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

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Drought-induced xylem embolism is a key process closely related to plant mortality during extreme drought events. However, this process has been poorly 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 significant 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.

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 autumn 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

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

et al. 2012), with adverse effects on all stages of plant growth and development (Jensen and Mogensen 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 for maize (*Zea mays* L.) in agricultural regions of Europe affected by the 2003 heat wave 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 and Zimmermann 2002, Wheeler and Stroock 2008, Trifilò 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). The scarcity of vulnerability curve assessments in crops is mainly due to their fragile stems and low hydraulic conductivity, making measurements technically more challenging. 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) has indicated that hydraulic failure also has a major effect on crops, within the physiological range of xylem pressure. Knowledge on the vulnerability to xylem embolism 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 (Cochard et al. 1994, Mencuccini and Comstock 1999, Saha et al. 2009, 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.

Here we focus 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 (*H.*

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 (1) the potential trade-off between growth traits and embolism resistance and (2) the link between embolism resistance and xylem anatomical traits.

Materials and methods

Plant material and experimental design

The study was performed on four sunflower (*H. annuus*) accessions: a robust 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 free of charge by the Centre Technique Interprofessionnel des Oléagineux Métropolitains (CETIOM), France. Plants were grown in a randomized complete block design with four blocks, including six plants per accession per block, giving a total of 96 plants [$(6 \times 4) \times 4$]. Seeds were sown on March 14, 2014, in pots filled with Peltracom substrate (Greenyard Horticulture, Gent, Belgium) containing peat, clay, plant fibers, volcanic 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 10–12 days after sowing), when the seedlings had reached a height of about 10–15 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, Fiume Veneto, Italy) was used to irrigate the pots. It was set to irrigate all the pots simultaneously, for 10 min every 12 h, 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 08:00 a.m. 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 VCs

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, Brea, CA). This large-diameter rotor was developed for long-vesseled species in order to avoid the so called 'open vessel' artifact that may favor exponential (r-shaped) VCs, as demonstrated several times in woody species with long vessels (Cochard et al. 2013, Martin-StPaul et al. 2014, Choat et al. 2015, Cochard et al. 2015, Torres-Ruiz et al. 2015, 2017). Prior to the main experiment, we collected six sunflower stems to test whether we were able to accurately assess VCs with the standard cavitron (rotor diameter of 27 cm). All VCs obtained with this standard rotor were r-shaped, while those obtained with the 42-cm large rotor were s-shaped (Fig. S1). We then performed maximum vessel lengths measurements on six additional 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 sunflower stems have a maximum vessel length of 23 cm, which confirms that VCs in this species cannot be adequately constructed using the 27 cm diameter rotor, where a significant proportion of open-cut vessels surpass the center of the plant segment or even permeate through its whole length.

Therefore all samples were re-cut under water at 42 cm and 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 m*M* KCl and 1 m*M* $CaCl_2$ 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 1022 *g*. 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 charge coupled device (CCD) camera (Scout sca640, Basler, Ahrensburg, Germany) coupled to custom-written software (Cavisoft version 4.0, BIOGECO, University of Bordeaux, France). After exposing the sample at the required speed during 2 min, hydraulic conductance was measured three times per speed step. The mean values were used to determine the percentage loss of hydraulic conductance (PLC) at each pressure, as follows:

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

where K_{max} is the maximum hydraulic conductance measured at low speed, i.e. at very high xylem pressure. 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):

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

where P_{50} (MPa) is the xylem pressure inducing a 50% loss of conductance and S (% MPa^{-1}) is the slope of the VC at the inflection 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 15–17 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, Wetzlar, Germany), for light microscopy (DM2500 microscope, Leica, Wetzlar, 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 min 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 s, and then subjected to dehydration by successive steps of at least 1 min 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 3 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) 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 (P_{50}, P_{12}, P_{88}, *S*, K_s) and growth (stem diameter and height) traits between accessions were assessed by one-way ANOVA. Correlations between variables were evaluated by calculating the Pearson correlation coefficient (*r*), and were considered to be significant if $P \leq 0.05$. Statistical analyses of the data were performed with SAS software (version 9.4, SAS Institute, Cary, NC).

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 ($F=20.96$, $P \leq 0.0001$; Table 2). The VCs of the four accessions followed a similar sigmoidal shape (Fig. 1). Embolism resistance (P_{50}) differed significantly between accessions ($F=45.59$, $P \leq 0.0001$), 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 ($F=33.95$, $P \leq 0.0001$), 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.

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 inflection 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

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	$1.6 \times 10^{-4} \pm 0.9 \times 10^{-4}$**ab** (16)	$1.4 \times 10^{-4} \pm 0.8 \times 10^{-4}$**b** (17)	$2.4 \times 10^{-4} \pm 0.9 \times 10^{-4}$**a** (16)	$1.7 \times 10^{-4} \pm 0.7 \times 10^{-4}$**ab** (15)

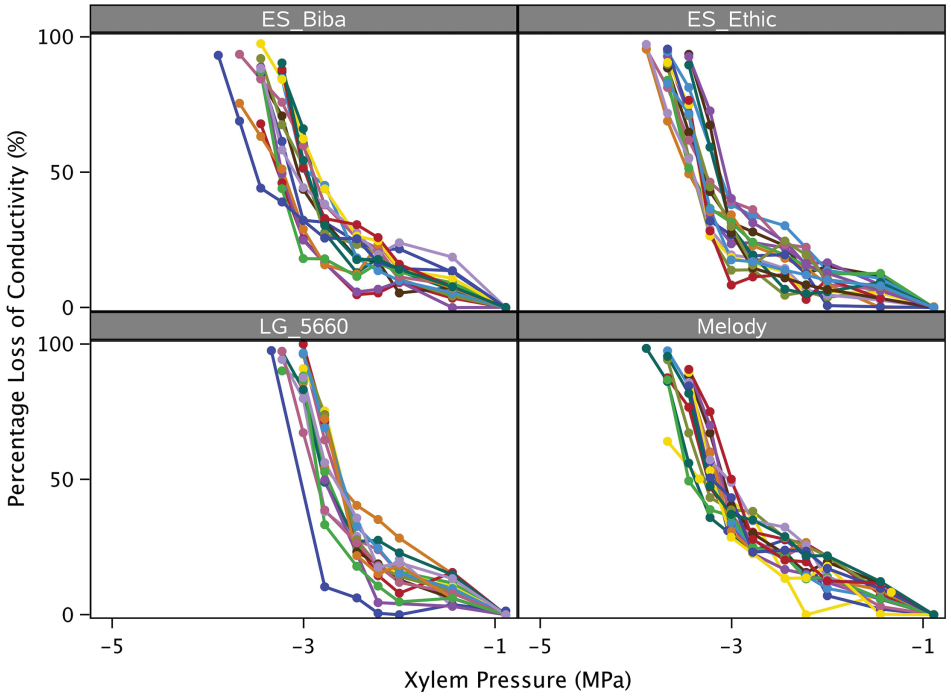


Fig. 1. Vulnerability curves (VCs) for individuals of the four sunflower accessions studied, for which xylem embolism was induced by in situ flow centrifugation according to the Cavitron technique. n = 16, 17, 16 and 15 for Melody, LG_5660, ES_Ethic and ES_Biba, respectively. VCs are expressed as the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

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} ($r = 0.54$; $P \leq 0.0001$), 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) ($r = -0.56$; $P \leq 0.0001$; Fig. 3B), indicating 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 (Table 1 and Table S1). P_{12} and P_{50} were strongly and negatively correlated with vessel density in both secondary and total

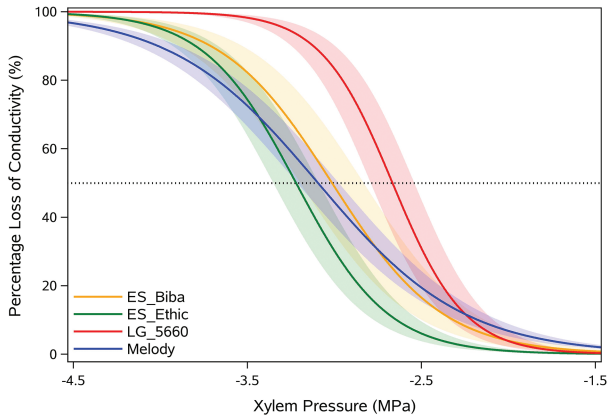


Fig. 2. Mean vulnerability curves (VCs) (± SE) for each of the four sunflower accessions studied. n = 16, 17, 16 and 15 for Melody, LG_5660, ES_Ethic and ES_Biba, respectively. VCs were generated by the in situ flow centrifugation (Cavitron) technique and are expressed as the percentage loss of hydraulic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

xylem (VD_{Tx} and VD_{Sx}), respectively (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 Table S1 in Appendix 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 *H. 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 -3 MPa) 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

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.

	Growth traits		Anatomical traits						
Hydraulic 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*

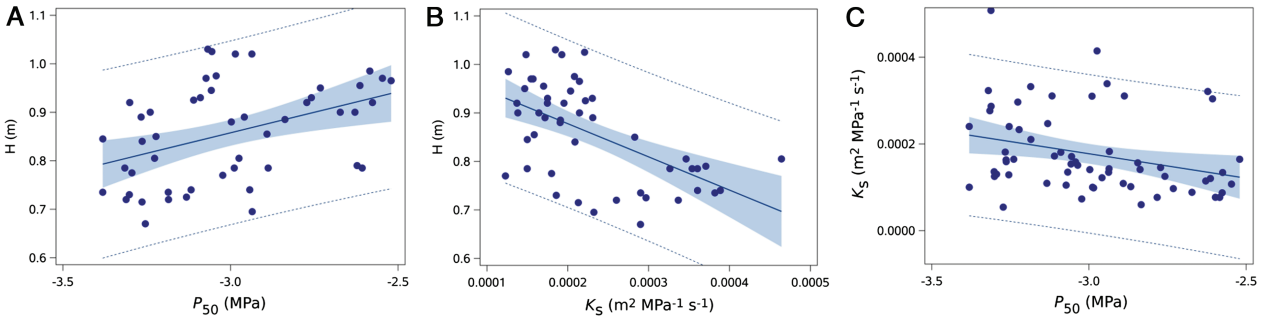


Fig. 3. Relationships between height (H, m), xylem embolism resistance (P_{50}, MPa) and xylem specific hydraulic conductivity (K_s, $m^2\ MPa^{-1}\ s^{-1}$). n = 49, 63 and 49 for panels A, B and C, respectively.

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.

In contrast with previous studies, xylem-specific hydraulic conductivity (K_s) was negatively correlated with both height and stem diameter. 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. These 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

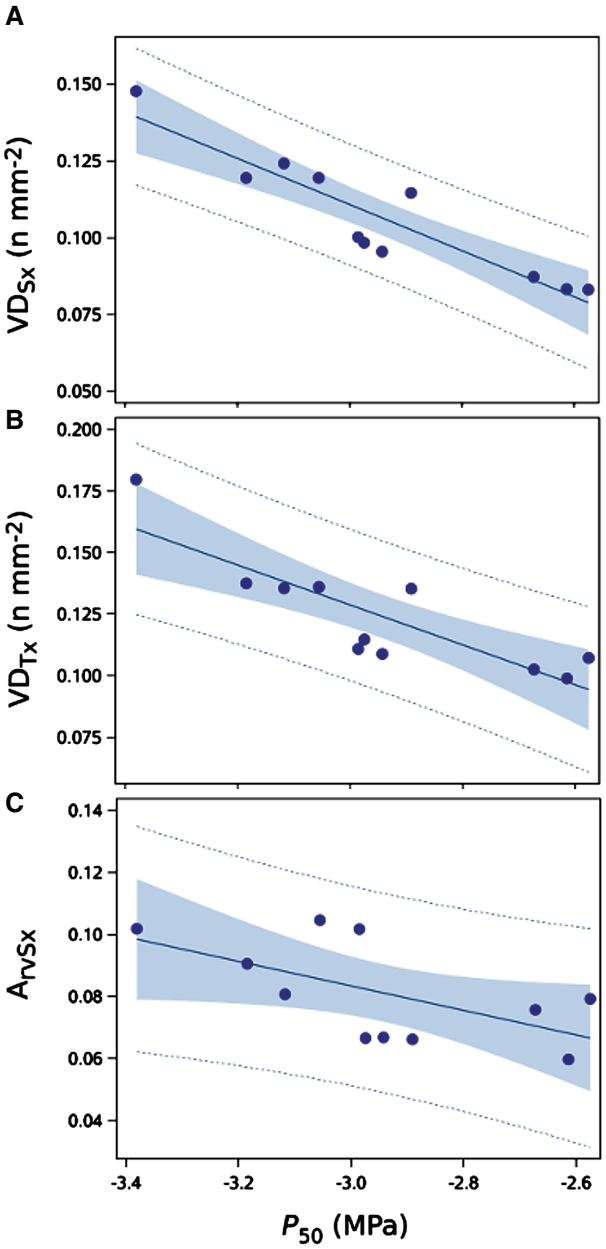


Fig. 4. Relationship between vessel density in secondary xylem (VD_{Sx}; A), vessel density in total xylem (VD_{Tx}; B), vessel lumen area relative to secondary xylem area (A_{rvSx}; C) and xylem embolism resistance (P_{50}, MPa). n = 11.

area relative to secondary xylem area (A_{rvSx}). Vessel density increased with decreasing vessel diameter in both primary and secondary xylem but those trends were not significant ($P = 0.10$ and $P = 0.09$ for D_{Sx} and D_{Px}, respectively). This might explain why we did not observe any correlation between embolism resistance traits and vessel diameter (equivalent circle diameter and hydraulically weighted vessel diameter, Table S1). 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) and woody (Awad et al. 2012) 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 more negative 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 (Hacke et al. 2001, Bouche et al. 2014) 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.

Ideally, plants should be able to maintain both the efficient conductivity and safety of the hydraulic system. 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. However, a large scale study recently reported little or no support for a safety-efficiency trade-off 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, Larter et al. 2017) 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). Indeed, Lens et al. (2011) and Hajek et al. (2014) found a positive relationship between K_s and relative vessel lumen area and vessel diameter, indicating

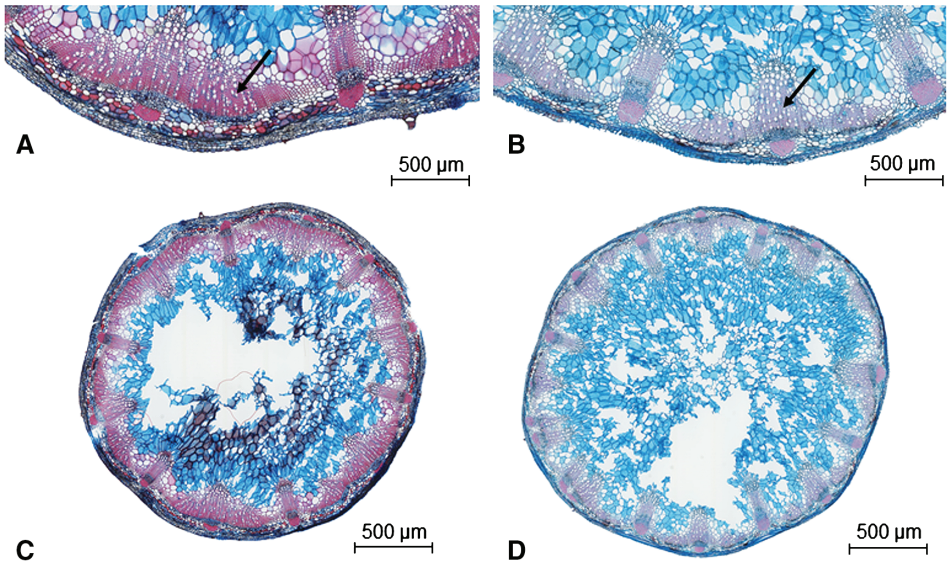


Fig. 5. Transverse sections of (A, C) the most resistant (ES_Ethic) and (B, D) the most vulnerable (LG_5660) sunflower accessions. Plants were grown in pots filled with Peltracom substrate placed in a greenhouse under full light and non-limiting growing conditions. They were sectioned with a sledge microtome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

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). 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. This suggests that (1) increases in lumen conductivity are not necessarily associated with increases in total conduit conductivity and (2) the potential prominent role of the pit resistivity of the conduit end walls in the water-conducting efficiency of vessels (about 56% according to Sperry et al. 2006). However, further studies are required to determine how pits can be efficient for water transport, leading to low values of pit resistivity, while also limiting air-seeding under high xylem tension.

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 (1) the variability of embolism resistance across a wider range of accessions, (2) the extent of the negative correlation between K_s and growth traits in crops and (3) 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. Non-significant ($P > 0.05$) correlations between anatomical and hydraulic traits.

Figure S1. Vulnerability curves (VCs) of six sunflower stems, for which xylem embolism was induced by in situ flow centrifugation according to the Cavitron technique.

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