ORIGINAL ARTICLE

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 programmes represents a critical step for effectively managing a crop's ability to maintain hydraulic conductivity and productivity under water deficit.

KEYWORDS
crop, drought, transgenic line, water deficit, xylem embolism

1 | INTRODUCTION

Whereas 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; Schauberger et al., 2017). For example, Lesk, Rowhani, and Ramankutty (2016) showed that droughts and extreme heat events between 1964 and 2007 reduced global cereal production by ca. 10% over this period, whereas 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 stricter 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 programmes have understandably searched to lower plant transpiration and improve crop water use efficiency (WUE), 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 (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, & Vermeylen, 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., 2007). The drought-induced increase in ABA content allows plants to conserve their water content by reducing transpiration and 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 signalling has agronomic promise (Helander, Vaidya, & Cutler, 2016), and transgenic approaches to increase ABA biosynthesis and signalling 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, Andrews, et al., 2007). Similarly, in *Arabidopsis* and wheat, activation of ABA signalling 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 WUE. 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 favourable yield outcome while efficiently using available water and optimizing growth.

It is understandable that breeding programmes 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 & Field, 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 (*Phallaris 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, Sperry, & Shao, 2009; Ryu, Hwang, Kim, & Lee, 2016; Tyree, Fiscus, Wullschleger, & 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 WUE in crops is important to ensure the success of these crop improvement strategies and help build an integrated view of the behaviour 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 WUE (Thompson, Andrews, et al., 2007). We hypothesized that the high levels of ABA would decrease the risk of hydraulic failure by increasing the hydraulic safety margin (HSM) 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.

2 | MATERIALS AND METHODS

2.1 | Plant materials

This study was centred on two homozygous true-breeding lines of *S. 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 harbours a construct with the *LeNCED1* transgene driven by the Gelvin Superpromoter (Thompson et al., 2000; Thompson, Andrews, et al., 2007). 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 Institut National de la Recherche Agronomique station of Villenave d'Ornon, France. Greenhouse conditions followed a 15:9-hr temperature cycle of 22:20°C with mean daily light intensity set at 2018 W m^{-2} and humidity maintained 24 hr a day at 65%. The protocol for seed germination and establishment followed recommendations detailed in Thompson et al. (2007) 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, Klasmann-Deilmann) and one-third perlite. As the high ABA content in the transgenic line delayed seed establishment by 5–8 days compared with 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.

2.2 | 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: $F = 0.01$, $p = .9210$; initial diameter: $F = 4.39$, $p = .0515$). Final plant height and stem diameter were recorded for the 30 plants included in this experiment, whereas aboveground biomass was harvested and weighed after being dried at 65°C for at least 48 hr on six well-watered plants per line that served as controls.

Gas-exchange measurements were conducted using an infrared gas analyser (GFS-3000, Heinz Walz GmbH, Effeltrich, Germany) equipped with CO_2, humidity, temperature, and light control modules. The conditions inside the sealed cuvette of 3 cm^2 were set as follows: 400 ± 5 ppm of CO_2, 60 ± 6% of relative humidity, cuvette temperature of 22°C, and light exposure of 1,200 μmol m^{-2} s^{-1}. Leaf vapour-pressure deficit 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 15 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 ABA 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 (8,000 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 analysed using a Waters Acquity ultra performance liquid chromatograph coupled to a Waters Xevo Triple quadrupole mass spectrometer (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%), and (15.7 min, 98%). Mass spectrometry was conducted in electrospray and multiple reaction monitoring scanning mode, in negative ion mode for the hormone. The capillary was set at 1.5 kV (negative mode), whereas 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 and 800 L h^{-1}, respectively), whereas 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 2 hr with aluminium foil and wrapped in a plastic bag.

Whole-plant transpiration as well as predawn and minimum midday water potential kinetics were monitored over a 4-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), whereas water potentials were determined following the same procedure than the one mentioned above.

2.3 | Vulnerability to embolism

2.3.1 | X-ray microtomography

Direct, non-invasive visualization of xylem embolism in 120-day-old tomato plants was conducted at the SOLEIL Synchrotron microcomputed tomography (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, that is at ca. 90 cm above the stem base. Dehydration was progressively induced in the 10 plants by stopping watering. X-ray projections were collected with a 50-m 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, Gouillart, Tafforeau, & Kieffer, 2014) and resulted in 2-bit volumic images with a 3.02-μm^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 2 hr with aluminium 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 centre 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, that is, the distinction between functional vessels from non-functional xylem conduits and fibres, and vessel diameter calculations were facilitated by using a final scan ("final cut") that was generated for each stem section, that is, 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, m^4 MPa^{-1} s^{-1}) of air-filled vessels of each cross section was calculated as

$$k_h = \Sigma \pi D^4 / 128\eta, \tag{1}$$

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

The theoretical percentage loss of hydraulic conductivity (PLC) was then determined as

$$PLC = 100.(k_h / k_{max}), \tag{2}$$

where k_{max} represents the theoretical hydraulic conductivity of all functional vessels as based on a cross section after the final cut.

2.3.2 | 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, m^2 MPa^{-1} 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-vesseled 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 hr 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 1-mM $CaCl_2$ was used as reference ionic solution, whereas 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, kg m^{-1} s^{-1} MPa^{-1}) were measured using the Cavisoft software (v. 5.2, University of Bordeaux). The PLC was determined at each pressure as follows:

$$PLC = 100.(1 - k_i/k_{max}), \qquad (3)$$

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

2.3.3 | Vulnerability curve fitting

Vulnerability curves, corresponding to PLC 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):

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

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.

2.4 | 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, that is, between plant nodes 5 and 6. Measurements followed the protocol described originally in Sperry, Donnelly, and Tyree (1988) and later corrected by Torres-Ruiz, Sperry, and 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; Practum224-1S, 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). Seventy-micrometre-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% Safranine 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 dried for at least 24 hr. 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, Scudiero, Schiavon, and Rolshausen (2017). Stem hydraulic conductivity (k_s) was determined from the slope of a plot water flow versus pressure gradient multiplied by stem length, whereas specific hydraulic conductivity (K_s) was calculated as the ratio of k_s to xylem cross-sectional area.

Root hydraulic conductivity (Lp_r, kg s^{-1} MPa^{-1} kg^{-1} 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 15 well-watered 50-day-old root systems per line that were manipulated. Once measurements were completed, roots were dried at 65°C for at least 24 hr 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 data logger (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 = .77).

2.5 | 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 (a) 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 (b) to 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 modelling the PLC dynamics of the wild type line using the g_s or Ψ_{50} values of the sp12 line.

2.6 | 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:

$$g_s = g_{smax} / \left(1 + \exp\left[S.\left\{\Psi - \Psi_{gs50}\right\}\right]\right). \qquad (5)$$

The water potential value corresponding to 10% g_s decline (Ψ_{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 (generalized linear model procedure). Differences in growth, physiological, hydraulic, and anatomical traits were further evaluated using a one-way analysis of variance procedure.

3 | RESULTS

3.1 | 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 μg g^{-1} DW in sp12 and WT,
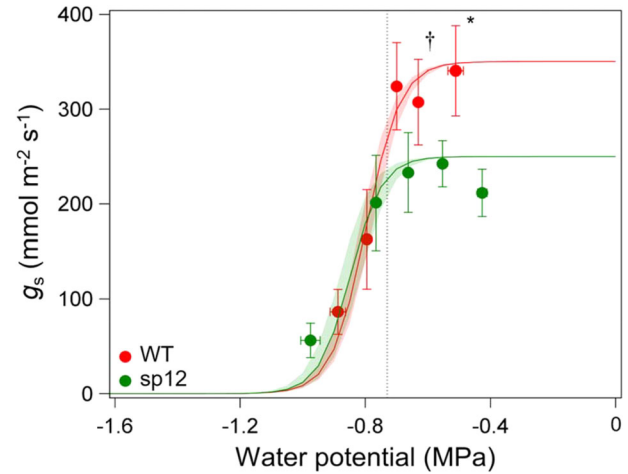

FIGURE 1 Kinetics of stomatal conductance (g_s) in 70-day-old wild type (WT) and sp12 plants during dehydration. g_s data were binned per minimum midday water potential (Ψ) classes (HBIN procedure and quantile method, SAS 9.4). Dots and error bars represent mean ± standard error for each Ψ class (n = 18 per class). Class 1: Ψ < −0.56 MPa, Class 2: −0.65 MPa < Ψ < −0.56 MPa, Class 3: −0.74 MPa < Ψ < −0.65 MPa, Class 4: −0.83 MPa < Ψ < −0.74 MPa, Class 5: Ψ < −0.83 MPa. *p < .05, †p < .1. The dotted line refers to the 10% g_s decline threshold (−0.7 MPa for both lines)

TABLE 1 Result of general linear model analyses for traits related to plant metabolism, physiology, and hydraulics for tomato wild type control and 9-cis-epoxycarotenoid dioxygenase overexpressing transformant (sp12)

| Trait | Factor | Source of variation | | | | | | | | |
| | | Line | | | Factor | | | Line x Factor | | |
		df	F	p	df	F	p	df	F	p
Metabolism										
Foliar ABA	Water deficit	1	8.36	.0049	1	27.00	<.0001	1	0.11	.7379
Physiology										
g_s	Water deficit	1	4.86	.0303	4	10.95	<.0001	4	1.26	.2933
A	Water deficit	1	4.30	.0415	4	9.28	<.0001	4	0.33	.8558
E	Water deficit	1	4.73	.0327	4	11.75	<.0001	4	1.07	.3795
WUE$_i$	Water deficit	1	0.39	.5352	4	8.68	<.0001	4	0.25	.9106
Hydraulics										
Ψ_{12}	Age	1	0.57	.4607	1	6.92	.0176	1	0.28	.6063
Ψ_{50}	Age	1	7.41	.0145	1	17.75	.0006	1	0.65	.4321
Ψ_{88}	Age	1	11.22	.0038	1	12.64	.0024	1	3.33	.0858
S	Age	1	2.36	.1432	1	0.25	.6212	1	1.24	.2807
Ψ_{pd}	Time	1	5.31	.0264	3	6.46	.0011	3	0.42	.7393
Ψ_{min}	Time	1	4.11	.0493	3	5.38	.0033	3	0.35	.7869

TABLE 2 Mean values (± standard error) of water potentials inducing 50% loss of conductivity (Ψ_{50}) in stems of tomato wild type control and 9-*cis*-epoxycarotenoid dioxygenase overexpressing transformant (sp12)

	MicroCT		In situ 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
p value		0.0001		0.0339		0.2164

Note. p values are from one-way analysis of variances.
Abbreviations: microCT, micro-computed tomography; WT, wild type control.
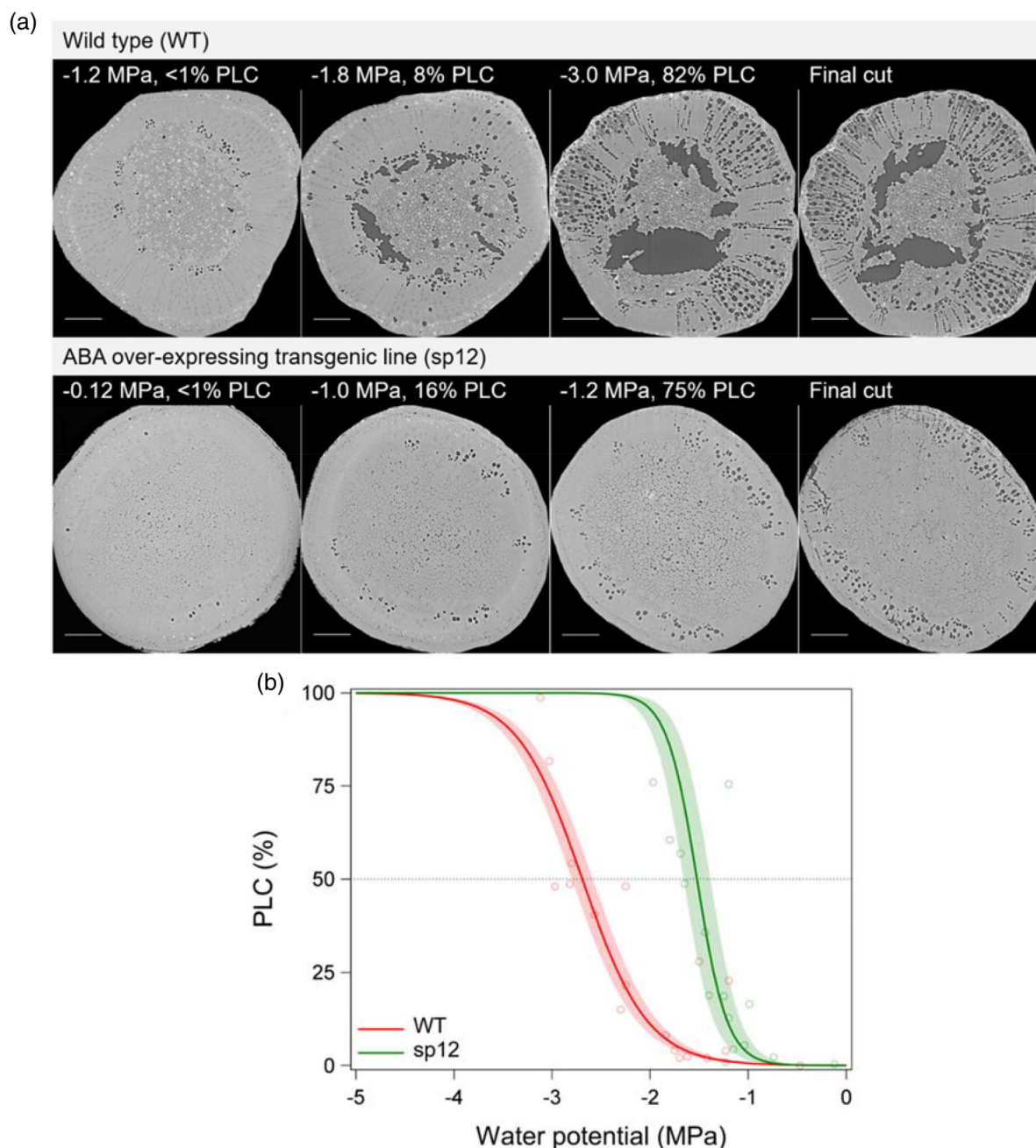

FIGURE 2 (a) X-ray micro-computed tomography (microCT) observations of xylem embolism in the main stem of dehydrating 120-day-old tomato wild type control (WT) and 9-*cis*-epoxycarotenoid dioxygenase overexpressing line (sp12). Embolized vessels appear in dark grey, whereas water-filled vessels appear in light grey. Scale bar = 500 μm. (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, that is, 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
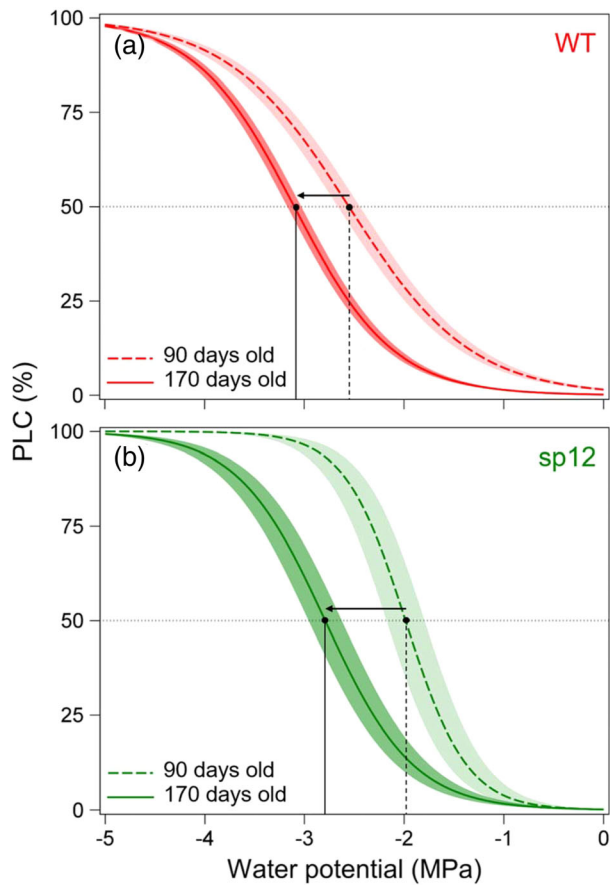
FIGURE 3 Mean vulnerability curves (VCs) for 90- and 170-day-old plants of (a) wild type (WT) and (b) sp12 plants obtained using the in situ flow-centrifugation method. $n = 5$ VCs from five individuals for both lines at 90-day-old stage. $n = 4$ and seven VCs for WT and sp12 at 170-day-old stage, respectively. See Figure S5 for corresponding raw data. PLC, percentage loss of hydraulic conductivity
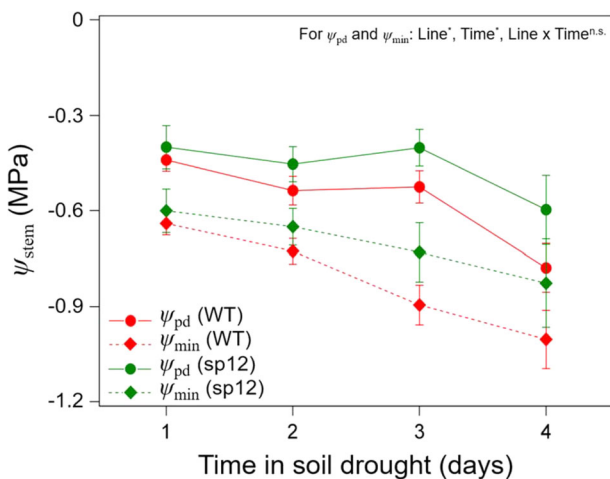

FIGURE 4 Kinetics of predawn and minimum midday leaf water potentials during dehydration of 70-day-old wild type (WT) and sp12 plants. Dots and error bars represent means ± standard error ($n = 6$ per line). *$p < .05$, n.s.: non-significant. See Table 1 for the results of the corresponding generalized linear models
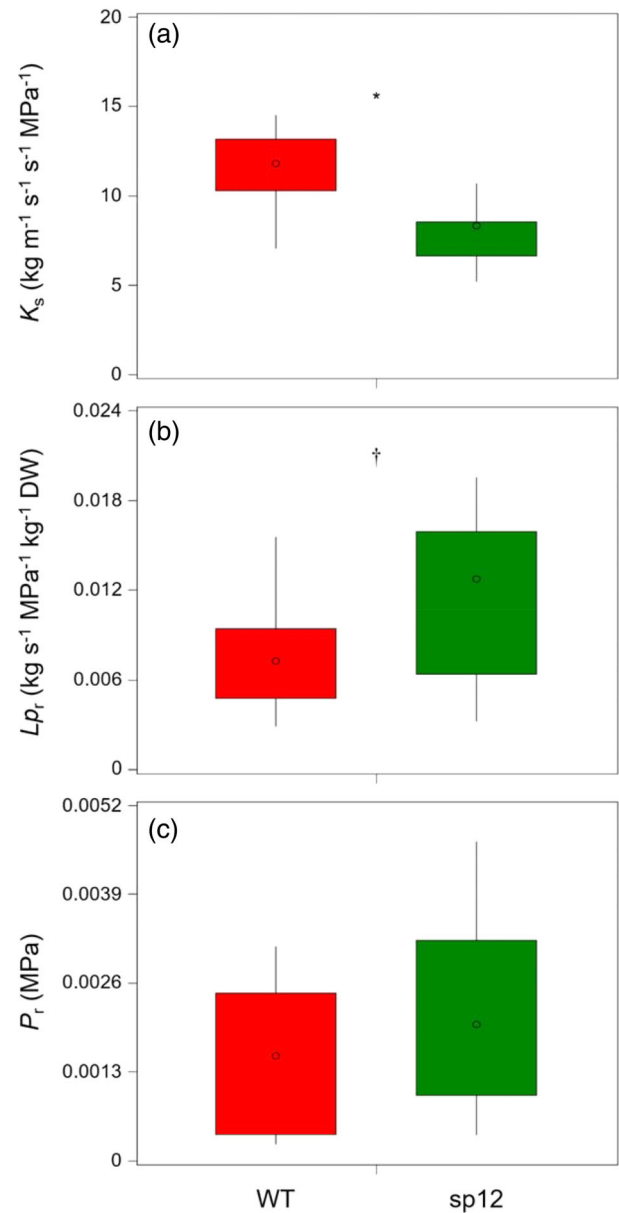

FIGURE 5 (a) Stem and (b) root hydraulic conductivity and (c) root pressure of well-watered 50-day-old wild type (WT) and sp12 plants. Stem hydraulic conductivity (K_s), measured on segments sampled between plant nodes 5 and 6, was normalized to xylem cross-sectional area. Boxes represent the 25th and 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. *$p < .05$, †$p < .1$

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; $p = .0081$; 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).
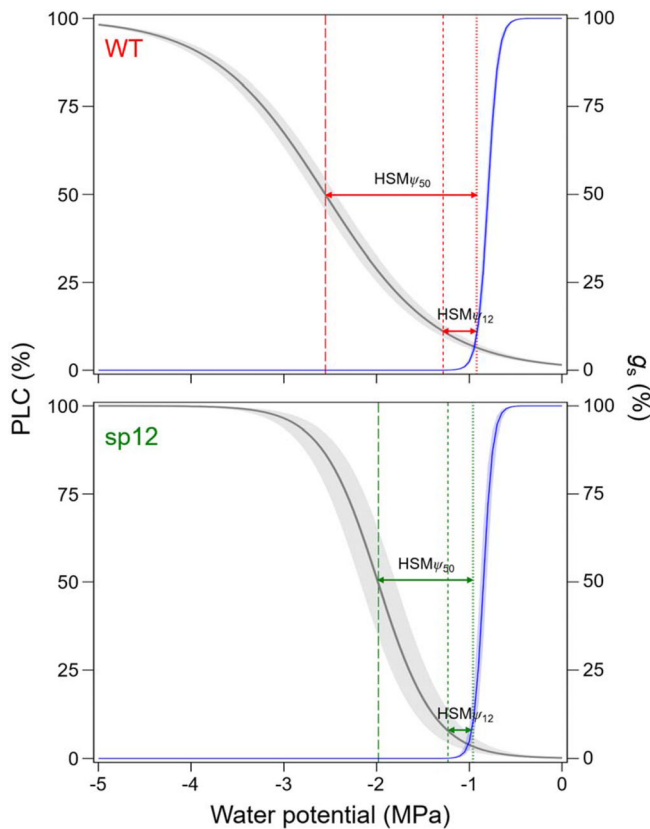
FIGURE 6 Hydraulic safety margins (HSM) for wild type and sp12 plants, corresponding to the difference between the pressure inducing 90% stomata closure (Ψ_{gs90}; dotted lines) and that inducing a given percentage loss of hydraulic conductivity. $HSM_{\Psi_{12}}$ and $HSM_{\Psi_{50}}$ were calculated for both Ψ_{12} (short dashed lines) and Ψ_{50} (medium dashed 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). WT, wild type
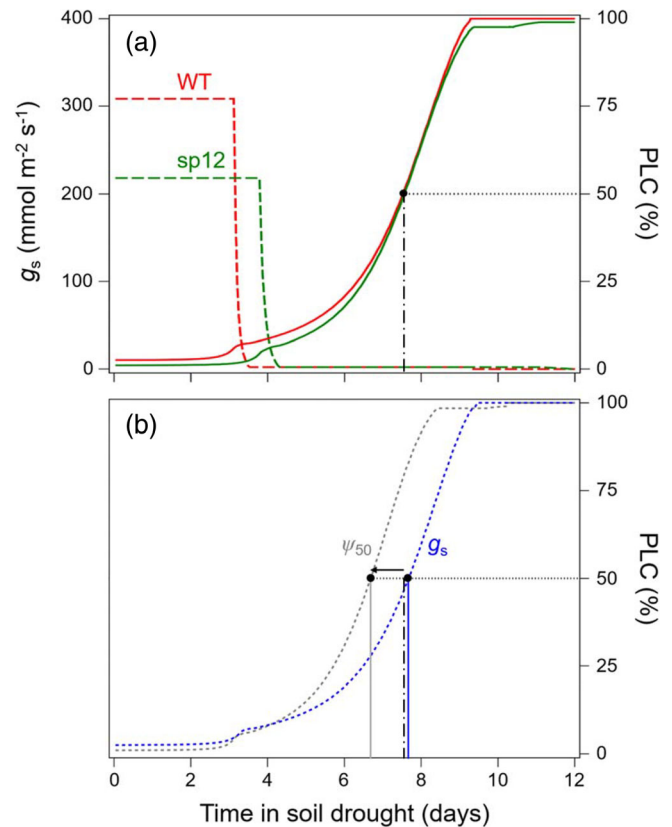

FIGURE 7 Daily simulated patterns of stomatal conductance (g_s) and loss of hydraulic conductivity (PLC) during the progression of a simulated soil drought (SurEau model; Martin-StPaul et al., 2017). (a) Declining g_s and increasing PLC modelled for both wild type control (medium dashed and solid red line, respectively) and 9-cis-epoxycarotenoid dioxygenase 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) value as the sp12 transgenic line

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_{smax}; mean = 229 ± 30 vs. 324 ± 46 mmol m^{-2} s^{-1} in sp12 and WT, respectively; p = .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 µmol m^{-2} s^{-1} in sp12 and WT, respectively; p = .0264) and transpiration per unit leaf area (E; mean = 2.17 ± 0.15 vs. 2.94 ± 0.21 mmol m^{-2} s^{-1} in sp12 and WT, respectively; p = .0049) compared with the wild type individuals. Consequently, the transgenic line operated with a greater intrinsic WUE (WUE$_i$, corresponding to the A/g_s ratio; mean = 0.049 ± 0.004 vs. 0.033 ± 0.005 µmol mmol^{-1} in sp12 and WT, respectively; p = .0214) and exhibited a significant 20% decrease in daily water use (mean = 0.43 vs. 0.57-kg H$_2$O day^{-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 (Ψ_{gs90}) 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$_i$.

3.2 | 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 (Ψ_{12} approximately −1.1 MPa; Figure 2a), with the loss of hydraulic conductivity occurring to reach 50% and 88% at Ψ_{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 = .0145, Table 1). The differences were also much larger at the early stage of plant growth, that is, at 90 days compared with 170 days old (Table 2). Both wild type and sp12 lines increased resistance to xylem embolism by 18% and 29% throughout plant development; 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.

3.3 | 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). Although 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 (Lp_r) compared with 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 with 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 = <.0001) highlighted the greater proportion of small vessels <60 μm in sp12 (p = .0015) and that of large vessels in the wild type (p = .0316; Figure S3b).

3.4 | Hydraulic safety margins

By expressing similar stomatal closure but higher vulnerability to xylem embolism than their wild type counterparts, sp12 plants had a narrower HSM (Figure 6). The HSM$_{\Psi12}$ and HSM$_{\Psi50}$, corresponding to the difference between Ψ_{gs90} and the Ψ_{12} or Ψ_{50} of 90-day-old plants, were reduced by 28% and 37% in the sp12 line, respectively (HSM$_{\Psi12}$ = 0.26 vs. 0.36 MPa and HSM$_{\Psi50}$ = 1.03 vs. 1.63 MPa in sp12 and WT). A similar result was found when defining the HSM as the difference between Ψ_{min} and Ψ_{50}, the HSM$_{\Psi min}$ being 26% lower for the sp12 than for the wild type plants (1.15 vs. 1.55 MPa).

3.5 | 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-hr difference in stomatal closure as wild type and sp12 lines reached Ψ_{gs90} ca. 80 and 95 hr 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 behaviour 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 hr earlier with higher Ψ_{50} (i.e., with greater vulnerability to xylem embolism; Figure 7b).

4 | DISCUSSION

Increased ABA production in the tomato sp12 transgenic line resulted in greater WUE under well-watered conditions but, contrary to what we hypothesized, also led to higher vulnerability to xylem embolism compared with 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 HSM, a lower capacity to maintain stem hydraulic conductivity, and subsequently a higher risk of mortality under drought.

In agreement with Thompson, Andrews, et al. (2007) 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 with 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, Shackel, and Mitcham (2011) when spraying tomato cultivars with exogenous ABA (500 mg L^{-1}). As expected, the sp12 transgenic line displayed greater intrinsic WUE 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 with the wild type

(Thompson, Andrews, et al., 2007), 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 because 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 HSM 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 because 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 WUE 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, Brodribb, & Choat, 2017), and wheat (Corso et al., in press). In addition, greater WUE 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 and 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. Whereas annuals such as tomato and wheat may not express variation in xylem embolism resistance among organs (Corso et al., in press; 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). Although 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 et al., 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, Cochard, Gil-Pelegrin, and Notivol (2011) found a correlation between greater hydraulic conductivity and lower embolism resistance in maritime pine, whereas 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 (Tardieu & Parent, 2017; Vandeleur et al., 2014). 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 with 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 (Mahdieh & 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; Knipfer, Eustis, Brodersen, Walker, & McElrone, 2015; Steudle, 2001). However, whereas 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).

5 | 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 HSM. This trade-off should be considered when targeting ABA accumulation in order to manipulate WUE, especially for perennial, rainfed cropping systems. Important questions remain, notably those surrounding the extent of variation in embolism resistance and HSM 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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SUPPORTING INFORMATION

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Short Abstract

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It will not be published as part of main.

We show that an ABA overproducing tomato line exhibits greater water use efficiency than its wild type counterpart but higher vulnerability to hydraulic failure through increased vulnerability to xylem embolism and a reduced hydraulic safety margin. This suggests that breeding programmes should integrate hydraulic traits for effectively managing a crop's ability to maintain hydraulic conductivity and productivity under drought.