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Changes of pH of solutions during perfusion through stem segments: further evidence for hydrogel regulation of xylem hydraulic properties?

A. GASCÓ, E. GORTAN, S. SALLEO and A. NARDINI*

Dipartimento di Biologia, Università di Trieste,Via L. Giorgieri 10, I-34127 Trieste, Italia

Abstract

Changes in hydraulic conductivity (K_h) and pH were measured in stem segments of laurel (*Laurus nobilis* L.) during perfusion with iso-osmotic solutions of KCl, NaCl and sucrose. Sucrose had no effect on K_h while 100 mM NaCl or KCl induced up to 22 and 35 % increase of K_h with respect to deionized water, respectively. Increases in K_h were accompanied by a sharp drop in pH from 6.0 (inlet solution) to 5.0 (outlet solution). The same effect was observed with both KCl and NaCl solutions but not in the case of sucrose. Also, similar changes of K_h and pH were observed for stems killed after immersion in hot water. Our results might provide further evidence for ion-mediated regulation of xylem hydraulic conductivity based on the hydrogel properties of pectins at the pit membrane level.

Additional key words: pit membranes, potassium, hydraulic conductivity, *Laurus nobilis* L.

Introduction

The solute composition of xylem sap is known to vary on both a diurnal and seasonal time scale (Schill *et al.* 1996, Siebrecht *et al.* 2003). Until recently, however, the ion concentration of xylem sap has been mostly studied in relation to the nutrient need of plants (*e.g.* Herdel *et al.* 2001) or to stomatal physiology (*e.g.* Gollan *et al.* 1992; Goodger *et al.* 2005) rather than to the xylem hydraulics per se. In the past, sharp drops in the hydraulic conductivity (K_h) of excised stems of conifers and angiosperms had been reported when water was used as perfusion fluid (*e.g.* Huber and Metz 1958). This effect was not attributed to water until Zimmermann (1978) found that dilute salt solutions injected into xylem prevented the drop in K_h caused by water. Other scientists became aware of the 'water effect' on K_h, especially during long-term hydraulic measurements lasting 6 h or more when continuous drop in K_h was observed until the stem seemed to be almost completely blocked. Acid solutions at pH between 2 and 3 were shown to prevent drop in K_h (Sperry *et al.* 1988, Tyree and Yang 1992) and interpreted as inhibiting microbial growth that had caused clogging of vessel-to-vessel pits. In most studies on plant hydraulics, either artificial pond water or 50 to 100 mM KCl have been used as perfusion solutions (*e.g.* Salleo *et al.* 1996, Brodribb *et al.* 2002) just ignoring the 'water problem'.

The influence of the ionic strength and composition of xylem fluids on xylem hydraulics was the objective of a study by Van Ieperen *et al.* (2000). The authors found that different salt solutions raised the K_h of *Chrysanthemum* stems by 5 to 8 % compared to deionized water. Solutes differing in cation or anion composition were tested showing that the hydraulic effect was due to the cation. By contrast, sugar solutions with similar osmotic potential and pH had no effect on K_h so that the final conclusion was that it was the presence of cations that determined the measured increase in K_h with respect to the K_h measured with water. Similar but more detailed experiments have been reported by Zwieniecki *et al.* (2001) on both excised stems of *Laurus nobilis* and single vascular bundles of *Nicotiana tabacum* which basically confirmed the results by Van Ieperen *et al.* (2000). The involvement of pit membranes in controlling xylem K_h was invoked as a possible explanation for the 'ionic effect'. Moreover, the hydrogel nature of pectins as major constituents of pit membranes was suggested to

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Abbreviation: K_h - hydraulic conductivity.

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* Corresponding author: fax (+39) 040 568855; e-mail: nardini@units.it

play a role in the observed increase in K_h. A consistent although still circumstantial evidence exists suggesting the interconduit pits as the control sites of the regulation of xylem hydraulic conductivity (Gascó *et al.* 2006, 2007).

Pectins are polysaccharides that can be found in a gel state when fully hydrated and in an amorphous solid state when dehydrated (Ryden *et al.* 2000). The degree of pectin hydration depends on the equilibrium between neutral carboxylic residues (due to methyl esterification and/or to their non-dissociated state) and negative charges exposed (Dähnert and Huster 1999). Cations can interfere with this equilibrium and cause some shrinking of pectins with consequent increase in the dimensions of pores in the pit membranes (Ryden *et al.* 2000, Willats *et al.* 2001). This, in turn, would increase the hydraulic conductance of stems.

All the above mentioned studies strongly suggest that changes in the ion concentration of xylem sap induce consistent changes in xylem hydraulics. It is still unknown, whether and to which extent solutions injected into the xylem can be changed during hydraulic measurements. This is an interesting point because eventual modifications *in itinere* of these solutions may provide some insight into the mechanism(s) underlying changes in the xylem hydraulic properties due to interactions of cations with the negatively charged pectin network (Willats *et al.* 2001).

In the present study, we report changes in hydraulic conductivity and pH of solutions during flow across laurel stems. In particular, the hypothesis was tested that the measured changes in the above mentioned variables are linked to changes of pit membrane porosity.

Materials and methods

All experiments were conducted between April and June 2005 on one-year-old twigs from 20-year-old plants of laurel (*Laurus nobilis* L.). Plants were growing in the Botanical Garden of the University of Trieste, Italy. Twigs about 50 cm long and 5 - 7 mm in diameter were cut off in the field under deionized water, transported to the laboratory and cut to the preferred length (12 cm) that was selected on the basis of vessel-length distribution measured using the silicone injection technique (Gascó *et al.* 2006). Segments 12 cm long had about 60 % conduits intact. Stem hydraulic conductivity (K_h) was measured using a *XYL'EM* apparatus (*Xylem Embolism Meter*, *Bronkhorst*, Montigny les Cormeilles, France). For technical details see Cochard *et al.* (2000).

The effect of iso-osmotic solutions of sucrose (200 mM), NaCl (100 mM) and KCl (100 mM) on K_h was measured. Solutions were filtered (0.1 μm) to prevent conduit clogging due to spurious particles and tested for pH was adjusted to 5.9 - 6.4, *i.e.* the same that measured for distilled water in the reservoir of the hydraulic apparatus. Samples were initially flushed with deionized water at a pressure (P) = 0.19 MPa for 10 min to remove embolism. K_h was measured as $(F/P) \times L$ where F is the measured flow rate, P is the pressure applied and L is the length of the stem segment. Flow rate was first measured at P = 9 kPa with deionized water and then with one of the other solutions. During measurements with deionized water, flow rates became stable within 10 min from the beginning of the experiment. All the measurements were corrected for viscosity and were performed at an air temperature of 20 ± 1 °C. Five measurements of K_h were made with each solution. During measurements, the pH of the inlet and outlet solutions was measured at 5 - 10 min intervals using a pH-meter (*Twin mod. B-213*, *Horiba Ltd.*, Kyoto, Japan). Samples of the solution flowing into stems were collected from the low pressure reservoir of the *XYL'EM* using a syringe. Outlet solutions were collected using plastic capsules fitted to the cut stem surface. To prevent contamination of inlet and outlet solutions with phloem fluids, 1 cm bark was removed at both ends of stem segments. Measurements of pH (see below) were made on 0.04 cm^3 samples collected using a micropipette.

To discriminate changes in pH during stem perfusion as due to vital xylem loading/unloading mediated by xylem-associated parenchyma cells (De Boer and Volkov 2003) from merely physico-chemical processes due to interactions with pectic polysaccharides of pit membranes, experiments were repeated on stem segments previously killed by immersing them in boiling water for 5 min. Killed stem segments were connected to the *XYL'EM* and K_h and pH were measured as described above. It has to be noted that pectins are relatively stable even when exposed to high temperatures (up to 100 °C) for 4 h (Thakur *et al.* 1997). Hence, it was unlikely that the boiling treatment induced significant modifications of pectins' structure in our samples.

Results

Three different solutions at iso-osmotic concentrations were compared for changes in K_h with respect to water (Fig. 1). No significant increase in K_h was measured during perfusion with sucrose solutions (ΔK_h was ranging between 0 ± 5 % compared to water). Both NaCl and KCl solutions, on the contrary, induced significant increase in K_h ranging between 22 ± 3 % for NaCl and 35 ± 4 % for KCl. It is of interest to note that the ionic effect of NaCl was significantly lower than that of KCl (by about 37 %). In other words, both cations modified K_h but the maximum effect was recorded with K^+.

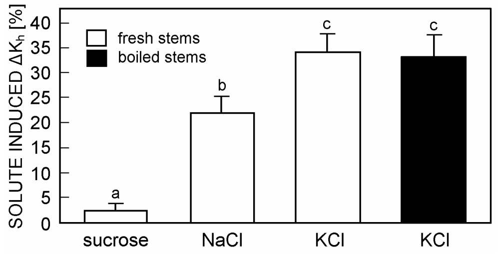

Fig. 1. Percentage increase in stem hydraulic conductivity (ΔK_h) as induced by iso-osmotic solutions (200 mM sucrose, 100 mM NaCl, 100 mM KCl) perfused through fresh or boiled stem segments 12 cm long compared to K_h measured with deionized water. Means ± SD ($n = 5$). Different letters indicate significant differences ($P < 0.05$) according to Tukey pairwise test.

The time course of changes in the pH measured during perfusion of stems 12 cm long with the three different iso-osmotic solutions was reported (Fig. 2). While pH of deionized water tended to become slightly more acidic (Fig. 2*A*) or remained approximately constant (Fig. 2*B*,*C*), the pH of the two KCl and NaCl solutions (Fig. 2*A*,*B*) became consistently more acidic while flowing through the samples. In the case of KCl, pH dropped from 6.0 (inlet) to 5.1 (outlet) in 15 min and further decreased to 4.8 after 30 more min. In the case of NaCl, pH decreased from about 5.9 (inlet) to about 4.9 (outlet) after about 20 min. On average, the pH of outlet saline solutions decreased by about one unity compared to that of the inlet solutions. No effect on pH was observed for the 200 mM sucrose solution (Fig. 2*C*).

Samples killed and then perfused with deionized water or with 100 mM KCl showed an effect of the cation on K_h very similar to that observed in vital stems (Fig. 1). K_h measured in killed samples injected with KCl, in fact, increased with the time up to about 33 % more than that measured with water. Changes in pH during perfusion of killed stems with KCl (Fig. 3) were similar to those measured in vital stems (pH decreased from 6.3 to 5.8). The amplitude of this pH change was, however, about one half of that measured in fresh stems.

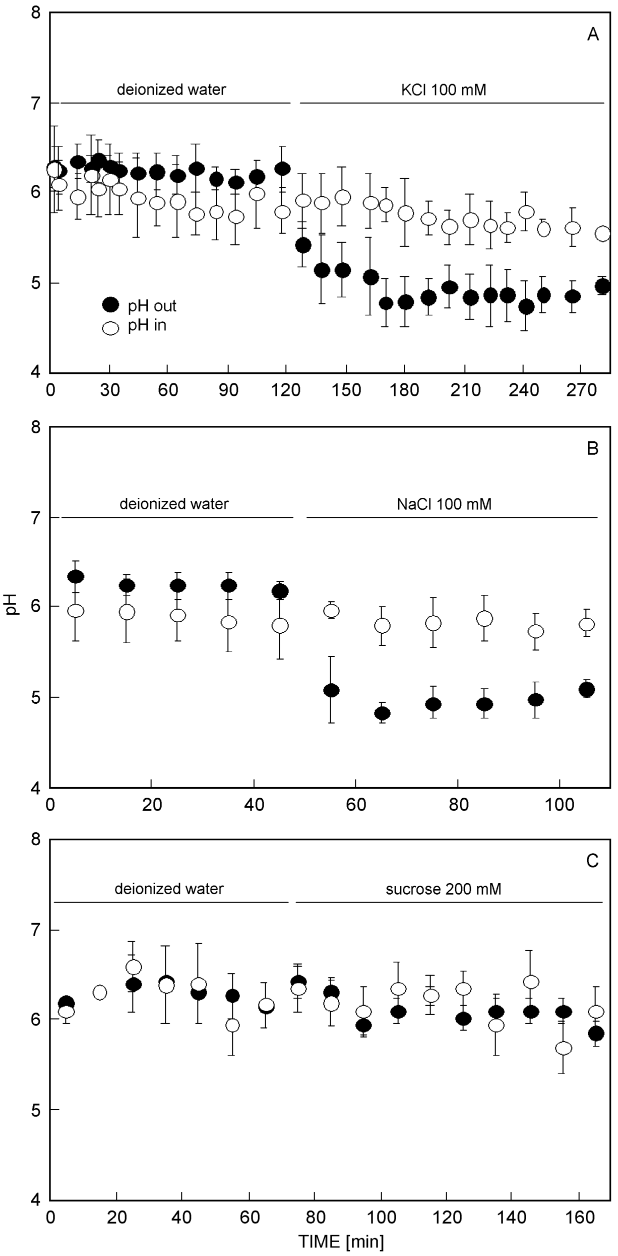

Fig. 2. Time course of changes in pH of outlet and inlet solutions during perfusion of stem segments (12 cm long) with deionized water, 100 mM KCl, 100 mM NaCl or 200 mM sucrose. Means ± SD ($n = 5$).

Discussion

The aim of the present work was to check eventual changes in pH of solutions during perfusion through stems leading to enhancement of stem K_h. Our data confirm the importance of cations (K^+ and Na^+ in the present study) for the hydraulic effect of saline solutions.

The recorded acidification of the outlet solutions is consistent with H^+ substituted for cations by non-dissociated carboxylic groups of pectic chains (Gascò *et al.* 2006). Some slight acidification due to CO_2 produced by the surrounding tissues could contribute to the acidification effect although outlet sucrose solutions (Fig. 2*C*) showed no detectable decrease in pH compared to the inlet ones. It has to be noted that after saturation of pectins with K^+ or Na^+ no further exchange with H^+ would be expected and, hence, equilibration of pH values of the inlet and outlet solutions but this was not the case. We have no explanation for this phenomenon which clearly deserves more studies. We can only speculate that other phenomenon, like electro-osmosis, might contribute to the acidification of perfused solution while crossing the samples.

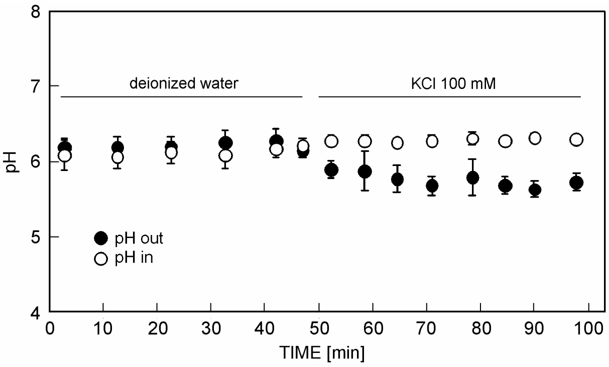

Fig. 3. Time course of changes in pH of outlet and inlet solutions during perfusion of stems (12 cm long) previously killed by immersion in hot water for 3 min with deionized water or 100 mM KCl. Means ± SD (*n* = 5).

The effect of Na^+ on K_h was significantly less than that of K^+ (22 % increase in K_h for 100 mM NaCl versus 35 % for iso-osmotic KCl solution, Fig. 1). Zwieniecki *et al.* (2001) have reported similar results during perfusion with 10 mM NaCl compared to KCl while no difference in this regard has been reported by Van Ieperen *et al.* (2000). Differences between these two solutions are difficult to explain on the basis of pectin chemistry in that Na^+ and K^+ have been reported to induce similar protonation degree of the exchange sites at similar experimental pK_a in polygalacturonanes of *Nitella* cell walls (Gillet *et al.* 1998) and to have similar fractions of binding sites at the same pH of the external solution. Quantitatively different effect of Na^+ versus K^+ might be related to different ion mass and mobility but our data do not provide any explanation for this phenomenon.

Pit membranes have classically been seen as water pathways for water flow from vessel to vessel (Fahn 1990) and only recently their functional role has been hypothesized to be more ample than thought (Zwieniecki *et al.* 2001). Our data corroborate the hypothesis that pits are effective control sites of regulation of the hydraulic conductivity of the xylem system through modification of pit membrane pectin neutrality. On the other hand, the possibility that other phenomena, like interactions of ions with electric charges exposed throughout xylem cell walls or some wound response are involved in the 'ionic effect', cannot be ruled out on the basis of available data.

The simultaneous changes in pH with the K_h increase in laurel stems points to a possible substitution of H^+ for K^+ or Na^+ and provides, in our opinion, further circumstantial evidence for the influence of these counterions on xylem hydraulics. In particular, K^+ is the most abundant ion in xylem sap (Siebrecht *et al.* 2003) so that regulation of xylem flow through changes in K^+ concentration seems to be reasonable. The effect of KCl on K_h of killed stems was very similar to that observed for vital stems from both a quantitative and a temporal point of view. This points to a physico-chemical basis for the ionic effect on K_h, however, ion secretion into xylem sap by xylem associated cells is strictly under metabolic and hormonal control (Tyree *et al.* 1999, De Boer and Volkov 2003, Salleo *et al.* 2004).

In conclusion, the ability of plants to regulate the hydraulic efficiency of their organs through ion exchange between living and dead tissues opens new insights into the regulation of plant water balance.

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