Hydrogel Control of Xylem Hydraulic Resistance in Plants

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Increasing concentrations of ions flowing through the xylem of plants produce rapid, substantial, and reversible decreases in hydraulic resistance. Changes in hydraulic resistance in response to solution ion concentration, pH, and nonpolar solvents are consistent with this process being mediated by hydrogels. The effect is localized to intervessel bordered pits, suggesting that microchannels in the pit membranes are altered by the swelling and deswelling of pectins, which are known hydrogels. The existence of an ion-mediated response breaks the long-held paradigm of the xylem as a system of inert pipes and suggests a mechanism by which plants may regulate their internal flow regime.

Xylem vessels, composed of dead cells, are well known for their passive role in water transport (*1*–*3*), and the possibility that these tubes might be capable of rapid flow control has been overlooked. The prevailing view among plant biologists is that xylem conduits have two hydraulic states: They either transport water at a constant resistance or are blocked by embolism (*2*, *4*). However, reports of short-term changes in xylem resistance (*5*, *6*) question this view. Over two decades ago, it was first observed that the hydraulic resistance of perfused stem segments was significantly decreased when deionized water was replaced by tap water (*7*). This finding has been recently confirmed (*8*). Initially, the effect of ions on xylem hydraulic resistance was seen as a methodological problem of limited biological importance (*9*). However, this phenomenon might substantially contribute to the regulation of water flow through plants. For example, an ion-dependent mechanism for altering the hydraulic resistance of the xylem could allow plants to compensate for increases in resistance due to cavitation.

We examined the effect of ion concentration

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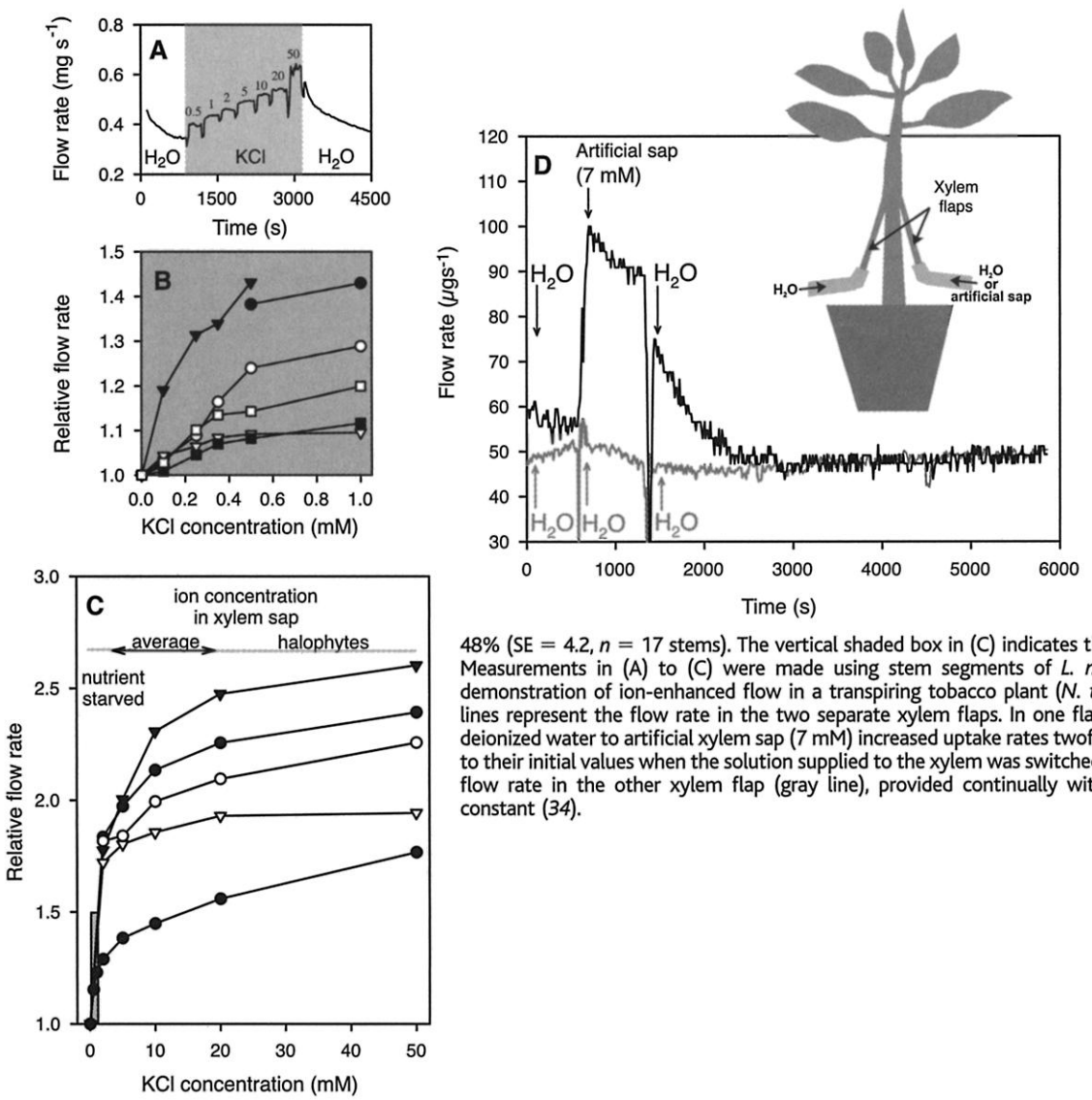

Fig. 1. Effect of KCl concentration on flow rate through xylem. (**A**) Time course of typical experiment showing a slow decline in flow rates with deionized water and increasing flow rates with KCl. Numbers above the curves indicate KCl concentration (mM). Sharp drops in flow rate between consecutive treatments reflect temporary pressure drops associated with switching between solutions. (**B** and **C**) Relative flow rate (flow rate with indicated solution/flow rate with deionized water) as a function of KCl concentration (0 to 1 mM and 0 to 50 mM, respectively). Symbols represent different stem segments. The average increase in flow rate in response to 10 mM KCl was 48% (SE = 4.2, n = 17 stems). The vertical shaded box in (C) indicates the concentration range of (B). Measurements in (A) to (C) were made using stem segments of *L. nobilis* (*33*). (**D**) Experimental demonstration of ion-enhanced flow in a transpiring tobacco plant (*N. tabacum*). The black and gray lines represent the flow rate in the two separate xylem flaps. In one flap (black line), switching from deionized water to artificial xylem sap (7 mM) increased uptake rates twofold, and uptake rates returned to their initial values when the solution supplied to the xylem was switched back to deionized water. The flow rate in the other xylem flap (gray line), provided continually with deionized water, remained constant (*34*).

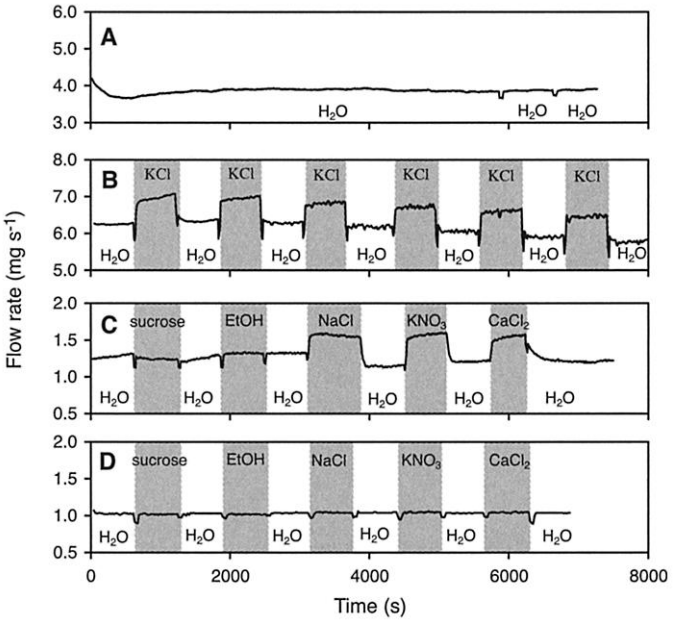

Fig. 2. Effect of solution composition on flow enhancement. Measurements were repeated with qualitatively consistent results for five different *L. nobilis* stem segments; a representative set of measurements is shown here (*33*). (**A**) Sustained perfusion of deionized water. Switching between deionized water containers does not alter the flow rate. (**B**) Effect of repeated changes between deionized water and KCl solution (10 mM). (**C**) Effects of solution composition (10 mM) on flow rate through stem segments. EtOH, ethanol. (**D**) Effects of solution composition (10 mM) on flow rate through a 74-cm-long plastic capillary tube (polyetheretherketone, 64 μm in diameter).

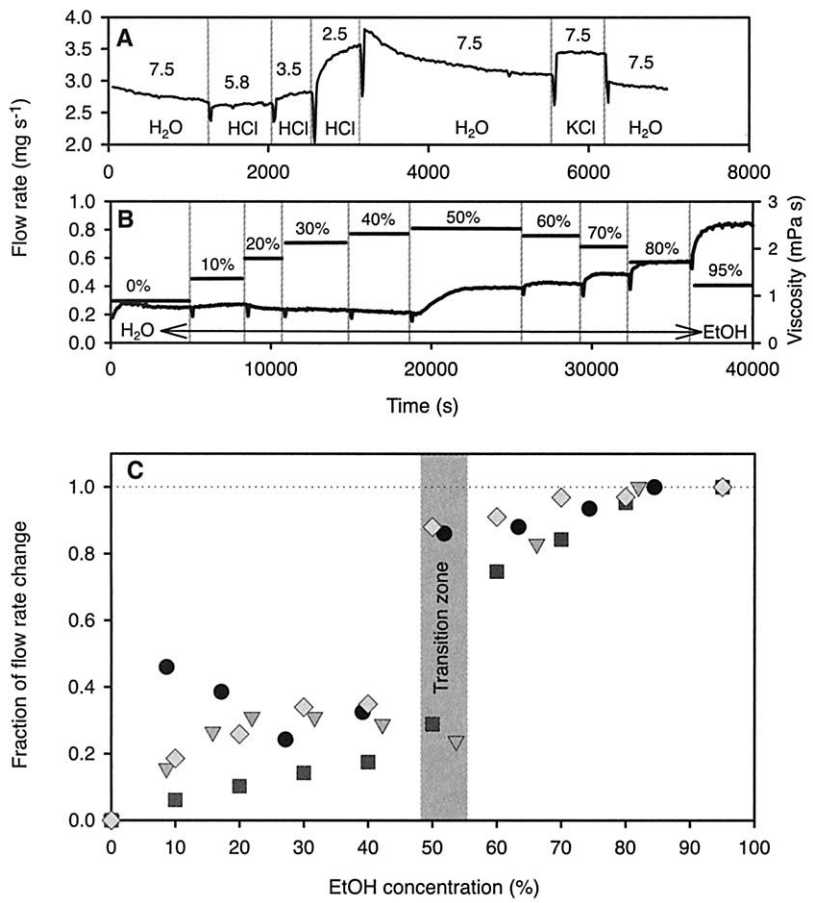

Fig. 3. Demonstration that hydrogels are responsible for ion-mediated flow enhancement in stems of *L. nobilis*. (**A**) Effect of pH on flow rate. Numbers above the curves indicate the solution pH. (**B**) Effect of nonpolar solvent on flow rate through stem segments maintained at 25°C. Percentages indicate the % ethanol concentration of the perfusing solution. Horizontal bars indicate solution viscosity. (**C**) Relative change in flow rate as a function of ethanol concentration. Flow rates were adjusted for viscosity (η) by multiplying flow rates by $\eta_{solution}/\eta_{water}$ (*35*). Symbols represent different stem segments. Measurements were scaled so that the maximum difference in flow rate = 1. In all cases, there was a marked increase in flow rate at ~50% ethanol.

on xylem hydraulic resistance by perfusing branch segments of *Laurus nobilis* with aqueous KCl solutions. Flow rates increased by up to 2.5 times as the KCl concentration was increased from 0 to 50 mM (Fig. 1, A and C). When the solution was replaced with deionized water, the flow rate declined to its initial value. The effect was most pronounced at low concentrations (0 to 10 mM), a range that encompasses the total ionic strength found in the xylem of transpiring plants (*10*–*12*). Because the driving force for water flow was held constant, changes in flow rate reflect changes in the hydraulic resistance of the xylem. We confirmed that this process operates in vivo by monitoring uptake into the partially split stem of a transpiring tobacco plant (Fig. 1D). Uptake increased twofold when one side of the stem was supplied with an artificial xylem sap solution (7 mM) and subsequently decreased to the initial rate when deionized water was resupplied. In contrast, uptake by the side that was continually supplied with deionized water remained constant throughout the experiment.

The increase in flow rate in response to KCl (10 mM) was both reversible and repeatable (Fig. 2, A and B). Perfusing stems with other dissociating solutes (NaCl, KNO_3, and $CaCl_2$) resulted in similar flow enhancements, although the time required to reverse the effect using deionized water depended on the identity of the solute; it was particularly long in the case of $CaCl_2$ (Fig. 2C). In contrast, flow rates were not affected by the same concentrations of nondisassociating solutes (sucrose and ethanol). Substituting a piece of plastic capillary tubing for the stem segment eliminated any effect of ion concentration on flow rate, indicating that the effect was not due simply to switching between solutions (Fig. 2D). Ion-mediated flow enhancement was not affected by flushing stems with boiling water for several hours or by freezing stems in liquid nitrogen before excision. This demonstrates that living cells do not cause the observed changes in xylem resistance, nor do they arise from a post-harvest wound response. We observed ion-mediated flow enhancement in all the species we examined (19 angiosperms, 5 conifers, and 3 ferns), indicating that this process is widespread among vascular plants. The enhancement ranged from <1.1 times in conifers to as high as 2.5 times in some angiosperm species.

The dynamics of ion-mediated flow enhancement are similar to those observed in the swelling and deswelling of hydrogels (*13*, *14*), which suggests that the observed changes in xylem resistance might be regulated in a similar manner. We tested whether hydrogels could be responsible for actuating ion-mediated changes in hydraulic resistance by examining the response to solution pH (which alters the effective ionization of the polymer network) and to polarity (which alters poly-

mer-polymer affinity). There was no significant effect of pH within the natural pH range of xylem sap (5.8 to 8.0) (*12*); however, low pH (2.5) led to markedly increased flow rates (Fig. 3A). The addition of ethanol to the perfusing solution had a small effect (other than through viscosity) until the concentration reached ~50%, at which point there was a marked decrease in xylem hydraulic resistance (Fig. 3, B and C). This is consistent with a hydrogel phase transition resulting from a change in polymer-polymer affinity as a result of the decreased solvent polarity (*14*).

Measurements of flow through individual xylem vessels (*15*) demonstrate that the effect of ions on xylem resistance occurs only in vessel-to-vessel connections (Fig. 4). Flow through a single vessel of *Fraxinus americana* was unaffected by the ionic concentration of the perfusing solution (Fig. 4E). In contrast, flow between adjacent vessels showed a strong response to 100 mM KCl (Fig. 4F). As water flows between vessels, it must cross a modified region of the primary cell wall known as the pit membrane (*16*). Water moves across this barrier through microchannels in the mesh of cellulose microfibrils, hemicelluloses, and pectins (*16*, *17*). Small changes in the radii of these microchannels will result in marked changes in hydraulic resistance. Among the cell wall constituents, pectins are known for their gel-like properties (*17*–*19*) and their shrinking and swelling behavior is used in drug delivery systems (*20*). The most abundant pectic residue, D-galacturonic acid, has a pK_a (where K_a is the acid constant) of 3.23 (*19*), which is consistent with the observed step change in flow rate in response to pH (Fig. 3A). We suggest that shrinkage of the pectin matrix in response to ions increases the size of the microchannels in the pit membrane, decreasing their resistance to water flow (Fig. 4G).

Xylem resistance is an important determinant of water supply to leaves and thus can constrain rates of photosynthesis and growth (*21*–*25*). Because of the existence of negative pressures (*26*–*28*), water transport in the xylem is susceptible to cavitation (*29*). The ability to locally increase transport capacity via ion-mediated effects may contribute to whole plant function by compensating for increases in xylem resistance due to embolism. Ion-mediated control of xylem resistance is likely to involve the loading of K^+ ions into the xylem (*30*). The concentration of K^+ may increase markedly with transpiration rate (*10*), and it is strongly influenced by recycling from the phloem (*31*). An important role for ions in regulating xylem hydraulic properties improves our understanding of long-distance transport processes by linking nutrient status, water transport, and phloem activity. Hydrogels are increasingly used in engineering and medical applications (*13*, *32*). The effect of ions on xylem hydraulic resistance in plants describes a role for hydrogels in natural systems.

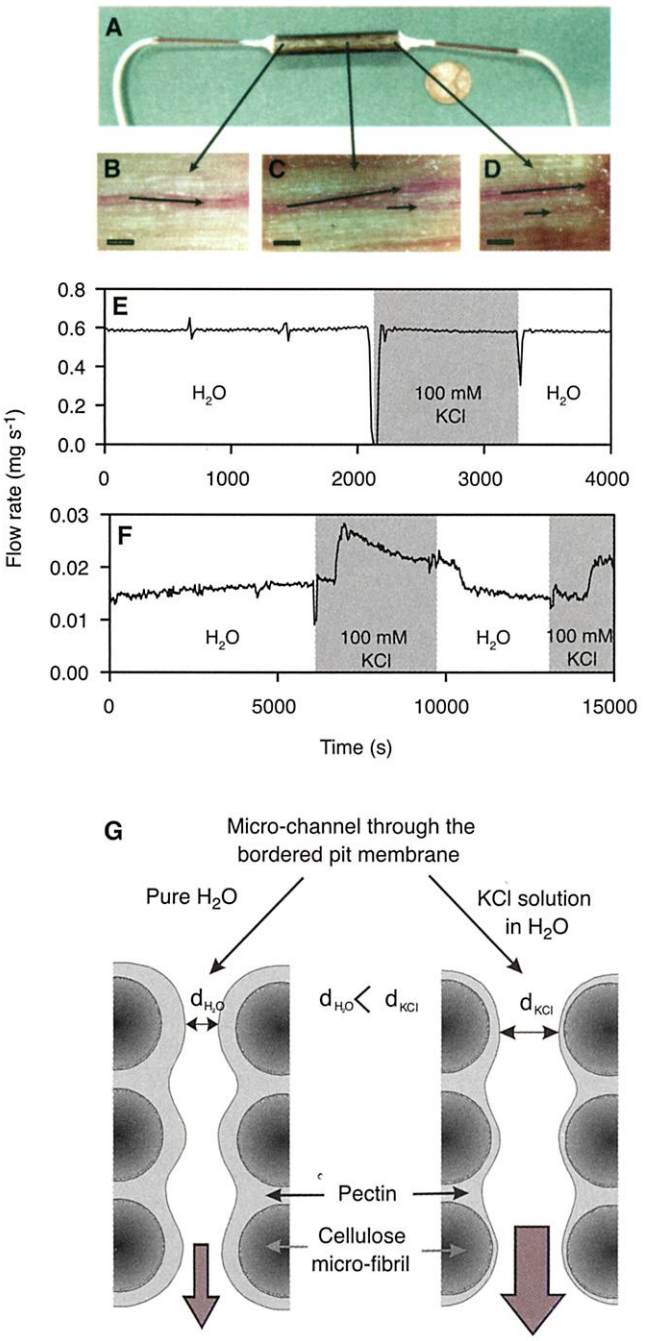

Fig. 4. Localization of ion effect on xylem hydraulic resistance to bordered pit membranes. (**A**) Stem segment of *F. americana*, with microcapillaries inserted into individual xylem vessels at either end (*15*, *36*). (**B** to **D**) Safranin dye perfusion shows that water enters the segment in a single vessel. This vessel is connected to a second vessel (C) that subsequently diverges (D). The long arrow indicates the open vessel; the short arrow indicates the diverging vessel. Scale bar, 100 μm. (**E**) The flow rate through the open vessel was not affected by KCl. (**F**) The flow rate through the diverging vessel, which requires water to flow across bordered pit membranes, increased with 100 mM KCl. The time lag between switching solutions and flow response corresponds to the time needed to flush the previous solution from the microcapillary. (**G**) Diagram showing how the deswelling of pectins may dramatically decrease hydraulic resistance by increasing the diameter of microchannels in the pit membrane.

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33. Stem segments (3 to 6 cm long and 2 to 4 mm in diameter) were cut under water and attached at one end to a manifold that permitted rapid switching between solutions of different compositions and ionic strengths. Stem segments were perfused at a constant delivery pressure (0.04 MPa) and the flow rate was measured by directing the outflow onto a balance. A long water-filled tube between the stem segment and the balance prevented changes in solution density from confounding measurements of volume flow rates. Before these measurements, stem segments were flushed with deionized water for 10 min at a delivery pressure of 0.2 MPa to remove emboli. Solutions were prepared using deionized water (pH 7.5). The pH of KCl solutions was adjusted to 7.5 using KOH; the pH of all other salt solutions varied between 5.7 and 6.5; HCl was used in pH experiments.
34. Xylem feeding experiments were made using 8-week-old tobacco plants (*Nicotiana tabacum*) growing in 2-liter pots in a greenhouse. Plants were placed in a growth chamber (1500 μM photosynthetically active radiation m^{-2} s^{-1} at 28°C with 380 parts per million CO_2 and 80% relative humidity) with the pot and ~20 cm of the stem protruding below the chamber. Two longitudinal flaps (~5 cm long by 3 mm wide) were cut in the xylem near the base of the stem (Fig. 1D). These flaps were connected to reservoirs located on two analytic balances so that uptake rates could be continuously monitored. Artificial sap consisted of potassium (189 μg ml^{-1}), calcium (89 μg ml^{-1}), phosphorus (50 μg ml^{-1}), chloride (10 μg ml^{-1}), sulfur (157 μg ml^{-1}), ammonium (4 μg ml^{-1}), nitrate (13 μg ml^{-1}), and magnesium (40 μg ml^{-1}), plus trace amounts of micronutrients (at a total concentration of ~7 mM) (*13*).
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36. Single-vessel measurements were made on short (~4-cm-long) segments of *F. americana* (Fig. 4, A to D). A glass microcapillary was inserted into a single vessel at one end, and safranin dye was pushed into the stem. Only segments in which the dye exited the segment from two separate vessels were used. Air was then pushed through the microcapillary into the stem segment to determine which of the two pathways was continuous. A second microcapillary was then glued to the other end of the open vessel, and the response to KCl was measured. This microcapillary was then removed and a final microcapillary was inserted into the other stained vessel, and the response to KCl was also measured for this pathway.
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