A safe breeding ground: genetically improved maritime pine for growth and stem form has more efficient but not more vulnerable xylem

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One sentence summary: Artificial selection in a pine tree enhanced hydraulic conductivity.

## **Abstract**

Through repeated cycles of selection and recombination, tree breeding programs deliver genetically improved varieties for a range of target traits such as biomass production, stem form, resistance to biotic stresses, wood properties, etc. However, in the context of increased, drought and heat waves, it is not yet known whether growth performance will impede drought resistance. To address this question, we compared the hydraulic properties, such as hydraulic efficiency and hydraulic safety, in four varieties over successive generations of genetically improved maritime pines (i.e. Pinus pinaster Aït.) for growth and stem form. We measured 22 functional traits related to hydraulic efficiency, hydraulic safety, xylem anatomy and wood density. We found that improved varieties presented higher hydraulic conductivity with larger tracheid lumen size and tracheid lumen fraction, and smaller wall thickness reinforcement and tracheid density, but not at the cost of reduced embolism resistance. The reported absence of trade-off between hydraulic conductivity and embolism resistance is a strong asset to improve biomass productivity, through increased hydraulic efficiency, without impacting drought resistance, and should enable new maritime pine varieties to cope with a drier climate. Our study is one of the first to reveal the hydraulic mechanisms over successive generations of genetic improvement for tree growth. It provides guidelines for sustainable forest management through breeding for other forest tree species.

**Keywords:** breeding program, drought, functional traits, hydraulic efficiency, hydraulic safety, maritime pine

## 1 Introduction

## 1.1 Tree breeding programs

Due to the high tree mortality induced by drought (Allen et al. 2010; Brodribb et al. 2020), and the rising world's population, natural forests will not be able to meet the foreseen demand for forest related products (Nabuurs et al. 2018). To fill global wood demand and decrease the supply from natural forests, highly-productive plantation forests, grown primarily for wood biomass, have been developed worldwide particularly since the 1950s.

In this context, two main factors of forest productivity have been considered and developed: the optimization of silvicultural practices and the use of genetically improved plant material (Corona 2019). Regarding the latter, recurrent selection schemes, a strategy whereby multiple breeding and production populations are produced to deliver genetic gains over successive generations, have been developed worldwide to improve stem volume growth and stem straightness (Pâques 2013; Zhao et al. 2021). The effect of high productivity on drought resistance and stand resilience is however not well documented.

## 1.2 Tree growth and xylem efficiency

The breeding system aims to improve growth, which could be explained by the underlying traits related to hydraulic efficiency, such as xylem specific hydraulic conductivity (hereafter hydraulic conductivity,  $K_s$ ).  $K_s$  indicates the capacity of water transport, high  $K_s$  advances high transpiration rate and stomatal conductance, and hence high growth rate (Domec and Gartner 2003). Nevertheless, the direct impact of artificial selection in a breeding system on hydraulic conductivity remains untested. Comparing the hydraulic conductivity among successive generations of breeding is therefore important to understand what mechanisms improve the growth of improved genetic materials.

Wood anatomy plays an important role in explaining hydraulic efficiency. The pits and tracheids are key xylem structures for water transport, as water moves through one tracheid to another tracheid in the transverse section and through the inter-conduit pit aperture from one tracheid to adjacent tracheid in tangential section (Hacke and Jansen 2009; Sterck et al. 2008). Trees with large pits and tracheids enable relatively high lumen fraction to xylem, thus having

high hydraulic conductivity (Rungwattana and Hietz 2018). However, a recent study found that there were no consistent relationships between pit/tracheid size and hydraulic conductivity (Song et al. 2022a). Instead, the pore in margo, overlapping tracheid tips or tracheid length probably determines hydraulic conductivity (Sperry et al. 2006). It remains needed to reveal the underlying mechanisms of hydraulic conductivity.

## 1.3 Trade-off between growth and drought resistance

Although a high growth rate is important for timber productivity in a drier future, selecting drought-tolerant species and populations is important for designing a resilient forest to cope with the increased frequency of drought events (Gleason 2015; Lamy et al. 2014; Song et al. 2022b). Recent forest dieback events have highlighted drought-induced embolism as a driving factor in tree mortality (Anderegg et al. 2016; Choat et al. 2018; Choat et al. 2012). Hydraulic safety, the ability of trees to maintain water supply to the aerial organs during prolonged drought is quantified as the xylem pressure inducing 50% loss of hydraulic conductivity ( $P_{50}$ ): high  $|P_{50}|$  means high resistance to drought-induced embolism. It is well known that breeding programs typically select genotypes with increased growth (Serrano-León et al. 2021), but it is not clear whether breeding for growth also affects drought resistance. Generally, trees with high  $K_s$  adapt a fast-growing strategy with high growth but at the cost of reduced  $|P_{50}|$  (Wang et al. 2022; Yao et al. 2021), because high  $K_s$  needs high relative lumen area to wall thickness. Consequently, there is a reduced carbon investment in conduit walls and the capacity of withstanding extreme xylem tensions will be weak (Hacke and Sperry 2001). Therefore, there is a hypothesized trade-off between hydraulic conductivity (or growth) and embolism resistance (Liu et al. 2019).

Within Scots pine, faster-growing trees had lower xylem embolism resistance (Sterck et al. 2012), however, other studies (e.g. in *Populus* species) found no intraspecific trade-off (Guet et al. 2015; Hajek et al. 2014), or even a positive relationship between both traits (Schuldt et al. 2016). Therefore, despite a conduit-level relationship between construction costs and embolism resistance, there is not a broad consensus on organ- or whole plant-level relationship between growth and embolism resistance, especially within a breeding context.

Embolism resistance is thought to be driven by the fine-scale structure of the interconduit pit anatomy. During drought, the spread of air through the xylem occurs by air-seeding from one tracheid to another through the inter-conduit pits (Tyree and Sperry 1989; Tyree and Zimmermann 2013). In broadleaf trees, microscopic air-bubbles seep through tiny pores in the pit membrane, whereas in gymnosperms the torus-margo structure acts as a valve (Hacke et al. 2004), where the impermeable torus can become deflected to seal the pit aperture when embolism occurs (Jansen and McAdam 2019). A flexible margo and a large torus diameter (relative to the pit aperture) lead to an effective sealing of the pit aperture. This likely contributes to the higher embolism resistance of conifers (Delzon et al. 2010). A reduction in pit aperture diameter, thereby increasing the torus overlap explains the variation of  $P_{50}$  across conifer species (Bouche et al. 2014; Hacke and Jansen 2009). While the pit size and torus-pit sealing underlie the differences in embolism resistance across species (Song et al. 2022a), it remains uncertain if they also contribute to the intraspecific differences in embolism resistance (Li et al. 2022).

Studying tree hydraulics will advance our understanding of tree growth and survival, facilitating a better prediction of biomass production under a drier future climate. Therefore, it is important to compare to what extent the improved generations of breeding for growth affect tree hydraulic safety and efficiency. Most plantations have been and are still done with genetically improved material. Especially maritime pine (*Pinus pinaster*) stands are widely planted over 4 million hectares in the western part of the Mediterranean region (Lamy et al. 2012; Marguerit et al. 2014). They play important roles in Mediterranean ecosystems due to high timber productivity with fast-growing characteristics. However, it has never been studied whether the artificial selection through recurrent breeding impacts the hydraulic properties of trees.

To compare the xylem resistance and efficiency of different maritime pine varieties (natural versus improved varieties) , we 1) compare the hydraulic traits (i.e., embolism resistance and hydraulic conductivity) of four varieties of *P. pinaster*, and 2) relate their

hydraulic traits to the underlying anatomical tracheid and pit traits. Here, we aim to answer the following two questions and hypotheses:

- 1) How has artificial selection affected the embolism resistance and hydraulic conductivity of maritime pine? We hypothesize that genetically improved maritime pine varieties have increased hydraulic conductivity allowing for higher gas exchange, but at the cost of reduced embolism resistance.
- 2) How do wood anatomical traits underlie hydraulic conductivity and embolism resistance in maritime pine under artificial selection? We hypothesize that high hydraulic conductivity of improved genetic material is associated with wider tracheids and pits, while high embolism resistance is triggered by strong valve effect via margo flexibility and torus overlap to limit air seeding.

## 2 Materials and Methods

#### 2.1 Maritime pine stands

Starting in the 1960s, the maritime pine breeding program has produced three series of seed orchards based on the local Landes provenance, with an increasing improvement in growth and stem straightness from VF1 or VSEL (Vigor and Form, 1st series) to VF3 (3rd series) (Mullin et al. 2011). While VF1=VSEL corresponds to the first generation of seed orchards (plus trees or progenies of plus-trees) with an increased volume of 15%, VF2 corresponds to a 1.5 generation (i.e. progeny tested plus-trees) with an increased volume of 30%, and VF3 corresponds to a 2.5 generation (i.e. progeny tested first-generation trees) with an increased volume of 40% (Bouffier et al., 2013). The second series VF2 (established during the 1980s-1990s) is gradually being replaced by the third series VF3, which entered into production in 2011–2015 (Bouffier et al., 2013)(Bouffier et al., 2013; GIS GPMF, 2014).

To compare the hydraulic properties (Table 1) of the aforementioned selected series (VSEL, VF2, VF3), we sampled and measured natural populations (VNAT) from southwestern France, and the three improved maritime pine varieties (VSEL, VF2 and VF3) in spring 2021. For samples collection, we selected even-aged, monospecific stands of maritime pine with an

understory consisting mainly of *Molinia caerulea* (L) Moench located in the Médoc natural park 35km west of Bordeaux in the Landes de Gascogne Forest (Fig. S1: Hourtin (45° 11′N; 1° 3′ W, south-west France). All stands were growing on the same soil (sandy podzol with a cemented Bh horizon limiting the root depth to -0.8 m) and climatic conditions (temperature climate, Fig. S1b-d) in which 57-59 individuals per variety were sampled within one or 4 four stands per variety (Table S1). To avoid intra-individual variation (considered very low in maritime pine for hydraulic traits, see Bouche et al. 2016 AFS), we selected one fully sun-exposed branch (*c*. 50 cm) per individual. To reduce water loss and cavitation, we wrapped the branches in wet papers and sealed in black plastic bags, and then transported the branches to the lab at University of Bordeaux. The stands had extremely low soil phosphorus and nitrogen levels, and an average pH-H<sub>2</sub>O of 4·0. The regional climate is maritime (mean annual temperature of 13°C, mean annual precipitation of 977 mm) with marked drought in summer (June–August, Fig. S1). Tree age ranged between 25 and 30 years old.

## 2.2 Embolism resistance and hydraulic conductivity

To compare embolism resistance and hydraulic conductivity among the four levels of genetically improved maritime pines (VNAT: unimproved material, VSEL, VF2, VF3: gradually improved materials), we used a standard "Cavitron" method (Cochard et al. 2005), improved by Burlett et al. (2022) by generating negative pressure (P in MPa) in branch segments. All measurements were carried out at the PHENOBOIS phenotyping platform (CavitPlace, University of Bordeaux, Talence, France). Samples were cut with a specific length of 27 cm and barks were removed to prevent resin fillings from barks. We infiltrated samples with a reference ionic solution of 10 mm 25 KCl and 1 mM CaCl<sub>2</sub> in deionized ultrapure water. To induce embolism in the xylem, we generated negative pressures by using a centrifugal force, which increased from -0.8 to -7 MPa (González-Muñoz et al. 2018). Meanwhile, hydraulic conductivity (K in m<sup>2</sup> MPa<sup>-1</sup> s<sup>-1</sup>) was recorded for 2 mins when samples were exposed to the corresponding pressure. The maximum xylem hydraulic conductivity (Kmax) was initially obtained when the pressure was near zero, and then the xylem specific hydraulic conductivity (hereafter referred to as hydraulic conductivity, Ks in kg m<sup>-1</sup> MPa<sup>-1</sup> s<sup>-1</sup>) was calculated by dividing

 $K_{\text{max}}$  by sample length and sapwood area (Larter et al. 2017). To fit the relationship of K and P, we firstly calculated the percentage loss of hydraulic conductance (PLC) and then used a sigmoid function (Delzon et al. 2010) as follows:

PLC = 100 
$$(1 - \frac{K}{K_{max}})$$
 (1)

$$PLC = \frac{100}{(1 + exp(\frac{S}{25}*(P - P_{50})))}$$
(2)

where  $P_{50}$  (MPa) is the xylem pressure when 50% of hydraulic conductivity is lost, S (% MPa<sup>-1</sup>) refers to the slope at the inflection point. Afterwards, xylem pressures (P in MPa) when 12% and 88% of conductance were derived and referred to as  $P_{12}$  and  $P_{88}$ . Individual values were used for further analysis.

## 2.3 Wood density

Wood density (WD in g cm<sup>-3</sup>) indicates the capacity of mechanical support and toughness construction (Poorter et al. 2018). To assess the difference of WD among these four varieties, we measured the wood density of the same samples (around 40-58 samples per variety, Table S1). We used a double-bladed saw to cut a branch segment with a thickness of 2 mm for each individual and then air-dried. Afterwards, we used an indirect-reading X-ray densitometer and measured the longest orthogonal radial density profiles by analyzing the scanned images with WinDENDRO software (Guay et al. 1992), inducing a series of wood density data from the bark to the pith. To get a clean dataset of wood density for all rings, we deleted the data corresponding to the bark and pith. To classify the earlywood and latewood, we used the two following criteria: 1) WindDENDRO automatically identified earlywood and latewood, and 2) the distribution curves were carefully checked with the images of the stem sections. For detailed methods, see Lamy et al. (2012). For each individual we calculated the mean wood density for earlywood (WD\_E, g cm<sup>-3</sup>) and latewood (WD\_L, g cm<sup>-3</sup>).

#### 2.4 Wood anatomy

To explore how wood anatomical traits differ among these four varieties, we kept two 2-3 cm segments for three to five individuals per variety for measuring pit and tracheid traits (Table S1). Small pit size and strong pit sealing contribute to high embolism resistance of conifer species (Song et al., 2021), we therefore measured pit traits related to pit size (i.e., pit aperture diameter, pit membrane diameter and torus diameter) and pit sealing (margo flexibility, torus overlap and valve effect) based on earlywood for three individuals per variety. We used a scanning electron microscopy at the PHENOBOIS platform (SEM, PhenomG2 pro; FEI, The Netherlands) to take pictures. Afterwards, we used ImageJ 1.53k to measure pit aperture diameter (DPA, µm), pit membrane diameter (DPM, µm) and torus diameter (DT, µm) for 30 pits per variety. Margo flexibility (MF) was calculated [MF=(DPM-DT)/DPM], and a high value indicates higher ability of a torus to be deflected against the pit aperture. To assess the sealing capacity of pit aperture during an embolism event, torus overlap was calculated by comparing the torus against the aperture [TO=(DT-DPA)/DT]. High margo flexibility and large torus overlap both contribute to a strong valve effect [VE=MF×TO], which is an estimation of the ability of a torus to seal the pit. These composite measurements have been previously described in Delzon et al. (2010) (see also Bouche et al. (2014); Song et al. (2022a)). All traits were averaged per individual.

To estimate the contribution of tracheids to water transport efficiency, we measured tracheid-level anatomical traits for five individuals per variety using light microscopy, i.e. tracheid diameter (D, μm), tracheid density (i.e., the tracheid number per area; TD, # mm<sup>-2</sup>), wall thickness (Tw, μm), thickness to span ratio (TSR, μm) and tracheid lumen fraction (i.e., the proportion of tracheid lumen area over the corresponding xylem area; TLF, μm μm<sup>-1</sup>). We used a sliding microtome to section samples to a thickness around 20 μm, and stained with safranine for 15 minutes, then washed with distilled water, ethanol 50%, 75%, 96% and 100%. Samples were then fixed and pictures were taken with the LAS V 3.8 software connected to a light microscope (DM2500M; Leica Microsystems, Wetzlar, Germany). For all images, tracheid traits were measured on a complete radial section using ImageJ version 1.53k (Schneider et al. 2012), and at least 200 tracheids per individual were measured. Although earlywood in conifers

contributes 90% of total hydraulic conductivity (Domec and Gartner 2002), we performed our measurements on both earlywood and latewood separately. Thickness to span ratio (TSR) was calculated as the square of double wall thickness over tracheid diameter (Song et al. 2022a), and hydraulic diameter was calculated based on the weighted tracheid diameter (Dh,  $\mu$ m) for both earlywood and latewood to correct the irregular tracheid shape (Poorter et al. 2010):

$$TSR = \left(\frac{2 \times Tw}{D}\right)^2 \tag{1}$$

$$Dh = \sqrt[4]{\frac{1}{n}\sum_{i=1}^{n} Di^4} \tag{2}$$

where Tw is wall thickness, D is the adjacent tracheid diameter, and Di is the ith measured tracheid diameter.

## 2.5 Statistical analyses

To assess whether different varieties of breeding intensity have different hydraulic traits (i.e., embolism resistance and hydraulic conductivity) and wood anatomical traits, two-way ANOVA (variety and stand) was performed to take into account all remaining residual effects related to stand. We also used post-hoc Tukey (HSD) tests to compare varieties one by one. To further evaluate how wood anatomical traits impact hydraulic strategy, we performed Pearson correlation and regression analyses between hydraulic and anatomical traits. To meet the normality and homogeneity of variance assumptions of residuals, data was transformed when necessary using a log or square root transformation. All data were analyzed using R version 4.1.2 (R Core Team 2021).

## 3 Results

## 3.1 Differences in hydraulic traits between varieties of genetically improved maritime pine

Hydraulic conductivity ( $K_s$ ) varied significantly among the four varieties of maritime pines (ANOVA, Fig. 1a, Table 2). The comparison of the estimated coefficients from the linear model showed that the VF2 and VF3 (coeff. of 0.23 and 0.21 kg.m<sup>-1</sup>.s<sup>-1</sup>.MPa<sup>-1</sup>, respectively) are

significantly larger than that of VNAT and VSEL (coeff. of 0 and -0.08 kg.m<sup>-1</sup>.s<sup>-1</sup>.MPa<sup>-1</sup>, respectively) with an intercept of 0.34 kg.m<sup>-1</sup>.s<sup>-1</sup>.MPa<sup>-1</sup>. However, no difference in  $K_s$  was detected between the two 'improved' varieties. The two varieties of genetically improved P. pinaster (i.e., VF2 and VF3) had significantly higher hydraulic conductivity compared with the natural variety (VNAT). Embolism resistance ( $P_{12}$ , Fig. 1c;  $P_{50}$ , Fig. 1b; and  $P_{88}$ , Fig. 1c) did not vary significantly among these four varieties. By contrast, VF3 had a higher slope of vulnerability curve than VNAT and VSEL (Fig. S2b). Additionally, there was no significant relationship between hydraulic conductivity and embolism resistance (Fig. S3).

## 3.2 Differences in wood traits between varieties of genetically improved maritime pine

Both wood density of earlywood (ANOVA, Fig. 2a) and latewood (ANOVA, Fig. 2b) varied significantly across the four varieties (Table 2). The wood density of earlywood was significantly lower for the natural population compared with the other three genetically improved generations, while the wood density of latewood for VNAT, VF2 and VF3 was significantly lower than VSEL. In summary, there was a trend that genetically improved generations even had a higher earlywood density than unimproved material, but no clear trend was apparent for latewood.

We then compared the underlying wood anatomical traits such as the size of tracheids and pits that play a role in hydraulic function. Anatomical traits of earlywood varied significantly among the different varieties (Fig. 3, Table 2). The most improved variety (VF3) had larger hydraulic diameter compared to VNATA and VSEL (Dh\_E, Fig. 3a), a higher lumen area to whole area ratio (TLF\_E, Fig. 3e) than VSEL, a smaller tracheid density (TD\_E, Fig. 3b) than VSEL, and a smaller thickness to span ratio (TSR\_E, Fig. 3d) than VNAT and VSEL. For the latewood tracheid traits, there was no significant variation across the four varieties(Fig. 3f-j). Overall, we observed a trend for artificial selection to increase hydraulic diameter and lumen ratio, and to decrease tracheid density and thickness to span ratio in the earlywood, rather than in the latewood.

Among the four varieties there were no significant differences of pit membrane structure and function such as pit membrane diameter (DPM, Fig. 4a), torus diameter (DT, Fig 4b), aperture diameter (DPA, Fig. 4c), torus overlap (TO, Fig 4e) and valve effect (VE, Fig 4f)

(Table 2). Margo flexibility only varied significantly across two varieties, i.e., VF2 had significantly lower margo flexibility than VSEL (Fig. 4d).

#### 3.3 Relationships between wood and hydraulic traits

The significant differences in hydraulic conductivity across these four varieties of improved material were further explained by the tracheid traits of earlywood, but not by those of latewood (Fig. 5, Table 3). Especially, hydraulic conductivity ( $K_s$ ) increased with the hydraulic diameter (N=20,  $R^2$ =0.36, P=0.001) and lumen ratio (N=20,  $R^2$ =0.36, P=0.001), decreased with tracheid density and thickness to span ratio of the earlywood (Fig. 5a-d, Table 3), but had no relationships with pit aperture size (Fig. 5e) and latewood tracheid values (Fig. S4). Embolism resistance increased with torus diameter and decreased with margo flexibility, but not related to other pit traits (Table S1).

## 4 Discussion

For the first time to our knowledge we tested whether the genetic selection of trees for better growth and straightness has had an impact on water transport efficiency and drought resistance in their vascular system. To this end we compared both efficiency and safety of hydraulic traits (i.e., hydraulic conductivity and embolism resistance) and wood anatomical traits among four varieties of maritime pines with increasing selection intensity. Our results evidence that the most improved varieties (VF2 and VF3) had higher hydraulic efficiency than the natural (VNAT) and the first generation selected variety (VSEL), which was mostly explained by larger tracheid size and higher tracheid lumen fraction. On the other hand, improved generations had no striking differences in embolism resistance, demonstrating that genetic improvement has not been at the expense of the drought resilience of the species. In other words, it enables high timber productivity, but no reduced embolism resistance to cope with drought events. Here, we discuss how the different varieties differ in hydraulic functions, how wood anatomical traits determine the high hydraulic conductivity of improved generation and the implication for sustainable forest management.

#### 4.1 The improved maritime pine increased xylem hydraulic conductivity

We hypothesized that the improved generations would have higher hydraulic conductivity, since high xylem hydraulic conductivity improves water transport, and hence high stomatal conductance and growth rate (Brodribb et al. 2009). As expected, we found improved varieties (VF2 and VF3) had higher hydraulic conductivity (i.e.  $K_s$ ) than the natural variety (VNAT, Fig. 1a). Therefore, the improved generations adopt an acquisitive strategy to grow faster thanks to high  $K_s$  and this strategy is not at the cost of reduced embolism resistance (i.e.  $|P_{50}|$ ), demonstrating the lack of a trade-off between  $K_s$  and  $|P_{50}|$  in maritime pine. Previous studies failed to find this trade-off across species (Gleason et al. 2016; Larter et al. 2017), and as well as the lack of intraspecific trade-off (Lamy et al. 2014; Sáenz-Romero et al. 2013). The control by a different combination of traits may explain the lack of this trade-off, since  $K_s$  is positively determined by the fourth power of tracheid size (Tyree and Ewers 1991), tracheid length, margo pores and total area of inter-conduit pits (Pittermann et al. 2006) Schulte et al. 2015). These characteristics enable conifer trees to grow fast with high hydraulic efficiency (ref). In comparison,  $|P_{50}|$  is positively characterized by the strong valve effects through a flexible margo and large torus overlap to seal the pit aperture when embolism occurs (Delzon et al. 2010; Song et al. 2022a), which is strikingly different from the above-mentioned factors that affect K<sub>s</sub>. Thus, the decoupled relationship between hydraulic conductivity and embolism resistance allows plants to grow faster without losing their capacity to tolerate drought events.

## 4.2 Mechanisms underlying hydraulic conductivity

We expected that the improved generations for growth would have lower wood density (WD), since improved generations grow faster they should have wider tracheid lumens to support increased water transport, carbon gain and growth (Poorter et al. 2010; Poorter et al. 2019). Nevertheless, our findings challenge this assumption: instead, the improved generations have higher wood density for earlywood (Fig. 2a), suggesting that breeding higher hydraulic conductivity does not reduce wood construction cost in maritime pine. Considering that mechanically stronger wood can support against implosion by negative pressure (Hacke et al. 2001), this result indicates that xylem collapse or conduit implosion is not affected by artificial

selection. The lack of relationship between hydraulic conductivity and wood density, has been also reported for angiosperms (Schumann et al. 2019) and other gymnosperms (Song et al. 2022a). We therefore conclude that hydraulic conductivity is likely to be determined by anatomic conduit and pit traits both for gymnosperms and angiosperms, rather than wood density.

We further investigated whether anatomical trait values could explain the high hydraulic conductivity of improved varieties. It was expected that hydraulic conductivity would increase with tracheid size, pit size and lumen fraction, and decrease with tracheid density, since large tracheids and pits allow for more efficient water transport. We indeed found that the most improved variety (VF3) had significantly larger tracheids than wild material (VNAT) and even first generation of breeding (VSEL). Moreover, the linear regression reveals that hydraulic conductivity increased with earlywood hydraulic diameter and tracheid lumen fraction, decreased with thickness to span ratio and tracheid density (Fig. 5a-d), supporting the theory that large conduits and lumen areas favor water transport (Russo et al. 2010). However, the different generations did not differ in latewood tracheid trait values. Besides latewood tracheid traits values did not correlate with hydraulic conductivity, due to the fact that earlywood contributes to 90% of total flow for hydraulic conductivity of conifer species (Domec and Gartner 2002).

We also failed to find a positive effect of pit aperture size on hydraulic conductivity (Fig. 5e). Large tracheid size and high tracheid lumen fraction enable large amounts of water transport for plants, thus leading to high hydraulic conductivity (Rungwattana and Hietz 2018; Tyree and Ewers 1991). Although some studies did not find a positive relationship between tracheid size and hydraulic conductivity (Larter et al. 2017; Song et al. 2022a), the other tracheid anatomical traits still need to be further investigated, such as tracheid length and margo porosity. The lack of relationship between pit aperture and hydraulic conductivity may indicate that hydraulic conductivity is mainly determined by tracheid-level traits, while pit aperture has a stronger link with embolism resistance. This is also in line with the lack of trade-off between hydraulic conductivity and embolism resistance. Our study stresses the importance of tracheid traits for predicting hydraulic conductivity of improved breeding generations.

#### 4.3 Maritime pine breeding programme had no impact on embolism resistance

Contrary to our hypothesis, the genetically improved maritime pine generations do not display a lower resistance to embolism than non-improved varieties (similar  $|P_{50}|$ ). We indeed found high stability of embolism resistance among the four studied varieties of maritime pines with increasing breeding selection for height growth and stem straightness, indicating that breeding programme has no effects on the drought resistance of the vascular system of P. pinaster. Contrary to P. sylvestris for which fast-growing trees adopt an acquisitive strategy at the cost of reduced embolism resistance (Sterck et al. 2012), we failed to find a reduced embolism resistance in fast-growing maritime pines. Our result is consistent with other studies showing a lack of trade-off between growth and embolism resistance among conifer species (Song et al. 2022a). This result implies the lack of trade-off between growth and embolism resistance is not only true across species but also within species. We found similar values of P<sub>50</sub> among the four varieties of improved material, probably owing to the fact that  $P_{50}$  is a genetically constrained trait as reported by (Lamy et al. 2011; Lamy et al. 2014). The authors demonstrated that  $P_{50}$  is a canalized trait in maritime pine by showing that between-population variability of  $P_{50}$  was significantly lower than would be expected under a hypothesis of genetic drift alone. Their quantitative genetics analysis also showed that  $P_{50}$  presented a significant heritability (above 0.4), higher than that estimated for other traits such as growth. However, we did not study different genotypes of  $P_{50}$  and  $K_s$  in arid environments (e.g. in Southern Spain), where phenotypic plasticity might lead to differences. We assume that  $P_{50}$  would be still phylogenetically controlled across species and  $K_s$  would be smaller in more arid environments. However, it still needs to be tested in more arid areas.

We further tested whether the breeding process had an impact on pit traits, and whether pit traits related to size and sealing could explain embolism resistance. We found that artificial selection had no impact on pit size and pit sealing, but had limited impacts on margo flexibility. We found no effect of pit dimension traits on embolism resistance, likely because of the low variability in P50 and pit traits themselves (e.g. pit aperture size ranged from 4.16 to 4.54  $\mu$ m). Hence, pit trait values explained no variation of  $P_{50}$ . The interspecific values of  $P_{50}$ 

could be different (Ahmad et al. 2018; Bartlett and Sinclair 2021; Li et al. 2009) or the same across crop cultivars (Stiller et al. 2003), but few studies assessed tree species. Our study suggests that improved breeding could have no impacts on embolism resistance both on crop cultivars and tree species. It confirms the approach used to obtain better performing and more resilient genotypes to better adapt to increasing drought during the vegetative period.

## 4.4 Implication for future study and forest management

We found that the highest yielding generations did not have reduced resistance to drought, a benefit for designing a climate-smart forest with high timber production that can mitigate the climate warming impacts via carbon sequestration (Nabuurs et al. 2018). Given that the increased intensity and frequency of drought events have triggered tree mortality worldwide (Brodribb et al. 2020; Choat et al. 2018; McDowell et al. 2022), it is urgent to select tree species with high drought resilience to reduce mortality risk (DeSoto et al. 2020; Song et al. 2022c), which is critical for maintaining a resilient forest ecosystem. Based on our results we are confident about the possibility of selecting genotypes with higher drought resistance without impacting growth performance.

Breeding programme is an effective method to maintain a highly productive forest, providing guidelines for sustainable forest management. Different genotypes within and across species have been studied for comparing their embolism resistance (Larter et al. 2017; Sáenz-Romero et al. 2013; Song et al. 2022a), but the relationship between embolism resistance and growth remains equivocal (Guet et al. 2015). The negative relationships found in *Cedrus libani* (Ducrey et al. 2008) turns out to be positive across poplar hybrids (Fichot et al. 2010), or even disappear in both angiosperms (Guet et al. 2015; Kumar et al. 2022) and gymnosperms (Song et al. 2022a; Sterck et al. 2012). Providing guidelines for species selection with high drought resistance and high growth is thus urgently needed to better design the silviculture system adapted to each pedoclimatic situation. Our study shows that breeding programmes can provide genetic material with high productivity in a drier climate.

#### **5 Conclusions**

We compared the hydraulic strategies of different varieties of *P. pinaster* corresponding to increasing intensity of breeding for height growth and stem straightness. Compared to natural populations (unimproved material), the improved varieties had higher hydraulic conductivity, with wider tracheid lumens and narrower tracheid walls, higher lumen fraction and lower tracheid density, but no reduction of embolism resistance. The decoupled relationship between hydraulic conductivity and embolism resistance enables improved material with high hydraulic efficiency and growth to cope with more frequent and intense drought events. Our study is the first study to reveal the hydraulic mechanisms of different generations within a tree breeding scheme. It provides guidelines for forest management and breeding strategies for future studies and other forest tree species.

#### **Conflict of Interest**

The authors declare no conflict of interest.

#### **Author Contributions**

XPB and SD initiated the research project. XPB and YS contributed to data collection, YS analyzed the data. SD, ML and CP contributed to the improvements of data analyses, and YS wrote the first draft that was edited by all authors

# Supplementary data

Supplementary data for this article are available at Tree Physiology Online.

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# **Table and Figure legends**

**Table 1** Overview of 23 hydraulic traits, abbreviations and units.

Туре	Trait name	Abbreviation	Units
Embolism	Xylem pressure inducing 12% loss in hydraulic conductance	$P_{12}$	MPa
	Xylem pressure inducing 50% loss in hydraulic conductance	<b>P</b> <sub>50</sub>	MPa
resistance	Xylem pressure inducing 88% loss in hydraulic conductance	P <sub>88</sub>	MPa
	Slope	S	- /
Hydraulic	Xylem specific hydraulic conductivity	Ks	Kg m <sup>-1</sup> s <sup>-1</sup> MPa <sup>-1</sup>
conductivity			
Wood	Wood density (earlywood)	WD_E	g cm <sup>-3</sup>
vvoou	Wood density (latewood)	WD_L	g cm <sup>-3</sup>
Tracheid	Hydraulic diameter (earlywood)	Dh_E	μm
Tracticia	Hydraulic diameter (latewood)	Dh_L	μm
	Tracheid density (earlywood)	TD_E	# mm <sup>-2</sup>
	Tracheid density (latewood)	TD_L	# mm <sup>-2</sup>
	Wall thickness (earlywood)	Tw_E	μm
	Wall thickness (latewood)	Tw_L	μm
	Thickness to span ratio (earlywood)	TSR_E	μm μm <sup>-1</sup>
	Thickness to span ratio (latewood)	TSR_L	μm μm <sup>-1</sup>
	Tracheid lumen fraction (earlywood)	TLF_E	$\mu m^2  \mu m^{-2}$
	Tracheid lumen fraction (latewood)	TLF_L	$\mu m^2  \mu m^{-2}$
Pit	Pit membrane diameter	DPM	μm
110	Torus diameter	DT	μm
	Pit aperture diameter	DPA	μm
	Margo flexibility	MF	-
	Torus overlap	TO	-
	Valve effect	VE	-

**Table 2** The differences in hydraulic traits related to four varieties of *Pinus pinaster* with increasing breeding intensity or study stand using two-way ANOVA. Bold and underlying values indicate significant differences. For trait abbreviations see Table 1.

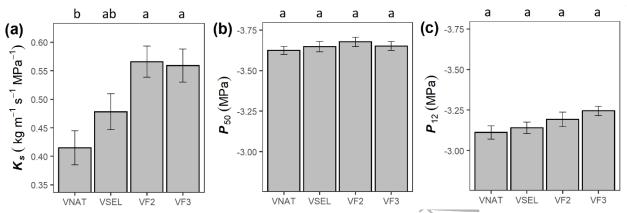
Trait	Variety		Stand	Stand			
	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value			
$P_{12}$	F <sub>3,224</sub> =2.39	0.07	F <sub>4,224</sub> =1.30	0.27			
<b>P</b> <sub>50</sub>	$F_{3,224}$ =0.53	0.66	F <sub>4,224</sub> =2.79	0.03			
P <sub>88</sub>	$F_{3,224}=1.48$	0.22	F <sub>4,224</sub> =1.72	0.15			
Slope	$F_{3,224}=3.93$	<0.01	F <sub>4,224</sub> =0.51	0.73			
Ks	$F_{3,224}=8.42$	<0.001	F <sub>4,224</sub> =7.64	<0.001			
WD_E	$F_{3,177}$ =11.01	<0.001	F <sub>6,177</sub> =0.97	0.45			
WD_L	F <sub>3,176</sub> =11.01	<0.001	F <sub>6,176</sub> =1.90	0.08			
Dh_E	F <sub>3,13</sub> =6.17	<0.01	F <sub>3,13</sub> =0.53	0.67			
TD_E	$F_{3,13}$ =3.71	0.04	F <sub>3,13</sub> =0.17	0.91			
Tw_E	$F_{3,13}$ =0.57	0.64	F <sub>3,13</sub> =0.94	0.45			
TSR_E	$F_{3,13}$ =6.66	<0.01	F <sub>3,13</sub> =0.94	0.45			
TLF_E	$F_{3,13}$ =10.62	<0.001	F <sub>3,13</sub> =1.10	0.38			
Dh_L	<i>F</i> <sub>3,13</sub> =1.17	0.36	F <sub>3,13</sub> =0.84	0.50			
TD_L	$F_{3,13}$ =1.78	0.20	F <sub>3,13</sub> =0.36	0.78			
Tw_L	$F_{3,13}$ =0.11	0.95	F <sub>3,13</sub> =0.20	0.90			
TSR_L	$F_{3,13}$ =0.10	0.96	F <sub>3,13</sub> =0.58	0.64			
TLF_L	$F_{3,13}$ =0.49	0.69	F <sub>3,13</sub> =0.34	0.80			
DPM	F <sub>3,6</sub> =0.91	0.49	F <sub>2,6</sub> =0.26	0.78			
DT	F <sub>3,6</sub> =1.35	0.35	F <sub>2,6</sub> =0.47	0.64			
DPA	F <sub>3,6</sub> =0.67	0.60	$F_{2,6}$ =0.04	0.96			
MF	F <sub>3,6</sub> =5.41	0.04	F <sub>2,6</sub> =1.37	0.32			
ТО	F <sub>3,6</sub> =1.55	0.30	$F_{2,6}=2.43$	0.17			
VE	$F_{3,6}$ =4.51	0.06	F <sub>2,6</sub> =2.70	0.15			

**Table 3** Pearson correlations among embolism resistance, hydraulic conductivity and tracheid traits (N=20, five individuals per variety). Bold and underlying values indicate significant correlations (*P*<0.05). For trait abbreviations see Table 1.

Trait	$\mathbf{K}_{s}$	$ P_{12} $	<b>P</b> <sub>50</sub>	P <sub>88</sub>	Slop	Dh_	TD_	Tw_	TSR_	TLF_	Dh_	TD_	Tw_	TSR	TLF
S					е	E	E	E	E	Е	L	L	L	_L	_L
Ks	1.0														
	0													,	
$ P_{12} $	0.3	1.0													
1	0	0													
<i>P</i> <sub>50</sub>	0.1	<u>0.7</u>	1.0												
	5	<u>6</u>	0											)	
P <sub>88</sub>	-	-	٥.	1.0											
	0.1 7	0.1	<u>0.5</u>	1.0											
Slop	/	1	<u>6</u>	0											
e	0.3	0.7	0.1	<u>-</u> 0.7											
	3	<u>5.7</u> <u>4</u>	7	1	1.00										
Dh_			_	-							Y				
E	<u>0.6</u>	0.1	0.0	0.2		1.0									
	<u>2</u>	3	3	2	0.22	0									
TD_	=	-				=									
E	<u>0.5</u>	0.0	0.2	0.4	-	<u>0.8</u>	1.0								
т	<u>4</u>	4	3	0	0.26	<u>9</u>	0								
Tw_ E	0.2	0.3	0.3	0.0	_	0.1	01	, , 7							
E	3	0.5 4	0.5	1	0.29	3	0.1	1.00							
TSR_		-	-	_	0.23		-	1.00							
E	<u>0.6</u>	0.2	0.1	0.1		0.5	<u>0.5</u>	<u>0.6</u>							
	4	5	2	5	0.31	0.5 8	<u>1</u>	0	1.00						
TLF_						<b>)</b>	<u>=</u>								
E	<u>0.6</u>	0.1	0.0	0.1		<u>0.7</u>	<u>0.7</u>	-	Ξ						
	<u>1</u>	8	7	4	0.22	<u>3</u>	<u>4</u>	0.24	<u>0.61</u>	1.00					
Dh_	0.2	0.0	-			0.4	- 0.4				1.0				
L	0.2	0.0	0.2	<u>0.4</u> 5	0.33	0.4 3	0.4	0.04	0.19	0.47	1.0 0				
TD_L		~ \	٥	<u>3</u>	0.55	- -	1	0.04	0.19	<u>0.47</u>	-				
ID_L	0.3	0.0	0.2	0.4	_	0.5	0.6	_		_	0.5	1.0			
	1	8	5	9	0.33	4	5	0.42	0.06	0.32		0			
Tw_				_		_	-				-	_			
L	0.0	0.0	0.3	<u>0.4</u>	-	0.2	0.1	<u>0.4</u>			0.3	0.1	1.0		
	2	9	7	<u>6</u>	0.26	0	1	<u>7</u>	0.23	0.10	5	9	0		
TSR_						-					=				
L	0.0	0.1	<u>0.4</u>	<u>0.5</u>	-	0.0	0.1			-	<u>0.7</u>	0.1	<u>0.8</u>		
TIF	1	2	<u>5</u>	<u>4</u>	0.29	9	2	0.20	0.17	0.16	<u>4</u>	5	<u>3</u>	1.00	
TLF_	0.2	0.0	0.4	<u>-</u> 0.6	0.38	0.3	0.4	0.13	_	0.38	0.7	<u>-</u> 0.4	<u>-</u> 0.4		1.00
	0.2	0.0	0.4	<u>0.0</u>		0.5	0.4		-		<u>U.7</u>	<u>0.4</u>	<u>U.4</u>	=	

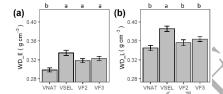


**Fig. 1** Mean values of a)  $K_s$  (xylem specific hydraulic conductivity), b)  $P_{50}$  (xylem pressure inducing 50% loss in hydraulic conductance), and c)  $P_{12}$  (xylem pressure inducing 12% loss in hydraulic conductance) for four varieties of *Pinus pinaster* (n= 57-59 individuals per variety with increasing breeding intensity, i.e. VNAT, VSEL, VF2, VF3). The bars indicate standard error.

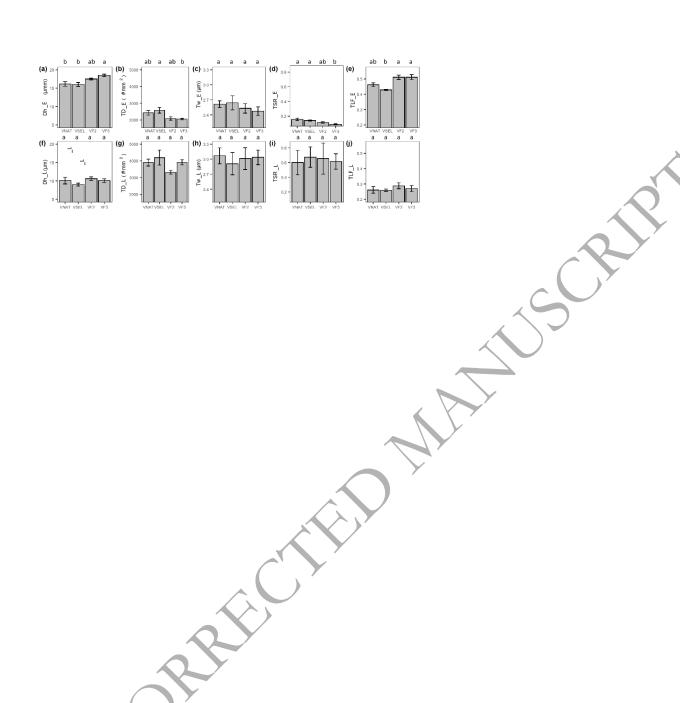


Different letters indicate significant differences (P<0.05) using the post-hoc HSD test.

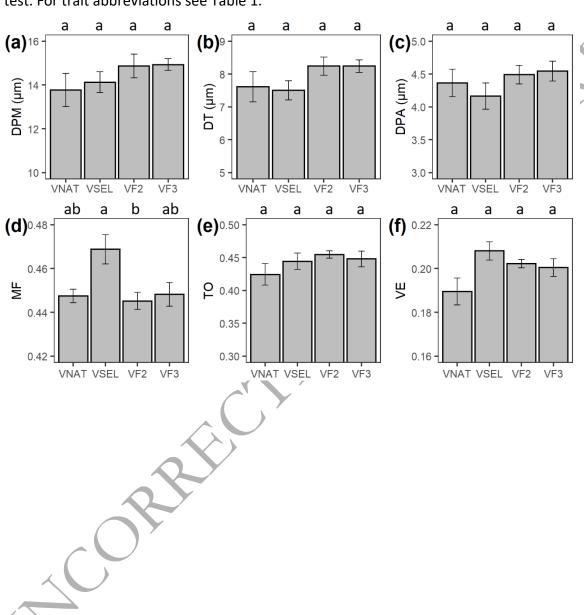
**Fig. 2** Mean values of wood density for a) earlywood and b) latewood among four varieties of *Pinus pinaster* (n= 40-57 individuals per variety with increasing breeding intensity, i.e. VNAT, VSEL, VF2, VF3). The bars indicate standard error. Different letters indicate significant differences (*P*<0.05) using the post-hoc HSD test.



**Fig. 3** Mean values of tracheid traits based on earlywood (top panel, a-e) and latewood (bottom panel, f-j) for four varieties of *Pinus pinaster* (n= 5 individuals per variety with increasing breeding intensity, i.e. VNAT, VSEL, VF2, VF3): a) the hydraulic diameter of earlywood, b) tracheid density of earlywood, c) wall thickness of earlywood, d) thickness to span ratio of earlywood, e) lumen to xylem ratio of earlywood, f) hydraulic diameter of latewood, g) tracheid density of latewood, h) wall thickness of latewood, i) thickness to span ratio of latewood, and j) lumen to xylem ratio of latewood. The bars indicate standard error. Different letters indicate significant differences (*P*<0.05) using the post-hoc HSD test. For trait abbreviations see Table 1.



**Fig. 4** Mean values of pit size (a-c) and pit sealing (d-f) based on earlywood for four varieties of *Pinus pinaster* (n= 3 individuals per variety with increasing breeding intensity, i.e. VNAT, VSEL, VF2, VF3): a) DPM, pit membrane diameter; b) DT, torus diameter; c) DPA, pit aperture diameter; d) MF, margo flexibility; e) TO, torus overlap; and f) VE, valve effect. The bars indicate standard error. Different letters indicate significant differences (*P*<0.05) using the post-hoc HSD test. For trait abbreviations see Table 1.



**Fig. 5** Bi-variate relationships between hydraulic conductivity, earlywood tracheid traits (a-d) and pit aperture diameter (e): a). hydraulic diameter of earlywood (Dh\_E), b). tracheid density of earlywood (TD\_E), c). thickness to span ratio of earlywood (TSR\_E), d). tracheid lumen fraction of earlywood (TLF\_E), and e) pit aperture diameter (DPA). Each symbol represents mean trait values over three or five individuals per population with increasing breeding intensity, i.e. VNAT, VSEL, VF2, VF3. The bars indicate standard error. None significant regression is not shown. Significant regression lines (P<0.05) and 95 confidence intervals (grey), R<sup>2</sup> and P-value are shown.

