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ORIGINAL ARTICLE

Role of sucrose and phloem–xylem interaction in recovery of water status and hydraulic dehydration impacts in tobacco plants (*Nicotiana tabacum*)

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Abstract
The role of phloem–xylem interaction via sucrose exchanges in recovery of dehydration impacts, specifically xylem embolism, has not been directly investigated thus far. Most previous studies were indirect approaches leading to suggestive conclusions. We hypothesized that a block in phloem loading and so no exchange of sucrose with xylem affect tolerance and recovery of tobacco plants (*Nicotiana tabacum*) during dehydration and after the rehydration phase. Transgenic *N. tabacum* (*αNtSUT1*-antisense) plants, which showed impaired phloem loading and high accumulation of soluble sugars in leaves, were compared to the wild-type (WT) plants. The water status, osmotic adjustments, leaf turgor, stomatal conductance, xylem cavitation, and stem xylem sucrose content were determined during dehydration and after the rehydration phases. Results showed that retention of sucrose outside phloem conduits highly improved water status, osmotic adjustment and turgidity of the source leaves in the transgenics during drought period. However, no impact occurred on stomata function and tolerance to xylem cavitation in α*NtSUT1*. After the rehydration period, WT plants with free phloem transport and phloem–-xylem exchange of sucrose recovered better their water status, leaf turgidity, stomatal conductance and xylem functioning than *αNtSUT1* plants. The accumulation of sucrose in leaves of transformants ameliorated their tolerance to water deficit by reinforcing the osmotic adjustment mechanism at the leaf level. However, lack of sucrose in phloem sieve resulted in impairment of hydraulic recovery of xylem from drought of *αNtSUT1* after rehydration. This suggests a crucial role of the phloem–-xylem exchange of sucrose in refilling of embolized xylem vessels.

Keywords Cell turgor · Drought · Phloem loading · Sucrose · Xylem resilience

Introduction

Sucrose is involved in several physiological processes in plants, both in normal and in stress conditions. Under drought or salt stress conditions, sucrose may serve as an osmoregulator, improving water supply, and as an osmoprotectant for membranes and macromolecules (Eggert et al. 2016; Ennajeh et al. 2009; Sami et al. 2016). The changes of sucrose allocation, metabolism, and transport in leaves and roots of water-stressed soybean seedlings contribute to their drought resistance (Du et al. 2020). Sucrose is synthesized in photosynthesizing leaves and is subsequently transported and distributed via the phloem to other parts of the plant (Liu et al. 2012). Perturbation or block of the long-distance transport of sucrose may affect all physiological processes relying on this disaccharide. Impaired sucrose transport via phloem sieve elements and so no exchange with xylem may result in disturbances of the plant water status and xylem hydraulic functioning.

The efficiency of the mechanisms maintaining plant water balance and functional water transport systems is crucial for plant development and survival. Indeed, stomatal function, osmotic adjustments and resilience to xylem embolism are important mechanisms during drought period as

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well as throughout the rehydration phase. The resistance to drought depends on timely stomatal closure (Martin-StPaul et al. 2017). Osmotic adjustments play a key role in plant adaptation to dehydration through turgor maintenance and osmoprotection of vital cellular functions (Blum 2017).

The xylem assures long-distance transport and allocation of water and mineral nutrients throughout the whole plant. Thus, the structural and functional integrities of this tissue are crucial for plant development, productivity and survival. In severe drought, the increased tension of xylem sap is responsible for xylem embolism (Tyree and Zimmermann 2002), which results in hydraulic dysfunction and systemic physiological disturbance, and finally to the death of some parts or the whole plant (Brodersen and McElrone 2013). Plants develop different strategies to withstand the embolism impact on the functionality of their xylem system, (1) avoiding embolism, (2) reversing embolism, or (3) producing new xylem vessels (Brodersen and McElrone 2013; Nardini et al. 2011).

However, in vascular plants, energy costs and time needs to develop functional xylem system are high. Hence, avoiding xylem dysfunction (protection) and resuming its function after embolism (restoration) seem to be the suitable strategies of adaptation under constraining conditions. Numerous key mechanisms may accomplish these strategies including morpho-anatomical, physiological and biochemical processes. Early stomatal closure may delay the reach of xylem water tension threshold inducing cavitation (Ennajeh et al. 2008; Sevanto 2018). Other mechanisms require orchestrated activities of different organs, tissues or cells to assure suitable responses for xylem protection or reparation. In severe drought, the increase of xylem water tension is sensed by the phloem tissue, which reacts by adjusting osmotically its water potential to match that of the xylem (Sevanto 2018). The interruption of phloem pathway by bark girdling resulted in a decrease in branch xylem hydraulic conductance (Zwieniecki et al. 2004). This suggests a functional link between phloem and xylem hydraulic systems mediated by changes in solute exchanges between the two conductive tissues (Zwieniecki et al. 2004). The girdled stems cannot repair the embolized xylem vessels (Salleo et al. 1996). Indeed, phloem can be involved in the refilling process of embolized xylem vessels (Bucci et al. 2003; Salleo et al. 1996, 2006).

In xylem-cavitated stems, the loss of total hydraulic conductance of the stem can be compensated by two different physiological mechanisms. The first is realized by the enhancement of hydraulic conductance of persisting functional vessels by secreting some ions by xylem parenchyma cells into the xylem conduits (Trifilo et al. 2008). Several woody species increase the osmotic attractive force on water molecules in their xylem vessels by concentration of the xylem sap (Ewers et al. 2001; Hacke and Sperry 2003; Sauter et al. 1973). This mechanism increases water movements into persistent no-embolized xylem conduits and enhances hydraulic conductance of these conduits hydraulically connected to the transpiration stream (Brodersen et al. 2010; Salleo et al. 2004). The second mechanism consists to the reparation (refill) of embolized xylem conduits by pressurizing (Klein et al. 2018). Hydrostatic pressure can be built by osmotic gradient generated through the secretion of solutes by living vessel-associated cells into the embolized xylem vessels pulling water into these conduits (Bucci et al. 2003; Nardini et al. 2011; Perrone et al. 2012). The mechanism is possible according to the 'pit membrane osmosis' hypothesis where semi-permeable cell walls act as osmotic membranes (Hacke and Sperry 2003; Ooeda et al. 2016). Solutes include inorganic ions and organic molecules, such as ions, sugars, proteins, or polysaccharides (Améglio et al. 2004; Salleo et al. 2004; Tyree et al. 1999). Thus, the reparation of embolized xylem vessels requires a source of water and a source of energy (Holbrook and Zwieniecki 1999; Secchi and Zwieniecki 2011).Water moves from phloem to xylem according to the established gradient of water potential strongly enhanced by difference in osmotic potential between these two tissues, following active sugar translocation into cavitated xylem conduits (Améglio et al. 2004; Nardini et al. 2011). Starch-to-sugar conversion is a key event during recovery from the embolism (Nardini et al. 2011; Salleo et al. 2009). The chemical analysis of sap extracted from embolized xylem vessels showed increased cations and sugar levels, presumably related to sucrose–cation co-transport activity (Secchi et al. 2011; Secchi and Zwieniecki 2012). The sucrose may play a key role during the protection and the refilling mechanisms of embolized xylem vessels. H^+-sucrose transporters (SUT) have been proposed to act as specific sensors in sieve elements (Barker et al. 2000). Additionally, up-regulation of the sucrose release in embolized stems suggests its role in refilling as an osmoticum or source of energy for increased respiration (Brodersen and McElrone 2013). Thus, sucrose concentration in the xylem may act as the embolism sensor, and it could be used as the stimulus triggering protective mechanisms (Brodersen and McElrone 2013). Most of the cited studies here are indirect approaches to appreciate the role of sucrose exchanged from phloem to xylem in xylem embolism—refilling.

The objective of the present work was a direct investigation of the role of the phloem–xylem interaction via sucrose exchange on plant water balance and xylem functioning during drought and recovery phases. The importance of the phloem–sucrose in tolerance to drought-induced embolism and its involvement in the refilling of cavitated xylem vessels during the rehydration phase were examined. It was hypothesized that the exclusion of sucrose from phloem sieve elements and so no exchange with xylem perturbs the plant's water balance and affects hydraulic xylem function

and refilling during the dehydration and rehydration periods. Transformants showing sucrose transport deficiency can be used to assess this disaccharide's function in plant resilience to xylem embolism.

In the present study, we used transgenic tobacco plants *Nicotiana tabacum* (*αNtSUT1*-antisense) with reduced expression of the sucrose–proton symporter NtSUT1 (α*NtSUT1*). These plants demonstrated blockage in the sucrose translocation from the mesophyll to the phloem in mature leaves. The quantitative analysis of the transgenic tobacco lines has been previously characterized and published in the original paper from 1998 (Bürkle et al. 1998), and all different transgenic lines behaved similarly. In our study, water relations, stomatal conductance, xylem embolism and sucrose content in stem xylem were determined in $\alpha NtSUT1_{55}$ plants subjected to water deficit followed by a rehydration period, and compared to the same parameters in the wild-type plants.

Materials and methods

Plant material

We used transgenic plants of *Nicotiana tabacum* (*αNtSUT1*-antisense) which demonstrated impairment in the sucrose translocation from the mesophyll to the phloem in mature leaves (Bürkle et al. 1998). Therefore, we chose one representative line number 55 ($\alpha NtSUT1_{55}$), which is one of the strongest inhibited lines to perform qualitative analysis regarding drought stress. The line $\alpha NtSUT1_{55}$ shows increased levels of sucrose in mature leaves (21.5 mmol m^{-2} compared to 3.8 mmol m^{-2} in tobacco WT leaves), more than tenfold levels of monosaccharides (glucose, fructose) and doubled amounts of starch (Bürkle et al. 1998). The sucrose export rate is dramatically reduced in all tested *αNtSUT1* lines (in line $\alpha NtSUT1_{55}$ to only 0.1 µmol m^{-2} s^{-1} compared to 3.7 µmol m^{-2} s^{-1} in WT plants). The rate of photosynthesis dropped from 14.1 µmol m^{-2} s^{-1} in WT plants to only 4.3 µmol m^{-2} s^{-1} in line $\alpha NtSUT1_{55}$ (Bürkle et al. 1998). In the present study, the behavior of $\alpha NtSUT1_{55}$ plants was compared to that of WT plants during drought and re-watering periods.

For both plant types, 14-week-old plants were grown in 5 l pots soil under 500 µmol m^{-2} s^{-1} light intensity with a 14/10 h light/dark cycle and a 25 °C/20 °C light/dark temperature cycle. Plants were watered daily and supplied with Rorison's nutrient solution containing 2.2 mM nitrogen (Hewitt 1966). According to Bürkle et al. (1998), SUT1 inhibition in $\alpha NtSUT1_{55}$ plants provoked severely reduced growth, especially in roots, resulting in a large increase in the shoot/root ratio. At leaf level, $\alpha NtSUT1_{55}$ exhibited the phenotypic effects of retarded leaf development. After that, when the leaves of this line reached full expansion, a progressive development of chlorosis appeared in interveinal regions. In our study, plants of both tobacco lines were 14-week-old. The reproductive part (flowers) was present in WT, but not in transgenic type. However, their vegetative parts were comparable in size and leaves had roughly the same area (Fig. 1). In addition, the number of leaves by plant was approximately the same, and so transpiring areas were identical. For each plant type, experiments were performed in triplicate consisting of six individual plants each.

Plants were subjected to drought stress by not watering until reaching predawn leaf water potential (Ψ_{pd}) of − 2.2 MPa. At this Ψ_{pd} value, tobacco plants wilted and suffered from drought stress. From this water deficit intensity ($\Psi_{pd} = -2.2$ MPa), plants were rewatered and the rehydration phase started and lasted 6 days. At regular measurement intervals (each 3 days) during the dehydration period, and at the end of the rehydration phase, several physiological and biochemical parameters were measured. At each measurement interval, three plants per tobacco type were randomly picked and Ψ_{pd}, Ψ_s, Ψ_p and g_s were measured. In addition, on

Fig. 1 Phenotype of *Nicotiana tabacum* transformant (α*NtSUT1*) used in the experiments compared to wild type (WT). Picture depicts representative samples of 14-week-old plants grown individually in soil. Leaves of the α*NtSUT1* plants suffer from accumulation of the soluble sugars and starch due to the block in phloem loading

the same plants, Ψ_{xylem}, PLC in stems and sucrose content in stem xylem samples were determined.

Determination of the water status in plants

The predawn leaf water potential (Ψ_{pd}) was measured before the onset of light, while the water potential in xylem (Ψ_{xylem}) was determined by the covered-leaf technique. The leaf was enclosed in an aluminum bag for at least 2 h before measurement to prevent leaf water loss and thus allow leaf and xylem water potentials at the base of the bag to equilibrate. Ψ_{pd} and Ψ_{xylem} were measured using a Scholander pressure chamber (Sky Instruments, Powys, UK) (Scholander et al. 1965).

The osmotic potential (Ψ_s) was determined by the method of Nobel (Nobel 1991) on the same plant leaves used for measuring the Ψ_{pd}. To obtain the cell extracts, discs of 0.5 cm diameter collected from inter-vein zones of the fresh leaves were enclosed in a 0.5 ml Eppendorf tube perforated at its base. The tube was immerged in liquid nitrogen for a few seconds and allowed to thaw for 5 min. Three freeze–thaw cycles were performed for each sample. The perforated Eppendorf was placed in another 2 ml non-perforated tube which was centrifuged at 8000×*g* for 15 min at 4 °C. The obtained cell extracts were used to determined Ψ_s using the freezing-point osmometer (Dr. Knauer GmbH & Co. KG, Berlin, Germany).

The turgor potential (Ψ_p) was calculated as the difference between Ψ_{pd} and Ψ_s:

$$\Psi_p = \Psi_{pd} - \Psi_s.$$

Stomatal conductance

The stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) was measured on the mature leaves using a portable AP4 Leaf porometer (Delta-T Devices, Cambridge, UK). Measurements were performed 2 h after the onset of light in saturating light conditions at temperatures of 25 °C. Each measurement was repeated in triplicates for each of the three leaves per plant ($n=9$). A total of three plants per measurement interval were used.

Xylem embolism

The aboveground part of each tobacco plant was separated from the roots and the stem was excised at its base under water. This stem sample was then bagged in a large black plastic bag and covered with a moist sheet for at least 2 h, so that the water potential within each stem sample equilibrated and no gradient existed. Prior to hydraulic conductance (K) measurements, relaxation of xylem tension was realized by cutting a 5 cm increment both from the apical and basal end alternatively underwater every 10–20 s. The process was conducted until the stem sample neared 15 cm of length. The obtained relaxed stem sample was then divided into five segments of 3-cm-long samples longer than tobacco xylem vessels 2–4 mm (Hepworth and Vincent 1998). K of stem segments was measured gravimetrically with a conductance apparatus (Sperry et al. 1988). The hydraulic conductance was measured before (K initial, K_i) and after (K maximum, K_m) water refilling. Stem segments were excised under water to prevent any cutting artifact. Their cut ends were re-cut with a sharp razor blade. One of the cut ends was then attached to the hydraulic apparatus. Flow rates were measured with an analytical balance (± 0.1 mg) interfaced to a computer. The measurement filtered solution was 20 mM KCl and 1 mM $CaCl_2$ in ultrapure water. The measurement delivery pressure was about 6 kPa. The solution flowed from a beaker sitting on the balance through the stem segment, which was kept under water during the measurements. We measured Ki at low pressure (6 kPa). To measure Km, air obstructing stem xylem vessels was removed by applying a series of 10-s hydraulic pressure flushes (0.2 MPa) until the measured values of Km remained constant between flushes. K_i and K_m values allowed calculating the percent loss of hydraulic conductance (PLC) as:

$$\text{PLC}\ (\%) = \left(1-K_i/K_m\right) * 100.$$

Xylem embolism in the stem was quantified by the PLC. Vulnerability curves (VCs) were constructed by plotting the PLC values against Ψ_{xylem}. The value of PLC, due to the air blockage, is an indirect estimate of the percentage of the embolized vessels (Cochard et al. 2000).

Sucrose content

The sucrose content in stem xylem samples was quantified in all plants. Stem samples of 5 cm length were taken from the basal part of the plants. The preparation of stem xylem sample for sucrose quantification was realized according to the method described by Pan et al. (1993). All tissues other than xylem were eliminated to prevent any contamination and avoiding the overestimation of sucrose content. The tissues external and internal to the xylem (epidermis, cortex, external phloem, cambium, internal phloem and pith) were eliminated by a scalpel blade. The cleaned stem xylem sample was then treated with liquid nitrogen and stored at − 20 °C. Before sucrose quantification, the weight of each cleaned stem xylem sample was determined. The sucrose was extracted in 80% ethanol at

70 °C for 30 min and its content assayed enzymatically as described by Stitt et al. (1989).

Statistical analysis

The experimental setup was arranged as a completely randomized design with three replicates. To test whether the transgenic line differs significantly from the WT in our experimenta the *t* test with a cutoff level of $\alpha = 0.05$ was used. Analysis of variance with the GLM procedure and the Duncan post hoc test ($P = 0.05$) were applied using SAS software (SAS Institute 1999). For WT × αNtSUT1 comparisons, we compared the mean values for each parameter and not the fitting models.

Results

Plant–water relations

The leaf water potential before the onset of light decreased according to the time of drought stress application in both *αNtSUT1* and WT (Fig. 2A). However, the decrease was less pronounced in *αNtSUT1* than in WT. The difference between *αNtSUT1* and WT was even more apparent after 6 days of dehydration. The most severe drought stress threshold, expressed as Ψ_{pd} (− 2.2 MPa), was reached already after 15 days in WT and after 21 days in *αNtSUT1*. This may indicate a higher capacity to maintain water status in the transgenic plants than in wild type. Ψ_{pd} measurements confirmed the visual appearance of plants starting from the 6th day of drought stress (Fig. 2B). *αNtSUT1* plants appeared well turgid and had a better water status than the WT plants which wilted severely.

After 6 days of rehydration, the wild-type plants recovered from the drought stress, as indicated by the Ψ_{pd} measured in the controls before stress application. However, *αNtSUT1* only partially recovered their water status by 55% of the lowest Ψ_{pd} and referring to the initial value.

Figure 3A depicts the trend of the osmotic potential (Ψ_s) in leaves of *αNtSUT1* compared to WT during the water stress period and after rehydration. The Ψ_s decreased progressively during the dehydration period, but more severely in α*NtSUT1* than in WT plants. In *αNtSUT1* plants, the lowest value of Ψ_s was reached earlier (after 12 days) than in WT plants (after 15 days), and it was maintained at the lowest level during severe drought stress and rehydration periods. The rehydration allowed the WT plants to recover their Ψ_s by 73% of the lowest Ψ_s and referring to the initial value. However, Ψ_s of the transgenic *αNtSUT1* plants recovered only 25% of the lowest Ψ_s value, which points to impairment in the recovery process. Indeed, Ψ_s in *αNtSUT1* plants was not significantly different between the end of drought stress and rehydration periods.

The turgor potential (Ψ_p) was plotted as a function of Ψ_{pd} during the drought period and after rehydration (Fig. 3B). At the beginning of the drought (3 days), the Ψ_p increased in *αNtSUT1*, but decreased in WT. This later type plants lose their cell turgor starting from the 3rd day of not watering. They showed Ψ_p near zero and sometimes slightly below throughout the whole drought stress period. In WT, the plant turgor loss measurements matched our observations that the leaves wilted severely during the drought. However, in *αNtSUT1* the Ψ_p increased significantly reaching 1.12 MPa after 15 days of drought. After that, Ψ_p decreased but still remained higher than in non-stressed plants. It was concluded that the cell turgor decreased upon the drought stress application in WT, but it was maintained with even increasing pressure in *αNtSUT1* plants (Fig. 3B). After rehydration, Ψ_p indicated recovery of WT, but *αNtSUT1* showed re-increase in Ψ_p even higher than the unstressed plants.

Vulnerability to xylem embolism

The curves of vulnerability to xylem embolism indicated that both *αNtSUT1* and WT have a comparable tolerance response to cavitation (Fig. 4). No significant difference ($P = 0.9486$) existed between their PLC values for the same Ψ_{xylem} during the dehydration phase (Table 1). The xylem water potential inducing 50% of PLC ($\Psi_{50\%}$), was − 1.18 MPa and − 1.29 MPa for WT and α*NtSUT1*, respectively. At severe water stress (Ψ_{xylem} around − 2.2 MPa), both tobacco plant types showed the highest value of PLC. It was 94% for WT and 81% for transgenic. So, there were no influences of sucrose phloem–xylem exchanges on tolerance to xylem embolism during the dehydration phase. However, WT showed the phenotype regarding the ability to refill its embolized xylem vessels. After 6 days of rehydration, PLC was around 40% in WT plants. Therefore, these plants recuperated around 60% of hydraulic functionality of their xylem. However, rehydrated *αNtSUT1* plants exhibited high level of PLC (80%). So these plants recuperate just 20% of their xylem hydraulic conductance. Xylem hydraulic conductance was significantly different between rehydrated plants of WT line and *αNtSUT1* line ($P = 0.0094$). This indicated the limited ability of transformants to recover the functionality of their xylem system. The differences in xylem functional recovering after drought-induced embolism between WT and *αNtSUT1* may be related to the difference in the efficacy of their xylem refilling mechanisms depending to sucrose accumulation.

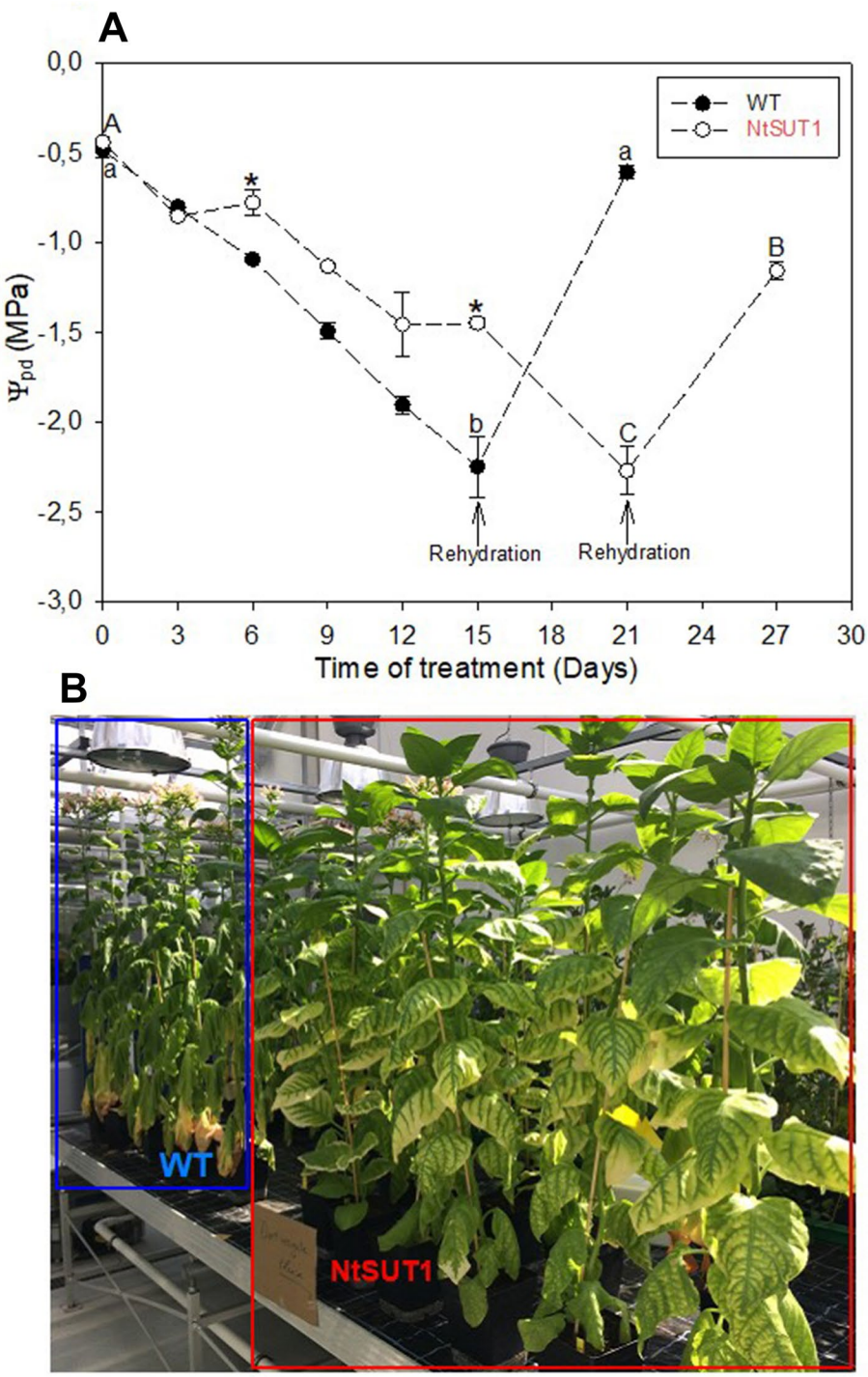

Fig. 2 **A** Predawn leaf water potential (Ψ_{pd}, MPa) measured before the onset of light, presented as a function of time (days) of water treatment; wild-type (WT) and transgenic (α*NtSUT1*) *N. tabacum* plants subjected to drought stress, followed by a rehydration period. Rehydration started upon the reach of the critically low Ψ_{pd} after 15 and 21 days, for WT and α*NtSUT1* plants, respectively. Experiments were performed in at least three biological replicates; the error bars represent the SE. An asterisk indicates the time points when differences between WT and α*NtSUT1* were determined to be significant ($P \leq 0.05$). The Ψ_{pd} at the beginning and at the end of the drought stress period, as well as after the rehydration phase, were compared. Statistical analysis based on the Duncan post hoc test is presented by small letters for WT and capital letters for transgenic. The different letters on circle points indicate significant difference between the three dates for the same plant type ($P \leq 0.05$). **B** Representative examples of WT and α*NtSUT1* plants after 6 days of the dehydration phase

Stomata functioning

Under normal watering conditions, both *αNtSUT1* and WT showed similar stomatal conductance (g_s) (Fig. 5). In water-deficiency conditions, stomata opening was also similar in *αNtSUT1* and WT. The g_s show a fast decrease at the beginning of the drought period and the complete closure of the stomata was observed at Ψ_{pd} of − 1 MPa. However, following rehydration, *αNtSUT1* did not show recovery in g_s observed in WT. The recovery of g_s in the transformant plants was only 38% compared to the control plants. Indeed, the difference between unstressed and rehydrated plants was no significant within WT genotype ($P = 0.2641$), but was highly significant within *αNtSUT1* genotype ($P = 0.0009$) (Table1).

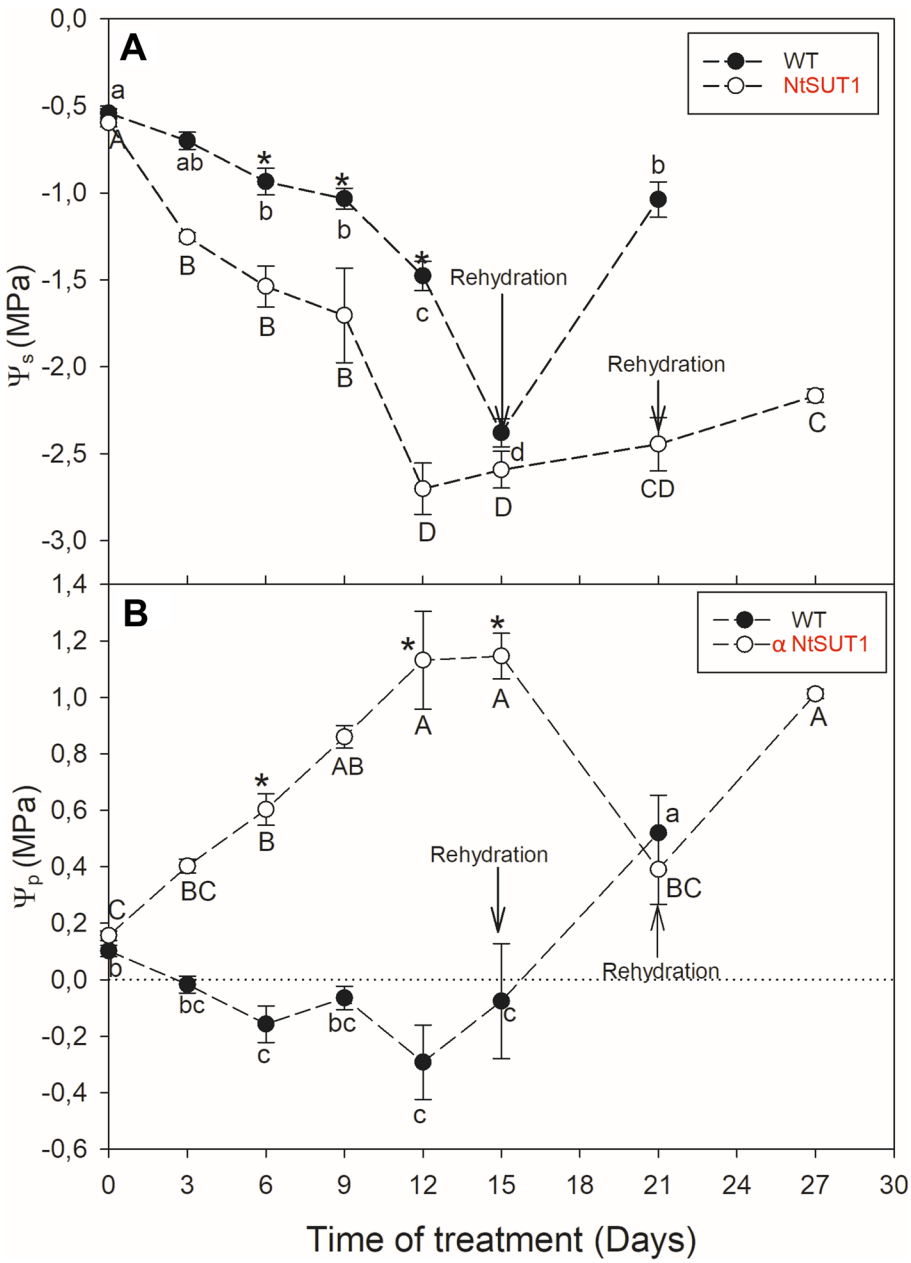

Fig. 3 **A** Osmotic potential (Ψ_s, MPa) and **B** turgor potential (Ψ_p, MPa) as a function of time (days) in wild-type (WT) and the transgenic (α*NtSUT1*) plants subjected to drought stress, followed by the rehydration period. Experiment was performed in at least three biological replicates; the error bars indicate SE. An asterisk indicates significant difference between WT and α*NtSUT1* ($P \leq 0.05$). Ψ_s measured at different time points were compared. Statistical analysis based on the Duncan post hoc test is presented by small letters for WT and capital letters for transgenic. Letters indicate significance of the difference between Ψ_s values for the same plant type ($P \leq 0.05$)

Sucrose accumulation in stem xylem

Moderate drought stress triggered an increase of the sucrose content in the xylem both in α*NtSUT1* and WT (Fig. 6). However, under severe drought conditions ($\Psi_{pd} < -1$ MPa), a significant ($P = 0.0001$) decrease was observed in the transgenics, whereas in the wilds the sucrose content increased and remained at high level even under severe stress. The sucrose content was low in the stem xylem of both *αNtSUT1* and WT upon rehydration.

Discussion

The tolerance to drought requires an integrative response involving various morpho-anatomical, physiological, biochemical and metabolic mechanisms. Some of them are based on synchronized processes and interaction and exchanges between cells, tissues and organs. The influence of phloem–xylem interaction specifically via sucrose exchanges on plant water status, stomatal functioning and refilling of xylem embolism was studied in *N. tabacum* plants during

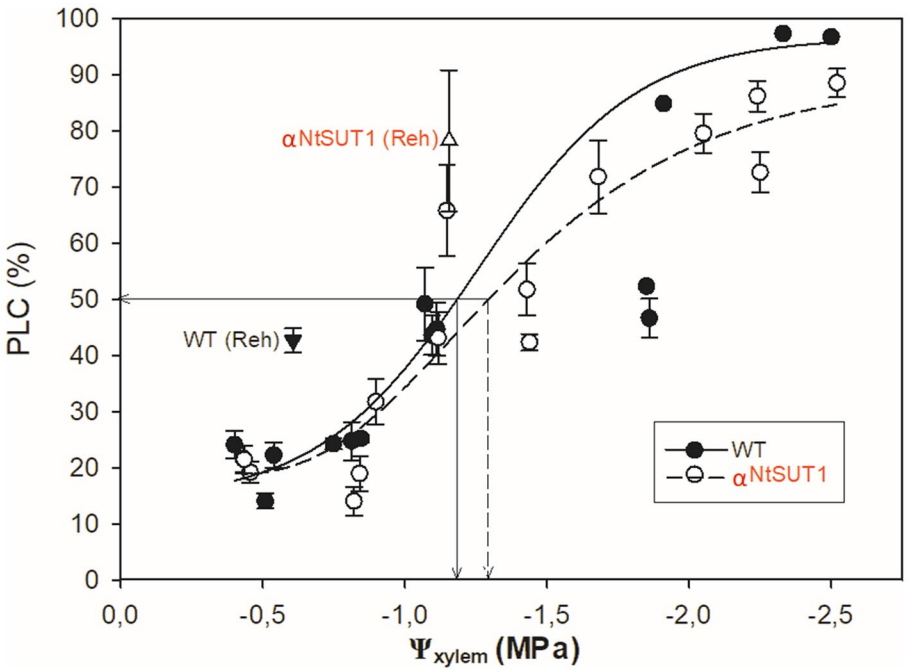

Fig. 4 Vulnerability curves (VCs) of drought-induced xylem cavitation in wild-type (WT) and transgenic (α*NtSUT1*) plants. The recovery capacity of the xylem function after cavitation was also evaluated by measuring the percent loss of the hydraulic conductance (PLC) following the rehydration period (Reh). The fitting of VCs did not include the Reh points. Arrows indicate the xylem water potential (Ψ_{50}) inducing 50% of PLC, vertical solid arrow is relative to WT plants and dashed arrow for α*NtSUT1* plants. Each point is the average of at least three replicates and the vertical bars indicate SE

Table 1 *P* values of comparison of the percent loss of hydraulic conductivity (PLC) and stomatal conductance (g_s) between water-stressed plants (Stressed) of WT and *αNtSUT1* tobacco genotypes, and between unstressed and rehydrated (Reh) plants within WT genotype and within *αNtSUT1* genotype

		PLC	g_s
Stressed	WT × *αNtSUT1*	0.9486 NS	0.4584 NS
Unstressed × Reh	WT	0.0137*	0.2641 NS
	αNtSUT1	0.0094**	0.0009**

Note: NS, *, ** indicate differences: not significant or significant at $P \leq 0.05$ or at $P \leq 0.01$, respectively

the dehydration and rehydration phases. Our approach was a direct estimation of the effects of phloem–xylem exchanges on some key physiological mechanisms involved in plant response to drought. For this purpose, the sucrose transporter antisense plants *αNtSUT1* (Bürkle et al. 1998) were used. These transgenic plants show strongly reduced expression of sucrose transporter *NtSUT1*, which is the main phloem loader in *N. tabacum* source leaves (Doidy et al. 2012). Thus, the *αNtSUT1* plants were characterized by reduced sucrose loading capacity into their phloem conduits and so reduced sucrose exchanges with xylem.

Impacts of phloem unload and phloem–xylem exchanges of sucrose during dehydration

During the dehydration phase, the water status of *αNtSUT1*, characterized by leaf Ψ_{pd}, was less affected than that of the WT plants. To explain this difference, two key defense mechanisms participating in the preservation of the plant water status under drought, stomatal regulation and osmotic adjustment, were assessed. Stomatal closure is the earliest plant response to water deficit (Schroeder et al. 2001). Osmotic adjustment is a common mechanism adopted by most plant species under limiting water conditions (Dichio et al. 2003; Ennajeh et al. 2006; Madan et al. 1994; Ouledali et al. 2018). In our study, both *αNtSUT1* and WT exhibited similar stomatal conductance during the water deficit period. They closed their stomata during early stress and the stomatal leaf transpiration was stopped. Thus, the difference in the water status between *αNtSUT1* and WT was not related to their stomatal function. However, the Ψ_s of leaves decreased more rapidly and acutely in *αNtSUT1* plants compared to WT. The Ψ_s decrease could be related to the block of transport of sucrose into the phloem conduits and its accumulation in leaves. Bürkle et al. (1998) demonstrated that the concentration of soluble sugars increased in leaves of transgenic tobacco line $\alpha NtSUT1S35_{55}$. This physiological process allowed the *αNtSUT1* plants to accomplish more efficient osmotic adjustment than WT plants. The extent of the osmoregulation is species and even cultivar dependent (Ennajeh et al. 2006; Teskey and Hinckley 1986). The enhanced osmoregulation in *αNtSUT1* leaves could explain the retention of the turgor in leaf tissue. Upon drought conditions, the Ψ_p in *αNtSUT1* leaves was increased, while it decreased in the WT plants. Accumulation of sucrose in leaf mesophyll of *αNtSUT1* plants can increase the osmotic attractive force (of the leaf) on water. Thus, the hydration of leaves of *αNtSUT1* was improved. The *αNtSUT1* plants could create an osmotic adjustment at the *organ level*:

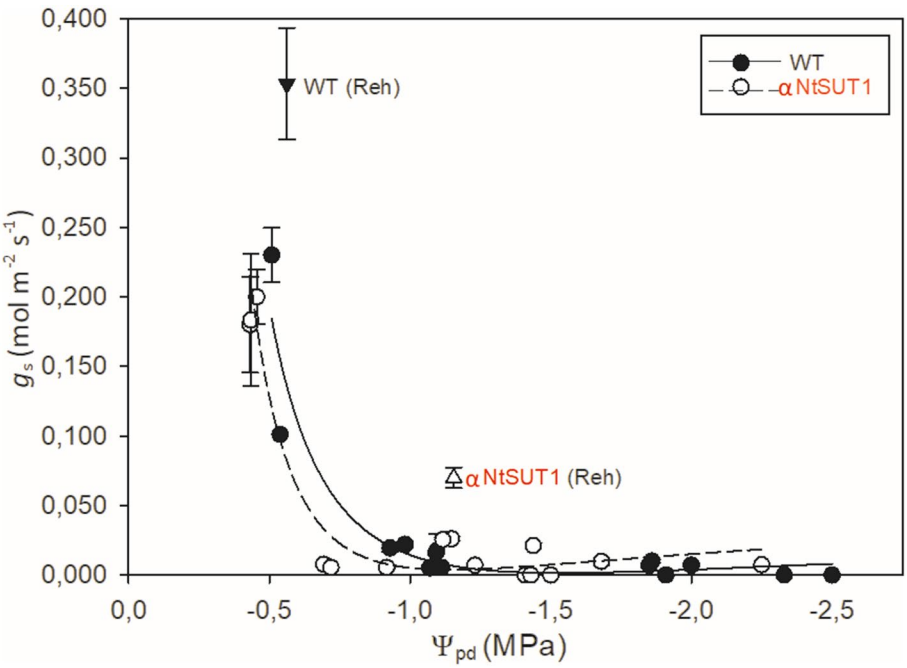

Fig. 5 Stomatal conductance (g_s, mol m^{-2} s^{-1}) as a function of the predawn leaf water potential (Ψ_{pd}, MPa) in α*NtSUT1* and wild type (WT) subjected to drought stress. The g_s was measured after the rehydration period (Reh). Each point is the average of at least three replicates and the vertical bars indicate SE

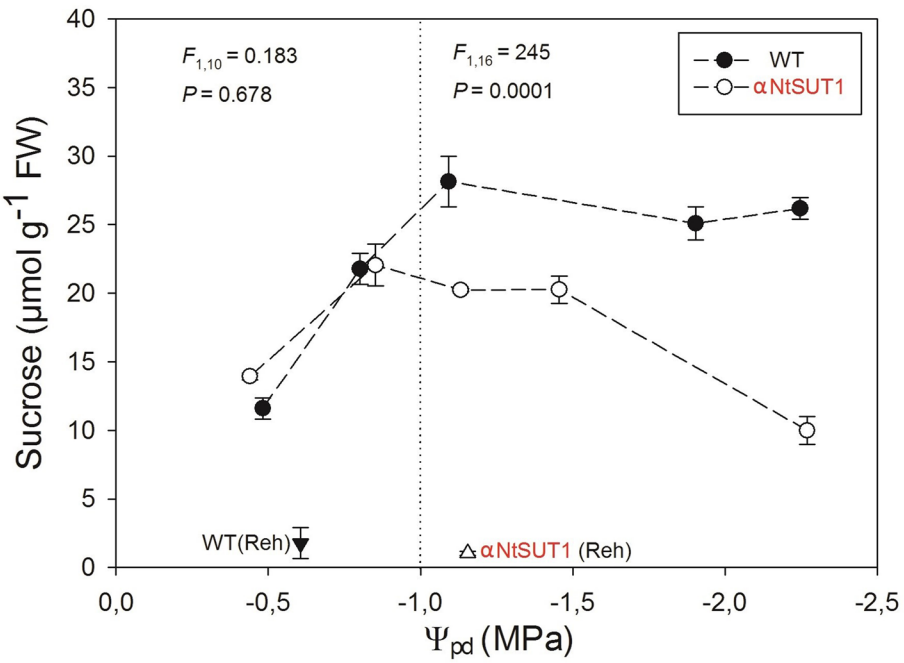

Fig. 6 Sucrose content (µmol g^{-1} FW) in the stem xylem as a function of predawn leaf water potential (Ψ_{pd}, MPa) in wild-type (WT) and transgenic (α*NtSUT1*) tobacco plants subjected to water stress. The sucrose content was also quantified after the rehydration period (Reh.). Each point is the average of at least three replicates and the vertical bars indicate SE

i.e."leaf osmoregulation", which enhanced the turgor of leaf tissue (Fig. 3B). The higher efficiency of osmotic adjustment mechanism in *αNtSUT1* compared to WT can explain its great ability to maintain leaf tissue turgor (high Ψ_p) under drought stress conditions.

The importance of the sucrose accumulation in leaves of another Solanaceous species (potato) as a response to salt stress conditions has been described previously (Asensi-Fabado et al. 2015). An additional stress-induced interaction partner of plant sucrose transporters is the protein disulfide isomerase StPDI1 that interacts with all three sucrose transporters from potato, namely SUT1, SUT2 and SUT4 (Eggert et al. 2016). Transgenic plants with reduced expression of StPDI1 severely suffer from abiotic stresses such as salt or drought stress and do not recover after re-watering (Eggert et al. 2016). Interaction of sucrose transporter StSUT1 with several stress-induced proteins points ton the importance of sucrose partitioning for stress tolerance and recovery.

Concerning the xylem hydraulic characteristics, we demonstrated that there was no significant difference in the vulnerability to drought-induced xylem embolism between *αNtSUT1* and WT. Ψ_{50} in *αNtSUT1* was only 0.1 MPa lower compared to WT. So during water stress perception, tobacco did not use sucrose to avoid dehydration-induced xylem cavitation. Our hypothesis that sucrose phloem–xylem exchange in WT plants may enhance their tolerance to xylem embolism compared to *αNtSUT1* plants is not valid. It seems that tobacco involved more ions for osmosis than sugars to resist to xylem cavitation. Indeed, when stressed, the plant induces multiple defense mechanisms in addition to osmoregulation. These mechanisms use specific new-synthesized metabolites suitable for withstanding dehydration. To synthesize these metabolites, the plant may use sugars like sucrose as an element in their synthesis pathway or to offer required carbonic skeleton. Also when stressed, the plant increases its respiration and requires more energy, and sucrose may be a source of this energy.

Thus, during dehydration stress, the tobacco plant uses its sucrose pool preliminarily to synthesize new metabolites or to produce energy required for defense mechanisms than to resist xylem embolism, because other solutes like ions may be used to avoid xylem cavitation. In addition, the sucrose transport is a complex process involving various partners (Eggert et al. 2016), which may make the process slower and more energy costly than ion transport specifically under stress conditions. All preview arguments may explain the absence of sucrose-dependant difference concerning tolerance to xylem embolism during water stress by the two tobacco lines.

On the other hand, despite early and complete closure of the stomata, both WT and transgenic plants exhibited high vulnerability to xylem cavitation. Recently, a lack of correlation between the water potential thresholds of stomatal closure and the embolism induction was established in several species (Martin-StPaul et al. 2017). Stomatal closure does not completely eliminate supplementary embolism (Brodribb et al. 2016; Hochberg et al. 2017).

Role of phloem–sucrose in recovering the dehydration impacts

After the rehydration phase, *αNtSUT1* and WT showed different behaviors in terms of the assessed physiological and biochemical parameters. The WT plants showed higher recovery rate regarding their water status than *αNtSUT1*. Grapevine exhibited inter-specific variability in responses to drought stress that involves maintenance/recovery of xylem transport capacity coordinated with root pressure and gas exchange responses (Knipfer et al. 2015). In WT tobacco, the efficient hydraulics of the xylem allowed retention of water transport in the whole plant. The sucrose could be influencing the stomatal behavior indirectly through its impact on the leaf tissue turgidity, or directly through its interaction with abscisic acid, the principal controller of the stomatal aperture (Secchi and Zwieniecki 2011). The sucrose transporters (SUT) are important regulators in plant abiotic stress tolerance that use an ABA-signaling pathway, which might be crossed with sucrose signaling (Dong et al. 2015). Sucrose could also affect the refilling of the cavitated xylem vessels. Sugars and ions from living elements of xylem and phloem cells are involved in xylem refilling (Nardini et al. 2011; Salleo et al. 2004).

Because of the high energy demands and time to build a new conductive system of xylem, it is strategically important for woody species to repair their pre-existent embolized xylem. The non-destructive method of frequency domain reflectometry (using frequency domain sensors) proved xylem embolism refilling in large tree trunks (Hao et al. 2013). In addition, X-ray computed microtomography measurements proved xylem embolism repair in grapevine (Brodersen et al. 2010). In our study, no technical artifact existed in the experimental protocol of embolism measuring. Indeed, in several woody species, PLC hydraulic measurements are in agreement with PLC estimated by non-destructive imaging technique X-ray microtomography (microCT) (Nardini et al. 2017; Nolf et al. 2017; Torres-Ruiz et al. 2014). However, signs of X-ray-induced damages were observed in herbaceous crop plants (Savi et al. 2017). So in herbaceous species, hydraulic measurements of samples excised under tension, providing standard sampling and handling protocols, are more reliable. In our study, standard hydraulic technique was used to quantify xylem embolism.

Plants adopted metabolically active embolism repair mechanisms (Brodersen and McElrone 2013). It is well documented that several ions and metabolites are implicated in the xylem refilling after embolism (Secchi and Zwieniecki 2012). Accumulation of sucrose in drought-embolized plants testifies the participation of this metabolite in the defense response. Sucrose is involved in embolism signaling, osmotic energy and expression of the embolism-induced genes (Secchi and Zwieniecki 2011). The phloem–sucrose pool may be implicated in refilling of the embolized xylem vessels. In our current study, the difference in PLC between WT and transgenic tobacco plants at the end of the recovery period is mainly due to different efficiency in the xylem vessel refilling mechanism between the two tobacco lines. This difference may not be related to slower process. In another Solanaceous species (tomato), a significant drop of PLC was observed within 100 min following re-watering. A full embolism recovery in severely stressed tomato plants was observed within 24 h following re-watering (Secchi et al. 2013). So in our case, PLC was quantified after 6 days of re-watering, a period sufficient for any embolism recovery to happen. Our results indicated that the xylem embolism

repair was retarded in transgenic plants unlike WT plants. Indeed, the recuperation degree of xylem hydraulic functioning was 60% and 20% in rehydrated WT and *αNtSUT1* plants, respectively. These percentages were deduced by referring to the PLC levels that were 40% and 80% in WT and *αNtSUT1* plants, respectively, at the end of the rehydration period. Thus, tobacco exhibited inter-line variability in xylem embolism repair. In several woody species, xylem refilling exhibited inter-specific variability and depended on timescale (Hacke and Sperry 2003).

On the other hand, despite the higher recuperation of xylem functioning in WT plants compared to transgenic ones, this recovery is partial even with the restitution of pre-stress plant water potential. A similar hydraulic system recuperation failure was established in several woody species, specifically at short term (Hacke and Sperry 2003; Choat et al. 2019). In WT tobacco plants, the physiological mechanism involving osmotic attraction of water by sucrose secretion in embolized vessels seems insufficient for complete xylem refilling. This mechanism should be strengthened by positive root pressure. No refill of embolized xylem vessels may be also attributed to their plugging by gel and resin. The failure of complete embolism repair in herbaceous like tobacco was also observed in woody plants (Choat et al. 2019).

The xylem refilling mechanism may involve orchestrated functions and interactions between xylem and phloem. The two tissues are hydraulically connected and they tend to be in water balance (Thompson and Holbrook 2003). The elimination of the phloem induced the decrease of the osmotic concentration of the xylem sap and a reduction of the hydraulic conductance. This suggests functional connection and solute exchanges between the two conducting tissues (de Boer and Volkov 2003). In our study, we suggested that a portion of the phloem sucrose could be diverted into the embolized xylem vessels of the WT plants triggering osmotic attractive force on water molecules allowing their refill. The osmosis mechanism may take place in xylem conduits when their pit walls become semi-permeable and act as osmotic membranes according to the hypothesis of 'pit membrane osmosis' (Oertli 1993). The sucrose secretion into the xylem conduits was proven by the significant increase of its content in the xylem of the WT plants, specifically under severe drought stress. In tomato plants (*Solanaceae*), soluble sugars are involved in xylem embolism refilling, and the leaf starch pool may be used as source of energy for embolism refilling during recovery (Secchi et al. 2013).

In conclusion, the difference in water relations and xylem hydraulic characteristics between WT and transgenic plants during drought and after recovery might be principally attributed to different phloem sucrose distribution. During the dehydration phase, the lack of sucrose transport into phloem and its accumulation in the photo-assimilating leaf tissue enhanced the osmotic adjustments in leaves of *αNtSUT1* plants. This process highly improved leaf turgidity of the transformants. Indeed, in drought stress conditions, cell turgor in *αNtSUT1* was higher than that in WT plants, but in water-sufficient conditions it was the same in both plant types. However, the block of sucrose transport into the phloem and the lack of exchange with xylem do not affect stomatal conductance or vulnerability to xylem cavitation in *N. tabacum* plants exposed to drought stress conditions. The importance of the phloem–xylem interaction via sucrose exchanges became even more apparent during the recovery phase, allowing higher recovery rate of water status, stomatal conductance and xylem conductance in WT plants than in *αNtSUT1* plants. The exchange of sucrose between phloem and xylem may highly participate in the refilling of the embolized xylem vessels.

The retention of sucrose in photo-assimilating leaf tissue was more advantageous under drought conditions for sufficient hydration and turgor maintenance. However, free photoassimilate distribution in the whole plant and possible phloem–xylem exchanges of sucrose were more beneficial during the recovery phase. In the first case, it is accomplished by an efficient osmotic adjustment in leaves, but in the second case it may involve xylem refilling after embolism.

Author contribution statement ME and CK conceived and designed the research. ME conducted experiments. RE contributed to measuring some parameters. ME and CK analyzed and interpreted the data and drafted the manuscript.

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Code availability Not applicable.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

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