for quantitative trait loci (QTL) analysis.

Wood density and radial growth

All branches used for embolism resistance measurement were cut into 2cm longitudinal pieces and microdensity profile was obtained using X-ray densitometry (Polge, 1966). Two orthogonal (longest and shortest axes,) radial density profiles were obtained by analyzing the scanned images with WinDENDRO software (Guay et al., 1992). Data from these two sections were averaged and the mean used as a new variable. Ring limits (Fig. S1) were determined automatically, checked manually and then corrected with this software. For each genotype, a series of traits (including early and late wood densities and ring widths, see Table 1) was obtained for all the rings and then averaged for statistical and QTL analyses. When replicates (i.e. several branches per genotype) were available, the average was used for subsequent analysis.

Statistical analysis and QTL mapping

Classical descriptive statistics were generated using R (R core team, 2013) for the studied traits measured on the 477 F2 trees, i.e. mean, standard deviation, and coefficient of variation. A correlation matrix was obtained using Pearson correlation coefficients (R package *Hmisc*) to build a network (R package *Qgraph*) showing the relationship between variables. For QTL detection we used two software: (i) the multiple QTL mapping procedure implemented in the R package *qtl* (Broman et al., 2003) (function *stepwiseqtl* with the imputation method and a maximum of five QTLs for forward selection). In total, 1000 permutations were performed for each trait with the function *scantwo*, to estimate the type I error rate at genome level. A threshold error rate of 5% was used to define significant QTLs. The 95% Bayesian credible interval for each QTL was calculated using the function *bayesint* with default parameter (Manichaikul et al., 2006). The effects of the QTL as well as the percentage of the explained phenotypic variance were also calculated. Additive (a) and dominance (d) effects of a QTL were also calculated; (ii) the Multiple Interval Mapping (MIM) procedure (Jansen and Stam, 1994) implemented in MultiQTLV2.6 (Haifa, Israel, 2005; [http://www.multiqtl.com/]) following the approach described in Marguerit et al. (2014). Since both methods provided similar outcomes we only report here the result from the *R* package.

Results and discussion

Genotyping (248 SNPs mapped on the 12 linkage groups of the genetic map of accession H12) and phenotyping data (see material and method section) are provided in Table S1 for the 477 offspring of the F2 mapping population. The average, variance and distribution of each trait are presented in Table S2 and Fig. S2.

Hydraulic safety is not linked with wood density

Phenotypic variability and correlations among traits

All vulnerability curves for each genotype follow a sigmoid shape with the air-entry (P_{12}) around -3.15+0.27MPa as illustrated in Fig. 1A. The mean and standard deviation of the main variable P_{50} is -3.70 ± 0.21 MPa, i.e. similar to what was found in an earlier study (Lamy et al., 2011). The total amount of variability observed in our study is much larger than the variability observed across ten different Maritime pine ecotypes (Lamy et al., 2014) (Fig. 1B). However, as already observed for this species, the coefficient of variation for P_{50} is remarkably low (CV_P=5.6%

against 10.6% for mean wood density, 14.5% for mean ring width and 26.8% for mean ring area). The slope of the vulnerability curve (*S*) was relatively steep (99% MPa^{-1}) compared to those reported for conifer species in general but in the range of values observed for pine species (Delzon et al., 2010). As slope values >50% MPa^{-1} indicate a very fast rate of embolism, maritime pine can be considered as a vulnerable species to runway embolism. However, the coefficient of variation of *S* is high (CV_P=29%) showing the large variability between genotypes. The phenotypic correlations among variables (Table S3) show that the four variables of hydraulic safety (P_{50}, P_{12}, P_{88}) are positively correlated to each other. In particular there is a strong correlation between P_{50} and P_{12} (r=0.80) or P_{88} (r=0.77). Besides, there is no significant correlation between P_{50} and *Slope*. CV_P for mean wood density was around 10.5%, which agrees with a previous investigation with different Maritime pine ecotypes (Lamy et al., 2012). Both early- and late-wood densities were on the same range and highly correlated (r=0.8) to each other. CV_P for mean ring area was 14% and that for mean ring width was twice higher. Both traits were highly correlated (r=0.97). However, wood density- and growth-related traits segregated almost independently in two different clusters (Fig. 2 and Table S3) suggesting the absence of significant trade-off between carbon allocated to cell wall thickening (density) on the one hand and cell division and expansion (growth) on the other hand.

Lack of relationship between embolism resistance and wood density

No significant correlations between embolism resistance (P_{12}, P_{50}, P_{88}, *S*) and wood density (EWD_av, LWD_av, WD_av) were found in the present study, suggesting independence between these traits (Fig. 2 and Table S3). This result suggests that in this family, the more embolism resistance genotypes did not have the denser wood. This finding that has already been observed at the intraspecific level (Lamy et al., 2012) contradicts the main theory suggesting that dense wood is supposed to be less vulnerable due to a smaller lumen, higher conduit wall thickness and reduced endangerment through cell wall implosion (Hacke et al., 2001; Jacobsen et al., 2005). A mechanical constraint on tracheid anatomy was the premise of this theory as embolism resistant species generally experienced more negative xylem water potential and require to build tracheids with a higher thickness to span ratio to resist mechanical stresses. Indeed, Bouche et al. (2014) reported a significant trade-off between hydraulic and mechanical safety across conifer species with a strong link between increasing embolism resistance and increasing thickness to span ratio of tracheids. In addition, wood density is well known to correlate with the thickness to span ratio of conifers (Pitterman et al., 2006). However, a direct link between wood density and embolism resistance at both the inter- and intra-specific levels is still not convincingly confirmed. In conifers, those relationships were found across species (Hacke et al., 2001; Hacke and Jansen, 2009) and within a given species (Rosner et al., 2014; Rosner, 2017) but several studies reported lack of such correlations (Martínez-Vilalta et al., 2009; Corcuera et al., 2011; Lamy et al., 2012; Gleason et al., 2016). On a physiological perspective, the lack of link between wood density and embolism resistance in maritime pine is not surprising given the mechanistic role of the pit membrane in preventing embolism formation (Cochard et al., 2009; Delzon et al., 2010; Pittermann et al., 2010). Indeed, the best trait explaining the variability in embolism resistance across species is the ratio of torus to pit aperture diameter, so-called torus overlap (Bouche et al., 2014). More insights into the morphological, physiological or developmental mechanisms underlying the observed variability in cavitation resistance could then follow an approach consisting in finely studying the two extremes of the phenotypic distribution as reported for oak for water-use efficiency (Roussel et al., 2009a, b).

QTLs for cavitation resistance and wood density do not coincide

Following the mapping procedure described in Bartholomé et al. (2016), the linkage map of accession H12 included 248 SNP markers on 12 linkage groups (Table S1), corresponding to the haploid number chromosomes of the maritime pine genome. The length of the map was 1,754cM (146cM/linkage group on average) resulting into a density of 1 SNP every 7 cM, a density that is considered appropriate for QTL mapping.

No study has yet identified consistent QTLs for xylem water transport safety because it requires a high-throughput phenotyping method in a dedicated facility to measure a sufficient number of genotypes and reach a satisfactory statistical detection power. In this study we took advantage of the Cavitplace, a platform for hydraulic trait measurement at the University of Bordeaux (Talence, France) to screen the highest number ever of full-sibs with a single family, in order to dissect the genetic architecture of these traits and provide novel information regarding its genetic architecture and positional coincidence with wood density QTLs. As a result, highly significant QTLs were detected for all tested traits with high statistical support.

Considering the variability in the number of rings between the F2s (ranging from 2 to 5), we first analyzed the effect of this variable on embolism resistance traits. We only considered the

87, 328, 61 genotypes respectively with 2, 3 and 4 rings, because only one genotype presented 5 rings. Analysis of variance shows that the number of rings does not affect P_{12} and P_{50} (P-values > 0.05), while it barely affects P_{88} (P-value=0.0217) and had a significant impact (P= 0.0018) on S (Table S4). Therefore, while the full dataset (477 genotypes) was kept for QTL analysis for all the traits, we also performed the analysis for the 328 genotypes with only 3 rings for S. Since the results did not differ between both analyses we only report here the results for the whole dataset.

For P_{50}, five QTLs were detected on five different linkage groups (LG1, LG4, LG6, LG9, and LG10, Fig. 3). The phenotypic variance explained by each QTL (PEV) ranged from 3% to 6.5%, altogether accounting for 23% of the phenotypic variation (Table S5). Noteworthy, the same QTLs were detected with the restricted dataset based on three rings (data not shown). For P_{88}, four of these five QTLs were detected on the same chromosomal location of LG1, LG4, LG9 and LG10, and an additional one was detected in LG7. For both traits the strongest QTL (up to 10.3% of PEV) coincided on LG4. Two QTLs on LG6 and LG8 were detected for P_{12}. The former overlapped the confidence interval (CI) of the P_{50}-QTL on the same chromosome. Finally, a single significant QTL was found in LG4 for S with CI overlapping that of P_{50} and P_{88}.

For mean wood density three QTLs were detected on LG1, LG2 and LG8 (Fig. 3). The overall PEV amounted to 10.3% (Table S5). The QTL on LG1 was detected for both early wood (EW) and late wood (LW) on the same position, while that of LG2 and LG8 were found for EW only. These results suggest common genetic factor controlling basic wood properties whatever the type of wood considered, as well as specific molecular mechanisms differentiating EW vs. LW. Thus, none of the QTL for cavitation resistance coincided with that of wood density.

Conclusion

Comparison of QTL locations between embolism resistance and wood density related traits failed to detect co-locating regions. This result is in agreement with the absence of phenotypic correlations between both traits in this family. Whether this result would still hold true in other genetic backgrounds will have to be investigated, but the lack of phenotypic and genetic correlations across families of five ecotypes (Lamy et al., 2012) strongly support the lack of causality between these two traits in this species. Non-competing physiological mechanisms for wood density and cavitation resistance is interesting from a breeding perspective, as it leaves the possibility of selecting genotypes with high wood density and resistant to severe drought. This pattern should however be assessed at the trunk level where embolism resistance was found to be related to wood density, as reported by Rosner et al. (2017) in Norway spruce trunkwood at different cambial age.

Table 1: List of traits measured in this study.

Measured with the Cavitron	**Abbreviation**
Xylem pressure inducing 50% loss of hydraulic conductance (MPa) *(hydraulic safety = drought tolerance)*	P_{50}
Xylem pressure inducing 12% loss of hydraulic conductance (MPa) *(air-entry)*	P_{12}
Xylem pressure inducing 88% loss of hydraulic conductance (MPa)	P_{88}
Slope of the vulnerability curve *(hydraulic safety = embolism rates)*	***Slope***
Measured using X-ray microdensidometry	
Late wood density (kg.m^{-3})	***LWD_av***
Late wood ring surface (mm^{2})	***LWarea_av***
Late wood ring width (mm)	***LWwidth_av***
Late wood percent (%)	***LWpercent_av***
Early wood density (kg.m^{-3})	***EWD_av***
Early wood ring surface (mm^{2})	***EWarea_av***
Early wood ring width (mm)	***EWwidth_av***
Mean wood density (kg.m^{-3})	***WD_av***
Mean ring surface (mm^{2})	***area_av***
Mean ring width (mm)	***width_av***
Mean heterogeneity=Average of LWD - Average of EWD (kg.m^{-3})	***het_av***
Measured with a caliper	
Branch diameter (mm)	***branch_diam***

Figure captions:

Figure 1: Vulnerability curves of the F2 trees (**A**, red line: fitted mean, grey dark: fitted standard error, light grey: individual raw data) together with vulnerability curves obtained from a range of maritime pine ecotypes (**B**, Cazorla, Landais, Porto-vecchio, Tamjout correspond to four natural populations of the Saint Alban common garden described in Harfouche and Kremer, (2000) and, Bayubas de Abajo, Coca, Erdeven, Mimizan, Oria, San-Cipriano, Tamrabta are seven natural populations from the common garden described in Lamy et al. 2011).

Figure 2: Network of Pearson correlation coefficients between the studied traits. Each node represents a variable and each edge a correlation. The width of the edge indicates the strength of the correlation. Only significant coefficients (*P*-values < 0.05) are shown here. The negative correlations are in gray and the positive correlations in red.

Figure 3: QTL map for cavitation and wood density related traits. QTLs are shown along the 12 linkage groups (1 to 12) for the traits listed in Table 1. The confidence interval of each QTL is indicated in orange with a color gradient ranging from the peak to the end of the confidence interval.

Supplementary information

Table S1: Genotypic and phenotypic data.

Table S2: Descriptive statistics for the traits listed in table 1.

Table S3: Significant Pearson correlation coefficients for cavitation (P_{50}, P_{88}, P_{12}, *Slope*), branc diameter (branch_diam), wood density (LWD_av, EWD_av, het_av, WD_av) and radial growth (LWarea_av, LWwidth_av, LWpercent_av, EWarea_av, EWwidth_av, area_av) related traits in the F2 mapping population (*P*-value <0.05).

Table S4: **A**. Analysis of variance (cavitation related traits as dependent variable and number of rings as independent variable). **B**. Descriptive statistics for groups of sample with two rings, three rings or four rings.

Table S5: QTL results from the analysis of the F2 mapping population for the traits listed in table 1. Position (cM) of QTLs on linkage groups (LG) with flanking SNP markers listed in table S1, log10 likelihood ratio scores (LOD), genome wide *P*-value, 95% Bayesian credible intervals with marker interval, % variance explained by each QTL (PEV), and total PEV, genotypic class means and standard errors, additive-dominance effects are presented.

Figure S1: X-ray image of the radial section of a maritime pine branch. The dark grey color of early wood is related to its lower density compare to late wood indicated by a narrower whiter area.

Figure S2: Distribution of the studied traits listed in table 1.

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Figure 1: Vulnerability curves of the F2 trees (**A**, red line: fitted mean, grey dark: fitted standard error, light grey: individual raw data) together with vulnerability curves obtained from a range of maritime pine ecotypes (**B**, Cazorla, Landais, Porto-vecchio, Tamjout correspond to four natural populations of the Saint Alban common garden described in Harfouche and Kremer, (2000) and, Bayubas de Abajo, Coca, Erdeven, Mimizan, Oria, San-Cipriano, Tamrabta are seven natural populations from the common garden described in Lamy et al. (2011).

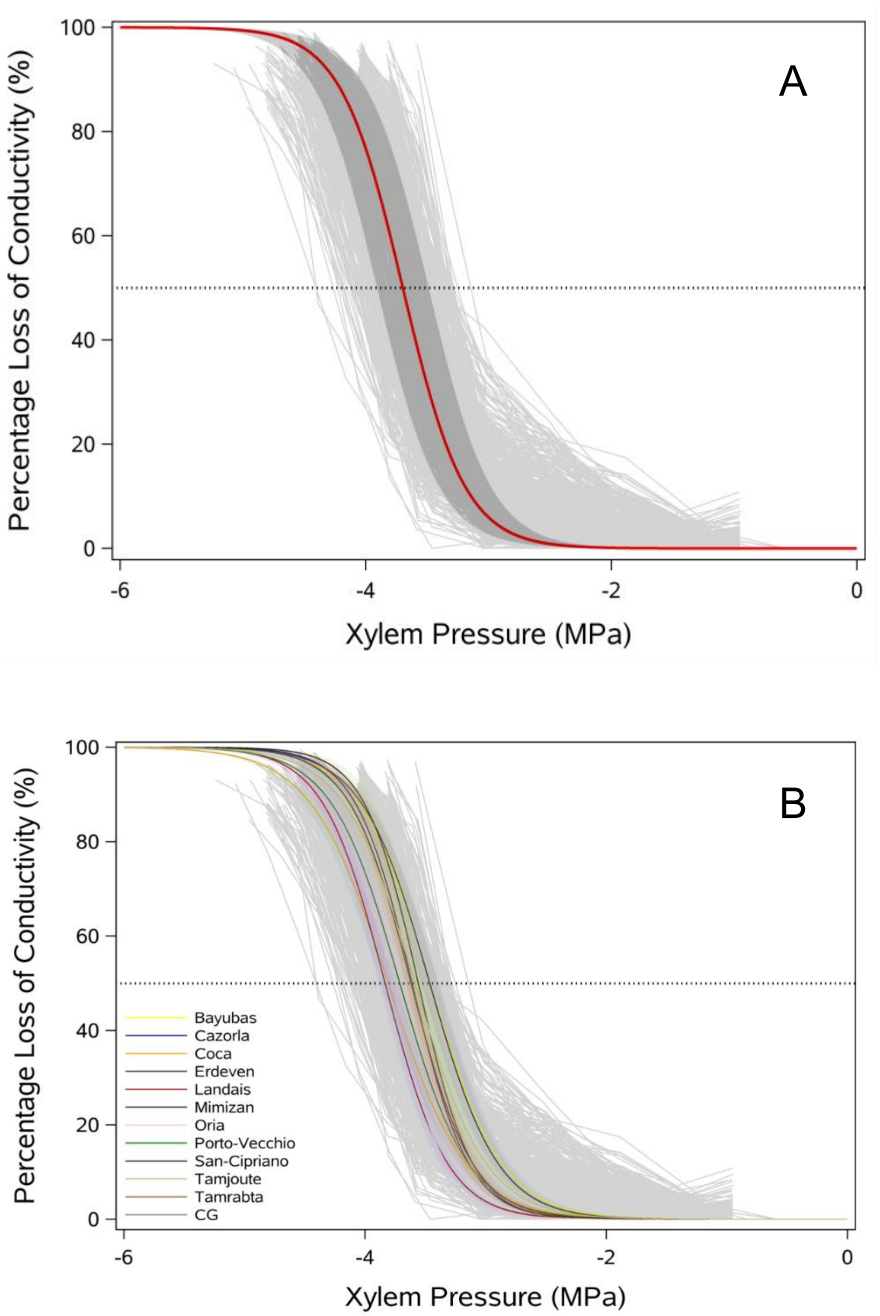


Figure 2: Network of Pearson correlation coefficients between the studied traits. Each node represents a variable and each edge a correlation. The width of the edge indicates the strength of the correlation. Only significant coefficients (*P*-values < 0.05) are shown here. The negative correlations are in gray and the positive correlations in red.

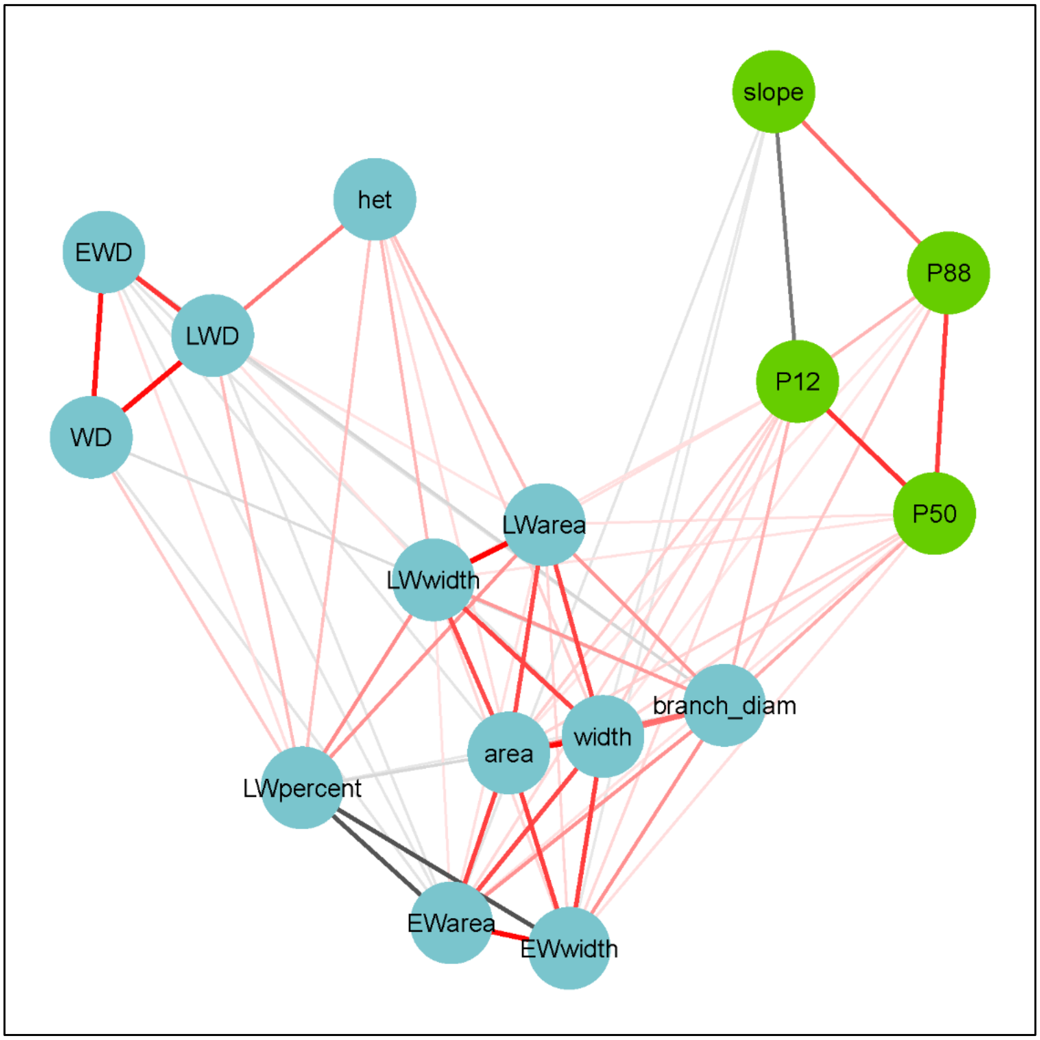
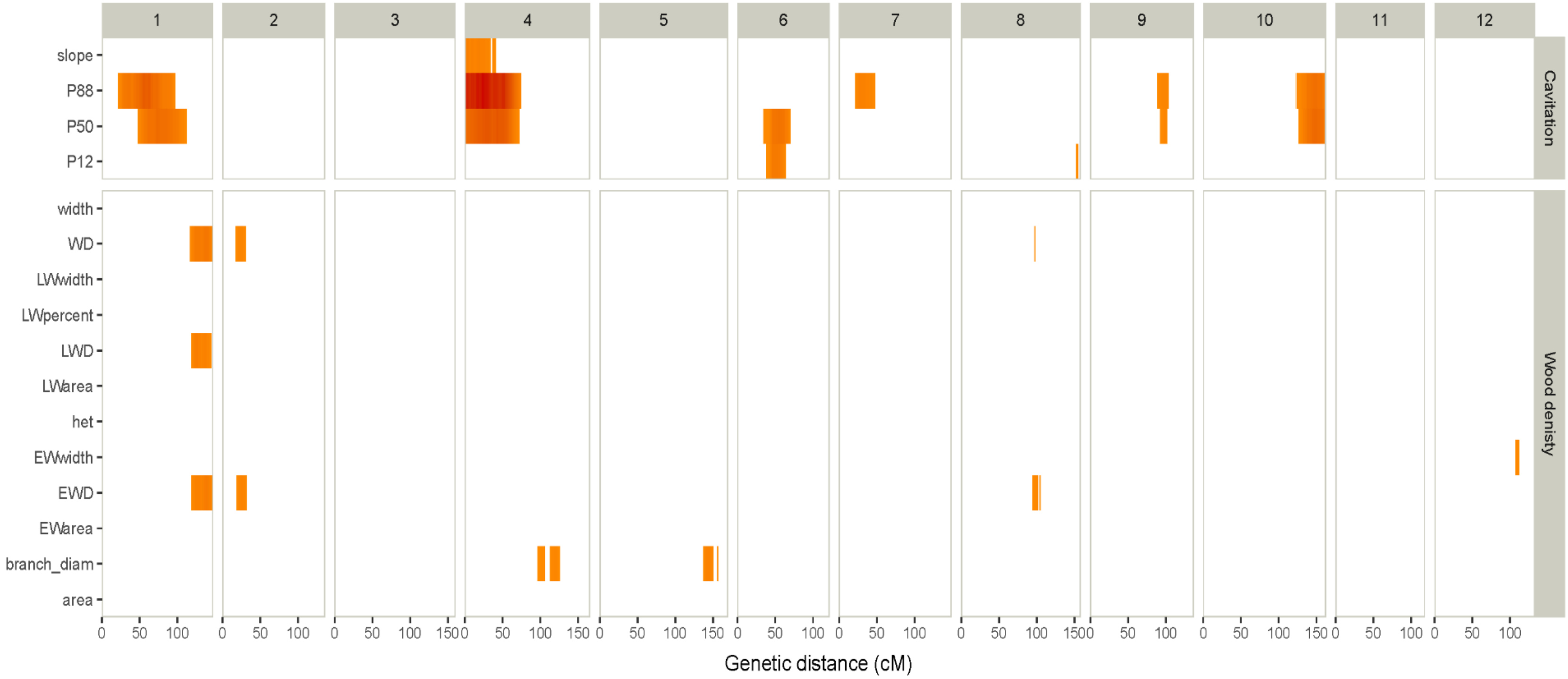


Figure 3: QTL map for cavitation and wood density related traits. QTLs are shown along the 12 linkage groups (1 to 12) for the traits listed in Table 1. The confidence interval of each QTL is indicated in orange with a color gradient ranging from the peak to the end of the confidence interval.



Supplementary information

Table S1: Genotypic and phenotypic data. (Data could be provided on demand)

Table S2: Descriptive statistics for the traits listed in table 1.

Traits	**P50**	**P12**	**P88**	**slope**	**branch_diam**	**LWD_av**	**LWarea_av**	**LWwidth_av**	**LWpercent_av**	**EWD_av**	**EWarea_av**	**EWwidth_av**	**het_av**	**WD_av**	**area_av**	**width_av**
nbr.val	465	465	465	465	465	466	466	466	466	466	466	466	466	466	466	466
nbr.na	12	12	12	12	12	11	11	11	11	11	11	11	11	11	11	11
min	-4.251	-3.771	-5.28	38.428	4.88	0.284	2.919	0.815	19.075	0.216	0.811	0.429	0.007	0.261	2.662	0.859
max	-3.178	-2.148	-3.515	234.635	9.32	0.549	19.783	2.509	85.75	0.444	17.196	2.261	0.154	0.483	14.415	2.095
range	1.073	1.623	1.765	196.207	4.44	0.265	16.864	1.694	66.675	0.228	16.385	1.832	0.147	0.222	11.753	1.236
median	-3.701	-3.181	-4.23	96.505	7.58	0.397	8.393	1.588	48.247	0.314	7.4145	1.47	0.083	0.356	8.12	1.549
mean	-3.699933	-3.147105	-4.25277	98.972955	7.5146882	0.3972597	8.6373519	1.6048798	48.80014807	0.3144635	7.6402768	1.4719742	0.0827961	0.3558455	8.1388391	1.5384292
SE.mean	0.0096854	0.012523	0.0122293	1.3437006	0.0417026	0.0019785	0.1332118	0.0130624	0.510883698	0.0016614	0.1379791	0.0139867	0.0010808	0.0017456	0.1011144	0.0103446
CI.mean.0.95	0.0190327	0.0246087	0.0240317	2.6404924	0.0819493	0.0038879	0.2617717	0.0256686	1.003926684	0.0032648	0.2711398	0.027485	0.0021239	0.0034302	0.1986977	0.020328
var	0.0436201	0.0729233	0.0695435	839.57209	0.8086831	0.0018241	8.2693533	0.0795114	121.6270032	0.0012863	8.8718139	0.0911629	0.0005444	0.0014199	4.7644366	0.0498672
std.dev	0.2088543	0.2700432	0.263711	28.97537	0.8992681	0.04271	2.8756483	0.2819777	11.02846332	0.0358651	2.978559	0.3019319	0.0233316	0.0376815	2.182759	0.2233096
coef.var	-0.056448	-0.085807	-0.062009	0.2927605	0.1196681	0.1075116	0.3329317	0.1757002	0.225992415	0.1140517	0.3898496	0.2051204	0.2817961	0.105893	0.2681905	0.1451543
skewness	-0.090702	0.564199	-0.407894	0.9213723	-0.261898	0.1014238	0.4106431	0.0115472	0.221617939	-0.018864	0.3878128	-0.223004	-0.095484	0.0119548	0.0885417	-0.230671
skew.2SE	-0.400526	2.4914237	-1.801204	4.068651	-1.156505	0.4483516	1.8152787	0.0510452	0.979678794	-0.08339	1.7143558	-0.985805	-0.422096	0.0528472	0.3914054	-1.019698
kurtosis	-0.351155	0.2292819	0.355305	1.6349223	-0.5745	0.2743173	-0.002732	-0.240316	0.020341513	0.2689728	-0.095225	0.1482016	0.1797559	0.349072	-0.156857	0.0597896
kurt.2SE	-0.776966	0.5073095	0.7861486	3.6174321	-1.271139	0.6076003	-0.00605	-0.532289	0.045055527	0.5957626	-0.210919	0.3282597	0.3981512	0.7731787	-0.347431	0.1324313
normtest.W	0.9959744	0.9783594	0.9895499	0.9567244	0.9856567	0.9940065	0.9851837	0.9969886	0.995750882	0.9938627	0.9873573	0.9924261	0.9963251	0.9916166	0.9972077	0.9947861
normtest.p	0.2843538	2.12E-06	0.0021481	1.92E-10	0.0001506	0.0632142	0.0001091	0.548108	0.240680416	0.0564674	0.0004552	0.0183482	0.3608435	0.0098387	0.6186437	0.1161574

Table S3: Significant Pearson correlation coefficients for cavitation (P_{50}, P_{88}, P_{12}, *Slope*), branc diameter (branch_diam), wood density (LWD_av, EWD_av, het_av, WD_av) and radial growth (LWarea_av, LWwidth_av, LWpercent_av, EWarea_av, EWwidth_av, area_av) related traits in the F2 mapping population (*P*-value <0.05).

	P50	P12	P88	slope	branch_diam	LWD	LWarea	LWwidth	LWpercent	EWD	EWarea	EWwidth	het	WD	area	width
P50																
P12	0.8															
P88	0.77	0.29														
slope		-0.53	0.58													
branch_diam	0.33	0.31	0.23													
LWD					-0.1											
LWarea	0.13	0.13			0.41	0.11										
LWwidth	0.12	0.12			0.4	0.12	0.99									
LWpercent					-0.09	0.27	0.43	0.45								
EWD					-0.16	0.8			0.13							
EWarea	0.12	0.16		-0.1	0.43	-0.11	0.13	0.12	-0.67	-0.1						
EWwidth	0.13	0.16		-0.09	0.43		0.17	0.16	-0.68		0.97					
het						0.55	0.26	0.29	0.26							
WD					-0.14	0.96			0.21	0.93	-0.11		0.3			
area	0.18	0.2	0.11		0.57		0.72	0.71	-0.15	-0.11	0.75	0.75	0.14			
width	0.17	0.19	0.1	-0.09	0.55		0.74	0.74	-0.16	-0.11	0.71	0.76	0.19		0.97	

Table S 4: A. Analysis of variance (cavitation related traits as dependent variable and number of rings as independent variable). **B**. Descriptive statistics for groups of sample with two rings, three rings or four rings.

A

Source P_{12}	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.31261047	0.03473450	0.47	0.8970
Error	467	34.77562535	0.07446601		
Corrected Total	476	35.08823583			

Source P_{50}	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.31957841	0.03550871	0.81	0.6069
Error	467	20.46539659	0.04382312		
Corrected Total	476	20.78497500			

Source P_{88}	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1.35033274	0.15003697	2.19	0.0217
Error	467	32.01024837	0.06854443		
Corrected Total	476	33.36058112			

Source S_{LOPE}	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	22147.2872	2460.8097	2.98	0.0018
Error	467	385726.7682	825.9674		
Corrected Total	476	407874.0554			

B

Traits	Mean	Std Deviation	Min	Max	N
P_{12} (2 rings)	-3.05	0.29	-3.60	-2.13	87
P_{12} (3 rings)	-3.21	0.27	-3.77	-2.15	318
P_{12} (4 rings)	-3.16	0.26	-3.64	-2.33	61
P_{50} (2 rings)	-3.70	0.21	-4.18	-3.26	87
P_{50} (3 rings)	-3.64	0.21	-4.25	-3.18	328
P_{50} (4 rings)	-3.72	0.20	-4.24	-3.23	61
P_{88} (2 rings)	-4.36	0.26	-5.28	-3.79	87
P_{88} (3 rings)	-4.08	0.26	-5.10	-3.52	318
P_{88} (4 rings)	-4.27	0.27	-4.99	-3.78	61
S_{LOPE} (2 rings)	85.85	21.38	38.30	138.35	87
S_{LOPE} (3 rings)	141.62	76.02	81.37	241.95	328
S_{LOPE} (4 rings)	98.9	28.85	49.69	197.51	61

Table S 5: QTL results from the analysis of the F2 mapping population for the traits listed in table 1. Position (cM) of QTLs on linkage groups (LG) with flanking SNP markers listed in table S1, log10 likelihood ratio scores (LOD), genome wide *P*-value, 95% Bayesian credible intervals with marker interval, % variance explained by each QTL (PEV), and total PEV, genotypic class means and standard errors, additive-dominance effects are presented.

	Trait	n	LG	Position	LOD	BCI	PEV	PEV tot	Add effect	Dom effect
Cavitation	P50	465	1	73	6.97	61 - 94	4.25	23.05	0.06044	0.03084
	P50	465	4	43	9.04	17 - 54.38	6.46		0.07314	0.02841
	P50	465	6	55	6.04	46 - 63	4.07		-0.06252	0.01396
	P50	465	9	100.69	4.07	88 - 104	3.05		-0.04139	-0.04669
	P50	465	10	148.29	7.15	140 - 158	4.72		-0.05809	-0.02975
	P12	465	6	49	5.48	45 - 60	4.84	7.19	-0.08438	-0.02451
	P12	465	8	154.57	3.49	134 - 157.85	2.51		0.06085	-0.00139
	P88	465	1	56	8.4	51 - 67	5.58	26.13	0.07673	0.06126
	P88	465	4	25	14.08	2 - 52	10.3		0.12273	0.03421
	P88	465	7	28	4.67	23 - 46	3.26		0.06004	0.02766
	P88	465	9	100.69	4.3	87 - 105	2.72		-0.04918	-0.05549
	P88	465	10	148	6.56	138 - 157	4.7		-0.08043	-0.03969
	slope	465	4	4	4.77	0 - 32	4.34		9.08926	0.32
Wood density	LWD_av	466	1	126	4.86	121 - 142	3.56		-0.01098	-0.00651
	EWD_av	466	1	139	5.18	123 - 145	4.85	11.75	-0.01152	0.00046
	EWD_av	466	2	26.32	4.41	18 - 40	3.93		0.01003	-0.00439
	EWD_av	466	8	97.56	4.01	90 - 121	3.34		0.00897	0.00382
	EWwidth_a	466	12	109.69	3.84	100 - 116	2.95		0.06914	-0.04371
	WD_av	466	1	139	5.72	123 - 143	4.33	10.33	-0.01121	-0.00074
	WD_av	466	2	26	4.2	11 - 40	3.34		0.00976	-0.00391
	WD_av	466	8	97.56	3.48	88 - 126	2.85		0.00873	0.00534

Figure S 1: X-ray image of the radial section of a maritime pine branch. The dark grey color of early wood is related to its lower density compare to late wood indicated by a narrower whiter area.

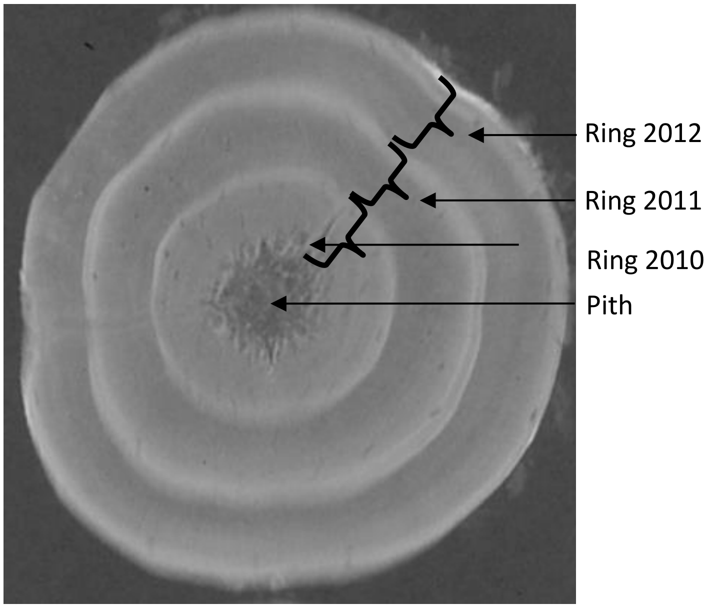
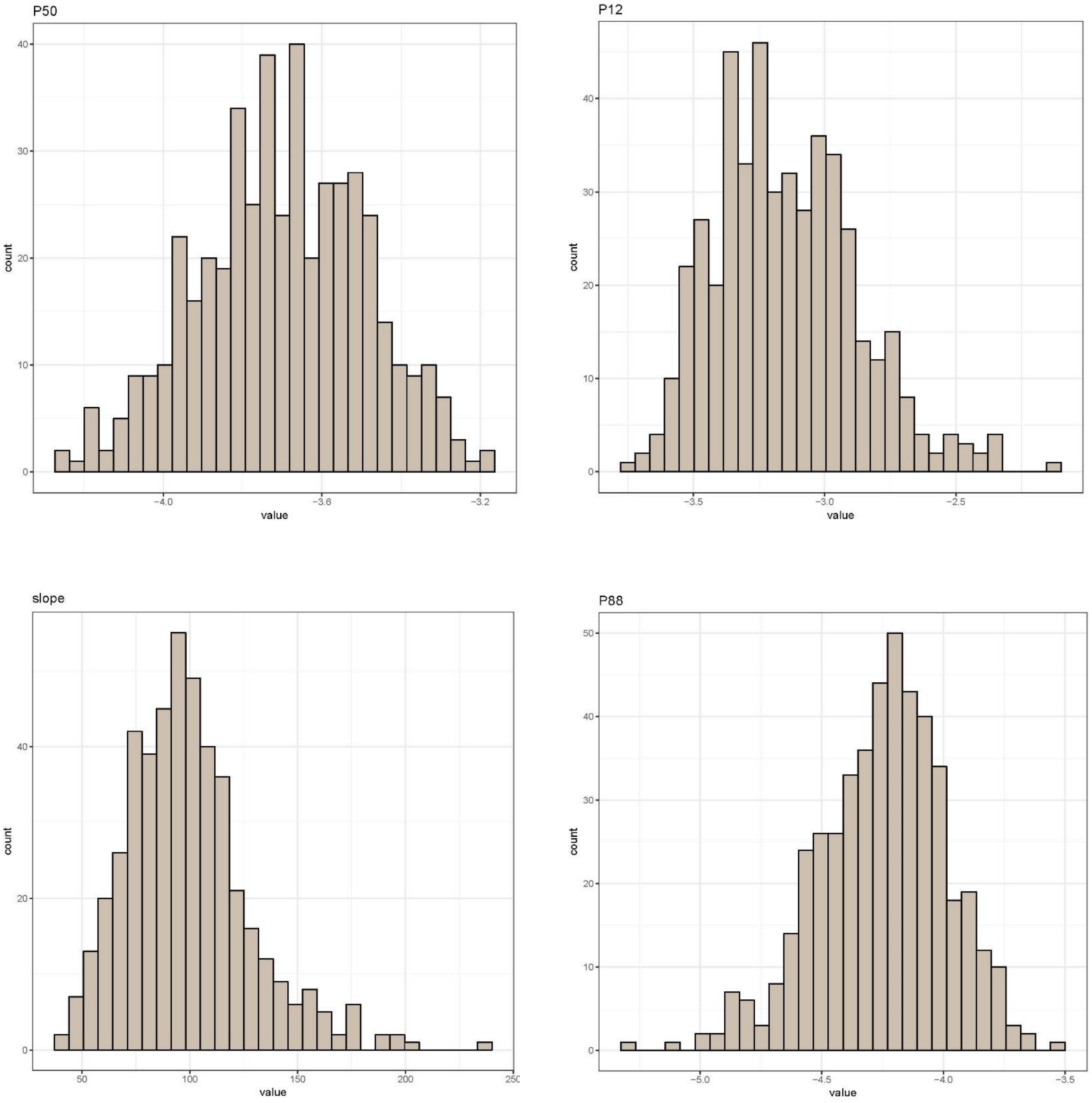
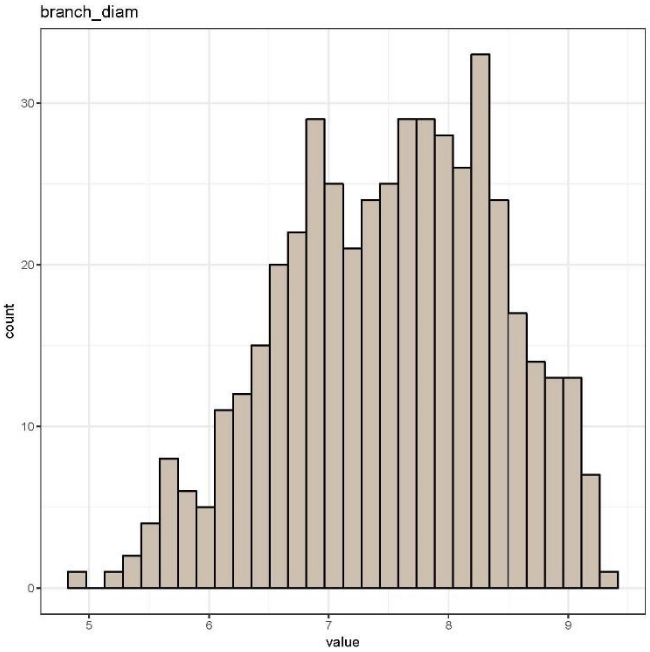


Figure S 2: Distribution of the studied traits listed in table 1.

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30
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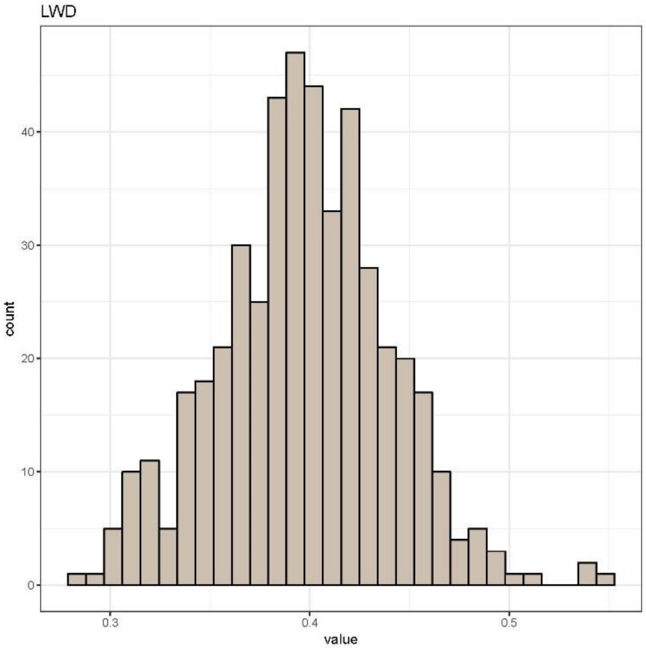
5
6
7
8
9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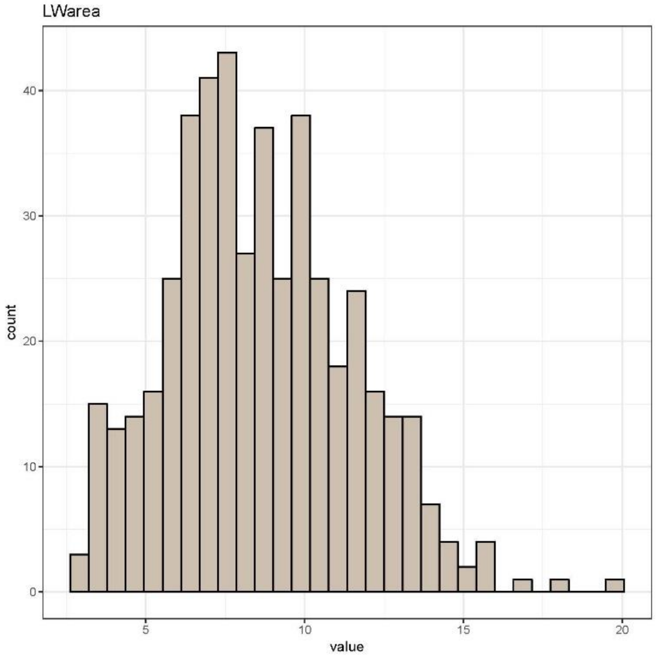
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15
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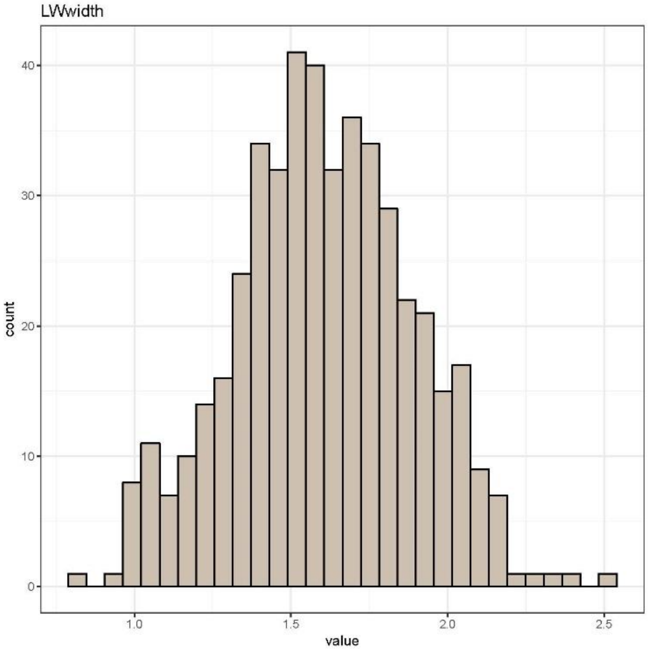
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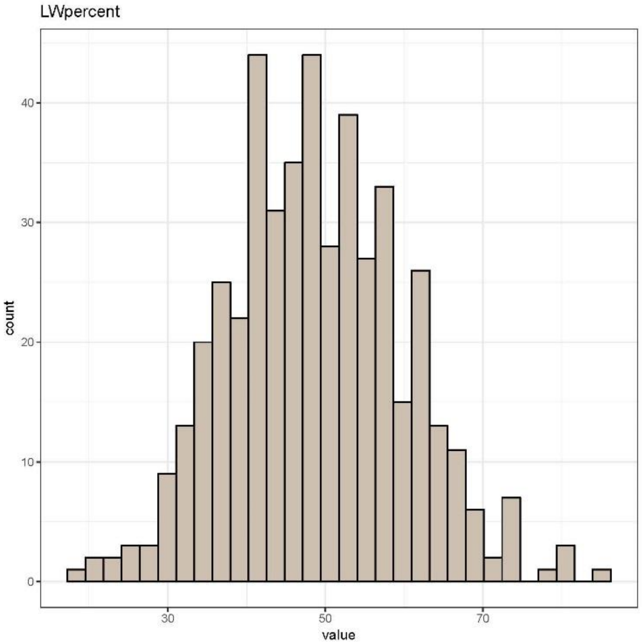
2.0
2.5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30
50
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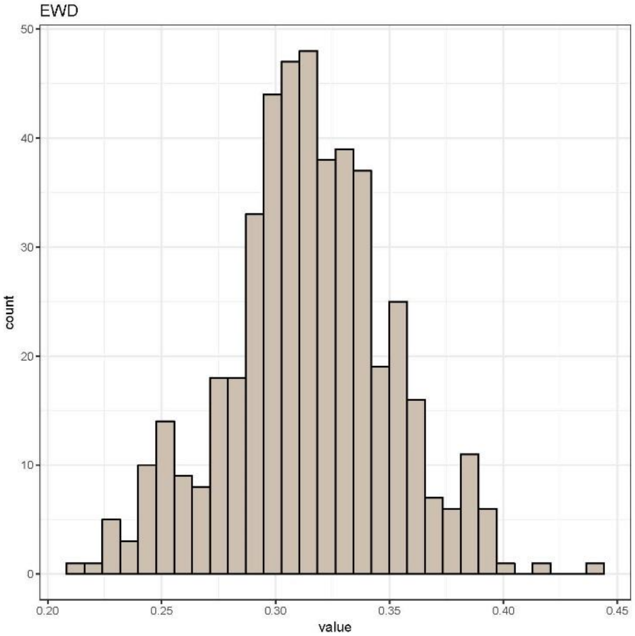
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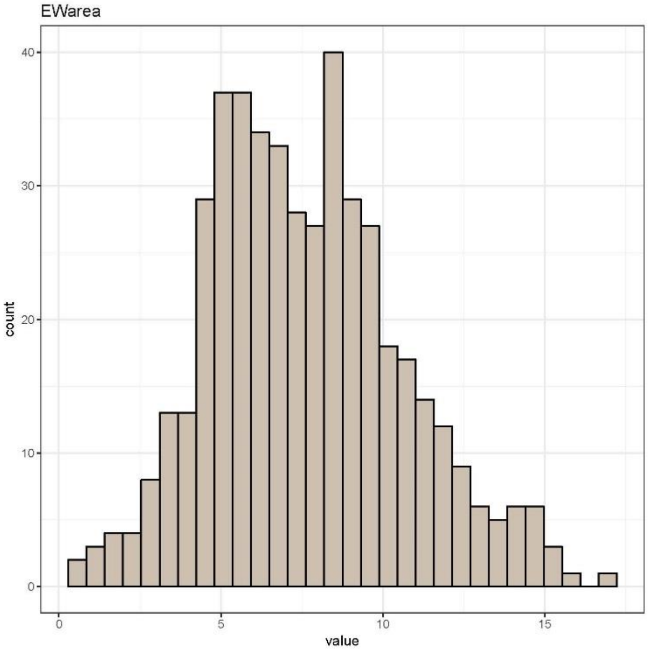
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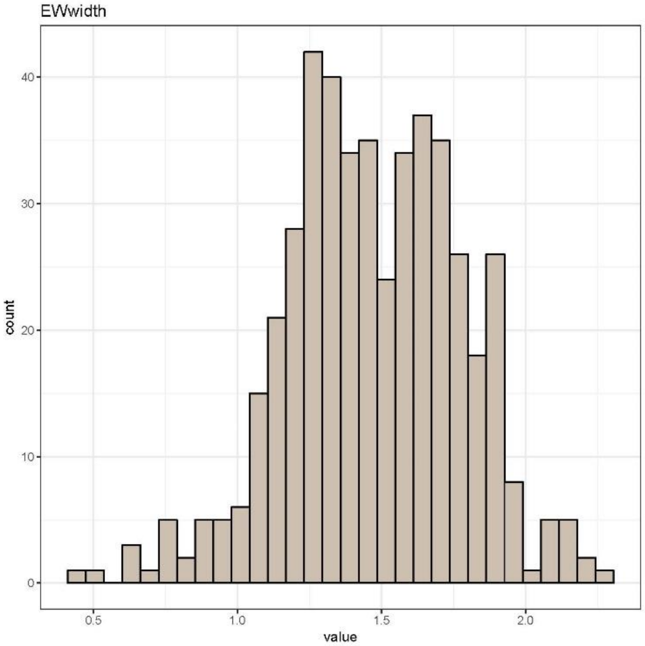
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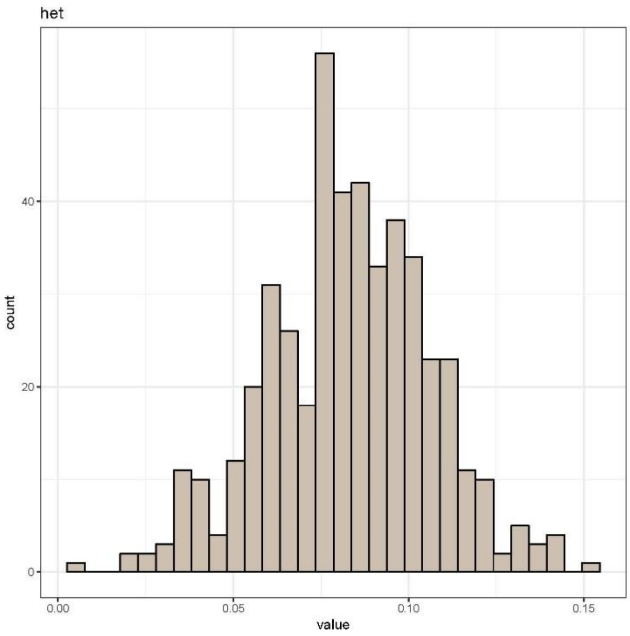
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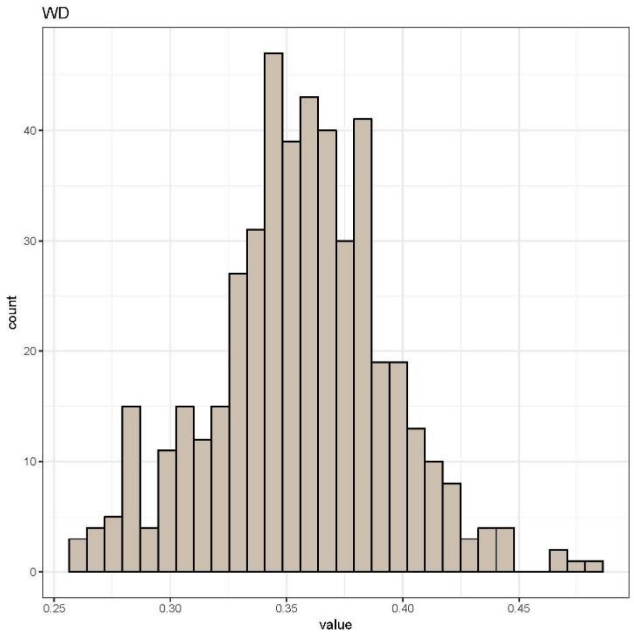
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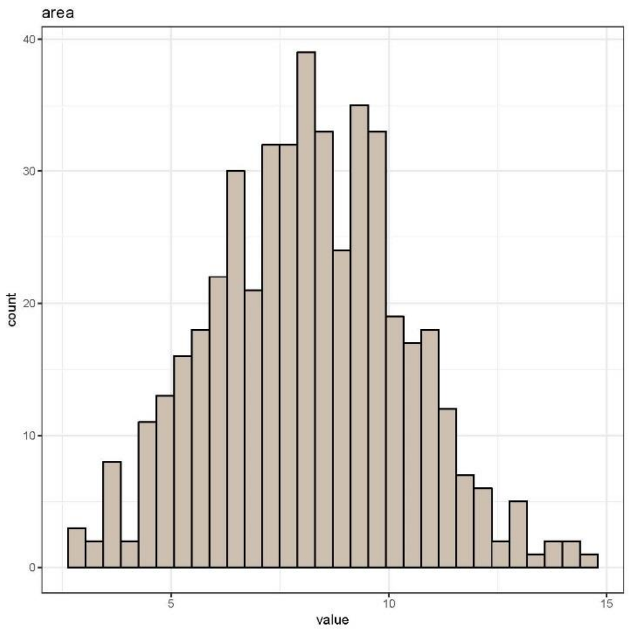
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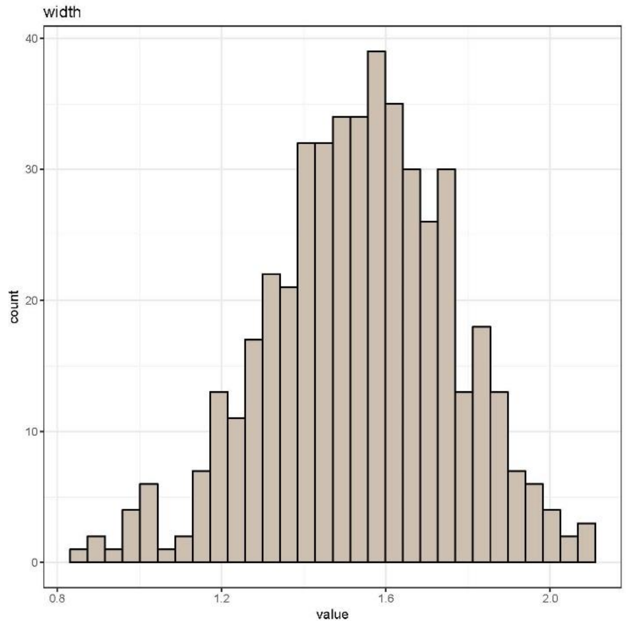
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Chapter 3:

Intra-specific variability of the physiological response to water stress in a full-sib Maritime pine family (*in preparation*)

Intra-specific variability of the physiological response to water stress in a full-sib Maritime pine family

This work was performed in the framework of the ANR project WUEtree - Understanding the genetic and environmental determinisms of water use efficiency to improve sustainability of forest tree plantations (Project ID: ANR-13-ADAP-0012) - as a collaborative project with Marina de Miguel Vega.

Abstract

In the context of climate change and increasing water stress due to drought events, tree breeders are considering new selection criteria to dispose of genotypes that can resist droughts and maintain productivity under changing environmental conditions. Prior to such a program, there is an urgent need to assess the intra-specific variability of functional traits related to survival and growth. We performed a multi-trait analysis of leaf and xylem functioning including seasonal measurements of photosystem II fluorescence, gas exchange and stem hydraulic traits on a full-sib family of provenance hybrid of maritime pine (*Pinus pinaster* Ait.) established in Cestas (France). Xylem vulnerability to embolism was assessed on 480 individuals of inter "Morocco **x** Landes" and "Corsica **x** Landes" families and leaf functioning traits were performed on a subsample consisting of 108 individuals from the "Morocco **x** Landes" family. The mean P_{50} values varied from -3.2 MPa to -4.2 MPa and were in average of -3.6 and -3.8 MPa for "Corsica x Landes" and "Morocco x Landes" families, respectively. The efficiency of both photosystem II and stomatal conductance declined with increasing seasonal water stress, resulting in a rapid decrease in assimilation rate. We found no evidence of trade-off between xylem resistance to embolism (P_{50}) and leaf functioning traits indicating the possibility to consider selection for both efficient and resistant genotypes in future.

Introduction

Ongoing climate change is rapid compared to past climate change questioning the ability of long-lived organisms such as trees to adapt to the changes. For example, the rate of warming increased from 0.097 ° C per decade in the early 20th century (1901-1950) compared to 0.254 ° C between 1979 and 2012 (Hartmann et al. 2013). Watering regimes are also expected to increase in intensity and number of drought events (Meel and Tabaldi 2004). Maintaining species in the new environment will be possible provided populations have adaptive capacities to withstand growing constraints (Davis and Shaw 2001). To assess the adaptive capacity of trees to climate change or to select trees that are better suited to new conditions, the phenotypic and genetic diversity of many individuals should be assessed at inter- and intra-specific levels.

The study of the intra-specific variability of ecophysiological traits is less developed on forest trees (Olivas-García et al 2000, Gutierrez et al., 2002, Bigras 2005) compared to fruit trees (Petridis et al., 2012, Pettigrew and Turley 1998, Sofo et al 2009, Viljevac et al., 2013). Forest studies have focused on morphological features (SLA, leaf shape) and growth (e.g. Martinez Meier et al., 2008). However, in order to understand the variability of responses in a changing environment, to identify or select suitable genotypes or populations that are resistant to increasing stresses and / or capable of maintaining efficient functioning in their new environment, a detailed analysis of the functioning of forest trees is paramount.

In the analysis of the resistance to water stress, recent developments have demonstrated that xylem vulnerability to cavitation is a good indicator of the resistance of species to drought (Brodribb et al. 2010, Choat et al. 2012, Urli et al. 2013). At the intra-specific level, first analyses evaluating phenotypic and genotypic variability of cavitation resistance (Lamy et al., 2012, 2014) suggested that it was possible to identify individuals more resistant to water stress. Apart from resistance to drought and cavitation, water and carbon plant functioning in response to drought is of interest to investigate in order to select genotypes that can maintain some production functions under changing environments. Reduction of carbon assimilation in response to water stress is resulting from either stomatal closure or biochemical limitations. Both limitations are changing with the magnitude and duration of water stress, the biochemical limitation inducing 50% loss of carbon assimilation under severe stress (Grassi and Magnani 2005). The maintenance of photosynthetic capacities in response to water stress was identified as an adaptive character by comparing two Nothofagus species (Piper and al. 2007). Moreover, at the intra-specific level, families were shown to have distinct responses to drought, maintaining their leaf functioning (photosynthesis, stomatal opening) in the event of water stress, as shown by Bigras (2005) on spruce (*Picea glauca*) or Gilbert et al. (2011) on soybean. A recent study made it possible to distinguish poplar clones by differentiating their photosynthetic capacities (Guo et al., 2010). However, there also appears to be a trade-off between resistance to water stress (cavitation, defoliation) and foliar functional capacities (photosynthesis, water use efficiency, growth) at the inter-specific level (Fu et al., 2012) or intra-specific level (Bigras 2005): the most resistant species or families also demonstrated the most limited growth.

Maritime pine (*Pinus pinaster* Aït.) is a Mediterranean tree species, whose range is expanding from Morocco in the South to the Aquitania region - France in the North and East, to Greece in the West. Its different provenances are presenting variability in some characters such as more drought resistant in Spain and Morocco, more productive in the Landes region, higher stem straightness in Corsica (Harfouche and Kremer 2000). In France, the species represent 6% of its forested area, 5% of its standing volume and more than 26% of its annual wood production (IGN 2016). A breeding program was initiated in the 60s to improve volume production and stem rectitude (Bouffier et al. 2009). As a consequence, in 2010, 25% of Maritime pine plantations in the Landes de Gascogne forests were corresponding to one of the two selected varieties. In the context of climate change and increasing water stress, breeding is considering new selection criteria related to the plant response to water availability and other strategies such as provenance crossings. Prior to such a program, it is compulsory to analyse the intra-specific variability of functional traits related to water and carbon functioning.

The objective of this study was to investigate the intra-specific variability within a Maritime pine tree family in response to water availability by performing a multi-trait analysis of leaf and xylem functioning through seasonal measurements of photosystem II fluorescence, gas exchange and stem hydraulic properties. Our objective was to evaluate the level of variability of these traits, as well as identifying potential trade-offs between these characters. This will be the first step towards selecting genotypes having the ability to sustain changes in water availability by resisting to drought, while maintaining the same growth rates during pre or post water stress phases.

Materials and Methods

Site and sampling

Analyses were performed on adult *Pinus pinaster* in the field, to avoid working on potted seedling in limited soil environment. The Abiogen trial (Figure 1) is a full-sib family essay planted in 2007 in Cestas (France) as a repeated block design: each block contains full-sibs from either Morocco x Landes, Corsica x Landes or Corsica x Morocco families (see Lagraulet 2015 for more details). The study was performed in spring (April, May) and summer (July) of 2015 and 2016 on two blocks of the Morocco x Landes family, corresponding to 108 trees (except for gas exchanges in summer 2015, performed on 40 individuals).

During each campaign, we sampled and measured 10 to 12 trees per day. Leaf physiological measurements were performed on 1-year-old needles selected on the southern branch of the 2- or 3-year-old whorl. Before sunrise, 4 pairs of needles were collected per tree, put into tubes closed with a cap, where wet paper placed inside the tube maintained saturated air humidity to avoid plant transpiration.

Lab measurements

Immediately arriving at the lab, one pair of needles was used for predawn water potential measurements using a Scholander pressure bomb. The second pair was used to perform dark adapted fluorescence measurements using a PAM 2100 (Heinz Walz GmbH, Effeltrich, Allemagne) to get maximum F_m and minimum F_0 fluorescence values. The third and fourth pairs were cut under water at the base and put into water, for further gas exchanges and fluorescence measurements. After at least one hour under lamps, fluorescence of light adapted needles was measured using the PAM 2100 to get maximum and minimum levels of fluorescence of light adapted needles, F_m' and F_0'. Using these measurements, fluorescence parameters of maximum efficiency of photosystem II (Fv/Fm), actual efficiency of photosystem II (yield), electron transport rate ETR and non-photochemical quenching (NPQ) were calculated according to equations and definitions of Roháček (2002).

Gas exchanges were performed with a Walz GFS 3000 (Heinz Walz GmbH, Effeltrich, Allemagne) to get steady-state values of Assimilation (A, µmol.m^{-2}.s^{-1}), transpiration (E, mmol.m^{-2}.s^{-1}) and stomatal conductance (gs, mmol.m^{-2}.s^{-1}). Conditions within the measuring chamber were air CO2 concentration of 400 ppm, air temperature of 20°C and relative humidity of 70% to get a constant air VPD, PAR light intensity of 1800 µmol.m^{2}.s^{-1}. Water use efficiency and instantaneous water use efficiency were calculated as the ratio of A to E or gs, respectively.

After gas exchange measurements, needles were measured in diameter / length using an electronic caliper (10-2mm) and a ruler (mm) to estimate their leaf area, then placed in an over to reach constant dry weight (g) to get SLA estimations.

A total of 480 individuals were sampled for xylem hydraulic measurements during two campaigns, first from October 2014 to February 2015 and second from January 2016 to March 2016. In order to measure the same number of rings in each sample, branches were collected on the 2012 whorl to further measured 2 year-old growth units (2013 during the 1st campaign, 2014 during the second campaign). For each individual, a branch fully exposed to the sun with a basal diameter less than 1 cm was collected early in the morning. All needles were immediately removed, branches were labeled and placed in black bags, wrapped in wet paper towels to avoid transpiration and dessication. They were then kept wet and cool (3°C) until measurement within maximum three weeks after field sampling. Prior to embolism resistance measurement, branches were cut under water to a standard length of 27 cm, and bark was removed with a razor blade.

Xylem vulnerability to embolism was assessed using the CAVITRON, a centrifuge technique following the procedure described by Cochard (Cochard 2002; Cochard et al. 2005) at the high-throughput phenotyping platform for hydraulic traits (CavitPlace, University of Bordeaux, Talence, France). Centrifugal force was used to establish negative pressure in the xylem and to provoke water stress-induced embolism, using a custom-built honeycomb rotor (Sam Precis 2000, Bordeaux, France) mounted on a high-speed centrifuge (Sorvall RC5, USA). This technique enables measurement of the hydraulic conductance of a branch under negative pressure. Xylem pressure (P) was first set to a reference pressure (-0.5 MPa) and maximal conductance (Ks) was determined by measuring the flux of a reference ionic solution (10 mM KCl and 1mM CaCl2 in deionized water) through the sample. The centrifugation speed was then set to a higher value for 3 min to expose the sample to a more negative pressure. Conductances were measured three times for each step, and the average value was used to compute the percent loss of xylem conductance (PLC in %). The procedure was repeated for at least eight pressure steps with a -0.3 to -0.5 MPa step increment until PLC reached at least 90%. Rotor velocity was monitored with a 10 rpm resolution electronic tachymeter and xylem pressure was adjusted to about -0.02 MPa. We used the Cavi_soft software (version 4.0, for the first campaign and version 5.0 for the second campaign, BIOGECO, University of Bordeaux) for conductance measurements.

Data and statistical analyses

Hydraulic measurements were analysed using vulnerability curves (VC), the percent loss of xylem conductivity plotted as a function of xylem pressure (MPa). A sigmoid function (Pammenter and Van der Willigen, 1998) was fitted to the VC from each sample using the following equation:

$$PLC = \frac{100}{\left[1 + \exp\left(\frac{S}{25} x \left(P - P_{50}\right)\right)\right]} \qquad (1)$$

where P50 (MPa) is the xylem pressure inducing 50% loss of hydraulic conductivity and S (% MPa -1) is the slope of the vulnerability curve at the inflexion point.

The xylem-specific hydraulic conductivity (K_s, m^2 MPa^{-1} s^{-1}) was calculated by dividing the maximum hydraulic conductivity measured at low speed by the sapwood area of the sample.

Correlation between ecophysiological traits were performed on the 108 individuals of the Morocco x Landes family that were used for both stem hydraulic measurements and leaf physiological measurements (40 individuals only, for gas exchanges in July 2015). Analyses were performed using the SAS Proc CORR (SAS 9.4, SAS Institute Inc., Cary, NC, USA).

Results

Both years were corresponding to two different water availability conditions (Figure 2): in spring 2015 and 2016, predawn leaf water potential remained high (close to 0)) and drop to more negative values during the summer, especially in 2015 where it reached -1.5 MPa. As a result of decreasing water potential, leaf functioning decreased, whatever the trait. Assimilation rate decreased rapidly (Figure 3A) as a consequence of both a decrease in the efficiency of photosystem II (Figure 3B) and in stomatal conductance (Figure 3C). The decrease in efficiency of photosystem II was marked on light adapted needles as the actual yield of light efficiency decrease from 30 to 10%. The potential efficiency of photosystem II, Fv/Fm, slightly decreased with water potential indicating that the photosynthesis apparatus was partly damaged in response to water stress.

The decrease in both assimilation and stomatal conductance resulted in a decrease in water use efficiency, in response to water stress (Figure 3D), as assimilation rate presented a steeper decrease in response to decreasing water potential.

All physiological leaf traits presented a large variability between genotypes (Figure 3, Table 1). Traits related to water functioning (E, gs, WUE, WUEi) were more variable under water-stressed conditions in July 2015, compared to spring 2015 or 2016, whereas traits related to photosynthesis (ETR, yield, A) were less variable under stressed conditions.

Vulnerability curves are all s-shaped (Figure 4) and P_{50} values vary from -3.2 MPa to -4.2MPa. Figure 5 shows that more than 70% of the genotypes has a P_{50} value ranging in between -3.5 and -3.9MPa. The average P_{50} values were -3.6 and -3.8MPa for “Corsica x Landes” and “Morocco x Landes” families, respectively. The difference between families is highly significant (p<0.0001). There was no trade-off between hydraulic safety (P_{50}) and hydraulic efficiency (K_s) for both Corsica x Landes ($P = 0.836$) and Morocco x Landes ($P = 0.1867$) families (Figure 7).

Overall, there was no correlation between xylem resistance to cavitation quantified using P_{50} and the leaf functioning traits we measured in the Morocco x Landes family (Table 2), neither under stressful nor under non-stressful water conditions. The only significant correlations were observed under the non-stressful conditions of April 2015: a weak positive relationship was observed with photosynthetic variables (ETR, Yield) and a negative one with water use efficiency (WUE, WUEi). However, these relationships were not significant through similar water conditions in 2016, nor under stressful conditions (July 2015-16). In particular, it is worth noticing that there is no significant and consistent trade-off (negative relationship) between plant functioning and plant resistance. However, there was a significant linear relationship between stem hydraulic conductivity and the actual efficiency of photosystem II under non stressful conditions in spring 2015: plants with the highest water conductivity also presented the highest light use efficiency (Figure 6).

Discussion - conclusion

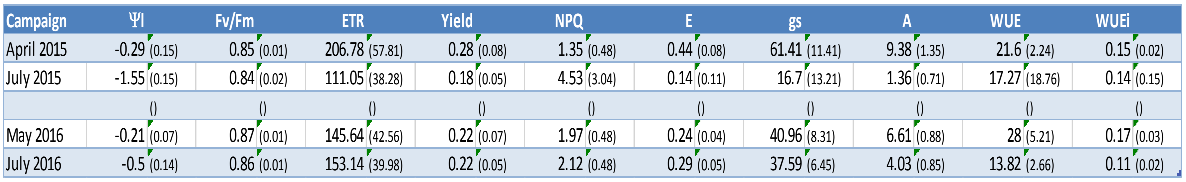
Through the analysis of two years of physiological measurements under well-watered and water-stressed conditions, we investigated the variability of leaf traits related to transpiration and assimilation in a full-sib Maritime pine family. This variability was also analysed according to P50 values measured on stem xylem, without evidencing proof of any trade-off between plant functioning and plant resistance to cavitation.

Our results showed that decreasing water potential resulted in rapid decrease in assimilation rate due to decrease in efficiency of photosystem II and in stomatal conductance within maritime pine. Water deficit-induced decrease in photosynthetic productivity was also observed by López et al (2009) who studied the responses to water deficit in seedlings of *Pinus canariensis* from five provenances under controlled conditions. Transpiration control for ensuring nutrients supply and regulating leaf temperature, and maintaining CO_2 entry of into the leaf, are the main functions of stomata. Photosynthesis is primarily restrained by stomatal closure in response to water deficit. (Flexas and Medrano 2002), affecting plant growth and temperature regulation under drought conditions. The magnitude of the variability of the leaf physiological traits within the Landes x Morocco maritime pine family was of the same magnitude than the one observed for similar traits within a Spanish maritime pine full-sib family (Oria x Pontevedra, de Miguel et al. 2011). The decrease in maximum efficiency of PSII with water-stress was minimum compared to the one observed in provenances of *Pinus canariensis* submitted to drought (from 0.77 to 0.4, Lopez et al. 2009), or in *Pinus halepensis* (from 0.82 to 0.6, Prieto et al. 2009); this would indicate that the photosynthetic apparatus of *P. pinaster* was structurally less affected by the water stress compared to the one of *P. canariensis* or *P. halepensis*.

Under non stressful conditions, there was a significant relationship between stem hydraulic conductivity and the actual efficiency of PSII; this is showing that the efficiency of leaf functioning traits is strongly affected by the supply capacity of the hydraulic system. Our results are in agreement with Brodribb and Field, (2000) who also found a strong relationship between hydraulic supply of water (hydraulic conductivity) to leaves and maximum photosynthetic capacity (quantum yield) in a community of rainforest comprising of conifers and angiosperms suggesting that the maximum photosynthetic rate of leaves was constrained by their vascular supply. Similar results showing significant positive correlations between leaf-specific hydraulic conductivity of upper branches and maximum rates of net CO_2 assimilation per unit leaf area and stomatal conductance (gs) were also found across 20 species of canopy trees (Santiago et al, 2004). The variability within the Maritime pine family was of the same magnitude than the one observed at the inter-specific level both on coniferous and broadleaved trees (Brodribb and Field 2000). Our results indicate that such a relationship exists not only at interspecific but also at intraspecific level.

On the contrary, during the stress, hydraulic conductivity was not linked to leaf physiology, and overall the resistance to cavitation of the stem was not correlated to functional leaf traits. Since both Pine species present a similar vulnerability to cavitation as assessed by their P50 value, -3.9 for *P. pinaster* (Bouche et al. 2016) and -3.9 / - 4 MPa for *P. canariensis* (Lopez et al. 2013) under temperate climate, this suggests that there is no relationship between the xylem vulnerability and the leaf functioning. The absence of trade-off between resistance and functioning and the existence of a large variability in the traits would leave open the possibilities for a breeding program, to select efficient and resistant genotypes of Maritime pine. To go further into these considerations, investigations could however focus on different genetic material, and should consider the genetic determinisms of this variability, through association genetics studies.

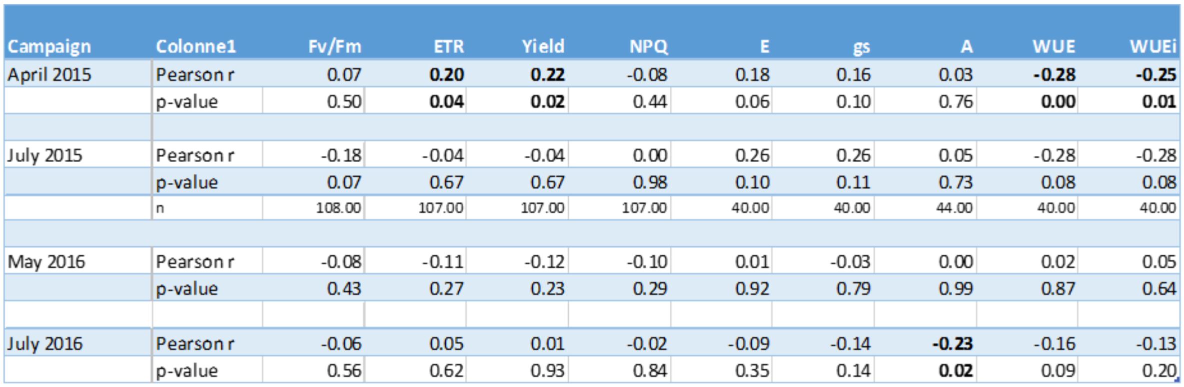
Table 1: Mean (SD) of leaf physiological traits per campaign



Campaign	Ψl	Fv/Fm	ETR	Yield	NPQ	E	gs	A	WUE	WUEi
April 2015	-0.29 (0.15)	0.85 (0.01)	206.78 (57.81)	0.28 (0.08)	1.35 (0.48)	0.44 (0.08)	61.41 (11.41)	9.38 (1.35)	21.6 (2.24)	0.15 (0.02)
July 2015	-1.55 (0.15)	0.84 (0.02)	111.05 (38.28)	0.18 (0.05)	4.53 (3.04)	0.14 (0.11)	16.7 (13.21)	1.36 (0.71)	17.27 (18.76)	0.14 (0.15)
	()	()	()	()	()	()	()	()	()	()
May 2016	-0.21 (0.07)	0.87 (0.01)	145.64 (42.56)	0.22 (0.07)	1.97 (0.48)	0.24 (0.04)	40.96 (8.31)	6.61 (0.88)	28 (5.21)	0.17 (0.03)
July 2016	-0.5 (0.14)	0.86 (0.01)	153.14 (39.98)	0.22 (0.05)	2.12 (0.48)	0.29 (0.05)	37.59 (6.45)	4.03 (0.85)	13.82 (2.66)	0.11 (0.02)

Ψl predawn leaf water potential (MPa), Fv/Fm maximum efficiency of PSII, ETR electron transport rate, Yield actual efficiency of PSII, NPQ non photochemical quenching, E leaf transpiration rate (mmol.m^{-2}.s^{-1}), gs stomatal conductance (mmol.m^{-2}.s^{-1}), A assimilation rate (µmol.m^{-2}.s^{-1}), WUE water use efficiency (µmol.mmol^{-1}), WUEi intrinsic water use efficiency (µmol.mmol^{-1}).

Table 2: Correlation between P50 and leaf physiological traits.



Campaign	Colonne1	Fv/Fm	ETR	Yield	NPQ	E	gs	A	WUE	WUEi
April 2015	Pearson r	0.07	**0.20**	**0.22**	-0.08	0.18	0.16	0.03	**-0.28**	**-0.25**
	p-value	0.50	**0.04**	**0.02**	0.44	0.06	0.10	0.76	**0.00**	**0.01**
July 2015	Pearson r	-0.18	-0.04	-0.04	0.00	0.26	0.26	0.05	-0.28	-0.28
	p-value	0.07	0.67	0.67	0.98	0.10	0.11	0.73	0.08	0.08
	n	108.00	107.00	107.00	107.00	40.00	40.00	44.00	40.00	40.00
May 2016	Pearson r	-0.08	-0.11	-0.12	-0.10	0.01	-0.03	0.00	0.02	0.05
	p-value	0.43	0.27	0.23	0.29	0.92	0.79	0.99	0.87	0.64
July 2016	Pearson r	-0.06	0.05	0.01	-0.02	-0.09	-0.14	**-0.23**	-0.16	-0.13
	p-value	0.56	0.62	0.93	0.84	0.35	0.14	**0.02**	0.09	0.20

Fv/Fm maximum efficiency of PSII, ETR electron transport rate, Yield actual efficiency of PSII, NPQ non photochemical quenching, E leaf transpiration rate (mmol.m^{-2}.s^{-1}), gs stomatal conductance (mmol.m^{-2}.s^{-1}), A assimilation rate (µmol.m^{-2}.s^{-1}), WUE water use efficiency (µmol.mmol^{-1}), WUEi intrinsic water use efficiency (µmol.mmol^{-1}). Except when indicated otherwise (n), number of data used for the analyses was 108. Significant correlations ($p < 0.05$) are indicated in bold characters.

Figure captions:

Figure 1: Abiogen full-sib family essay. Each block contains individuals (dots) of a full-sib family. Measurements were performed on two blocks of the Landes x Morocco family (bold letters).

Figure 2: Predawn leaf water potential (average + standard deviation) during each campaign (April and July 2015, May and July 2016)

Figure 3: Leaf functioning as a function of predawn leaf water potential: (A) assimilation (A, µmol.m-2.s-1). (B) Potential efficiency of photosystem II (Fv/Fm, blue dots) and actual efficiency of PSII (Yield, red dots). (C) Stomatal conductance (gs, mmol.m-2.s-1). (D) Intrinsic water use efficiency (WUEi, µmol.mmol-1).

Figure 4: Vulnerability curves obtained on 480 individuals from the two studied families growing in common garden. Each color represents a different individual.

Figure 5: Frequency distribution of P_{50} (pressure inducing 50 % loss of hydraulic conductance) in both "Corsica x Landes" and "Morocco x Landes" families of maritime pine.

Figure 6: Actual efficiency of photosystem II for light adapted needles (Yield) as a function of xylem specific hydraulic conductivity (Ks, kg.m-1.s-1.MPa-1).

Figure 7: Relationship between hydraulic safety (P_{50}, MPa) and hydraulic efficiency ((K_s, m^2 MPa^{-1} s^{-1}) across Corsica x Landes (blue) and Morocco x Landes (orange). N = 240 for each Corsica x Landes and Morocco x Landes families.

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Figure 1:

Abiogen full-sib family essay. Each block contains individuals (dots) of a full-sib family. Measurements were performed on two blocks of the Landes x Morocco family (bold letters).

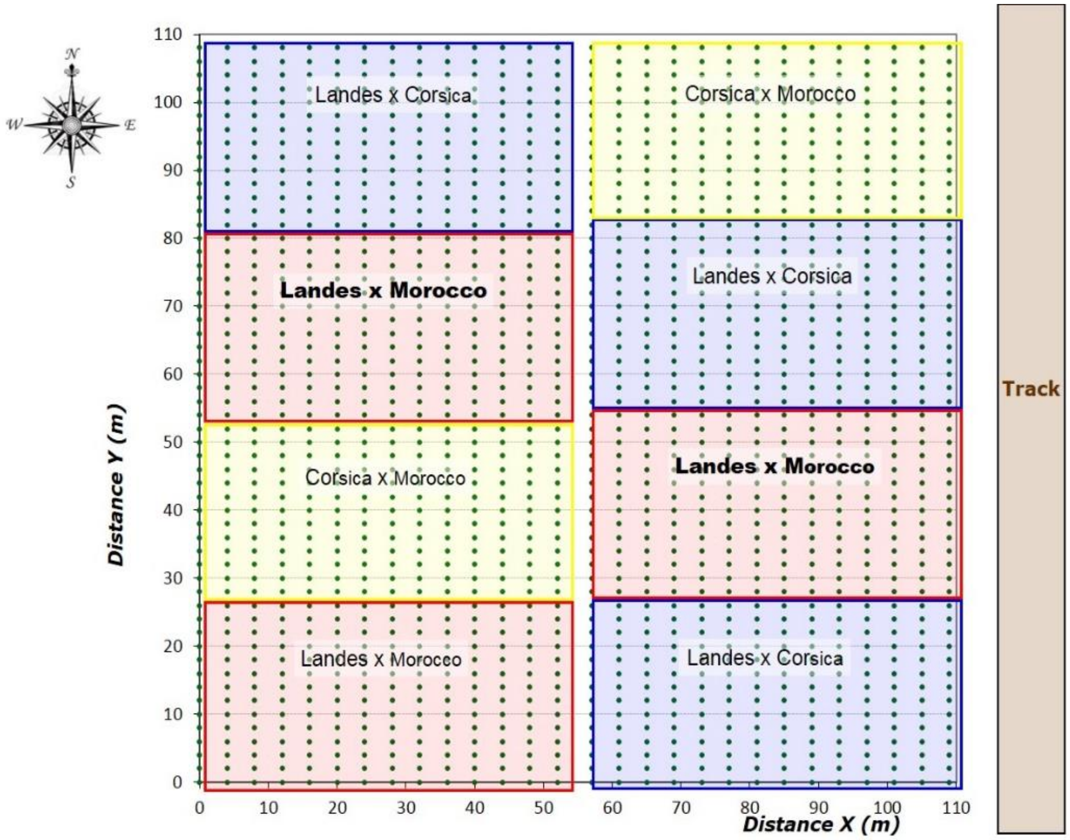


Figure 2:

Predawn leaf water potential (average + standard deviation) during each campaign (April and July 2015, May and July 2016).

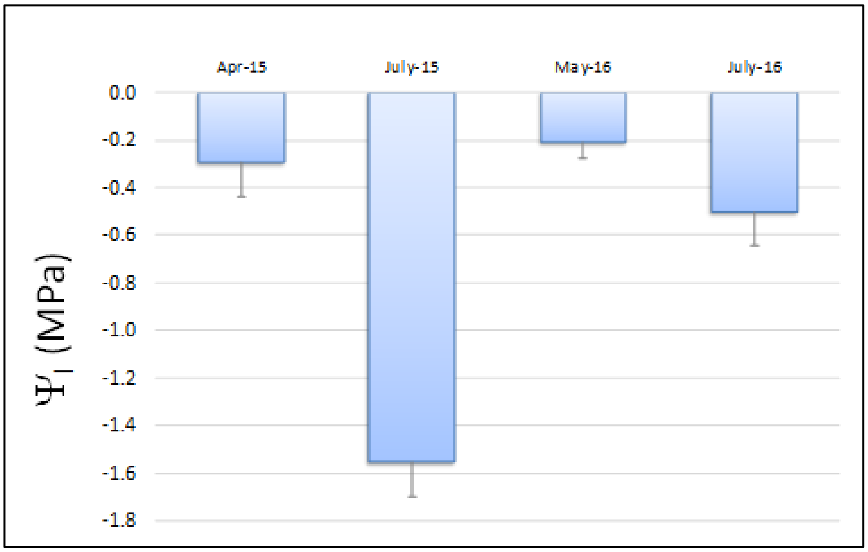


Figure 3:

Leaf functioning as a function of predawn leaf water potential: (A) assimilation (A, µmol.m-2.s-1). (B) Potential efficiency of photosystem II (Fv/Fm, blue dots) and actual efficiency of PSII (Yield, red dots). (C) Stomatal conductance (gs, mmol.m-2.s-1). (D) Intrinsic water use efficiency (WUEi, µmol.mmol-1).

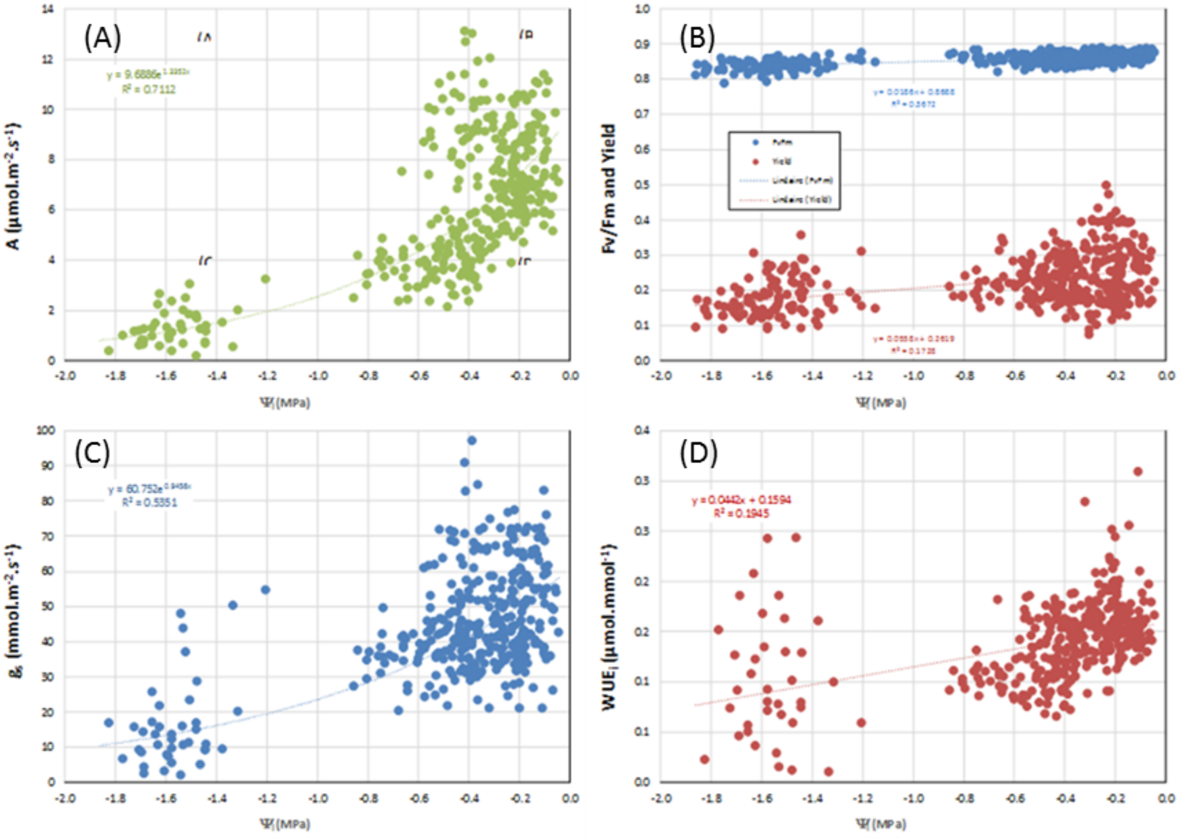


Figure 4:

Vulnerability curves obtained on 480 individuals of inter "Morocco **x** Landes" and "Corsica **x** Landes" families of maritime pine (*Pinus pinaster* Ait.) established in Cestas (France). Each color represents a different individual.

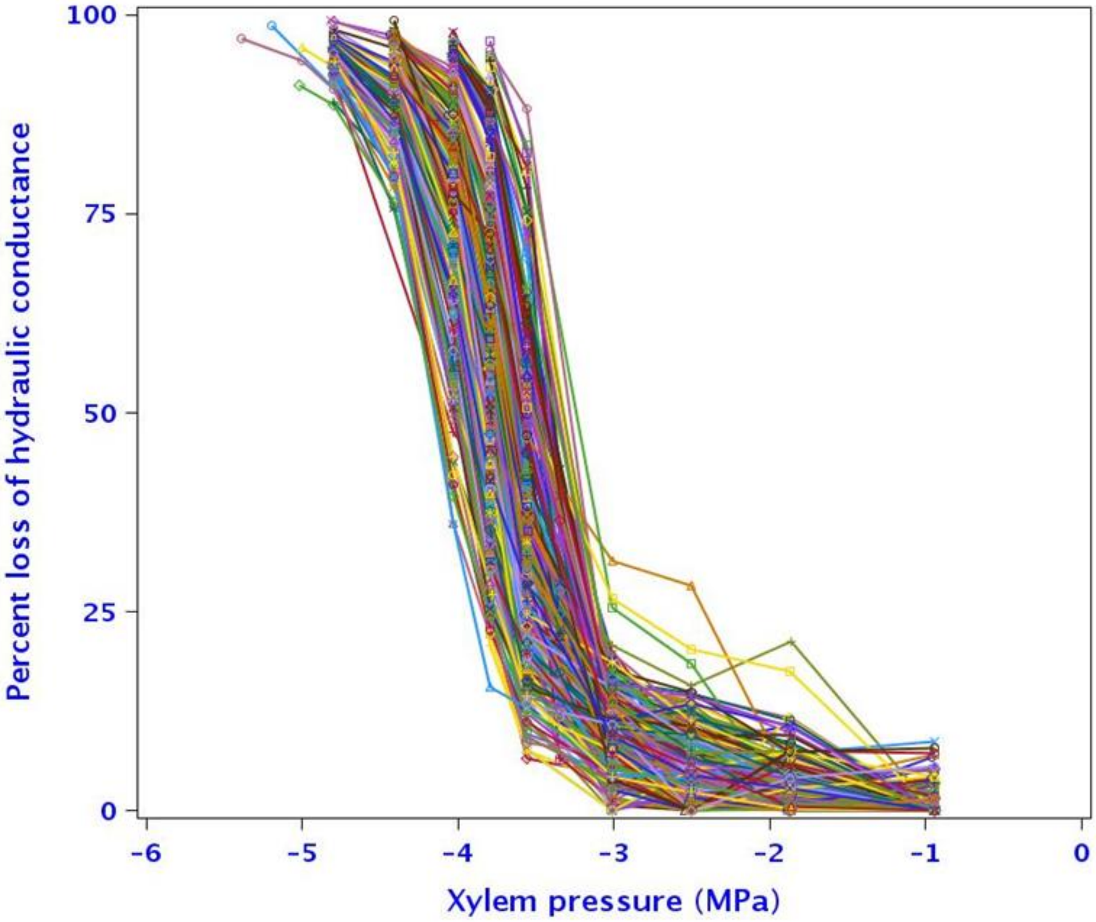


Figure 5:

Frequency distribution of P_{50} (pressure inducing 50 % loss of hydraulic conductance) in both“Corsica x Landes” and “Morocco x Landes” families of maritime pine (*Pinus pinaster* Ait.).

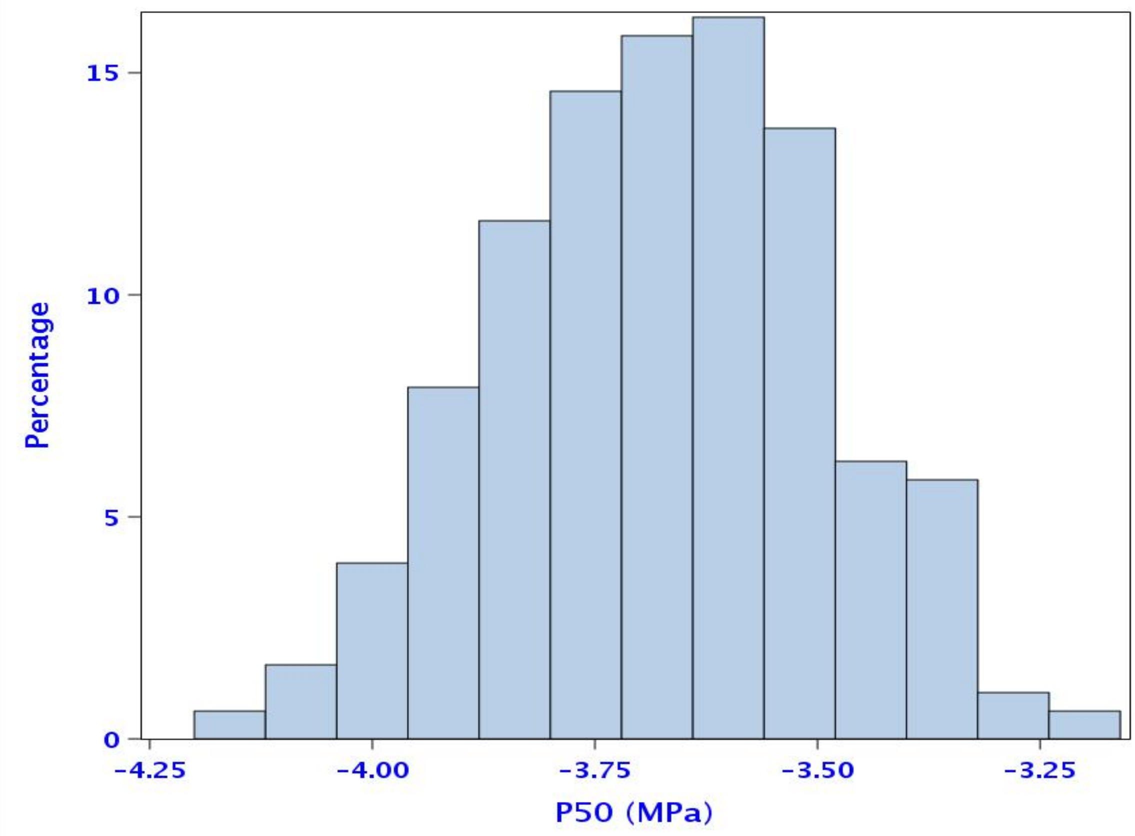


Figure 6:

Actual efficiency of photosystem II for light adapted needles (Yield) as a function of xylem specific hydraulic conductivity (Ks, kg.m-1.s-1.MPa-1).

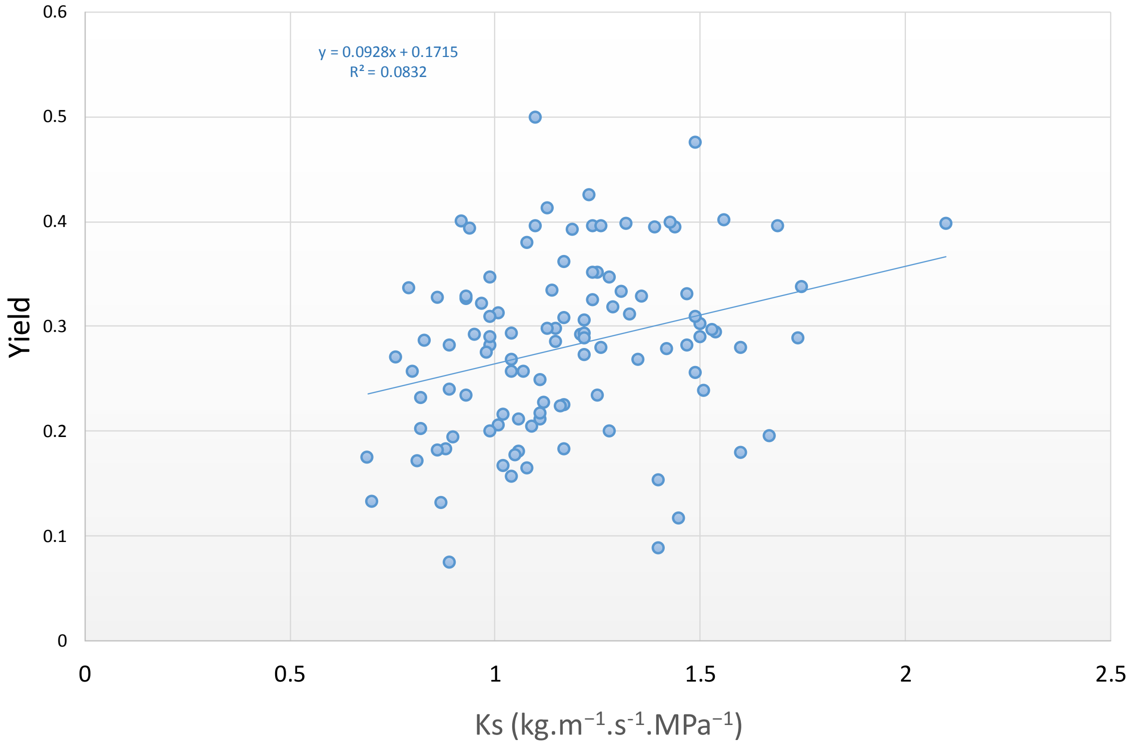
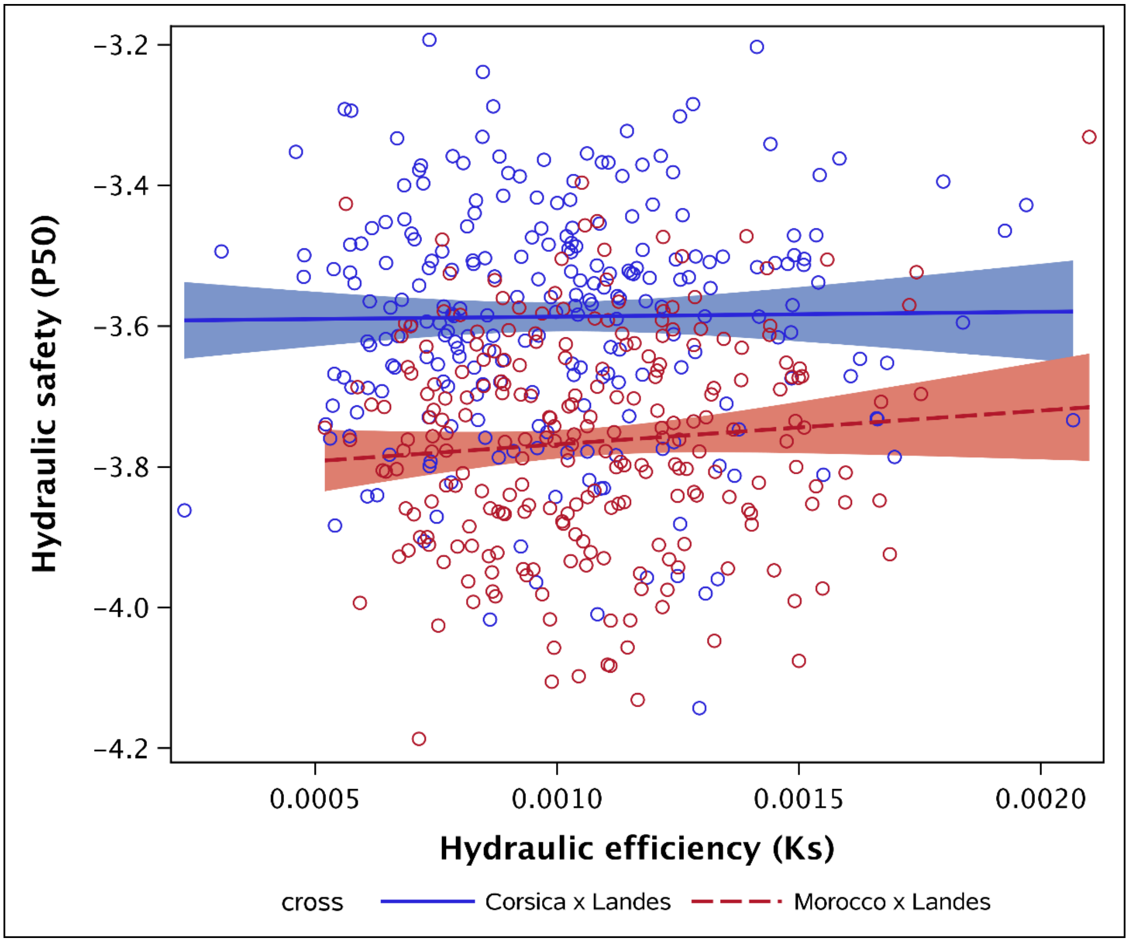


Figure 7: Relationship between hydraulic safety (P_{50}, MPa) and hydraulic efficiency ((K_s, m^2 MPa^{-1} s^{-1}) across Corsica x Landes (blue) and Morocco x Landes (orange). N = 240 for each Corsica x Landes and Morocco x Landes families.



Discussion

Discussion

The aim of this project was to investigate the intraspecific variability in embolism resistance in annual crop and conifer tree species and to find the relationship between hydraulic traits and traits related to wood density, xylem anatomy, genetics and leaf physiology.

In Part I, we provide a novel examination of variation in vulnerability to drought in sunflower. Few studies have considered the possibility of using embolism resistance to find more drought resistant crop varieties. We therefore investigated and determined vulnerability to embolism across four sunflower accessions growing under same environmental conditions with significant variations in P_{50} (pressure inducing 50% loss of hydraulic conductivity) ranging between -2.67 and -3.22 MPa. Moreover, the study of interactions between hydraulic traits, growth traits and xylem anatomical traits highlighted the potential trade-offs between hydraulic safety and growth but not between hydraulic safety and efficiency. The thorough tests of correlation between anato-morphological features and vulnerability provides a complete picture of the key factors interacting to determine P_{50}. We indeed found that several anatomical traits, such as vessel density and vessel lumen area correlated with embolism resistance.

In part II we provide new insight into the genetic determinism of resistance to embolism at intraspecific level in a large-sized three-generation inbred pedigree of *Pinus pinaster*. We dissected the genetic architecture of embolism related traits (hydraulic safety) and wood density related traits, and tested the relationship between wood density and xylem safety at the phenotypic and QTL level. Mean P_{50} value was found to be around -3.70 ± 0.21 MPa in both chapter 2 (Corsica x Landes) and chapter 3 (Corsica x Landes and Morocco x Landes families) with considerably low variability for P_{50} (CV_P=5.6%). There were a lack of relationship between hydraulic safety and hydraulic efficiency, wood density and leaf functional traits. The results also explained that embolism resistant genotypes in this family are not dependent on denser wood as no QTL co-located between P_{50} and wood density.

Vulnerability curves assessment in long vesselled species

For the construction of vulnerability curves (VCs) in sunflowers, from which the embolism resistance of a sample is estimated, notably from the inflexion point, i.e., the pressure at which 50% of total xylem hydraulic conductance is lost (P_{50}, in Mega Pascal), and the slope of the curve

(S, in % MPa^{-1}), we use 42 cm rotor diameter. This large-diameter rotor was developed for species having long vessels in order to avoid the so called 'open vessel' artifact that may favor exponential (r-shaped) vulnerability curves. This has been demonstrated several times in woody species with long vessels (Cochard et al., 2013; Martin-StPaul et al., 2014; Torres-Ruiz et al., 2014, 2017; Cochard et al., 2015; Choat et al., 2016). Prior to the main experiment, we collected six sunflower stems to test whether we were able to accurately assess vulnerability curves with the standard cavitron (rotor diameter of 27 cm). All VCs obtained with this standard rotor were r-shaped while all VCs obtained with the 42 cm large rotor were s-shaped (see Figure. 1). Then, we performed maximum vessel lengths measurements on six stems by injecting air at 2 bars and cutting the apical end of the water-immersed stem section until the air bubbles emerged. This procedure allowed us to find that the sunflower stems have a maximum vessel length of 23 cm and therefore confirm that the VCs cannot be assessed with the standard cavitron. Indeed a significant proportion of open-cut vessels surpass the center of the plant segment or even permeate through its whole length when using a 27 cm large rotor.

Embolism resistance is typically assessed by decreasing xylem pressures and determining the corresponding losses in hydraulic conductivity (K_s). However, by using centrifuge technique, it is not possible to determine with certainty whether this loss in K_s reflects patterns of embolism that occur *in situ* or whether it comes from an artefact due to the presence of open vessels. The issue of this open-vessel artefact may be avoided and the process of embolism formation be evaluated with greater reliability by the use of alternative techniques that enables the direct observation of xylem embolism in a non-invasive, unbiased manner, such as MRI (Holbrook et al., 2001; Kaufmann et al., 2009; Choat et al., 2010) and X-ray micro tomography (Cochard et al., 2015; Torres-Ruiz et al., 2015, 2016; Bouche et al., 2016; Brodribb et al., 2016; Choat et al., 2016; Torres-Ruiz et al., 2017).

Intraspecific variability in embolism resistance in sunflower and maritime pine

Mean value of embolism resistance (P_{50}) for four sunflower accessions measured was - 2.99 ± 0.15 MPa. There was a significant intraspecific variability in resistance to embolism among four accessions ranging from - 2.67 to - 3.22 MPa, between the most vulnerable and the most resistant accessions, respectively.

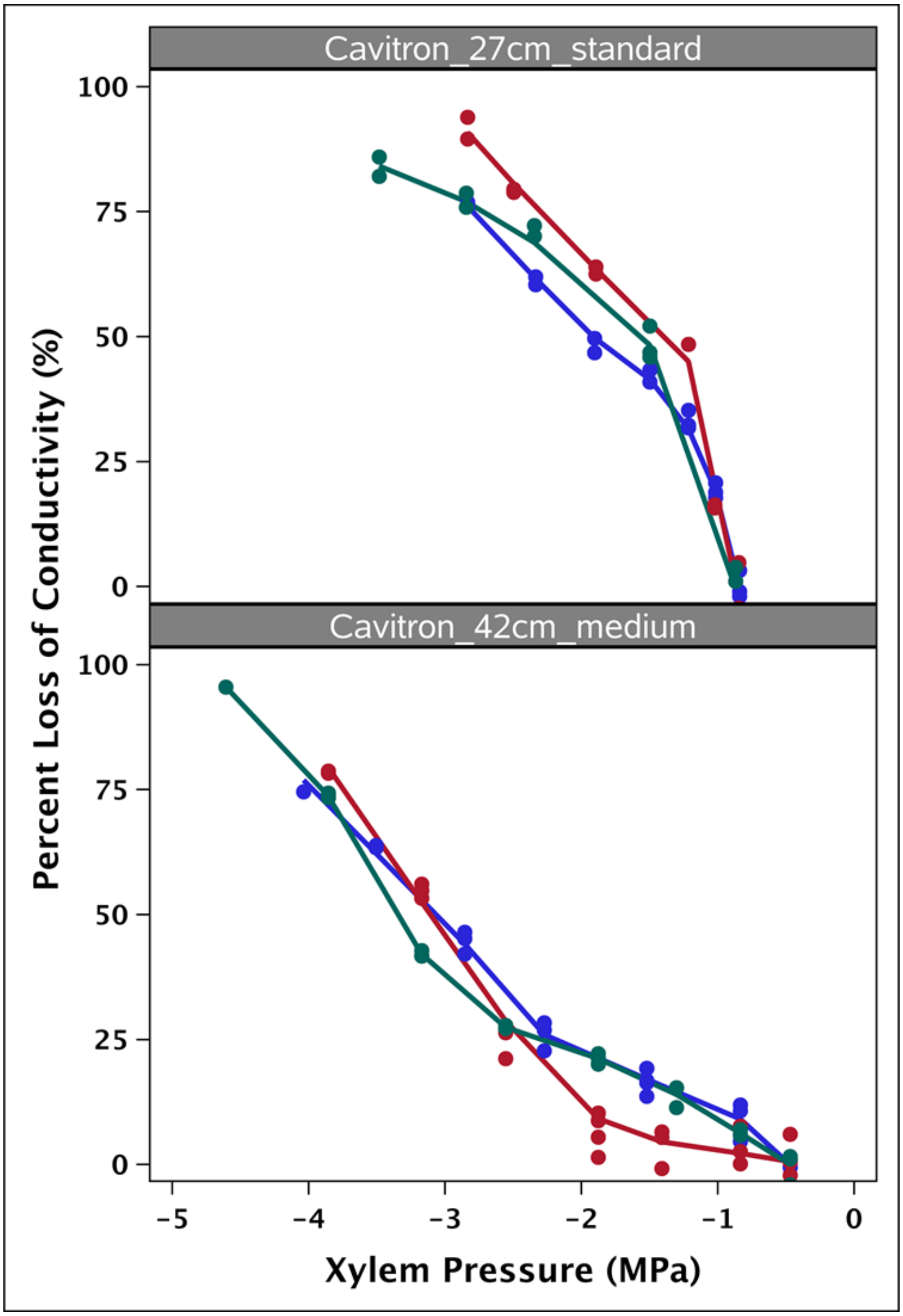


Figure: 1. Vulnerability curves (VCs) of six sunflower stems, for which xylem embolism was induced by in situ flow centrifugation according to the Cavitron technique. Three stems were measured with the standard rotor design (27cm, top panel) and three with the medium size rotor (42cm, bottom panel). VCs are expressed as the percentage loss of hydraulic conductivity (PLC) as a function of xylem pressure. This figure shows the discrepancy between the two rotor sizes: all VCs were r-shaped with the 27cm diameter rotor and s-shaped with the 42 cm diameter rotor, leading to a difference of 2MPa between them. The r-shaped curves (top panel) result from an open vessel artifact (Torres-Ruiz et al., 2014, 2017).

The difference in P_{50} values between the most resistant sunflower accession, the early-sown ES_Ethic, and the most vulnerable accession, the late-sown LG_5660 was found to be 0.55 MPa. Apart from showing significant intraspecific differences in vulnerability to embolism, these findings may also help in terms of developing selection and breeding strategies in future for drought prone areas on the basis of the greater resistance of specific sunflower accessions to xylem embolism. In comparison to many other crop species, sunflower is better able to resist drought events and is often cultivated in arid regions, however, like many other oil seed crops, sunflower is also sensitive to water stress, especially at the germination stage (Ahmad et al., 2009). Stiller and Sperry, (2002) reported variation in P_{50} (from -0.8 to -3 MPa) in a single sunflower genotype subjected to drought and re-watering cycles with mean P_{50} value -3.0 ± 0.1 MPa under well-watered conditions, which is quite similar to one found in our study. Despite the critical role of xylem resistance to embolism in plant survival during drought events (Anderegg et al. 2015), this trait is less studied in crops (Tyree et al., 1986; Sperry et al., 2003) and hence in sunflower. However, in some studies, decrease in leaf water potential in various sunflower lines has been observed due to water deficit (Ashraf and O'Leary, 1996). Leaf water potential in sunflower normally ranges between - 0.48 and - 1.74 MPa under different agro-climatic conditions (Prasad et al., 1985; Rachidi et al., 1993), however, under reduced water supply, it can further drop as below as - 3.0 MPa (Wise et al., 1990). This species seems therefore to have a null safety margin and works close to its hydraulic failure limit as already reported for trees by Choat et al. (2012). Moreover, it has been reported that dwarf sunflower lines are more resistant to drought than tall lines, showing a smaller decrease in leaf osmotic potential in response to drought stress (Angadi and Entz, 2002a). Maintenance of turgor plays a significant role in stress tolerance of plants due to its role in stomatal regulation, and hence photosynthesis (Ludlow et al., 1985). It has been shown that sunflower cultivars with higher turgor potential under water stress resulted in less decrease in yield (Angadi and Entz, 2002b). However, Ashraf and O'Leary, (1996) demonstrated that stress tolerance was not associated with leaf turgor potential. Indeed, the importance of stomata in understanding the overall hydraulic and leaf functioning performance cannot be under estimated as the closure of stomata is the primary limitation to photosynthesis in response to water deficit (Flexas & Medrano 2002) and play a key role in plant drought resistance. The timing of stomatal closure and xylem embolism during plant dehydration, as well as the fate of embolized organs, is a topic of hot debate in current years. While some favor the view that complete stomatal closure precedes the onset of xylem embolism, others argue that the two processes occur simultaneously and are accompanied with daily xylem refilling. In a recent meta-analysis related to stomatal response to drought and embolism resistance (Martin-StPaul et al., 2017), it was shown that the range of variation of water potential at which stomata close is much smaller when it is considered in the light of the full range of embolism resistance, indicating the absence of functional coordination between stomatal closure and the onset of embolism formation. Thanks to the development of a new optical technique (Brodribb et al., 2016), Hochberg et al. (2017) observed the complete stomatal closure before the appearance of embolism in the leaves of grapevines (*Vitis vinifera* L.) by several days. They further reported that the basal leaves once embolized, were shed, and prevented further water loss, thereby protecting the hydraulic integrity of younger leaves and the stem. I propose here to use this technique to test whether sunflowers also close their stomata before the onset of cavitation and also to quantify the leaf hydraulic safety margin between accessions (difference between the water potential inducing 90% stomatal closure and the potential leading to 12% of embolism in leaf veins).

The mean P_{50} value measured for 477 *Pinus pinaster* F2 genotypes "Corsica x Landes" provenance hybrid, growing in common garden, sampled between November 2012 and April 2013 was -3.70 MPa (Chapter 2) and are very similar to the mean P_{50} value for "Corsica x Landes" and "Morocco x Landes" families comprising a total of 480 genotypes sampled and measured during two campaigns, from October 2014 to February 2015 and from January 2016 to March 2016, which was 3.67 MPa (Chapter 3). The mean P_{50} values measured here are similar to what was found in an earlier study (Lamy et al. 2011 (P_{50}, -3.93 MPa), 2014 (P_{50}, 3.7 MPa).

Considerably low value (CV_P=5.6%) of coefficient of variation for P_{50} for "Corsica x Landes" and more than 70% of the genotypes for "Corsica x Landes" and "Morocco x Landes" families with P_{50} value ranging between -3.5 and -3.9 MPa shows that the intraspecific variability in embolism resistance in maritime pine is very low, which shows they could be more similar for this trait than expected under genetic drift as previously shown by Lamy et al., (2011, 2014). This evolutionary stasis could be due to uniform selection (Lamy et al., 2011), with all populations selected towards an optimum value, which is, therefore, not under climate control. It is worth noting that some species show phenotypic plasticity for embolism resistance, with a slight trend of increasing P_{50} with increasing aridity in natural populations of *P. canariensis* (López et al., 2013) and in *Fagus* *sylvatica* (Aranda et al., 2014; Schuldt et al., 2015). Similarly López et al. (2016) found that the intraspecific variability of hydraulic traits was largely due to phenotypic plasticity in six populations of Canary Island pine (*Pinus canariensis*). Vulnerability to embolism showed a plastic response in the driest provenance trial located in the ecological limit of the species. This trait was significantly correlated with annual precipitation, temperature oscillation and drought period at the origin of the populations, indicating that vulnerability to cavitation was directly related to survival in the dry and the xeric provenance trials, showing its importance in determining drought resistance. Contrasting results for variation of xylem embolism resistance at intraspecific level, as for example, moderate to low levels of intraspecific variation (P_{50}, -2.21 to -2.97 MPa) in poplar (*Populus* sp) (Hajek et al., 2014), and no significant variability in P_{50} in European beech (*Fagus sylvatica* L.) (Hajek et al., 2016) emphasized the need to further investigate this trait at broader spectrum. At broader evolutionary levels there is significant variability in P_{50} (Maherali et al., 2004; Pittermann et al., 2012; Bouche et al., 2014). On the other hand, at intraspecific level there is generally low variability (Delzon et al., 2010) and little genetic differentiation for xylem embolism resistance (Lamy et al., 2011; Sáenz-Romero et al., 2013).

Trade-offs with hydraulic traits

Safety/growth and safety/efficiency trade-offs

In sunflower we found a trade-off between P_{50} and growth-related traits i.e. height but not between hydraulic safety and xylem efficiency (Chapter 1), showing that shorter plants had greater embolism resistance. Previous studies show conflicting results regarding the possible existence of such a trade-off between P_{50} and growth-related traits. Positive relationship between embolism resistance and growth related traits was reported in poplar (Fichot et al., 2010) and Scot pine (Sterck et al., 2012). However, Cochard et al. (2007) found a close relationship between xylem vulnerability and productivity in poplar and willow clones. Several recent studies found no evidence of a trade-off between vulnerability to embolism and growth-related traits (Hajek et al., 2014; Guet et al., 2015; Hajek et al. 2016). In maritime pine family, we did not find any relationship of embolism resistance with wood density (Chapter 2) and with leaf functioning traits (Chapter 3), indicating that embolism resistant genotypes are independent on denser wood. It has been reported that xylem embolism resistance increases at the cost of slower growth as plant either has to allocate more carbon for thicker cell walls to construct denser wood (Hacke et al. 2001) or to construct foliar and axial tissues for increased canopy carbon gains and growth rate (Wikberg and Ogren, 2004, Ducrey et al. 2008). Increased survival is the evident advantage of xylem safety during drought events while increased carbon allocation to leaves relative to sapwood area is achieved by xylem efficiency which allows rapid growth rate and optimizing rates of photosynthesis in competitive environment (Santiago et al., 2004; Poorter et al., 2010). Thus plants need a balanced state between embolism resistance (xylem safety) and water transport (xylem efficiency) to their photosynthetic organs for better performance (Tyree & Zimmermann, 2002; Hacke et al., 2006). As plants get advantages from a water transport system that is both efficient as well as safe from embolism at the same time, variability in both efficiency and safety are expected to reflect ecological and evolutionary differences among species (Sperry, 2003; Baas et al., 2004). Indeed, it is generally believed that there is a trade-off between xylem safety and hydraulic efficiency (Lens et al., 2011). However, in our study we found no trade-off between these two traits at intraspecific level in both sunflower and maritime pine, which are in agreement with those found by Gleason et al. (2015) who showed no or weak trade-off between safety and efficiency at interspecific level across a wide range of angiosperm and gymnosperm species. It may be due to reason that in gymnosperms, safety seems to arise largely by the amount of overlap between torus relative to the size of pit aperture having minimum influence on efficiency (Delzon et al., 2010; Pittermann et al., 2010; Bouche et al., 2014). In angiosperms, mechanical stability is largely decoupled from wood density, vessel lumen fraction, and therefore from hydraulic efficiency, resultantly a strong tradeoff between hydraulic efficiency and mechanical safety across angiosperms could not be expected.

Relationship between hydraulic traits and xylem anatomy

We found that both the point of air entry during embolism formation (P_{12}) and the xylem pressure inducing a 50% loss of conductance (P_{50}) were strongly influenced by the vessel density in secondary xylem (VD_{Sx}) and total xylem (VD_{Tx}) (Chapter 1). These results indicate that higher production of vessels per unit xylem area increases the embolism resistance in sunflower. Increased embolism resistance by the production of more number of vessels has also been reported by Schuldt et al. (2015) for European beech. They found that this relationship was stronger at air entry point (P_{12}) as we found in case of sunflower. However, in poplar no such close relationship between vessel density and embolism resistance was found (Hajek et al., 2014). Another xylem anatomical trait “vessel grouping index” found closely related to vessel density (Lens et al., 2011) has also been reported to closely related with xylem embolism at the point of air entry (P_{12}) Schuldt et al. (2015). These results in a way support the assumption put forwarded by Carlquist, (1966) that in species adapted to water-limited environments having a xylem tissue composed of nonconductive fibers and no vasicentric tracheids, vessels should group to a larger degree to maintain the conductive flow path by pathway redundancy in case if the largest vessel of the group embolized.

We also found strong correlations between P_{12} and both inter vessel double-wall thickness in primary xylem (T_{vwPx}) and the thickness-to-span ratio of vessels in the primary xylem ($TD^{-1}{}_{Px}$), which suggest that these two traits are important to directly prevent embolism formation or to protect the vascular system from higher tensions. A higher thickness-to-span ratio has been reported to strengthen the vessel walls against implosion and the relationship between increased embolism resistances with increasing thickness-to-span ratio has also been reported in conifers (Hacke et al., 2001; Bouche et al. 2014). Previous studies (Pittermann et al., 2005; Sperry et al., 2006) show that species resistant to cavitation have thicker tracheid walls relative to lumen area. Whereas Pittermann et al. (2006b) and Sperry et al. (2006) explained that variation in the thickness to span ratio was determined by lumen diameter and not by the cell wall thickness. Bouche et al. (2014) showed that embolism resistant species had thicker tracheid walls with only minor reduction in lumen diameter (conduit size) and minimum impact on hydraulic conductivity. According to Maherali et al. (2004) and Choat et al. (2012) higher embolism resistance is associated with lower negative sap pressure, tracheids with a higher thickness to span ratio are required to resist mechanical stresses. From a functional point of view, thicker walls relative to lumen area do not improve drought-induced embolism resistance as this phenomenon occurs at the bordered pit level. Embolism resistance might, therefore, be indirectly linked to thickness to span ratio.

The relationship between higher levels of lignification and greater embolism resistance has been reported in woody plants (Awad et al., 2012) as well as in herbaceous plants (Lens et al., 2013; Tixier et al., 2013; Lens et al., 2016). However, on the contrary we found no relationship between P_{50} and greater stem tissue lignification, indicating that the development of embolism-resistant stems does not involve lignification of tissues. Our results are supported by previous studies showing that achievement of higher levels of embolism resistance is not always dependent on increased lignification of the xylem tissues (Watkins et al., 2010; Pittermann et al., 2011).

Relationship between xylem specific hydraulic conductivity and leaf physiological traits

We found a significant linear relationship between stem hydraulic conductivity and the actual efficiency of photosystem II under non stressful conditions. It was found that plants with the highest water conductivity also presented the highest light use efficiency resulting in the highest photosynthetic productivity (Chapter 3). The significant relationship between stem hydraulic conductivity and the actual efficiency of Photosystem II under non stressful conditions show that the efficiency of leaf functioning traits is strongly affected by the capacity of the hydraulic system to transport water. Similar results were also found by Brodribb and Field, (2000) demonstrating a strong relationship between hydraulic supply of water (hydraulic conductivity) to leaves and maximum photosynthetic capacity (quantum yield) in a community of rainforest comprising of conifers and angiosperms suggesting that the maximum photosynthetic rate of leaves was constrained by their vascular supply. This study illustrates that the photosynthetic capacity of leaves is related to the hydraulic conductance of the supporting xylem tissue under optimal conditions. Similar results showing significant positive correlations between leaf-specific hydraulic conductivity of upper branches and maximum rates of net CO_2 assimilation per unit leaf area and stomatal conductance (gs) were also found across 20 species of canopy trees (Santiago et al., 2004). The variability within the Maritime pine family was of the same magnitude as the one observed at the inter-specific level both on coniferous and broadleaved trees (Brodribb and Field 2000). Our results indicate that such a relationship exists not only at interspecific but also at intraspecific level. This relationship between hydraulic efficiency and photosynthetic productivity is understandable. Loss of water through transpiration is monitored by opening and closing of stomata, which is governed by the turgor of the guard-cells. During drought, plants can restrict loss of water by regulating overall stomatal conductance at the cost of reduced photosynthesis during drought. If stomata are kept fully open during drought, leaf and xylem water potentials (Ψ_L and Ψ_X, respectively) would decrease rapidly to a point where permanent damage to photosynthetic apparatus can occur through embolism. Plants avoid this area of dangerous water-potential by closing stomata, thereby stopping water-flow to the leaves, consequently photosynthesis is reduced drastically by restricting carbon entry. The relationship between hydraulic conductivity and leaf functioning traits under stressful conditions have been studied extensively (Brodribb et al., 2003; Domec et al., 2008) however, this relationship is required to be investigated under non-stressful conditions. As previously discussed that no relationship between the xylem vulnerability and the leaf functioning was found in maritime pine which is also supported by the similar P_{50} values -3.9 MPa for *P. pinaster* (Bouche et al. 2016) and -3.9 / - 4 MPa for *P. canariensis* (Lopez et al., 2013) under temperate climate. This absence of trade-off between resistance to embolism and leaf functioning traits and the existence of a high variability in the physiological traits would pave a way to open the possibilities for a breeding program and to select efficient and resistant genotypes of Maritime pine by focusing on different genetic material and should consider the genetic determinisms of this variability through integration of genetics studies.

Implications and future perspectives

The results presented in this thesis greatly improved our understandings of the intraspecific variability in hydraulic traits and their association with other traits like genetics, xylem anatomy and leaf functioning. Having knowledge in advance regarding the plant species which are under threat and are most vulnerable to drought-induced mortality, we can make efforts to prevent major impacts of future droughts and heatwaves on the performance of plant functioning. As climate disasters are the main reason for fluctuations in crop yields and occurrence of drought is one of the most widespread climate disasters affecting agricultural productivity (UNDP 2004; Dilley et al., 2005; Helmer and Hilhorst, 2006) and since many conifers are planted or ecologically dominant notably in the Northern Hemisphere, this knowledge is vital for managing forest and crop ecosystems in the context of climate change.

The intraspecific variation in resistance to drought-induced xylem embolism in sunflower explained in chapter 1 may be helpful to select drought-resistant accessions/genotypes, which will be of vital importance for future farming, particularly to take decisions for selection of suitable genotypes to grow in areas prone to drought. The study highlighted the potential tradeoffs between height and hydraulic safety and height and hydraulic efficiency and emphasized the need to intensively investigate the variability of embolism resistance across a wider range of accessions. Future implications regarding sunflower could envisage to investigate

(i) The possible existence of a trade-off between embolism resistance and yield potential. High yield potential is, indeed, the main objective of the most crop breeding programs, which may not be compatible with higher embolism resistance.

(ii) The relationship between embolism resistance and pit membrane thickness as higher embolism resistance has been attributed to inter-conduit pitting in *Zea mays* (Li et al., 2009).

(iii) Leaf hydraulic vulnerability to embolism using the new optical technique described by Brodribb et al. (2016). It is a new image processing technique that detects dynamic embolism by recording rapid changes in the transmission of light through the veins of leaf samples, thus enabling embolisms to be filmed and mapped during the development of leaf water stress. Plant photosynthetic performance can be well quantified on the basis of leaf hydraulic performance, which is a sensitive indicator of photosynthetic productivity (Sack and Scoffoni, 2013). Moreover, during water stress the vulnerability of leaves to hydraulic dysfunction has been demonstrated to provide valuable information regarding the timing of plant death, and the mechanisms of adaptation to increasing aridity (Blackman et al., 2010; Brodribb et al., 2014). Sunflower leaves are, therefore, required to investigate whether they are more vulnerable to embolism than stem and to test whether sunflower leaves close their stomata before the onset of embolism formation.

In maritime pine intraspecific variability is low (Lamy et al., 2011) and the comparison of QTL locations between embolism resistance and wood density related traits presented in chapter 2 failed to detect co-locating regions. Whether these results would still hold true, the lack of phenotypic and genetic correlations across families of five ecotypes (Lamy et al., 2012) strongly support the lack of causality between these two traits in this species. Finally, even if we detected strong QTL, the gain for future breeding program could be small or limited due to the low variability in P_{50} observed in maritime pine. Alternatively, we could find some interspecific hybrid to make a cross with *P. pinaster*. The best potential candidate for this would be *Pinus brutia* with P_{50} about -4.7 MPa. With its strong drought tolerance and control of apical dominance, this species is considered very important in the low/mid rainfall zone as its drought tolerance exceeds that of *P. pinaster*. It has already been tested as *P. pinaster* x *P. brutia* hybrid trial in 2005, east of York, Australia to improve drought tolerance and apical dominance of Maritime pine with survival rate of 88% at the age of one year (Butcher, 2007).

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