

Title: Over-accumulation of abscisic acid in transgenic tomato plants increases the risk of hydraulic failure

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ABSTRACT

Climate change threatens food security, and plant science researchers have investigated methods of sustaining crop yield under drought. One approach has been to overproduce abscisic acid (ABA) to enhance water use efficiency. However, the concomitant effects of ABA overproduction on plant vascular system functioning are critical as it influences vulnerability to xylem hydraulic failure. We investigated these effects by comparing physiological and hydraulic responses to water deficit between a tomato (*Solanum lycopersicum*) wild type control (WT) and a transgenic line overproducing ABA (sp12). Under well-watered conditions, the sp12 line displayed similar growth rate and greater water use efficiency by operating at lower maximum stomatal conductance. X-ray microtomography revealed that sp12 was significantly more vulnerable to xylem embolism, resulting in a reduced hydraulic safety margin. We also observed a significant ontogenic effect on vulnerability to xylem embolism for both WT and sp12. This study demonstrates that the greater water use efficiency in the tomato ABA overproducing line is associated with higher vulnerability of the vascular system to embolism and a higher risk of hydraulic failure. Integrating hydraulic traits into breeding programs represents a critical step for effectively managing a crop's ability to maintain hydraulic conductivity and productivity under water deficit.

KEYWORDS

Xylem embolism, tomato, water deficit, abscisic acid, hydraulic failure, transgenic line, crop, drought

INTRODUCTION

While food demands are growing due to the increasing world population, crop production and quality have suffered from increasing temperatures and considerable shifts in precipitation patterns (Ahmed & Stepp, 2016; Porter et al., 2014; Schauburger et al., 2017). For example, Lesk et al. (2016) showed that droughts and extreme heat events between 1964 and 2007 reduced global cereal production by ca. 10% over this period, while global maize and wheat production are projected to decline by 16% in South Asia and 17% in Africa by 2050 (Knox, Hess, Daccache, & Wheeler, 2012). Climate variability is also responsible for as much as a 60% year-to-year variability in crop yield, decreasing the stability of the world's food supply (Matiu, Ankerst, & Menzel, 2017; Ray, Gerber, MacDonald, & West, 2015). Maintaining high and constant crop yields under increasing water deficit conditions or water conservation policies is a serious challenge for the sustainability of agriculture in the future.

Water deficit represents the single most limiting factor of crop yield (Lobell & Field, 2007), thus breeding programs have understandably searched to lower plant transpiration and improve crop water use efficiency, especially under rainfed or deficit irrigation conditions. A substantial body of literature has addressed this matter, from examining the genetic aspects of increased leaf-level transpiration efficiency (TE or intrinsic WUE, WUE_i) to field-level plant (WUE) and plot (WUE_{ag}) efficiencies (Condon, Richards, Rebetzke, & Farquhar, 2002; Passioura, 2006; Passioura & Angus, 2010). Breeding for higher WUE has been found to be particularly beneficial in water-limited environments and when severe terminal drought periods occur during a crop's reproductive growth stage (Lopes, Araus, van Heerden, &

Foyer, 2011; Tardieu, Parent, Caldeira, & Welcker, 2014).

In this context, extensive work has been devoted to the plant hormone abscisic acid (ABA), which is considered the main chemical signal involved in stomatal regulation (Bauer et al., 2013; Mittelheuser & van Steveninck, 1969; Zhang & Davies, 1989). The correlation between increased ABA concentration and stomatal closure during progressive water deficit has long been documented in angiosperms (Bauerle, Whitlow, Setter, & Vermeulen, 2004; McAdam & Brodribb, 2015), and in various crops such as wheat (Henson, Jensen, & Turner, 1989; Saradadevi, Bramley, Siddique, Edwards, & Palta, 2014), maize (Tardieu et al., 1992), sunflower (Tardieu, Lafarge, & Simonneau, 1996) and tomato (Thompson et al., 2007a). The drought-induced increase in ABA content thus allows plants to conserve their water content by reducing transpiration, and thereby to postpone the inevitable reduction in leaf water potential. This was observed in tomato seedlings where the application of ABA delayed wilting (Vu, Kang, Kim, Choi, & Kim, 2015).

The use of chemical ABA receptor agonists to activate ABA receptors and to increase ABA signaling has agronomic promise (Helander, Vaidya, & Cutler, 2016), and transgenic approaches to increase ABA biosynthesis and signaling have been successful in enhancing WUE. Overexpression of the rate-limiting enzyme in ABA biosynthesis 9-*cis*-epoxycarotenoid dioxygenase (NCED) led to increased ABA accumulation (Thompson et al., 2000), lower stomatal conductance, and up to 79% higher gravimetric transpiration efficiency (another term for WUE) with only small effects on photosynthetic carbon assimilation or growth (Thompson et al., 2007a). Similarly, in *Arabidopsis* and wheat, activation of ABA signaling through manipulation of ABA receptors led to an increase in WUE of up to 40% without impacting growth significantly (Mega et al., 2019; Papacek, Christmann, & Grill, 2019; Yang et al., 2016). The use of candidate genes therefore holds huge promise for the production of crops with enhanced water use efficiency. However, translating the

manipulation of the ABA pathways into practical improvements in WUE and crop yield has either not been attempted, or has been challenging (Blum, 2015; Nuccio, Paul, Bate, Cohn, & Cutler, 2018). Challenges arise because stomatal limitations to photosynthesis can depress yield, or because ABA may have pleiotropic effects on plant growth and development. The variability of water deficit intensity and timing throughout growing seasons (Saradadevi, Palta, & Siddique, 2017; Vadez, Kholova, Medina, Kakkera, & Anderberg, 2014) may also require different ranges of stomatal regulation and other responses to achieve a favorable yield outcome while efficiently using available water and optimizing growth.

It is understandable that breeding programs have focused on surrogate or indicative traits such as carbon isotope discrimination, ABA content, osmotic adjustment, and rooting depth that theoretically relate to agronomic improvements in WUE, drought resistance, and yield under water limited environments (Blum, 2017; Blum & Tuberosa, 2018; Hu & Xiong, 2014; Kell, 2011). However, consideration should also be given to resistance to xylem embolism, which represents a plant's capacity to maintain its hydraulic functioning (i.e. hydraulic conductivity) that is essential for photosynthesis and productivity (Brodribb & Feild, 2000). Xylem embolism, i.e. hydraulic failure by air-entry into the water conducting xylem cells, disrupts water transport, induces a decrease in hydraulic conductivity, and can provoke tissue desiccation, growth decline, and ultimately plant death. Studies in plant hydraulics have mainly focused on woody species, but newly developed imaging tools such as X-ray microtomography (Cochard, Delzon, & Badel, 2015) and the optical vulnerability technique (Brodribb et al., 2016) allow for a better understanding of the thresholds of vulnerability to water deficit in herbaceous species. Some herbaceous grasses have been found to be more resistant to embolism than previously thought: the xylem pressure inducing a 50% loss of hydraulic conductivity (Ψ_{50}) ranged from -0.5 MPa in reed canary grass (*Phalaris arundinacea*) to -7.5 MPa in European feather grass (*Stipa pennata*) (Lens et al., 2016). The

few estimates of crop Ψ_{50} available so far range from -1.6 MPa in rice (Stiller, Lafitte, & Sperry, 2003) to -3 MPa in sunflower (Ahmad et al., 2017), but embolism resistance can vary significantly between different varieties within a crop species (Ahmad et al., 2017). The limited number of studies investigating crop hydraulics were carried out on maize (Cochard, 2002a; Li et al., 2009; Ryu, Hwang, Kim, & Lee, 2016; Tyree, Fiscus, Wullschlegel, & Dixon, 1986), sugarcane (Neufeld et al., 1992), sunflower (Ahmad et al., 2017; Cardoso, Brodribb, Lucani, DaMatta, & McAdam, 2018; Stiller & Sperry, 2002), and rice (Stiller et al., 2003; Stiller, Sperry, & Lafitte, 2005). These studies have highlighted the importance of resisting xylem embolism and maintaining hydraulic conductivity during periods of soil water deficit. For example, a recent study in wheat using the optical technique illustrated the terminal nature of high levels of xylem embolism, demonstrating that embolism formed at -2 MPa and more negative water potentials could not be repaired by rewatering the plants that had been previously exposed to water deficit (Johnson, Jordan, & Brodribb, 2018). Severe soil drying therefore carries the risk of irreversible xylem embolism and catastrophic loss of yield. Increasing our knowledge of how xylem embolism interacts with chemical and genetic strategies to improve water use efficiency in crops is important to ensure the success of these crop improvement strategies and help build an integrated view of the behavior of crops under drought.

This study investigated the concomitant effects of increased ABA production on both plant physiology and hydraulic functioning during progressive water deficit, using an ABA overproducing transgenic tomato (*Solanum lycopersicum*) line known to have improved water use efficiency (Thompson et al., 2007a). We hypothesized that the high levels of ABA would decrease the risk of hydraulic failure by increasing the hydraulic safety margin through the earlier stomatal closure that would both maintain less negative water potentials and delay water loss, while having no effect on xylem embolism resistance. We tested this hypothesis

by comparing the physiological and hydraulic responses of the ABA overproducing transgenic line and its wild type counterpart under well-watered and water deficit conditions.

MATERIALS AND METHODS

Plant materials

This study was centered on two homozygous true-breeding lines of *Solanum lycopersicum* L. cv. ‘Ailsa Craig’: the wild type control (WT) and the transgenic line sp12 that overexpresses the rate-limiting ABA biosynthetic gene *LeNCED1*. The sp12 line harbors a construct with the *LeNCED1* transgene driven by the Gelvin Superpromoter (Thompson et al., 2000; Thompson et al., 2007a). Seeds of both lines were provided by A. Thompson (Cranfield University, UK). All the plants used in the different experiments detailed below were grown in the same greenhouse located at the INRA station of Villenave d’Ornon, France. Greenhouse conditions followed a 15:9 h temperature cycle of 22:20 °C with mean daily light intensity set at 2018 W m⁻² and humidity maintained 24 h a day at 65%. The protocol for seed germination and establishment followed recommendations detailed in Thompson et al. (2007b) and Smeeton (2010). Briefly, wild type and sp12 seeds were first germinated onto filter paper soaked in distilled water and kept in the dark at 25 °C until radicle emergence, which respectively occurred after 3-4 and 7-8 days in the wild type and sp12 line. Seeds were then sown for establishment in 4-L pots filled with two-thirds compost (Substrat 4, Klsammann-Deilmann) and one-third perlite. As the high ABA content in the transgenic line delayed seed establishment by 5-8 days compared to the wild type, sowing of sp12 seeds in pots was advanced accordingly in order to synchronize the growth of both lines. The ages of tomato plants that are mentioned below were calculated from the day of establishment. All plants were irrigated daily to field capacity until the start of experiments.

Growth, gas exchange, ABA and WUE_i

The monitoring of stomatal conductance and gas exchange under increasing water deficit was carried out over a 12-day period on 70-day-old plants that were ca. 85 ± 6 cm tall and 6.5 ± 0.1 mm wide (no significant difference between lines; initial height: $F = 0.01$, $P = 0.9210$; initial diameter: $F = 4.39$, $P = 0.0515$). Final plant height and stem diameter were recorded for the 30 plants included in this experiment while aboveground biomass was harvested and weighed after being dried at 65 °C for at least 48 h on six well-watered plants per line that served as controls.

Gas-exchange measurements were conducted using an infrared gas analyzer (GFS-3000, Heinz Walz GmbH, Effeltrich, Germany) equipped with CO₂, humidity, temperature, and light control modules. The conditions inside the sealed cuvette of 3 cm² were set as follows: 400 ± 5 ppm of CO₂, $60 \pm 6\%$ of relative humidity, cuvette temperature of 22 °C and light exposure of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Leaf vapor-pressure-deficit (VPD) was maintained between 0.9 and 1.4 kPa. Measurements during each trial were performed daily between 08:00 and 11:00 solar time using exclusively mature leaves that were located at branch nodes 10-14. A total of fifteen plants were monitored per line including six well-watered controls and nine water-stressed. All the plants were randomly located within the greenhouse area dedicated to the experiment. Six to eight individuals per line and one to two leaves per individual were measured every day, with half corresponding to control plants and the other to stressed plants. Overall, 170 gas-exchange measurements were executed on 44 wild type and 45 sp12 plants.

Upon completion of daily stomatal conductance measurements, the sampled leaves were immediately harvested for measurements of foliar ABA content. They were frozen in liquid nitrogen and stored at -80 °C before being lyophilized and crushed. Analyses were carried out following a slightly modified protocol described in Li-Marchetti et al. (2015). For each sample, 1 mg of dry powder was extracted with 0.8 mL of acetone/water/acetic acid (80/19/1

v:v:v) before the addition of 1 ng of abscisic acid stable labelled isotope as internal standard. The extract was vigorously shaken for 1 min, sonicated for 1 min at 25 Hz, and shaken again for 10 min at 10 °C in a Thermomixer (Eppendorf®) before being centrifuged (8000 g, 10 °C, 10 min). The supernatants were then collected and the pellets re-extracted twice with 0.4 mL of the same extraction solution before being vigorously shaken for 1 min and sonicated 1 min at 25 Hz. After the centrifugations, the three supernatants were pooled and dried (Final Volume 1.6 mL). Each dry extract was then dissolved in 100 µL of acetonitrile/water (50/50 v/v), filtered, and analyzed using a Waters Acquity ultra performance liquid chromatograph coupled to a Waters Xevo Triple quadrupole mass spectrometer TQS (UPLC-ESI-MS/MS). The compounds were separated on a reverse-phase column (100*2.1 mm*3 µm particle size; Uptisphere C18 UP3HDO, Interchim, France) using a flow rate of 0.4 mL min⁻¹ and the following binary gradient with a column temperature set at 40 °C: (A) acetic acid 0.1% in water (v/v) and (B) acetonitrile with 0.1% acetic acid. For ABA, the binary gradient used was as follows: (time, % A): (0 min, 98%), (3 min, 70%), (7.5 min, 50%), (8.5 min, 5%), (9.6 min, 0%), (13.2 min, 98%), (15.7 min, 98%). Mass spectrometry was conducted in electrospray and Multiple Reaction Monitoring scanning mode (MRM mode), in negative ion mode for the hormone. The capillary was set at 1.5 kV (negative mode) while source block and desolvation gas temperatures were maintained at 130 °C and 500 °C, respectively. Nitrogen was used to assist the cone and desolvation (150 L h⁻¹ and 800 L h⁻¹, respectively) while argon was used as the collision gas at a flow of 0.18 mL min⁻¹.

The minimum midday stem water potential (Ψ_{\min}) of the monitored plants was also recorded daily around 13:00 solar time, using a Scholander pressure bomb (SAM Precis, Gradignan, France) to measure a leaf adjacent to those used for gas-exchange measurements that had been covered for at least two hours with aluminum foil and wrapped in a plastic bag.

Whole-plant transpiration as well as predawn and minimum midday water potential

kinetics were monitored over a four-day dry down following irrigation to field capacity, and six plants per line remained well-watered throughout the experiment as controls. Plants were weighed both at predawn and midday with a 0.01 g readability balance (S-6002, Denver Instrument, Bohemia, NY), while water potentials were determined following the same procedure than the one mentioned above.

Vulnerability to embolism

X-ray microtomography

Direct, non-invasive visualization of xylem embolism in 120-day-old tomato plants was conducted at the SOLEIL Synchrotron microCT PSICHÉ beamline (King et al., 2016). The main stem of six wild type and four sp12 plants, that were ca. 160 cm tall and 8 mm wide, was scanned throughout dehydration using a high flux (3.10^{11} photons mm^{-1}) 25 keV monochromatic X-ray beam, while being rotated from 0° to 180° using a continuous rotation mode. The high load capacity and free central aperture of the rotation stage allowed to select imaging cross sections near the middle of the main stems, i.e. at ca. 90 cm above the stem base. Dehydration was progressively induced in the ten plants by stopping watering. X-ray projections were collected with a 50-ms exposure time during rotation and recorded with an Orca-flash sCMOS camera (Hamamatsu Photonics K.K., Naka-ku, Japan) equipped with a 250 μm thick LuAG scintillator. The scan time was 75 s for each sample and yielded a stack of 1,500 TIFF image slices. Each stem section was scanned at the same location two to five times throughout dehydration of the tomato plants. Tomographic reconstructions were conducted using the Paganin method (Paganin, Mayon, Gureyev, Miller, & Wilkins, 2002) in PyHST2 software (Mirone, Brun, Gouillard, Tafforeau, & Kieffer, 2014) and resulted in 2-bit volumic images with a $3.02 \mu\text{m}^3$ voxel resolution.

Stem water potential (Ψ_{stem}) was measured before each scan ($n = 33$) with a Scholander

pressure bomb (Model 1000, PMS Instrument, Albany, OR, and SAM PreciS, Gradignan, France) using a leaf located below the scanned area and that had been covered for at least two hours with aluminum foil and wrapped in a plastic bag. Water potential determination was intentionally limited to measurements on a single leaf located below the scanned area as preliminary tests showed high consistency in water potential within each tomato plant. It thus allowed us to avoid possible water potential underestimation at the scan location given that a hydraulic disconnection can occur between the base of the stem and the top leaves after a certain amount of xylem embolism (Charrier et al., 2016).

Quantification of embolized vessels was conducted for each scan from a transverse cross section taken from the center of the scan volume. The theoretical hydraulic conductivity of a whole cross section (k_h) was determined by calculating the individual area and diameter of both air- and water-filled vessels. Vessel visualization, i.e. the distinction between functional vessels from non-functional xylem conduits and fibers, and vessel diameter calculations were facilitated by using a final scan ('final cut') that was generated for each stem section, i.e. each individual. Final cuts represented scans performed after samples were cut in air ca. 2 mm above the corresponding scanned stem volume, and where functional vessels with sap under tension immediately filled with air. Measurements were conducted manually with ImageJ software (Schneider, Rasband, & Eliceiri, 2012). The theoretical hydraulic conductivity (k_h , $\text{m}^4 \text{MPa}^{-1} \text{s}^{-1}$) of air-filled vessels of each cross section was calculated as:

$$k_h = \sum \pi D^4 / 128 \eta \quad (1)$$

, where D is the diameter of vessels (m) and η is the water viscosity ($1.002 \cdot 10^{-3} \text{ Pa s}$ at 20°C).

The theoretical loss of hydraulic conductivity (PLC, %) was then determined as:

$$\text{PLC} = 100 \cdot (k_h / k_{\max}) \quad (2)$$

, where k_{\max} represents the theoretical hydraulic conductivity of all functional vessels as based

on a cross section after the final cut.

In situ flow-centrifuge technique (MEGA-CAVITRON)

Vulnerability to embolism in WT and sp12 lines was also determined at the high-throughput phenotyping platform for hydraulic traits (Caviplace, University of Bordeaux, Talence, France). Preliminary tests using both standard (27-cm large rotor) and large (42-cm large) Cavitron (Cochard, 2002b; Cochard et al., 2005) proved unsuccessful to determine maximum stem hydraulic conductivity (k_s , $\text{m}^2 \text{MPa}^{-1} \text{s}^{-1}$) and construct vulnerability curves due to the open-vessel artefact (Torres-Ruiz et al., 2017). Measurements were consequently carried out using a Cavitron equipped with a 100-cm diameter rotor (DG-MECA, Gradignan, France), which permits proper embolism vulnerability determination in long-veined species (Charrier et al., 2018; Lamarque et al., 2018). Two sets of plants differing in their development stage were compared, including five 90-day-old individuals per line and four and seven 170-day-old WT and sp12, respectively. Plants were brought from the greenhouse to the laboratory early in the morning of each measurement day, wholly enclosed with plastic bags to avoid water loss by transpiration and high xylem tensions that could induce artefactual xylem embolism (Torres-Ruiz et al., 2015). The main stems were then kept in water for xylem relaxation during ca. 1 h prior to measurements, when they were cut under water to a standard length of 1 m. A solution of ultrapure and deionized water containing 10 mM KCl and 1mM CaCl_2 was used as reference ionic solution, while the centrifuge rotation speed was initially set to induce a xylem pressure of -0.8 MPa before being gradually increased to lower xylem pressures by -0.5 MPa. Hydraulic conductivities at every rotation speed (k_i , $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) were measured using the Cavisoft software (v. 5.2, University of Bordeaux). The percentage loss of hydraulic conductivity (PLC) was determined at each pressure as follows:

$$\text{PLC} = 100 \cdot (1 - k_i / k_{\max})$$

(3)

, where k_{\max} represents the maximum hydraulic conductivity measured at the first induced xylem pressure.

Vulnerability curve fitting

Vulnerability curves, corresponding to percentage loss of hydraulic conductivity as a function of stem water potential, were fitted using the NLIN procedure in SAS 9.4 (SAS, Cary, NC, USA) based on the following equation (Pammenter & Van der Willigen, 1998):

$$PLC = 100/(1+\exp(S/25.(\Psi-\Psi_{50}))) \quad (4)$$

, where Ψ_{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 pressures inducing 12% (Ψ_{12}) and 88% (Ψ_{88}) loss of hydraulic conductivity were calculated as follows: $\Psi_{12} = 50/S + \Psi_{50}$ and $\Psi_{88} = -50/S + \Psi_{50}$. One vulnerability curve was obtained per tomato plant in both microCT observations and centrifuge measurements. In other words, the Pammenter model was fitted on each vulnerability curve to obtain a Ψ_{50} and slope value per individual and subsequently an average value per line per method.

Hydraulic conductivity and root pressure

Hydraulic conductivity of unstressed tomato stems (K_s , kg m⁻¹ s⁻¹ MPa⁻¹) was obtained gravimetrically on nine 50-day-old plants per line using 40- to 60-mm-long segments from the same position along the stems, i.e. between plant nodes 5 and 6. Measurements followed the protocol described originally in Sperry et al. (1988) and later corrected by Torres-Ruiz, Sperry, & Fernandez (2012). Using deionized and filtered (0.2 µm filters) water as the perfusion solution, we recorded the water flow passing through the stem segments from an upstream reservoir to a downstream one that was connected to a high-precision analytical

balance (0.1 mg readability; Praxum224-1S, Sartorius, Göttingen, Germany) and interfaced with a computer. We used four different upstream reservoir heights corresponding to applied pressures of 8.1 to 4.2 kPa. The flow rate at the downstream balance was recorded at 5 s intervals with running means calculated from the last 10-15 records after reaching a steady state. Once hydraulic measurements were completed, stems were transferred in a 70% ethyl-alcohol solution and stored at room temperature until xylem vessel anatomy measurements (Ruzin, 1999). 70- μ m-thinned slices covering the entire stem cross-section were obtained using a WSL GSL-1 sledge-microtome (Gärtner, Lucchinetti, & Schweingruber, 2014), stained in a solution of 0.5% Safranin O (95% ethyl-alcohol) for 2 min and rinsed twice within a solution of 100% ethyl-alcohol for about 5 min. They were then transferred in xylene for 15 min, mounted between slide and cover slip in Histolaque LMR (Labo-Moderne, Paris, France) and let dry for at least 24 h. High resolution micrographs (ca. 500 nm/pixel) were obtained using a Nanozoomer 2.0 HT (Hamamatsu Photonics, Hamamatsu City, Japan) in bright-field mode. Measurements of stem tissues and xylem vessels were carried out on the entire section with ImageJ v1.52 software as described by Pouzoulet et al. (2017). Stem hydraulic conductivity (k_s) was determined from the slope of a plotwater flow versus pressure gradient multiplied by stem length, while specific hydraulic conductivity (K_s) was calculated as the ratio of k_s to xylem cross-sectional area.

Root hydraulic conductivity (L_{pr} , $\text{kg s}^{-1} \text{MPa}^{-1} \text{kg}^{-1} \text{DW}$) was measured as the water flow rate generated in response to applied pressures (Boursiac et al., 2005; Postaire et al., 2010). The tubing apparatus resembled the one aforementioned for K_s measurements, apart from the downstream balance that was this time connected to detopped roots that were previously fitted in a plastic tube filled with deionized and filtered water and placed into a pressure chamber. Four to five pressures ranging from 0.02 to 0.12 MPa were applied on each of the fifteen well-watered 50-day-old root systems per line that were manipulated. Once measurements were

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completed, roots were dried at 65 °C for at least 24 h before dry weight determination. Lp_r was calculated from the slope of a plot water flow versus pressure gradient, divided by the dry weight of root system.

Prior to Lp_r measurements, root systems were connected to a 30 Psi pressure probe transducer (26PCFFA6D, Honeywell, Morristown, NJ; Charrier et al., 2017) via a tube filled with deionized and filtered water. Root pressure (P_r , MPa) was recorded continuously using a high resolution datalogger (USB-TC-AI, Measurements Computing, Norton, MA) with mean values calculated from the steady state period that was usually reached after 10 min. P_r measurements were carried out on twelve 50-day-old plants per line that had similar root biomass of 2.5 ± 0.3 g ($P = 0.77$).

Simulated patterns of stomatal closure and loss of hydraulic conductivity

A simplified discrete-time soil-plant hydraulic model (SurEau; Martin-StPaul, Delzon, & Cochard, 2017) was used to (i) simulate for each line the temporal decline in g_s and the increasing loss of hydraulic conductivity during the progression of a simulated soil drought, and (ii) estimate the sensitivity of the loss of hydraulic conductivity dynamics to a change in either g_s or Ψ_{50} . For this latter objective, simulations were performed by modeling the PLC dynamics of the wild type line using the g_s or Ψ_{50} values of the sp12 line.

Statistical analyses

Prior to analyses, stomatal conductance measurements were first averaged per plant per day before being binned in equal classes of minimum midday water potentials (HBIN procedure with quantile method in SAS, version 9.4, SAS Institute, Cary, NC). The decline of stomatal conductance with decreasing water potentials was then fitted for each line using the NLIN procedure based on the following equation:

$$g_s = g_{smax}/(1+\exp(S.(\Psi-\Psi_{gs50}))) \quad (5)$$

The water potential value corresponding to 10% g_s decline ($\Psi_{stem} = -0.7$ MPa for both lines) was used as a threshold to differentiate well-watered from water deficit conditions. Overall differences between lines and across treatments (Ψ classes for foliar ABA content, g_s , A , E and WUE_i ; day since last watering for Ψ_{pd} and Ψ_{min} ; age for Ψ_{50} , Ψ_{50} , Ψ_{50} and S) were tested using a general linear model that included line, the respective treatment and their interaction term as fixed effects (GLM procedure). Differences in growth, physiological, hydraulic and anatomical traits were further evaluated using a one-way analysis of variance (ANOVA procedure).

RESULTS

ABA, growth, gas exchange and WUE_i

The transgenic plants showed higher foliar ABA level than WT (Table 1; mean = 3.8 ± 0.3 vs. 3.1 ± 0.2 $\mu\text{g g}^{-1}$ DW in sp12 and WT, respectively), due to a significant difference under well-watered conditions corresponding to water potential values inducing less than 10% g_s decline (i.e. $\Psi_{stem} > -0.7$ MPa; $P = 0.0081$; Figure S1). The level of foliar ABA increased similarly for both lines with increasing water deficit (significant water deficit but no significant line*water deficit effect; Table 1, Figure S1). Along the course of the experiment, WT and sp12 plants displayed a similar growth rate (Table S1, Figure S2a,b), which resulted in similar final above-ground biomass and total leaf area after a 70-day growing period (Table S1, Figure S2c-f).

The two lines expressed significant overall differences in gas exchange (Table 1). Under well-watered conditions, the sp12 plants had a 30% lower maximum stomatal conductance

(g_{max} ; mean = 229 ± 30 vs. 324 ± 46 $\text{mmol m}^{-2} \text{s}^{-1}$ in sp12 and WT, respectively; $P = 0.0031$; Figure 1). The ABA overproducing plants also showed a 15% and 26% decrease in the rate of assimilation (A ; mean = 8.6 ± 0.4 vs. 10.1 ± 0.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in sp12 and WT, respectively; $P = 0.0264$) and transpiration per unit leaf area (E ; mean = 2.17 ± 0.15 vs. 2.94 ± 0.21 $\text{mmol m}^{-2} \text{s}^{-1}$ in sp12 and WT, respectively; $P = 0.0049$) compared to the wild type individuals. Consequently, the transgenic line operated with a greater intrinsic water use efficiency (WUE_i , corresponding to the A/g_s ratio; mean = 0.049 ± 0.004 vs. 0.033 ± 0.005 $\mu\text{mol mmol}^{-1}$ in sp12 and WT, respectively; $P = 0.0214$), and exhibited a significant 20% decrease in daily water use (mean = 0.43 vs. 0.57 $\text{kg H}_2\text{O d}^{-1}$ in sp12 and WT, respectively; Table S1). Under water deficit conditions, stomatal conductance decreased in a similar way for both sp12 and wild type plants (significant water deficit but no significant line*water deficit effect; Table 1, Figure 1). The Ψ value at 90% stomatal closure ($\Psi_{\text{gs}90}$) was reached at -0.92 and -0.96 MPa for WT and sp12, respectively (Figure 1). Under water-stressed conditions, WT and sp12 expressed similar A , E and WUE_i .

Vulnerability to embolism

MicroCT observations revealed that the transgenic line was significantly more vulnerable to xylem embolism than its wild type counterpart (Table 2). The onset of xylem embolism (Ψ_{12}) in wild type plants occurred when Ψ_{stem} reached ca. -1.8 MPa (Figure 2a). The hydraulic conductivity then dropped steadily and reached critical thresholds of Ψ_{50} and Ψ_{88} at ca. -2.7 and -3.7 MPa, respectively (Figure 2b). In contrast, embolized xylem vessels in stems of sp12 plants were first observed at higher (i.e. less negative) water potentials ($\Psi_{12} \sim -1.1$ MPa; Figure 2a), with the loss of hydraulic conductivity occurring to reach 50% and 88% at $\Psi_{\text{stem}} = -1.5$ and -1.9 MPa, respectively (Figure 2b).

The overall difference in xylem embolism resistance between the wild type and sp12 plants

was confirmed by measurements carried out with the *in situ* flow-centrifuge technique ($P = 0.0145$, Table 1). The differences were also much larger at the early stage of plant growth, i.e. at 90 days compared to 170 days old (Table 2). Both wild type and sp12 lines increased resistance to xylem embolism by 18% and 29% throughout plant development, i.e. Ψ_{50} values decreased significantly between 90- and 170-day-old plants (significant age effect; Table 1, Figure 3). Similarly, the onset of xylem embolism (Ψ_{12}) dropped from -1.3 to -2.0 MPa between 90- and 170-day-old wild type individuals, and from -1.2 to -1.7 MPa between 90- and 170-day-old sp12 individuals.

Water potentials and hydraulic efficiency

A significant decrease was observed in predawn (Ψ_{pd}) and minimum midday (Ψ_{min}) water potentials during dehydration (Table 1, Figure 4). While this decrease did not differ between the two lines (no significant line*time effect), a significant difference was found between 70-day-old wild type and sp12 plants in both Ψ_{pd} and Ψ_{min} (Table 1). The sp12 plants maintained less negative water potentials with Ψ_{pd} and Ψ_{min} going down to -0.6 MPa and -0.8 MPa, respectively. The wild type in contrast displayed Ψ_{pd} and Ψ_{min} down to -0.8 MPa and -1.0 MPa, respectively.

After 50 days of growth, the sp12 plants displayed a statistically significant 30% reduction in stem hydraulic conductivity normalized to xylem area (K_s), and a 43% increase in root conductivity (L_{pr}) compared to the wild type (Table S1, Figure 5a,b). No difference was observed in root pressure (P_r) between the lines (Table S1, Figure 5c). Xylem vessel anatomical observations indicated that 50-day-old sp12 plants exhibited 22% less xylem area compared to wild type individuals of similar age (Table S1, Figure S3a). There was no overall difference in vessel density between the two lines (Table S1); however the significant line*vessel class interaction ($P = < 0.0001$) highlighted the greater proportion of small vessels

< 60 μm in sp12 ($P = 0.0015$) and that of large vessels in the wild type ($P = 0.0316$; Figure S3b).

Hydraulic safety margins

By expressing similar stomatal closure but higher vulnerability to xylem embolism than their wild type counterparts, sp12 plants had a narrower hydraulic safety margin (Figure 6). The $\text{HSM}_{\psi_{12}}$ and $\text{HSM}_{\psi_{50}}$, corresponding to the difference between $\Psi_{\text{gs}90}$ and the Ψ_{12} or Ψ_{50} of 90-day-old plants, were reduced by 28% and 37% in the sp12 line, respectively ($\text{HSM}_{\psi_{12}} = 0.26$ vs. 0.36 MPa and $\text{HSM}_{\psi_{50}} = 1.03$ vs. 1.63 MPa in sp12 and WT). A similar result was found when defining the hydraulic safety margin as the difference between Ψ_{min} and Ψ_{50} , the $\text{HSM}_{\psi_{\text{min}}}$ being 26% lower for the sp12 than for the wild type plants (1.15 vs. 1.55 MPa).

Simulated patterns of stomatal closure and loss of hydraulic conductivity

In simulations, the experimentally observed difference in maximum stomatal conductance between lines resulted in a 16-h difference in stomatal closure as wild type and sp12 lines reached $\Psi_{\text{gs}90}$ ca. 80 h and 95 h after the beginning of the drought period, respectively (Figure 7a). In terms of time, the model showed that both lines exhibited similar dynamics in the loss of hydraulic conductivity, with Ψ_{50} being reached after 7 days of water deficit (Figure 7a).

The sensitivity of the loss of hydraulic conductivity to a change in either g_s or Ψ_{50} was assessed by simulating the loss of hydraulic conductivity in the wild type when subjected to the same g_s or Ψ_{50} value as the transgenic line. We observed in simulated WT behavior that lower g_{smax} and delayed stomatal closure as found in the sp12 line had no effect on the PLC dynamics of the wild type (Figure 7b). However, 50% loss of hydraulic conductivity in the wild type was reached ca. 24 h earlier with higher Ψ_{50} (i.e. with greater vulnerability to xylem embolism) (Figure 7b).

DISCUSSION

Increased ABA production in the tomato sp12 transgenic line resulted in greater water use efficiency under well-watered conditions but, contrary to what we hypothesized, also led to higher vulnerability to xylem embolism compared to its wild type counterpart. This study provides a functional framework for testing how crop genotypes could respond to water deficit, suggesting that transgenic lines that overproduce ABA could have a narrower hydraulic safety margin, a lower capacity to maintain stem hydraulic conductivity, and subsequently a higher risk of mortality under drought.

In agreement with Thompson et al. (2007a) who first reported physiological characteristics of tomato wild type and transgenic sp12 lines, we found differences between the two genotypes under unstressed, well-watered conditions. Elevated ABA production allowed the transgenic line to operate at lower maximum stomatal conductance under well-watered conditions, supporting the large body of literature that has described the biochemical effect of ABA on guard cells and stomatal closure in a wide range of plant species including crops (Pantin et al., 2013; Saradadevi et al., 2014; Tardieu et al., 1992). Compared to the wild type line, the sp12 plants thus maintained lower leaf transpiration and higher stem water potentials, similar to the findings of Tonetto de Freitas et al. (2011) when spraying tomato cultivars with exogenous ABA (500 mg L⁻¹). As expected, the sp12 transgenic line displayed greater intrinsic water use efficiency as well as lower daily water use; however in this study, this was not translated into significantly greater whole-plant WUE because of high variation in biomass among plants (coefficient of variation of 25% for above-ground biomass). Previous work indicated that the long-term elevation of ABA accumulation in sp12 induced a 27% increase in whole-plant WUE compared to the wild type (Thompson et al., 2007a), without any effect on total fruit production (Smeeton, 2010). Other recent manipulations of ABA

content in *Arabidopsis* and wheat also managed to generate plants growing at both high WUE and high growth rates (Papacek et al., 2019; Yang et al., 2016; Yang et al., 2019).

Importantly, our study reveals for the first time that constitutive variation in ABA concentration is associated with changes in xylem vulnerability to embolism. The transgenic line was found to be 43% and 22% more vulnerable to xylem embolism in stems than the wild type after 90 and 120 days of growth, respectively. This is critical since direct, non-invasive observations of embolism thresholds in plants subject to dehydration-rehydration cycles have recently highlighted the lack of xylem embolism recovery under tension (Charrier et al., 2016 in grapevine; Choat et al., 2019 in eucalypts and pin oak; Johnson et al., 2018 in wheat; Knipfer et al., 2017 in laurel). Given that the sp12 transgenic plants showed a similar water potential threshold inducing stomatal closure to the wild type, their greater vulnerability to xylem embolism resulted in a reduced hydraulic safety margin and thereby increased vulnerability to hydraulic failure. This result is consistent with the fact that the magnitude of safety margins is more strongly associated with variation in vulnerability to embolism than with differences in water potentials inducing stomatal closure (Martin-StPaul et al., 2017). It also emphasizes that under water deficit conditions the sp12 transgenic line is likely to be unable to maintain its full hydraulic integrity (i.e. maximum hydraulic conductivity). Further research is clearly needed to resolve the mechanistic link between elevated ABA and increased embolism vulnerability, and a closer look at the underlying structural features of xylem vessels such as pit membranes should be considered. This is particularly important since SurEau simulations stressed that the ABA-related change in Ψ_{50} negatively impacted survival under drought. Indeed, model simulations demonstrated that when the wild type line was subjected to the same Ψ_{50} value as the transgenic line it showed earlier loss of hydraulic conductivity during drought (Figure 7b). Caution is thus necessary when exploiting ABA in

breeding for increased water use efficiency and maintained production in water-limited environments.

The sequence of physiological and hydraulic responses of tomato plants to increasing water deficit, manifested here by stomatal closure occurring before the onset of xylem embolism, concurs with patterns described in several crop species including crops such as sunflower (Cardoso et al., 2018), tomato (Skelton et al., 2017), and wheat (Corso et al., in revision). In addition, greater water use efficiency coupled with increased vulnerability to hydraulic failure observed in the ABA overproducing sp12 line provides new insight on how crops can cope with various degrees of water deficit, and highlights the challenges molecular breeding may face when searching for new water use efficient crop varieties, or the potential for unintended consequences when using ABA receptor agonists as agrochemicals. In this regard, the importance of the trade-off between WUE and xylem embolism resistance is likely to depend on the cropping system (e.g. annual vs. perennial, irrigated vs. rainfed). In annual, irrigated crops such as tomato, irrigation management must be controlled to prevent water potentials from reaching the onset of embolism, which may depend on varieties. To date, breeding for decreased water use and increased sustainability in these cases has therefore been associated with traits affecting plant water use rather than with those conferring greater survival (Blum 2014; Nuccio et al., 2018).

Yet, the effects of ABA overproduction on xylem embolism resistance described here could be more critical for rainfed (both perennial and annual) cropping systems, which may adapt to water deficit through the plasticity of numerous traits, such as rooting volume/depth, stomatal regulation, leaf osmotic adjustment, and increased resistance to xylem embolism. Cardoso et al. (2018) showed for example that sunflower plants grown under water deficit conditions exhibited higher resistance to xylem embolism and could face conditions that were lethal for unadjusted plants grown under well-watered conditions. While annuals such as

tomato and wheat may not express variation in xylem embolism resistance among organs (Corso et al., in revision; Skelton et al., 2017), some perennial plants such as grapevine have higher vulnerability to embolism in leaves in order to protect the hydraulic integrity in stems (Charrier et al., 2016; Hochberg et al., 2017). Future studies of plant hydraulic traits in conjunction with other morphological and physiological features in diverse cropping systems will be helpful to improve breeding in the context of climate change.

Two other general observations arose from the quantification of the resistance to xylem embolism in the wild type and transgenic lines. First, *in situ* flow-centrifuge measurements highlighted that the resistance to xylem embolism increased in both lines throughout plant development. Similar ontogenic changes in the resistance to xylem embolism have been observed in grapevine stems across the growing season (Charrier et al., 2018) as well as between juvenile and adult individuals of red oak (Cavender-Bares & Bazzaz, 2000), laurel (Lamarque et al., 2018), and Tasmanian bluegum (Lucani, Brodribb, Jordan, & Mitchell, 2019). While there is no clear explanation for these patterns, possible causes include increased lignification and changes in wood anatomical features through time (Lens et al., 2016; Li, Sperry, & Shao, 2016; Wheeler, Sperry, Hacke, & Hoang, 2005). Second, the wild type exhibited both higher stem hydraulic conductivity and greater resistance to xylem embolism, which suggests that there is no xylem safety-efficiency trade-off in the tomato lines we tested. Analyses in this regard have produced mixed results to date. At the intraspecific level, Corcuera et al. (2011) found a correlation between greater hydraulic conductivity and lower embolism resistance in maritime pine, while Ahmad et al. (2017) showed that sunflower accessions that were more resistant to xylem embolism also transported water more efficiently. The lack of trade-off between xylem safety and efficiency was further evidenced in Scots pine (Martínez-Vilalta et al., 2009) and European beech (Schuldt et al., 2016), as well

as at the interspecific level across a large pool of woody species distributed worldwide (Gleason et al., 2016).

Elevated ABA elicited differences in stem and root hydraulic conductivity between the wild type and the transgenic lines, in accordance with the feedback effects described between transpiration and plant hydraulic conductance (Vandeleur et al., 2014; Tardieu & Parent, 2017). Recent works have shown reductions in leaf hydraulic conductivity of detached leaves fed with exogenous ABA in both *Arabidopsis* (Pantin et al., 2013) and grapevine varieties (Coupel-Ledru et al., 2017), possibly through the regulation of aquaporin activity in the bundle sheath around leaf veins (Shatil-Cohen, Attia, & Moshelion, 2011). An alternative is that the decrease in K_s observed in the transgenic sp12 line may be due to the associated lower number of large diameter xylem vessels compared to the wild type (Figure S3), given that hydraulic properties of the xylem network strongly depends on wood anatomical features such as conduit diameter (Tyree & Ewers, 1991) and that a small increase in vessel diameter results in a large increase in conductivity (Hagen-Poiseuille law; Kotowska, Hertel, Rajab, Barus, & Schuldt, 2015; Nobel, 1991). The decrease in K_s in the sp12 line was balanced by an increase in root hydraulic conductivity (Lp_r). Sharipova et al. (2016) also found that the application of exogenous ABA to barley plants differing in ABA concentration increased Lp_r in all genotypes, likely through aquaporin activity modulation as in green bean (Aroca, Ferrante, Vernieri, & Chrispeels, 2006), maize (Parent et al., 2009) and tobacco (Mahdiah & Mostajeran, 2009). Yet, ABA effects on Lp_r are complex (Gambetta, Knipfer, Fricke, & McElrone, 2017) and it is difficult to understand how the results of the current study integrate with observations in soybean and grapevine where root hydraulic conductance is positively correlated to transpiration at the leaf- and plant-level (Gambetta et al., 2012; Vandeleur et al., 2009; Vandeleur et al., 2014). Finally, overproduction of ABA did not induce any changes in root pressure, which was of similar magnitude in sp12 and wild type lines. Root pressure has

been considered as a potential mechanism involved in both repair of embolized vessels and water supply to shoots in the absence of transpiration (Cao, Yang, Zhang, & Brodribb, 2012; Charrier et al., 2016; Ewers, Cochard, & Tyree, 1997; Steudle, 2001; Knipfer, Eutis, Brodersen, Walker, & McElrone, 2015). However, while root pressure has been reported to occur in a variety of crop species such as barley (Knipfer, & Fricke, 2010), maize (Gleason et al., 2017), rice (Stiller et al., 2003), sugarcane (Meinzer, Grantz, & Smit, 1991) and tomato (De Swaef, Hanssens, Cornelis, & Steppe, 2013), xylem repair in herbaceous and woody species has been seldom observed (Choat et al., 2019; Lens et al., 2016; Sperry, 2013).

Conclusion

Our results indicate that higher ABA content in the tomato transgenic line increases the vulnerability to hydraulic failure through increased vulnerability to xylem embolism and a reduction of hydraulic safety margin. This trade-off should be considered when targeting ABA accumulation in order to manipulate water use efficiency, especially for perennial, rainfed cropping systems. Important questions remain, notably those surrounding the extent of variation in embolism resistance and hydraulic safety margin size among varieties of a single crop species (Ahmad et al., 2017; Neufeld et al., 1992), and their relationship to genotypic variation in endogenous ABA concentrations.

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REFERENCES

- Aasamaa, K., Sober, A., Hartung, W., & Niinemets, U. (2002). Rate of stomatal opening, shoot hydraulic conductance and photosynthetic characteristics in relation to leaf abscisic acid concentration in six temperate deciduous trees. *Tree Physiology*, 22, 267-276.
- Ahmad, H. B., Lens, F., Capdeville, G., Burlett, R., Lamarque, L. J., & Delzon, S. (2017). Intraspecific variation in embolism resistance and stem anatomy across four sunflowers. *Physiologia Plantarum*, 163, 59-72.
- Ahmed, S., & Stepp, J. R. (2016). Beyond yields: Climate change effects on specialty crop quality and agroecological management. *Elementa-Science of the Anthropocene*, 4, 000092.
- Aroca, R., Ferrante, A., Vernieri, P., & Chrispeels, M. J. (2006). Drought, abscisic acid and transpiration rate effects on the regulation of PIP aquaporin gene expression and abundance in *Phaseolus vulgaris* plants. *Annals of Botany*, 98, 1301-1310.
- Barickman, T. C., Kopsell, D. A., & Sams, C. E. (2014). Exogenous foliar and root applications of abscisic acid increase in the influx of calcium into tomato fruit tissue and decrease the incidence of blossom-end rot. *HortScience*, 49, 1397-1402.
- Bauer, H., Ache, P., Lautner, S., Fromm, J., Hartung, W., Al-Rasheid, K. A., Sonnewald, S., Sonnewald, U., Kneitz, S., Lachman, N., et al. (2013). The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. *Current Biology*, 23, 53-57.
- Bauerle, W. L., Whitlow, T. H., Setter, T. L., & Vermeylen, F. M. (2004). Absciscic acid synthesis in *Acer rubrum* L. leaves: a vapor-pressure-deficit-mediated response. *Journal of the American Society for Horticultural Science*, 129, 182-187.
- Bell, D. B. (2011). Partial phenotypic reversion of ABA-deficient flacca tomato (*Solanum lycopersicum*) scions by a wild-type rootstock: normalizing shoot ethylene relations

- promotes leaf area but does not diminish whole plant transpiration rate. *Annals of Botany*, 108, 407-418.
- Blum, A. (2014). Genomics for drought resistance – getting down to earth. *Functional Plant Biology*, 41, 1191-1198.
- Blum, A. (2015). Towards a conceptual ABA ideotype in plant breeding for water limited environments. *Functional Plant Biology*, 42, 502-513.
- Blum, A. (2017). Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. *Plant, Cell & Environment*, 40, 4-10.
- Blum, A., & Tuberosa, R. (2018). Dehydration survival of crop plants and its measurement. *Journal of Experimental Botany*, 69, 975-981.
- Boursiac, Y., Chen, S., Luu, D. T., Sorieul, M., van den Dries, N., & Maurel C. (2005). Early effects of salinity on water transport in *Arabidopsis* roots. Molecular and cellular features of aquaporin expression. *Plant Physiology*, 139, 790-805.
- Brodribb, T. J., & Field, T. S. (2000). Stem hydraulic supply is linked to leaf photosynthetic capacity: evidence from New Caledonian and Tasmanian rainforests. *Plant, Cell & Environment*, 23, 1381-1388.
- Brodribb, T. J., Skelton, R. P., McAdam, S. A., Benaïme, D., Lucani, C. J., & Marmottant, P. (2016). Visual quantification of embolism reveals leaf vulnerability to hydraulic failure. *New Phytologist*, 209, 1403-1409.
- Cao, K. F., Yang, S. J., Zhang, Y. J., & Brodribb, T. J. (2012). The maximum height of grasses is determined by roots. *Ecology Letters*, 15, 666-672.
- Cardoso, A. A., Brodribb, T. J., Lucani, C. J., DaMatta, F. M., & McAdam, S. A. M. (2018). Coordinated plasticity maintains hydraulic safety in sunflower leaves. *Plant, Cell & Environment*, 41, 2567-2576.
- Cavender-Bares J., & Bazzaz, F. A. (2000). Changes in drought response strategies with

ontogeny in *Quercus rubra*: implications for scaling from seedlings to mature trees. *Oecologia*, 1, 8-18.

- Charrier, G., Burlett, R., Gambetta, G., Delzon, S., Domec, J-C., & Beaujard, F. (2017). Monitoring xylem hydraulic pressure in woody plants. *Bio-protocol*, 7, e2580.
- Charrier, G., Delzon, S., Domec, J-C., Zhang, L., Delmas, C. E. L., Merlin, I., Corso, D., King, A., Ojeda, H., Ollat, N., Prieto, J. A., Scholach, T., Skinner, P., van Leeuwen, C., & Gambetta, G. A. (2018). Drought will not leave your glass empty: low risk of hydraulic failure revealed by long-term drought observations in world's top wine regions. *Science Advances*, 4, eaao6969.
- Charrier, G., Torres-Ruiz, J. M., Badel, E., Burlett, R., Choat, B., Cochard, H., Delmas, C. E., Domec, J-C., Jansen, S., King, A., Lenoir, N., Martin-StPaul, N., Gambetta, G. A., & Delzon, S. (2016). Evidence for Hydraulic Vulnerability Segmentation and Lack of Xylem Refilling under Tension. *Plant Physiology*, 172, 1657-1668.
- Choat, B., Nolf, M., Lopez, R., Peters, J. M. R., Carins-Murphy, M. R., Creek, D., & Brodribb, T. J. (2019). Non-invasive imaging shows no evidence of embolism repair after drought in tree species of two genera. *Tree Physiology*, 39, 113-121.
- Cochard, H. (2002a). Xylem embolism and drought-induced stomatal closure in maize. *Planta*, 215, 466-471.
- Cochard, H. (2002b). A technique for measuring xylem hydraulic conductance under high negative pressures. *Plant, Cell & Environment*, 25, 815-819.
- Cochard, H., Damour, G., Bodet, C., Tharwat, I., Poirier, M., & Améglio, T. (2005). Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiologia Plantarum*, 124, 410-418.
- Cochard, H., Delzon, S. & Badel, E. (2015) X-ray microtomography (micro-CT): a reference technology for high-resolution quantification of xylem embolism in trees. *Plant, Cell &*

Environment, 38, 201-206.

- Condon, A. G., Richards, R. A., Rebetzke, G. J., & Farquhar, G. D. (2002). Improving intrinsic water-use efficiency and crop yield. *Crop Science*, 42, 122-131.
- Corcuera, L., Cochard, H., Gil-Pelegrin, E., & Notivol, E. (2011). Phenotypic plasticity in mesic populations of *Pinus pinaster* improves resistance to xylem embolism (P₅₀) under severe drought. *Trees*, 25, 1033-1042.
- Corso, D., Delzon, S., Lamarque, L. J., Cochard, H., Torres-Ruiz, J. M., King, A., & Brodribb, T. J. (in revision). Do xylem collapse or cavitation drive stomatal closure in *Triticum aestivum*?
- CoupeL-Ledru, A., Tyerman, S. D., Masclef, D., Lebon, E., Christophe, A., Edwards, E. J., & Simonneau, T. (2017). Absciscic acid down-regulates hydraulic conductance of grapevine leaves in isohydric genotypes only. *Plant Physiology*, 175, 1121-1134.
- De Swaef, T., Hanssens, J., Cornelis, A., & Steppe, K. (2013) Non-destructive estimation of root pressure using sap flow, stem diameter measurements and mechanistic modelling. *Annals of Botany*, 111, 271-282.
- Ewers, F. W., Cochard, H., & Tyree, M. T. (1997). A survey of root pressures in vines of a tropical lowland forest. *Oecologia*, 110, 191-196.
- Gambetta, G. A., Knipfer, T., Fricke, W., & McElrone, A. J. (2017). Aquaporins and root water uptake. In *Plant Aquaporins. Signaling and Communication in Plants* (eds F. Chaumont, S. Tyerman), pp 133-153. Springer, Cham.
- Gambetta, G. A., Manuck, C. M., Drucker, S. T., Shaghasi, T., Fort, K., Matthews, M. A., Walker, M. A., & McElrone, A. J. (2012). The relationship between root hydraulics and scion vigour across *Vitis* rootstocks: what role do aquaporins play? *Journal of Experimental Botany*, 63, 6445-6455.
- Gärtner, H., Lucchinetti, S., & Schweingruber, F. H. (2014). New perspectives for wood

anatomical analysis in dendrosciences: the GSL1-microtome. *Dendrochronologia*, 32, 47-51.

- Gleason, S. M., Wiggans, D. R., Bliss, C. A., Young, J. S., Cooper, M., Willi, K. R., & Comas, L. H. (2017). Embolized stems recover overnight in *Zea mays*: the role of soil water, root pressure, and nighttime transpiration. *Frontiers in Plant Science*, 8, 662.
- Gleason, S. M., Westoby, M., Jansen, S., Choat, B., Hacke, U. G., Pratt, R. B., Bhaskar, R., Brodribb, T. J., Bucci, S. J., Cao, K. F., et al. (2016). Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *New Phytologist*, 209, 123-136.
- Helander, J. D. M., Vaidya, A. S., & Cutler, S. R. (2016). Chemical manipulation of plant water use. *Bioorganic & Medicinal Chemistry*, 24, 493-500.
- Henson, I. E., Jensen, C. R., & Turner, N. C. (1989). Leaf gas exchange and water relations of lupins and wheat. III. Absciscic acid and drought-induced stomatal closure. *Australian Journal of Plant Physiology*, 16, 429-442.
- Hochberg, U., Windt, C. W., Ponomarenko, A., Zhang, Y. J., Gersony, J., Rockwell, F. E., & Holbrook, N. M. (2017). Stomatal closure, basal leaf embolism, and shedding protect the hydraulic integrity of grape stem. *Plant Physiology*, 174, 764-775.
- Hu, H., & Xiong, L. (2014). Genetic engineering and breeding of drought-resistant crops. *Annual Review of Plant Biology*, 65, 715-741.
- Johnson, K. M., Jordan, G. J., & Brodribb, T. J. (2018). Wheat leaves embolized by water stress do not recover function upon rewatering. *Plant, Cell & Environment*, 41, 2704-2714.
- Kell, D. B. (2011). Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. *Annals of Botany*, 108, 407-411.
- King, A., Guignot, N., Zerbino, P., Boulard, E., Desjardins, K., Bordessoule, M., Leclercq, N., Le, S., Renaud, G., Cerato, M., Bornert, M., Lenoir, N., Delzon, S., Perrillat, J-P., Legodec,

- Y., & Itié, J-P. (2016). Tomography and imaging at the PSICHE beam line of the SOLEIL Synchrotron. *Review of Scientific Instruments*, 87, 093704.
- Knipfer, T., & Fricke, W. (2010). Root pressure and a solute reflection coefficient close to unity exclude a purely apoplastic pathway of radial water transport in barley (*Hordeum vulgare*). *New Phytologist*, 187, 159-170.
- Knipfer, T., Cuneo, I. F., Earles, J. M., Reyes, C., Brodersen, C. R., & McElrone, A. J. (2017). Storage compartments for capillary water rarely refill in an intact woody plant. *Plant Physiology*, 175, 1649-1660.
- Knipfer, T., Eustis, A. J., Brodersen, C. R., Walker, M. A., & McElrone, A. J. (2015). Grapevine species from varied native habitats exhibit differences in embolism formation/repair associated with leaf gas exchange and root pressure. *Plant, Cell & Environment*, 38, 1503–1513.
- Knox, J., Hess, T., Daccache, A., & Wheeler, T. (2012). Climate change impacts on crop productivity in Africa and South Asia. *Environmental Research Letters*, 7, 034032.
- Kotowska, M. M., Hertel, D., Rajab, Y. A., Barus, H., & Schuldt, B. (2015). Patterns in hydraulic architecture from roots to branches in six tropical tree species from cacao agroforestry and their relation to wood density and stem growth. *Frontiers in Plant Science*, 6, 191.
- Lamarque, L. J., Corso, D., Torres-Ruiz, J. M., Badel, E., Brodribb, T. J., Burlett, R., Charrier, G., Choat, B., Cochard, H., Gambetta, G. A., Jansen, S., King, A., Lenoir, N., Martin-StPaul, N., Steppe, K., Van den Bulcke, J., Zhang, Y., & Delzon, S. (2018). An inconvenient truth about xylem resistance to embolism in the model species for refilling *Laurus nobilis* L. *Annals of Forest Science*, 75, 88.
- Lens, F., Picon-Cochard, C., Delmas, C. E., Signarbieux, C., Buttler, A., Cochard, H., Jansen, S., Chauvin, T., Doria, L. C., Del Arco, M., & Delzon, S. (2016). Herbaceous angiosperms

- are not more vulnerable to drought-induced embolism than angiosperm trees. *Plant Physiology*, 172, 661-667
- Lesk, C., Rowhani, P., & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature*, 529, 84-87.
- Li, S., Klepsch, M., Jansen, S., Schmitt, M., Lens, F., Karimi, Z., Schuldt, B., Espino, S., & Schenk, H. J. (2016). Intervessel pit membrane thickness as a key determinant of embolism resistance in angiosperm xylem. *IAWA Journal*, 37, 152-171.
- Li, Y., Sperry, J. S., & Shao, M. (2009). Hydraulic conductance and vulnerability to cavitation in corn (*Zea mays* L.) hybrids of differing drought resistance. *Environmental and Experimental Botany*, 66, 341-346.
- Li-Marchetti, C., Le Bras, C., Relion, D., Citerne, S., Huché-Thélier, L., Sakr, S., Morel, P., & Crespel, L. (2015). Genotypic differences in architectural and physiological responses to water restriction in rose bush. *Frontiers in Plant Science*, 6, 355.
- Lobell, D. B., & Field, C. B. (2007). Global scale climate-crop yield relationships and the impacts of recent warming. *Environmental Research Letters*, 2, 014002.
- Lopes, M. S., Araus, J. L., van Heerden, P. D. R., & Foyer, C. H. (2011). Enhancing drought tolerance in C4 crops. *Journal of Experimental Botany*, 62, 3135-3153.
- Lucani, C. J., Brodribb, T. J., Jordan, G. J., & Mitchell, P. J. (2019). Junevile and adult leaves of heteroblastic *Eucalyptus globulus* vary in xylem vulnerability. *Trees*, 33, 1167-1178.
- Mahdieh, M., & Mostajeran, A. (2009). Absciscic acid regulates root hydraulic conductance via aquaporin expression modulation in *Nicotiana tabacum*. *Journal of Plant Physiology*, 166, 1993-2003.
- Martin-StPaul, N., Delzon, S., & Cochard, H. (2017). Plant resistance to drought depends on timely stomatal closure. *Ecology Letters*, 20, 1437-1447.
- Martínez-Vilalta, J., Cochard, H., Mencuccini, M., Sterck, F., Herrero, A., Korhonen, J. F. J.,

- Llorens, P., Nikinmaa, E., Nole, A., Poyatos, R., & Ripullone, F. (2009). Hydraulic adjustment of Scots pine across Europe. *New Phytologist*, 184, 353-364.
- Matiu, M., Ankerst, D. P., & Menzel, A. (2017). Interactions between temperature and drought in global and regional crop yield variability during 1961-2014. *PLoS One*, 12, e0178339.
- McAdam, S. A. M., & Brodribb, T. J. (2015). The evolution of mechanisms driving the stomatal response to vapor pressure deficit. *Plant Physiology*, 167, 833-843.
- Mega, R., Abe, F., Kim, J. S., Tsuboi, Y., Tanaka, K., Kobayashi, H., Sakata, Y., Hanada, K., Tsujimoto, H., Kikuchi, J., Cutler, S. R., & Okamoto, M. (2019). Tuning water-use efficiency and drought tolerance in wheat using abscisic acid receptors. *Nature Plants*, 5, 153-159.
- Meinzer, F. C., Grantz, D. A., & Smit, B. (1991). Root signals mediate coordination of stomatal and root hydraulic conductance in growing sugarcane. *Australian Journal of Plant Physiology*, 18, 329-338.
- Mirone, A., Brun, E., Gouillart, E., Tafforeau, P., & Kieffer, J. (2014). The PyHST2 hybrid distributed code for high speed tomographic reconstruction with interaction reconstruction and a priori knowledge capabilities. *Nuclear Instruments and Methods in Physics Research*, 324, 41-48.
- Mittelheuser, C. J., & van Steveninck, R. F. M. (1969). Stomatal closure and inhibition of transpiration induced by (RS)-abscisic acid. *Nature*, 221, 281-282.
- Neufeld, H. S., Grantz, D. A., Meinzer, F. C., Goldstein, G., Crisosto, G. M., & Crisosto, C. (1992). Genotypic variability in vulnerability of leaf xylem to cavitation in water-stressed and well-irrigated sugarcane. *Plant Physiology*, 100, 1020-1028.
- Nobel, P. S. (1991). *Physicochemical and environmental plant physiology*. UK: Academic Press.

- Nuccio, M. L., Paul, M., Bate, N. J., Cohn, J., & Cutler, S. R. (2018). Where are the drought tolerant crops? An assessment of more than two decades of plant biotechnology effort in crop improvement. *Plant Science*, 273, 110-119.
- Paganin, D., Mayon, S. C., Gureyev, T. E., Miller, P. R., & Wilkins, S. W. (2002). Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object. *Journal of Microscopy*, 206, 33-40.
- Pammenter, N. W., & Van der Willigen, C. (1998). A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiology*, 18, 589-593.
- Pantin, F., Monnet, F., Jannaud, D., Costa, J. M., Renaud, J., Muller, B., Simonneau, T., & Genty, B. (2013). The dual effect of abscisic acid on stomata. *New Phytologist*, 193, 65-72.
- Papacek, M., Christmann, A., & Grill, E. (2019). Increased water use efficiency and water productivity of arabidopsis by abscisic acid receptors from *Populus canescens*. *Annals of Botany*, 225, 1-9.
- Parent, B., Hachez, C., Redondo, E., Simonneau, T., Chaumont, F., & Tardieu, F. (2009). Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach. *Plant Physiology*, 149, 2000-2012.
- Passioura, J. (2006). Increasing crop productivity when water is scarce – from breeding to field management. *Agricultural Water Management*, 80, 176-196.
- Passioura, J., & Angus, J. F. (2010). Increasing productivity of crops in water-limited environments. *Advances in Agronomy*, 106, 37-75.
- Porter, J. R., Xie, L., Challinor, A. J., Cochrane, K., Howden, S. M., Iqbal, M. M., Lobell, D. B., & Travasso, M. I. (2014). Food security and food production systems. In *Climate change 2014: Impacts, adaptation, and vulnerability. Part A: global and sectoral aspects*.

- Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change (eds C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, et al.), pp 485-533. Cambridge University Press, Cambridge.
- Postaire, O., Tournaire-Roux, C., Grondin, A., Boursiac, Y., Morillon, R., Schaffner, A. R., & Maurel, C. (2010). A PIP1 aquaporin contributes to hydrostatic pressure-induced water transport in both the root and rosette of *Arabidopsis*. *Plant Physiology*, 152, 1418-1430.
- Pouzoulet, J., Scudiero, E., Schiavon, M., & Rolshausen, P. E. (2017). Xylem vessel diameter affects the compartmentalization of the vascular pathogen *Phaeomoniella chlamydospora* in grapevine. *Frontiers in Plant Science*, 8, 1442.
- Ramachandran, P., Wang, G., Augstein, F., de Vries J., & Carlsbecker, A. (2018). Continuous root xylem formation and vascular acclimation to water deficit involves ABA signalling via miR165. *Development*, 145, dev159202.
- Ray, D. K., Gerber, J. S., MacDonald, G. K., & West, P. C. (2015). Climate variation explains a third of global crop yield variability. *Nature Communications*, 6, 5989.
- Ruzin, S. E. (1999). Plant microtechnique and microscopy (vol. 198). UK: Oxford University Press.
- Ryu, J., Hwang, B. G., Kim, Y. X., & Lee, S. J. (2016). Direct observation of local xylem embolisms induced by soil drying in intact *Zea mays* leaves. *Journal of Experimental Botany*, 67, 2617-2626.
- Saradadevi, R., Bramley, H., Siddique, K. H. M., Edwards, E., & Palta, J. A. (2014). Contrasting stomatal regulation and leaf ABA concentrations in wheat genotypes when split root systems were exposed to terminal drought. *Field Crops Research*, 162, 77-86.
- Saradadevi, R., Palta, J. A., & Siddique, K. H. M. (2017). ABA-Mediated stomatal response in regulating water use during the development of terminal drought in wheat. *Frontiers in Plant Science*, 8, 1251.

- Schauberger, B., Archontoulis, S., Arneth, A., Balkovic, J., Ciais, P., Deryng, D., Elliott, J., Folberth, C., Khabarov, N., Muller, C., Pugh, T. A., Rolinski, S., Schaphoff, S., Schmid, E., Wang, X., Schlenker, W., & Frieler, K. (2017). Consistent negative response of US crops to high temperatures in observations and crop models. *Nature Communications*, 8, 13931.
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671-675.
- Schuldt, B., Knutzen, F., Delzon, S., Jansen, S., Müller-Haubold, H., Burlett, R., Clough, Y., & Leuschner, C. (2016). How adaptable is the hydraulic system of European beech in the face of climate change-related precipitation reduction? *New Phytologist*, 210, 443-458.
- Sharipova, G., Veselov, D., Kudoyarova, G., Fricke, W., Dodd, I. C., Katsuhara, M., Furuichi, T., Ivanov, I., & Veselov, S. (2016). Exogenous application of abscisic acid (ABA) increases root and cell hydraulic conductivity and abundance of some aquaporin isoforms in the ABA-deficient barley mutant Az34. *Annals of Botany*, 118, 777-785.
- Shatil-Cohen, A., Attia, Z., & Moshelion, M. (2011). Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: a target of xylem-born ABA? *The Plant Journal*, 67, 72-80.
- Skelton, R. P., Brodribb, T. J., & Choat, B. (2017). Casting light on xylem vulnerability in an herbaceous species reveal a lack of segmentation. *New Phytologist*, 214, 561-569.
- Smeeton, R. C. (2010). An evaluation of the effects of over-production of ABA on whole plant water use, growth and productivity. PhD thesis, 250 pp. University of Warwick and University of Nottingham.
- Sperry, J. (2013). Cutting-edge research or cutting-edge artefact? An overdue control experiment complicates the xylem refilling story. *Plant, Cell & Environment*, 36, 1916-1918.

- Sperry, J. S., Donnelly, J. R., & Tyree, M. T. (1988). A method for measuring hydraulic conductivity and embolism in xylem. *Plant, Cell & Environment*, *11*, 35-40.
- Steudle, E. (2001). The cohesion-tension mechanism and the acquisition of water by plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology*, *52*, 487-875.
- Stiller, V., & Sperry, J. S. (2002). Cavitation fatigue and its reversal in sunflower (*Helianthus annuus* L.). *Journal of Experimental Botany*, *53*, 1155-1161.
- Stiller, V., Lafitte, H. R., & Sperry, J. S. (2003). Hydraulic properties of rice and the response of gas exchange to water stress. *Plant Physiology*, *132*, 1698-1706.
- Stiller, V., Sperry, J. S., & Lafitte, R. (2005). Embolized conduits of rice (*Oryza sativa*, Poaceae) refill despite negative xylem pressure. *American Journal of Botany*, *92*, 1970-1974.
- Tardieu, F., & Parent, B. (2017). Predictable ‘meta-mechanisms’ emerge from feedbacks between transpiration and plant growth and cannot be simply deduced from short-term mechanisms. *Plant, Cell & Environment*, *40*, 846-857.
- Tardieu, F., Lafarge, T., & Simonneau, T. (1996). Stomatal control by fed or endogenous xylem ABA in sunflower: interpretation of correlations between leaf water potential and stomatal conductance in anisohydric species. *Plant, Cell & Environment*, *19*, 75-84.
- Tardieu, F., Parent, B., Caldeira, C. F., & Welcker, C. (2014). Genetic and physiological controls of growth under water deficit. *Plant Physiology*, *164*, 1628-1635.
- Tardieu, F., Zhang, J., Katerji, N., Bethenod, O., Palmer, S., & Davies, W. J. (1992). Xylem ABA controls the stomatal conductance of field-grown maize subjected to soil compaction or soil drying. *Plant, Cell & Environment*, *15*, 193-197.
- Thompson, A. J., Andrews, J., Mulholland, B. J., McKee, J. M. T., Hilton, H. W., Horridge, J. S., Farquhar, G. D., Smeeton, R. C., Smillie, I. R. A., Black, C. R., & Taylor, I. B. (2007a). Overproduction of abscisic acid in tomato increases transpiration efficiency and root

- hydraulic conductivity and influences leaf expansion. *Plant Physiology*, 143, 1905-1917.
- Thompson, A. J., Jackson, A. C., Symonds, R. C., Mulholland, B. J., Dadswell, A. R., Blake, P. S., Burbidge, A., & Taylor, I. B. (2000). Ectopic expression of a tomato 9-*cis*-epoxycarotenoid dioxygenase gene causes over-production of abscisic acid. *The Plant Journal*, 23, 363-374.
- Thompson, A. J., Mulholland, B. J., Jackson, A. C., McKee, J. M. T., Hilton, H. W., Symonds, R. C., Sonneveld, T., Burbidge, A., Stevenson, P., & Taylor, I. B. (2007b). Regulation and manipulation of ABA biosynthesis in roots. *Plant, Cell & Environment*, 30, 67-78.
- Tonetto de Freitas, S., Shackel, K. A., & Mitcham, E. J. (2011). Absciscic acid triggers whole-plant and fruit-specific mechanisms to increase fruit calcium uptake and prevent blossom end rot development in tomato fruit. *Journal of Experimental Botany*, 62, 2645-2656.
- Torres-Ruiz J. M., Cochard, H., Choat, B., Jansen, S., Lopez, R., Tomaskova, I., Padilla-Diaz C. M., Badel, E., Burllett, R., King, A., Lenoir, N., Martin-StPaul, N. K., & Delzon, S. (2017). Xylem resistance to embolism: presenting a simple diagnostic test for the open vessel artefact. *New Phytologist*, 215, 489-499.
- Torres-Ruiz, J. M., Jansen, S., Choat, B., McElrone, A. J., Cochard, H., Brodribb, T. J., Badel, E., Burllett, R., Bouche, P. S., Brodersen, C. R., Li, S., Morris, H., & Delzon, S. (2015). Direct x-ray microtomography observation confirms the induction of embolism upon xylem cutting under tension. *Plant Physiology*, 167, 40-43.
- Torres-Ruiz, J. M., Sperry, J. S., & Fernandez, J. E. (2012). Improving xylem hydraulic conductivity measurements by correcting the error caused by passive water uptake. *Physiologia Plantarum*, 146, 129-135.
- Tyree, M. T., & Ewers, F. W. (1991). The hydraulic architecture of trees and other woody plants. *New Phytologist*, 119, 345-360.

- Tyree, M. T., Fiscus, E. L., Wulschleger, S. D., & Dixon, M. A. (1986). Detection of xylem cavitation in corn under field conditions. *Plant Physiology*, 82, 597-599.
- Vadez, V., Kholova, J., Medina, S., Kakkera, A., & Anderberg, H. (2014). Transpiration efficiency: new insights into an old story. *Journal of Experimental Botany*, 65, 6141-6153.
- Vandeleur, R. K., Mayo, G., Shelden, M. C., Gilliam, M., Kaiser, B. N., & Tyerman, D. S. (2009). The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. *Plant Physiology*, 149, 445-460.
- Vandeleur, R. K., Sullivan, W., Athman, A., Jordans, C., Gilliam, M., Kaiser, B. N., & Tyerman, S. D. (2014). Rapid shoot-to-root signalling regulates root hydraulic conductance via aquaporins. *Plant, Cell & Environment*, 37, 520-538.
- Vu, N. T., Kang, H. M., Kim, Y. S., Choi, K. Y., & Kim, I. S. (2015). Growth, physiology, and abiotic stress response to abscisic acid in tomato seedlings. *Horticulture, Environment, and Biotechnology*, 56, 294-304.
- Wheeler, J. K., Sperry, J. S., Hacke, U. G., & Hoang, N. (2005). Inter-vessel pitting and cavitation in woody Rosaceae and other vessel led plants: a basis for a safety versus efficiency trade-off in xylem transport. *Plant, Cell & Environment*, 28, 800-812.
- Yang, Z., Liu, J., Poree, F., Schaeufele, R., Helmke, H., Frackenpohl, J., Lehr, S., von Koskull-Döring, P., Christmann, A., Schnyder, H., Schmidhalter, U., & Grill, E. (2019). Abscisic acid receptors and coreceptors modulate plant water use efficiency and water productivity. *Plant Physiology*. doi: 10.1104/pp.18.01238.
- Yang, Z., Liu, J., Tischer, S. V., Christmann, A., Windisch, W., Schnyder, H., & Grill, E. (2016). Leveraging abscisic acid receptors for efficient water use in *Arabidopsis*. *Proceedings of the National Academy of Sciences*, 113, 6791-6796.
- Zhang, J., & Davies, W. J. (1989). Sequential response of whole plant water relations to

prolonged soil drying and the involvement of xylem sap ABA in the regulation of stomatal behaviour of sunflower plants. *New Phytologist*, 113, 167-174.

TABLES

Table 1. Result of General linear model (GLM) analyses for traits related to plant metabolism, physiology, and hydraulics for tomato wild type control (WT) and NCED overexpressing transformant (sp12).

Trait	Factor	Source of variation								
		Line			Factor			Line x Factor		
		<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
<i>Metabolism</i>										
Foliar ABA	Water deficit	1	8.36	0.0049	1	27.00	< .0001	1	0.11	0.7379
<i>Physiology</i>										
<i>g</i> _s	Water deficit	1	4.86	0.0303	4	10.95	< .0001	4	1.26	0.2933
<i>A</i>	Water deficit	1	4.30	0.0415	4	9.28	< .0001	4	0.33	0.8558
<i>E</i>	Water deficit	1	4.73	0.0327	4	11.75	< .0001	4	1.07	0.3795
WUE _i	Water deficit	1	0.39	0.5352	4	8.68	< .0001	4	0.25	0.9106
<i>Hydraulics</i>										
<i>Ψ</i> ₁₂	Age	1	0.57	0.4607	1	6.92	0.0176	1	0.28	0.6063
<i>Ψ</i> ₅₀	Age	1	7.41	0.0145	1	17.75	0.0006	1	0.65	0.4321
<i>Ψ</i> ₈₈	Age	1	11.22	0.0038	1	12.64	0.0024	1	3.33	0.0858
<i>S</i>	Age	1	2.36	0.1432	1	0.25	0.6212	1	1.24	0.2807
<i>Ψ</i> _{pd}	Time	1	5.31	0.0264	3	6.46	0.0011	3	0.42	0.7393
<i>Ψ</i> _{min}	Time	1	4.11	0.0493	3	5.38	0.0033	3	0.35	0.7869

Table 2. Mean values (\pm SE) of water potentials inducing 50% loss of conductivity (Ψ_{50}) in stems of tomato wild type control (WT) and NCED overexpressing transformant (sp12). *P* values are from one-way ANOVAs.

	MicroCT		<i>In situ</i> flow-centrifugation			
	120 day-old		90 day-old		170 day-old	
	n	Ψ_{50} (MPa)	n	Ψ_{50} (MPa)	n	Ψ_{50} (MPa)
WT	6	-2.69 ± 0.14	5	-2.55 ± 0.11	4	-3.10 ± 0.07
sp12	4	-1.52 ± 0.11	5	-1.99 ± 0.19	7	-2.79 ± 0.16
<i>P</i> value	0.0001		0.0339		0.2164	

FIGURE LEGENDS

Figure 1. Kinetics of stomatal conductance (g_s) in 70-day-old wild type (WT) and sp12 plants during dehydration. g_s data were binned per minimum midday water potential (Ψ) classes (hbin procedure and quantile method, sas 9.4). Dots and error bars represent mean \pm SE for each Ψ class ($n = 18$ per class). Class 1: $\Psi < -0.56$ MPa, class 2: -0.65 MPa $< \Psi < -0.56$ MPa, class 3: -0.74 MPa $< \Psi < -0.65$ MPa, class 4: -0.83 MPa $< \Psi < -0.74$ MPa, class 5: $\Psi < -0.83$ MPa. * $P < 0.05$, † $P < 0.1$. The dotted line refers to the 10% g_s decline threshold (-0.7 MPa for both lines).

Figure 2. (a) X-ray microtomography (microCT) observations of xylem embolism in the main stem of dehydrating 120-day-old tomato wild type control (WT) and NCED overexpressing line (sp12). Embolized vessels appear in dark grey while water-filled vessels appear in light grey. Scale bar = 500 μ m. (b) Mean vulnerability curves (VCs), expressed as percentage loss of hydraulic conductivity (PLC), for each of the two lines ($n = 6$ and 4 for WT and sp12, respectively). Note that one VC was first obtained for each individual, i.e. the Pammenter model was fitted on each VC to get a Ψ_{50} and slope value per individual and subsequently an average per line. See Figure S4 for corresponding raw data.

Figure 3. Mean vulnerability curves (VCs) for 90- and 170-day-old plants of (a) wild type (WT) and (b) sp12 plants obtained using the *in situ* flow-centrifugation method. $n = 5$ VCs from 5 individuals for both lines at 90-day-old stage. $n = 4$ and 7 VCs for WT and sp12 at 170-day-old stage, respectively. See Figure S5 for corresponding raw data.

Figure 4. Kinetics of predawn and minimum midday leaf water potentials during dehydration of 70-day-old wild type (WT) and sp12 plants. Dots and error bars represent means \pm SE ($n =$

6 per line). * $P < 0.05$, n.s.: non-significant. See Table 1 for the results of the corresponding GLMs.

Figure 5. (a) Stem and (b) root hydraulic conductivity and (c) root pressure of well-watered 50-day-old wild type (WT) and sp12 plants. Stem hydraulic conductivity (K_s), measured on segments sampled between plant nodes 5 and 6, was normalized to xylem cross-sectional area. Boxes represent the 25th and 75th percentiles with whiskers referring to minimum and maximum observations. Dots represent means. $n = 9, 15$ and 12 per line for K_s , Lp_r and P_r measurements, respectively. * $P < 0.05$, † $P < 0.1$.

Figure 6. Hydraulic safety margins (HSM) for wild type and sp12 plants, corresponding to the difference between the pressure inducing 90% stomata closure (Ψ_{gs90} ; dotted lines) and that inducing a given percentage loss of hydraulic conductivity. $HSM_{\Psi_{12}}$ and $HSM_{\Psi_{50}}$ were calculated for both Ψ_{12} (shortdashed lines) and Ψ_{50} (mediumdashed lines) using the vulnerability curve of 90-day-old tomato plants (grey curves; see Figure 2). The stomatal conductance response (blue curves) to plant dehydration was obtained on 70-day-old plants (see Figure 3).

Figure 7. Daily simulated patterns of stomatal conductance (g_s) and loss of hydraulic conductivity (PLC) during the progression of a simulated soil drought (SurEau model; Martin-StPaul et al., 2017). (a) Declining g_s and increasing PLC modeled for both wild type control (medium dashed and solid red line, respectively) and NCED overexpressing transformant (sp12; medium dashed and solid green line, respectively). (b) Simulated loss of hydraulic conductivity in the wild type line when it was subjected to the same g_s (short dashed blue line) or Ψ_{50} (short dashed gray line) values as the sp12 transgenic line.

Figure 1

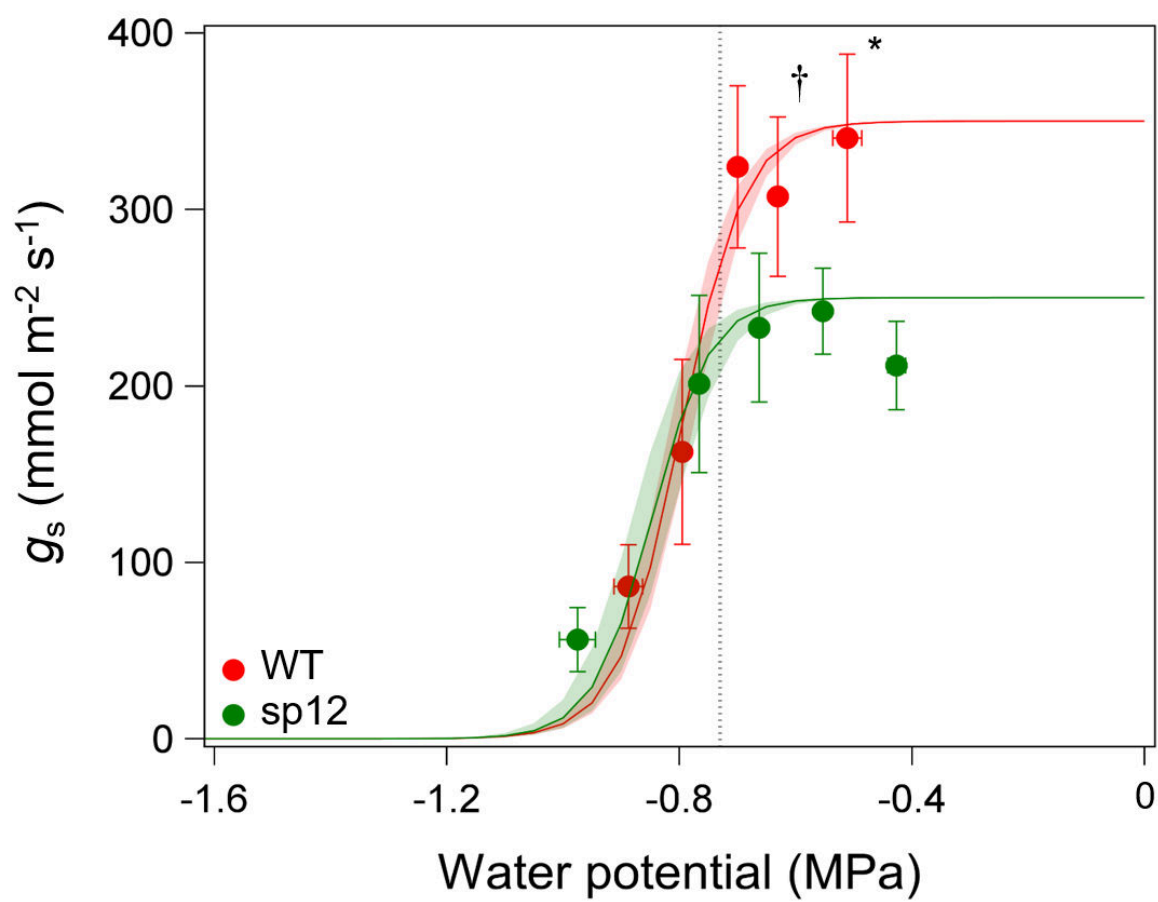


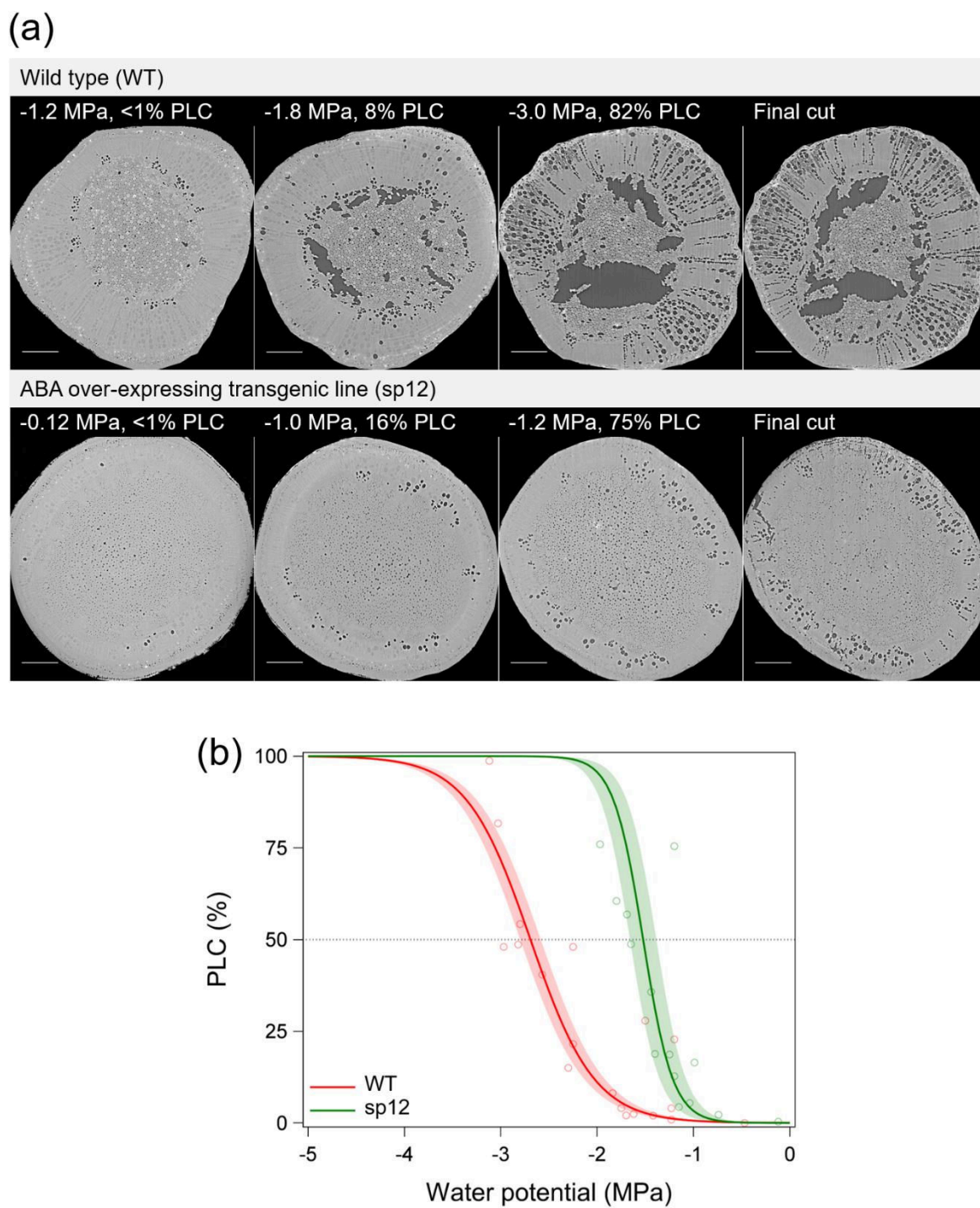
Figure 2

Figure 3

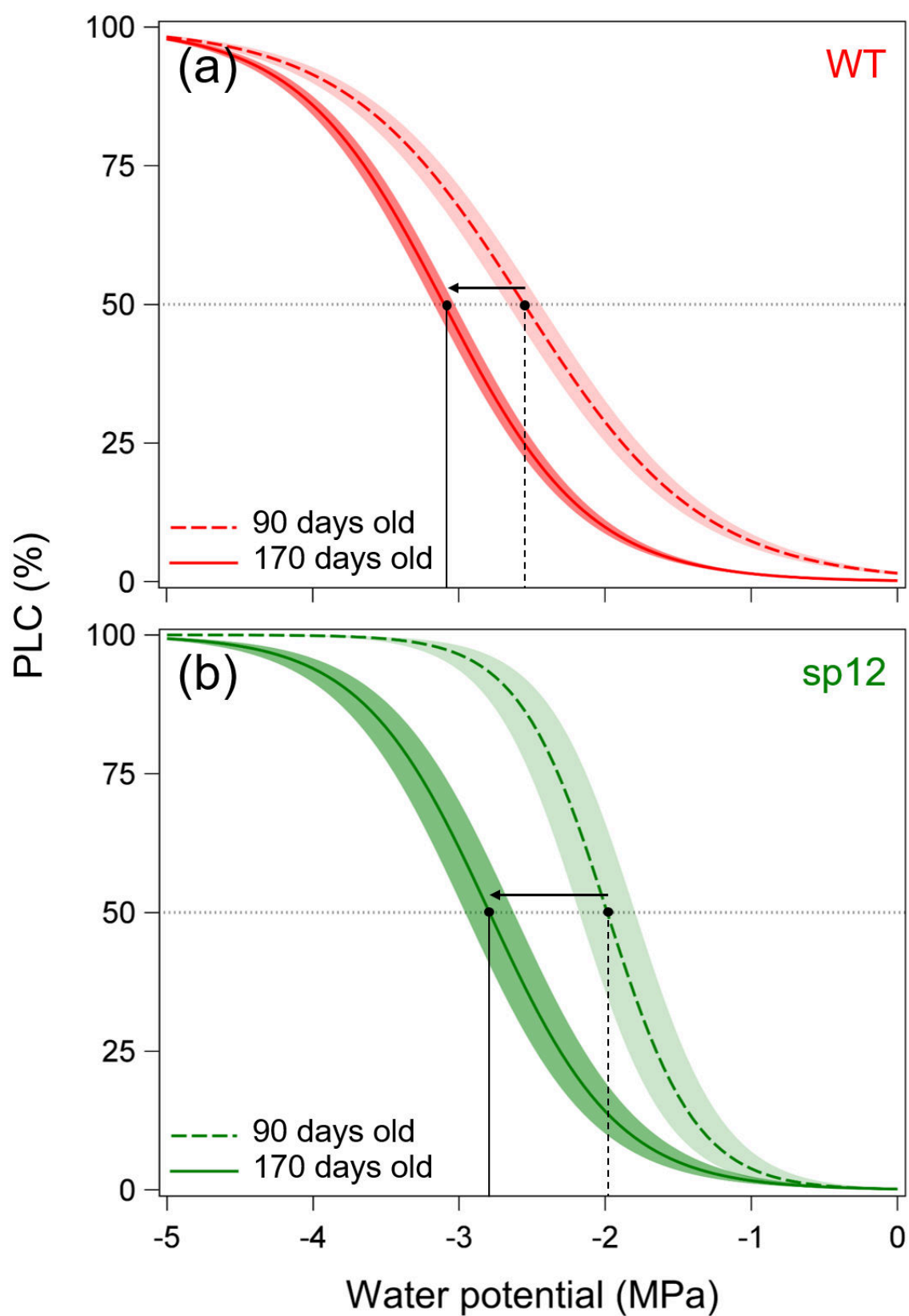


Figure 4

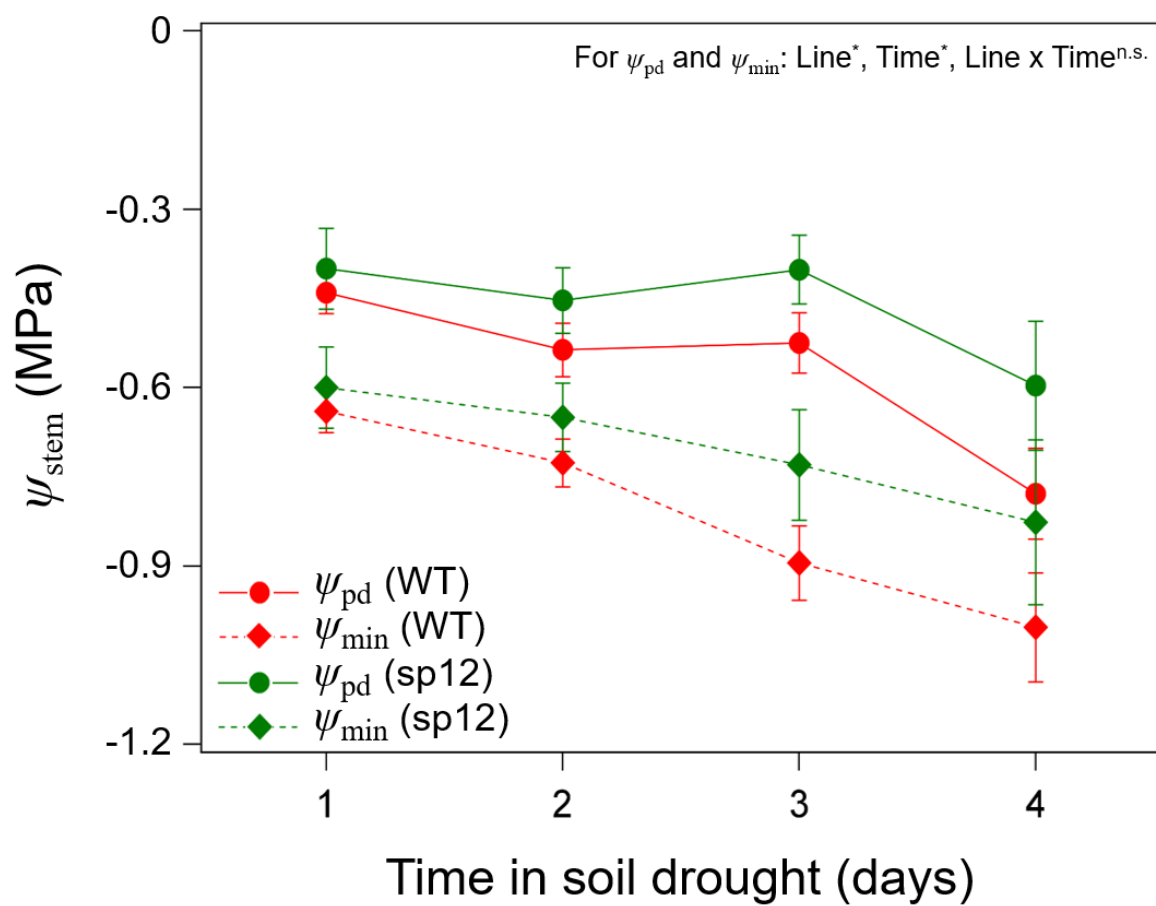


Figure 5

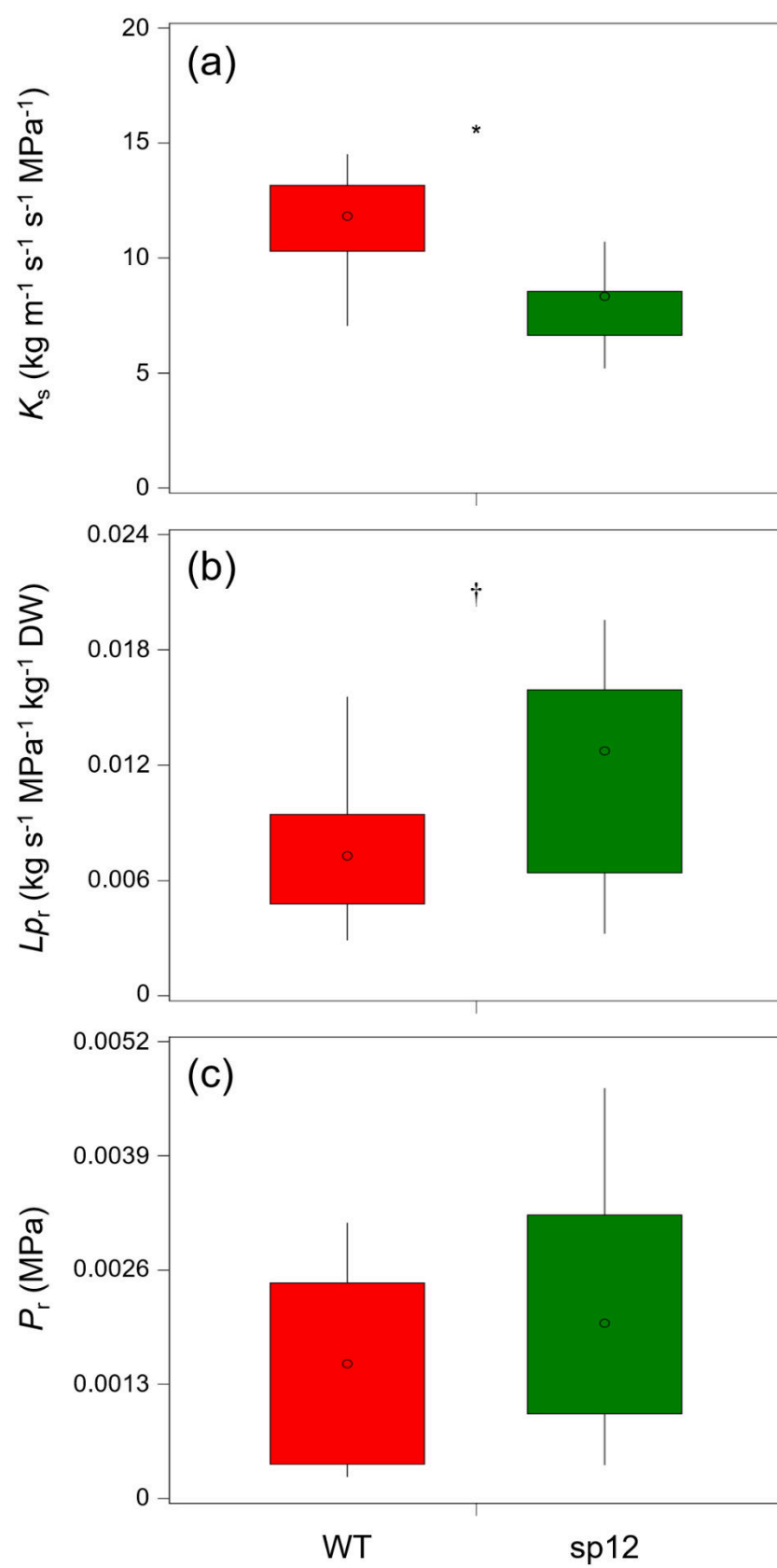


Figure 6

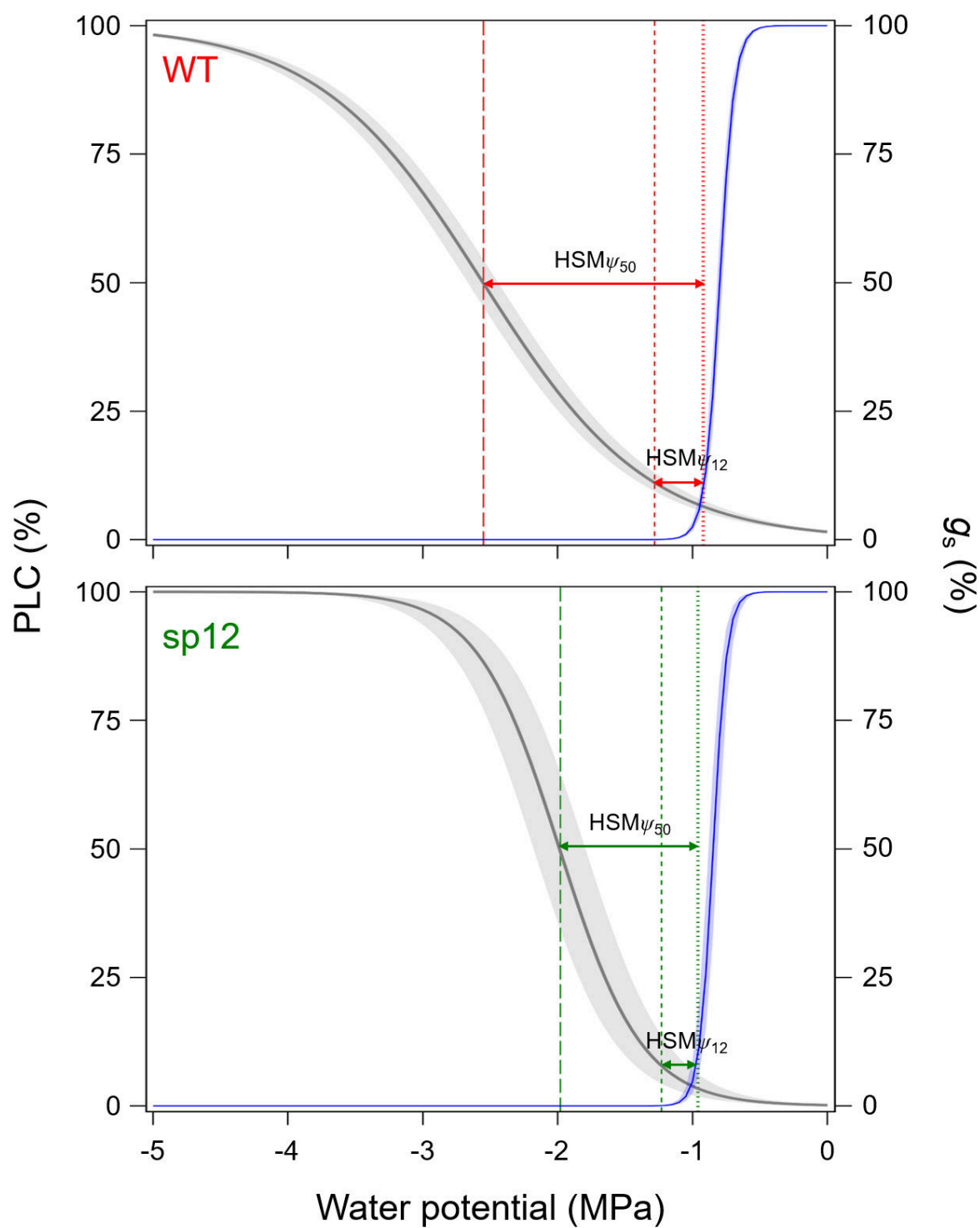
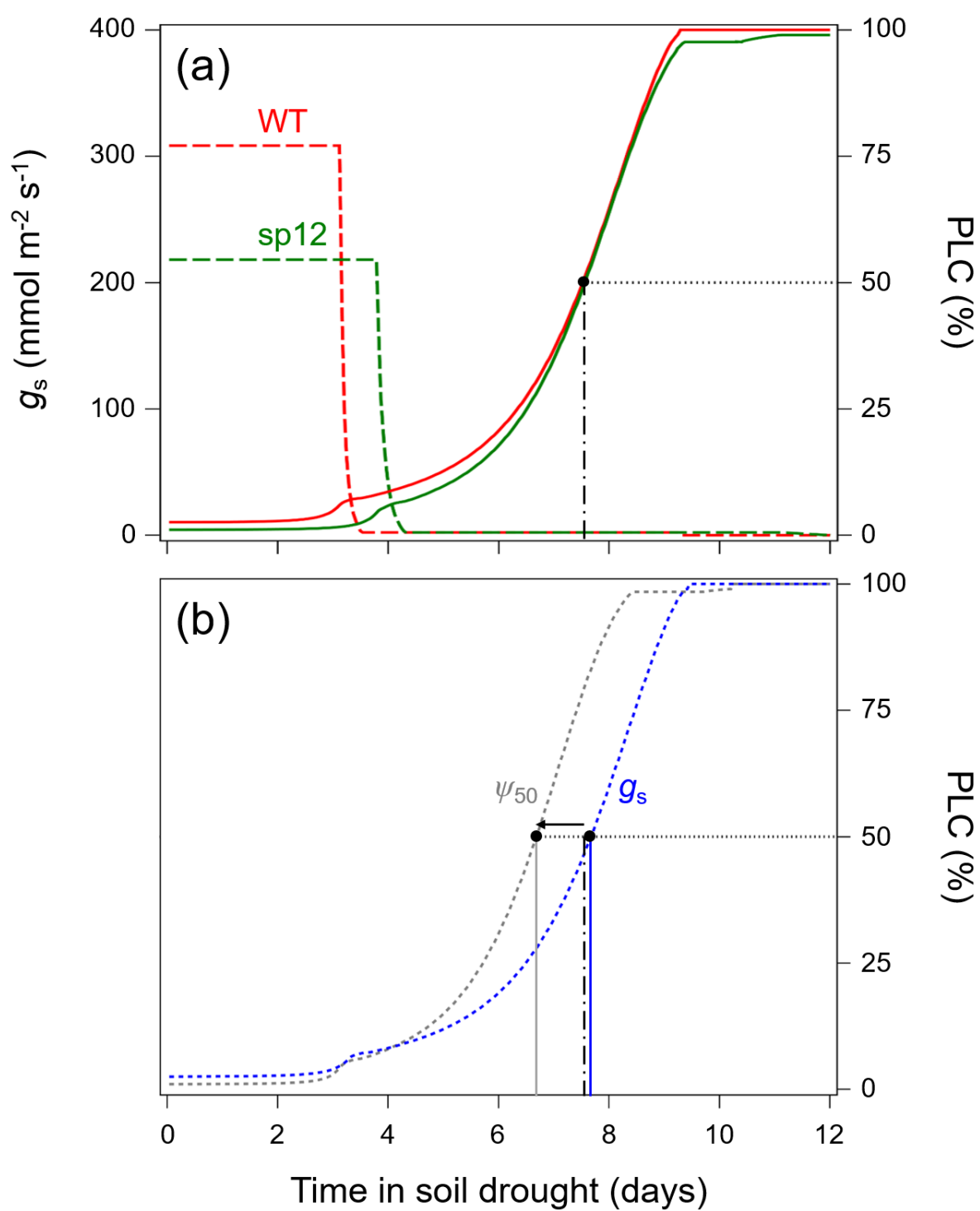


Figure 7



Over-accumulation of abscisic acid in transgenic tomato plants increases the risk of hydraulic failure

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Lamarque LJ, Delzon S, Toups H, et al., (2019) Over-accumulation of abscisic acid in transgenic tomato plants increases the risk of hydraulic failure. *Plant, Cell and Environment*, Volume 43, Issue 3, March 2020, pp. 548-562

<https://doi.org/10.1111/pce.13703>

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