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RESEARCH PAPER

Effect of sap salinity on hydraulic properties of the stem xylem of *Paraserianthes lophantha* grown in desert region

Effet de la salinité de la sève sur les propriétés hydrauliques du xylème de la tige de Paraserianthes lophantha cultivée en région désertique

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Abstract

Previous researches have shown that the hydraulic conductivity may be significantly increased with increasing ion concentration in the xylem sap. The main purpose of the present study was to investigate the impact of sap salinity on the hydraulic properties of *Paraserianthes lophantha* that grown in desert region. Isolated stem segments were used to (1) determine the changes in lateral hydraulic conductance when xylem fluid was changed from deionized water to salt solution; (2) evaluate the variation in the degree of intervessel contacts by changing the sap salinity; and (3) visualize the variations in the lateral vessel contacts by using a double-staining method. The results showed that the hydraulic conductance was significantly increased when changing from deionized water to salt solution. The degree of intervessel contacts was somewhat increased by salinity, and this was visually confirmed by observation of some new lateral contacts between vessels in the double-staining experiments. The results indicated that the degree of lateral contacts between vessels could be changed under sap salinity by increasing the porosity and permeability of intervessel pit pathways. Our results may contribute to understand the effect of salinity on the hydraulic properties of xylem in the desert species.

Keywords: Hydraulic conductance, intervessel contacts, pit membrane, sap salinity, xylem.

Résumé

Les recherches précédentes ont montré que la conductivité hydraulique peut être augmentée significativement avec l'augmentation de la concentration des ions dans la sève brute. L'objectif principal de la présente étude était d'examiner l'impact de la salinité de la sève sur les propriétés hydrauliques de Paraserianthes lophantha cultivée en région désertique. Des segments de tiges isolées ont été utilisés pour (1) déterminer les changements de conductance hydraulique latérale lorsque le fluide du xylème a été changé de l'eau déminéralisée à la solution de sel; (2) évaluer la variation dans le degré de contacts inter-vasculaires avec les variations de la salinité de la sève; (3) visualiser les variations des contacts latéraux des vaisseaux en utilisant la méthode de double coloration. Les résultats ont montré que la conductance hydraulique a été significativement augmentée lors du passage de l'eau déminéralisée à la solution de sel. Le degré de contacts inter-vasculaires a été quelque peu augmenté par la salinité et cela a été confirmé par l'observation visuelle de certains nouveaux contacts latéraux entre les vaisseaux dans les expériences de double coloration. Les résultats indiquent que le degré de contacts latéraux entre les vaisseaux pourrait être modifié sous la salinité de la sève par l'augmentation de la porosité et la perméabilité des ponctuations inter-vasculaires. Nos résultats peuvent contribuer à comprendre l'effet de la salinité sur les propriétés hydrauliques du xylème dans les espèces désertiques.

Mots-clés: *Conductance hydraulique, contacts inter-vasculaires, membranes des ponctuations, salinité de la sève, xylème.*

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Introduction

Several studies have demonstrated that intervessel pit membrane hydraulic conductance is sensitive to the xylem sap ion concentration. Conductance of intervessel pitting increases in response to an increase and decreases in response to a decrease in the xylem sap ion concentration (van Ieperen et al. 2000; Zwieniecki et al. 2001; van Ieperen and Van Gelder 2006; Gascó et al. 2007; Nardini et al. 2007; Schmitz et al. 2012; López-Portillo et al. 2012). Hypothetically, ion effects on water flow rates have been explained by the reaction of ions with the pectins in the pit membranes. Increasing ion concentration causes shrinkage of pectins, thereby increasing the porosity of the pit membranes and thus decreasing their resistance to water flow (Zwieniecki et al. 2001). However, because many angiosperm species do not contain pectins in the pit membranes, van Doorn et al. (2011) have proposed an alternative hypothesis explaining the ion effects. They suggested that the negatively charged polymers at the pit pores form a diffuse double layer of water that is less mobile and more viscous, which can physically reduce or inhibit the flow of water in the very narrow pores of the pit membranes. The increase of ion concentration would neutralize this electrostatic field of the pit pores and thus reducing its inhibitory effect. This, in addition to the shrinkage of all polymers that composed the pit membrane (lignins, hemicelluloses, cellulose and pectins if presented), will result in an increase in water flow (van Doorn et al. 2011).

Because intervessel connectivity within the xylem network is closely related to bordered pits (Zwieniecki et al. 2003), it is possible that the degree of intervessel contacts would be affected by ion concentrations. Variations in pit porosity and permeability may cause a change in the extent of intervessel contacts. If this true, then it implies that the amount of intervessel pit pathways that are functioning in water transport between vessels can be changed under the effect of ion concentrations. Since sap salinity increase considerably the size of intervessel pit pores (van Ieperen et al. 2000; Zwieniecki et al. 2001; Zwieniecki et al. 2003), it is possible that lateral contacts between vessels might be increased under high concentrations. To test this possibility, different experiments were performed on twigwood of *Paraserianthes lophantha*. Firstly, changes of lateral hydraulic conductance were evaluated when xylem fluid was changed from deionized water to salt solution. Secondly, we investigated the variations in the degree of intervessel contacts in response to the presence of ions in the perfusing solution. Thirdly, double-staining method was used to visualize and quantify the new intervessel pit pathways, if they presented.

1. Materials and methods

1.1. Plant material

Mature trees of the species *Paraserianthes lophantha (Willd.) I.C.Nielsen*. (Fabaceae) were selected in this investigation. Stem samples were collected in spring 2016 from the region of Touggourt. This region is located in the northeastern Sahara desert of Algeria. The climate along this area is arid to hyperarid, characterized by low rainfall and high rates of evapotranspiration. All samples were harvested in the morning and brought to the laboratory in wet plastic bags.

1.2. Effect of salt on hydraulic conductance

In this experiment, the variation of intervessel hydraulic conductance (i.e. lateral conductance) was determined when deionized water was replaced by salt solution. Sodium chloride solution (NaCl) was used to test for ion sensitivity of hydraulic conductance. Intervessel hydraulic conductance was measured according to the procedures described by Ellmore et al. (2010). Segments of 5 cm length were cut, under water, from the twigwood samples (Figure 1). Under a stereomicroscope and using a fast-setting, water-insoluble epoxy glue (Kafuter Epoxy AB Glue Adhesive, Guangdong Hhngda New Materials Technology Co., Ltd, Guangdong, China), the proximal inflow end of each segment was sealed except for a 60° section of the sapwood that was left unsealed (Figure 1). To completely block the axial flow path, the half of the distal outflow surface including the selected 60° section was completely blocked by glue. Thus, all solutions that were pushed through the unsealed 60° section at the proximal end should move laterally via intervessel pit membranes to exit from the distal surface (Figure 1).

To evaluate the percentile changes in lateral hydraulic conductance when changing from deionized water to salt solution, the proximal end of segment was attached to a hydraulic system that allowed switching between deionized water and salt solution. Deionized water was pushed at 100 KPa pressure, and the outflow from the distal surface was absorbed into lab tissue and weighed after 2 min of collection using electronic balance (model 210, Sartorius). The hydraulic conductance was determined as the mass–flow rate divided by the pressure applied (Tyree and Ewers 1991). Deionized water was then replaced by 50 mM

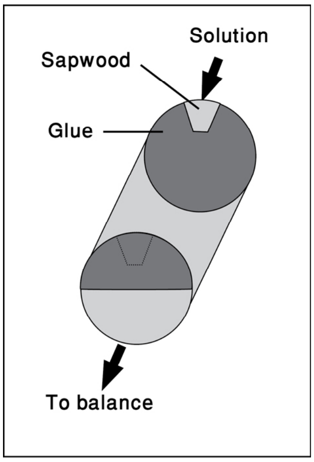

Figure 1. Schematic presentation of the method used to measure the hydraulic conductance in the stem xylem. The solution was pushed through 60° inlet in the proximal end and the flow rate from the distal outflow was measured.

NaCl and the positive pressure of 100 KPa was applied again. The rate of outflow was measured during every 2 min until a steady flow was observed. Finally, the 50 mM NaCl solution was replaced by a 100 mM NaCl solution and the measurements repeated.

1.3. Effect of sap salinity on the degree of lateral contacts

To check whether sap salinity is able to induce significant changes in the degree of intervessel connectivity of the stem xylem, the capacity of intervessel flow was compared between stem segments that flushed with deionized water and others that flushed with 100 mM NaCl. 7-cm-long segments were cut under water and their proximal ends were connected to a tubing system. Segments were then flushed with either deionized water or 100 mM NaCl solution for 15 min at 100 KPa pressure. After that, the degree of intervessel connectivity in each stem segment was measured by the 16-gauge needle method, as described by Halis et al. (Halis et al. 2013) (Figure 2). The piquant end of the metal needle was carefully eliminated. At the cut distal surface of segment, the cut needle was placed and fixed in a sapwood area (Figure 2) using the fast-setting, epoxy glue. The basal end was immersed in filtered (0.1 µm) toluidine blue O (1% w/v aqueous solution). A plastic tubing of suction pump was connected to the syringe needle, and by using a suction force of -100 KPa, the apoplastic dye was directly pulled, for 1 minute, through the open vessels that delimited by the syringe needle. To check for the distribution of dye in the xylem network, free-hand cross-sections were taken at 1 and 5 cm behind the distal cut end. Slides were then viewed under optical microscope, and the number of stained vessels was counted (Figure 2). The degree of intervessel connectivity was expressed as the number of stained vessels at 5 cm normalized by that observed at 1 cm from the outflow point.

1.4. Detection of new lateral pathways by double-staining method

Using two different apoplastic dyes, the pattern of intervessel connectivity within the sapwood was traced before and after flushing the stem segments with ionic solution. Stem segments of 7 cm length were cut under water, connected to plastic tubing and flushed with deionized water at 150 KPa for 15 min. 16-gauge needle was fixed with glue on the cut distal surface at a sapwood area (Figure 2). The proximal end was immersed in safranin-O dye (1% w/v aqueous solution). The plastic tubing of the suction pump was connected to the syringe needle and the suction force of -100 KPa was applied for 1 min. After that, the proximal end was transferred and immersed in 100 mM NaCl solution and the suction force was applied again for 10 min. Then, the proximal end was transferred and immersed in toluidine blue O (1% w/v aqueous solution) and the negative pressure was applied for 1 min. Finally, the tested segments were free-hand sectioned at 1 and 5 cm from the distal end, and the presence of the two dyes within the xylem network was checked microscopically. Motic Digital Microscope (DMB1-2MP, Motic Instruments Inc., Xiamen, China) was used to examine slides and to capture photomicrographic images.

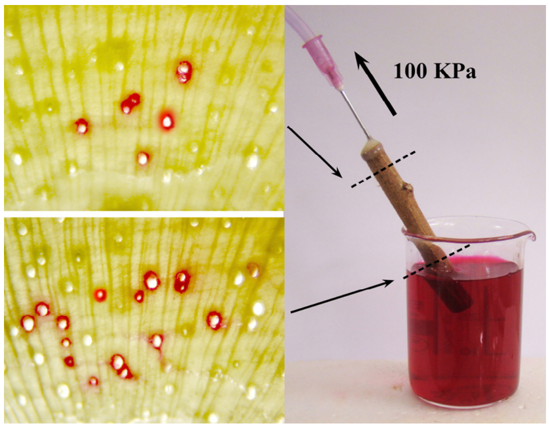

Figure 2. Showing how 16-gauge needle was used to study the lateral contacts between vessels. The needle was fixed, with epoxy glue, on the cut surface of the tested segment to delimit a small area of sapwood. Through the needle, a negative pressure could be applied to pull the dye solution through a small number of vessels. The degree of lateral contacts can be evaluated on the cross-sections.

1.5. Statistical analysis

All data were obtained at least in triplicate and presented as means ± standard deviation. The data were analyzed using SPSS software package (SPSS Ver. 15.0, SPSS Inc., Chicago, IL, USA). Different experimental groups were compared with the Student's t test. A probability level of $P \leq 0.05$ was considered to be statistically significant.

2. Results and Discussion

2.1. Hydraulic conductance

The effect of ion concentrations on hydraulic conductance within the stem segments are presented in Figure 3. In all cases, the hydraulic conductance increased significantly with salt solution in xylem. When changing from deionized water to salt solution, enhancement of the hydraulic conductance was 18.6±3.7% for 50 mM NaCl and 24.7±4.1% for 100 mM NaCl (Figure 3). Our results showed that the response of xylem hydraulic conductance to salinity strengths in the *Paraserianthes lophantha* follows the general pattern of ion-mediated increase in hydraulic conductivity that previously reported for other species (e.g. see Aasamaa and Sõber (2010) and references therein). Conductivity increased if stems were perfused with salt solution, while deionized water decreased substantially the conductivity (van Ieperen et al. 2000).

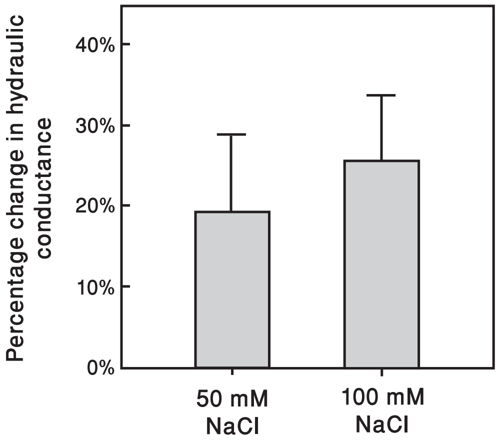

Figure 3. Percentile change in hydraulic conductance in response to changing the flowing solution from deionized water to 50 and 100 mM NaCl. Means are reported ± SD (n = 6).

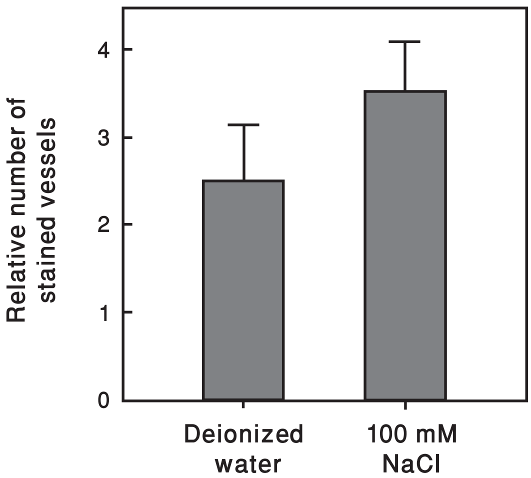

Figure 4. Effect of salt solution on the degree of intervessel connectivity (represented as the number of stained vessels at 5 cm normalized by that observed at 1 cm from the outflow surface). Values are means ± SD (n = 6 stem segments).

2.2. Degree of lateral contacts

The presence and extent of functional connections between sapwood vessels was investigated using 16-gauge needle that commonly used for blood donations. The dye solution was directly pulled through a small number of vessels that delimited by the syringe needle at the distal surface. Near the proximal surface, toluidine blue dye was observed not only in the selected vessels but also in many other neighboring vessels, which implied that the tension was transmitted via intervessel pitting from the selected vessels to others (see Figure 2). The number of stained vessels at the proximal surface relative to the number of vessels delimited by the syringe needle was considered as the degree of intervessel connectivity in the sapwood of our plant (Figure 2).

To determine the effect of low ionic strength on lateral continuity, stem segments were perfused with deionized water and their degree of intervessel connectivity was compared with segments that perfused with salt solution (100 mM NaCl). The results showed that salt solution slowly increased the degree of intervessel connectivity (Fig. 4; P=0.064, Student t-test) (Figure 4), which indicated that the presence of ions in the sap might elevate the number of functional contacts between vessels (Figure 4). This means that some intervessel contacts were closed or nonfunctional in the deionized water while in the salt solution these contacts became functional. Since the degree of intervessel connectivity is closely related to the lateral contacts between vessels through pit membranes, activation of some pit membranes could be the main cause of such increase in lateral intervessel connectivity (Burggraaf 1972; Fujii et al. 2010; Kitin et al. 2004; Kitin et al. 2009).

2.3. Double-staining experiment

The apoplastic dye (safranin-O) was sucked through a limited number of vessels with the help of 16-gauge needle. This dye moved within all vessels that were interconnected through intervessel pit pathways, and thus tracing the pathway of laterally interconnected vessels. When this pathway was perfused with salt solution for 10 min and stained again with toluidine blue O, we observed a difference in the course between the two dyes (Figure 5). Although the major of stained vessels appeared red-brown, some vessels were stained only by toluidine blue O and appeared blue-green in cross sections (Figure 5)). The blue-stained vessels were observed in nearly all samples examined in this experiment. The number of blue-stained vessels ranged from 1 to 5 (about 1 to 6 % of the total number of stained vessels). These vessels were randomly distributed within the vessel network (Figure 5). The presence of such blue-stained vessels indicated that some vessels were isolated from the suction force before treating with salt solution, but after the treatment, these vessels were subjected to the suction force since the dye solution moved only in the vessels that were under tension transmitted laterally via intervessel pit pathways.

Moreover, the double staining experiments provided another direct evidence for changes in the functionality of intervessel pits (Figure 5). In the first time, safranin-O dye moved freely within the network of laterally interconnected vessels. After treating this network with salt solution, the second dye (toluidine blue O) moved in the same pathway as safranin-O except for a few vessels that appeared with blue color. It was clearly that the pathways connecting those blue-stained vessels to others were completely closed or dysfunction, which prevented the transmission of tension needed for absorbing the safranin-O dye. Closing or dysfunction of pit membranes was the strong candidate, since intervessel pit membranes were the responsible for lateral intervessel connections (Zwieniecki et al. 2001; Nardini et al. 2007; Zwieniecki et al. 2003). Changes in the functionality of intervessel pit pathways could be a mechanism adopted in plants to regulate their degree of lateral intervessel connectivity. As the distribution of water and materials within woody plants largely depends on the pattern of lateral exchanges (Orians and Jones 2001; Zanne et al. 2010), regulation of intervessel connectivity through a mechanism of closing/opening pit pathways may provide plants with a large potential for fine regulation of water flow both within and between xylem sectors, and towards different plant organs.

Conclusion

In the present study, the effect of salt solution (ionic concentration) on the hydraulic characteristics of the stem xylem was investigated in *Paraserianthes lophantha*. Hydraulic and staining methods were used, and the results were consistent with the general pattern of ionic effect that demonstrated in the previous studies for other plant species. Moreover, our results suggested that the degree of lateral contacts between vessels could be changed due to the changes in the functionality of pit pathways. This phenomenon might be a mechanism used by plants for the adaptation to various ecological conditions. However, further researches on different species are needed to confirm the presence of such a mechanism in planta.

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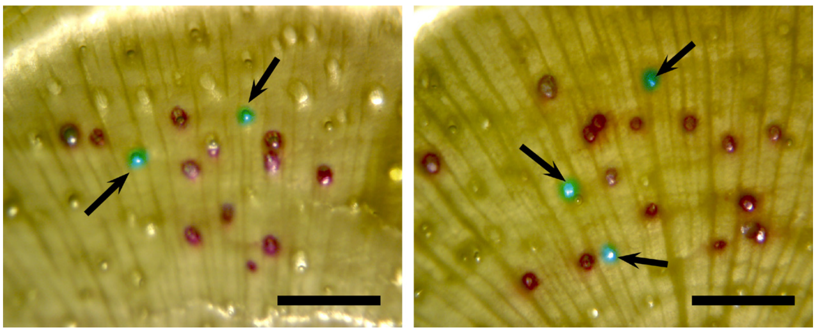

Figure 5. Double-staining method for detection of the variation in the lateral contacts in the vessel network. The panels show the cross sections at 5 cm from the suction point. The samples were treated with salt solution after the staining with safranin-O dye. The second dye (toluidine blue) was observed in new vessels, some vessels were stained only by toluidine blue. Scale bars = 200 µm.

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