

# Water transport in sunflower root systems: effects of ABA, $\text{Ca}^{2+}$ status and $\text{HgCl}_2$

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## Abstract

**Excised 20-d-old sunflower roots (*Helianthus annuus* L. cv. Sun-Gro 380) with different  $\text{Ca}^{2+}$  status were used to study the effects of root  $\text{Ca}^{2+}$  status and abscisic acid (ABA) on the exudation rate ( $J_v$ ), the hydraulic conductivity of the root ( $L_{p_r}$ ), the flux of exuded  $\text{Ca}^{2+}$  ( $J_{\text{Ca}}$ ), and the gradient of osmotic pressure between the xylem and the external medium.  $J_v$  and  $L_{p_r}$  increased in direct proportion to the  $\text{Ca}^{2+}$  status of the root. Addition of ABA (4  $\mu\text{M}$ ) at the onset of exudation in the external medium made  $J_v$  and  $L_{p_r}$  rise, and this effect also increased with the  $\text{Ca}^{2+}$  status. The effects of  $\text{HgCl}_2$  and its interaction with ABA on water transport in the root were also studied. Addition of  $\text{HgCl}_2$  (1  $\mu\text{M}$ ) 2 h after the onset of exudation in the external medium quickly inhibited  $J_v$ , independently of the presence of ABA in the root medium. The results recorded here point to the involvement of ABA and  $\text{Ca}^{2+}$  in the regulation of root water flow, as well as the existence of aquaporins in the cell membranes of sunflower roots.**

**Key words:** ABA, aquaporins, calcium, exudation rate, *Helianthus annuus*, hydraulic conductivity, sunflower.

## Introduction

Radial transport of water in the root occurs simultaneously via two parallel routes: cell to cell (including symplastic and transcellular pathways) and apoplast (Steudle and Frensch, 1996; Steudle and Peterson, 1998). The predominance of one route over the other depends on species, extent of root development and whether water absorption is determined by a hydrostatic or an osmotic gradient (Steudle and Frensch, 1996; Steudle and

Peterson, 1998). When osmotic gradients are present, the water flow is negligible in the apoplast (Quintero *et al.*, 1998; Steudle and Peterson, 1998) and transport occurs mainly from cell to cell (Steudle and Frensch, 1989; Steudle, 1994). In recent years, it has been shown that membrane-integral proteins, or aquaporins, act as water-specific channels (Chrispeels and Maurel, 1994; Maggio and Joly, 1995). The presence of aquaporins in roots (Yamamoto *et al.*, 1991; Niemietz and Tyerman, 1997) suggests that radial flow of water could be mainly transcellular (Chrispeels and Maurel, 1994; Maggio and Joly, 1995) and may be controlled by the opening or closing of these water channels (Chrispeels and Maurel, 1994; Steudle and Henzler, 1995).

The existence of a regulatory mechanism which controls radial transport of water in the root has yet to be determined (Quintero *et al.*, 1998). It is known that roots offer the greatest resistance to water flow (Weatherley, 1982), and that hydraulic conductivity of the root ( $L_{p_r}$ ) may be affected by diverse forms of abiotic stress, such as salinity (Munns and Passioura, 1984; Joly, 1989), anaerobiosis (Zhang and Tyerman, 1991), drought (North and Nobel, 1991) or nutritional stress (Radin and Matthews, 1989; Radin, 1990; Quintero *et al.*, 1998).

Among the endogenous factors related to water flow in plants, the role of abscisic acid (ABA) has received most attention. The role of ABA in the regulation of root water flow has yet to be firmly established, as experimental results have often been contradictory (Glinka, 1980; Fiscus, 1981; Markhart, 1984; BassiriRad and Radin, 1992). In isolated sunflower roots, ABA appears to promote exudation (Glinka, 1980; Fournier *et al.*, 1987; Quintero *et al.*, 1998), due to its effect on both the hydraulic conductivity of the root (Glinka, 1980; Quintero *et al.*, 1998) and the release of ions into the xylem vessels (Glinka, 1980). In contrast, in bean root systems, using

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a pressure chamber to increase water flux through the roots, no short-term effect of ABA was found on their hydraulic conductivity; and in the long-term, a decrease was even observed (Fiscus, 1981).

More is known about the role of ABA in regulating water flow in the shoot. ABA is synthesized in roots in response to water stress conditions, producing various effects in the shoot. One such effect is the opening of outward  $K^+$  channels, the loss of turgor and closing of stomata (Davies and Zhang, 1991; Chandler and Robertson, 1994). It has recently been reported that ABA induces an increase in the concentration of cytosolic  $Ca^{2+}$  in guard cells (Gilroy *et al.*, 1991; McAinsh *et al.*, 1990, 1992), preceding the closing of stomata (McAinsh *et al.*, 1990). The concentration of cytosolic  $Ca^{2+}$  in guard cells is essential for controlling the opening of the stomata (MacRobbie, 1997; McAinsh *et al.*, 1997), either through its effect on various plasma membrane  $K^+$  channels (Schroeder and Hagiwara, 1989) and the tonoplast (Ward and Schroeder, 1994) of guard cells, or on the ATPase of  $H^+$  in the plasma membrane of these cells (Kinoshita *et al.*, 1995).

Less is known about the effect of  $Ca^{2+}$  on water transport in the root system than in the shoot. It has been reported that salinity decreases hydraulic conductivity in maize roots, and that the addition of extra calcium to the salinized media causes ameliorative effects on hydraulic conductivity (Azaizeh and Steudle, 1991; Azaizeh *et al.*, 1992).

The object of the present work was mainly to study the effect of ABA and  $Ca^{2+}$  status on the water flow in sunflower root systems. The aim was to know whether ABA and  $Ca^{2+}$  are involved in regulating water transport across the root.

## Materials and methods

### Plant material and growth conditions

Sunflower seeds (*Helianthus annuus* L. cv. Sun-Gro 380, Eurosemillas S.A., Córdoba, Spain) were surface-sterilized in 0.5% (v/v) sodium hypochlorite for 1 min, and germinated in the dark for 4 d at 28 °C in perlite moistened with 5 mM  $CaCl_2$ . On the fourth day, the seedlings were put in a plant growth chamber with a relative humidity between 60% and 80%, a day/night temperature of 22/18 °C, a photoperiod of 14 h of light and a photosynthetic photon flux density of 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (fluorescent tubes, Sylvania cool-white VHO). The next day, the 5-d-old seedlings were transferred individually to glass flasks wrapped in aluminium foil. The flasks contained 790 ml of a standard nutrient solution with the following composition: 2.5 mM KCl, 2.5 mM  $Ca(NO_3)_2$ , 1.0 mM  $MgSO_4$ , 0.25 mM  $Ca(H_2PO_4)_2$ , 12.5  $\mu\text{M}$   $H_3BO_3$ , 1.0  $\mu\text{M}$   $MnSO_4$ , 1.0  $\mu\text{M}$   $ZnSO_4$ , 0.25  $\mu\text{M}$   $CuSO_4$ , 0.2  $\mu\text{M}$   $(NH_4)_6Mo_7O_{24}$ , and 10  $\mu\text{M}$  Fe-ethylenediamine-di-*o*-hydroxyphenylacetic acid.

In order to obtain plants with different  $Ca^{2+}$  levels, the plants were transferred (without rinsing their roots) 24 h before the exudation assay to a new nutrient solution with either

2.5 mM  $CaCl_2$  (control plants) or without calcium (low- $Ca^{2+}$  plants). The basic composition of this new solution was: 2.5 mM  $KNO_3$ , 2.5 mM  $NaNO_3$ , 0.5 mM  $KH_2PO_4$ , 1.0 mM  $MgSO_4$ , 12.5  $\mu\text{M}$   $H_3BO_3$ , 1.0  $\mu\text{M}$   $MnSO_4$ , 1.0  $\mu\text{M}$   $ZnSO_4$ , 0.25  $\mu\text{M}$   $CuSO_4$ , 0.2  $\mu\text{M}$   $(NH_4)_6Mo_7O_{24}$ , and 10  $\mu\text{M}$  Fe-ethylenediamine-di-*o*-hydroxyphenylacetic acid. In the experiments with  $HgCl_2$ , the plants were continuously kept in standard nutrient solution. In all cases, NaOH was used to adjust the pH of the nutrient solution to 5.5. In all assays, four plants were grown for each treatment.

The plants were grown in the growth chamber for 15 d. The nutrient solutions were continuously aerated using an air pump. The volume was adjusted daily to 790 ml. The nutrient solutions were renewed at day 7 and, in experiments with  $HgCl_2$ , also the day before the exudation assays.

### Exudate collection

The exudation experiments were performed with 20-d-old plants and were started 30 min after switching on the lights of the growth chamber. In the same flasks in which the plants had grown, the nutrient solution was changed for a new solution according to the type of assay. Abscisic acid (4  $\mu\text{M}$ , mixed isomers, Sigma) and  $HgCl_2$  (1  $\mu\text{M}$ ) were added to this new solution: ABA at the onset of exudation and  $HgCl_2$  2 h after the onset of exudation. Immediately after the change of solution, the plants were detopped 1 cm above the transition zone, and pieces of tightly fitting latex tubing were affixed to the cut stumps. The exudate from the xylem vessels was collected in test tubes during 3, 4 or 6 h according to the type of assay; during this period, the external medium of the root was continuously aerated using an air pump, and kept at 25 °C. The volume of exudate collected was determined by measuring the difference of weight of the test tube before and after the collection period. The exudates were individually frozen and stored at -20 °C. Afterwards, the roots were individually washed for 5 min in 150 ml of a cold 5 mM  $CaSO_4$  solution (5 °C) to allow exchange of the cell walls contents. Finally, the roots were also weighed, frozen and stored at -20 °C.

### Other analyses

The osmotic pressure of the exudates and of the external medium was determined by means of a thermocouple psychrometer (Decagon Devices, Inc., Pullman, WA, USA). The values of hydraulic conductivity of the roots ( $Lp_r$ ) were calculated using:

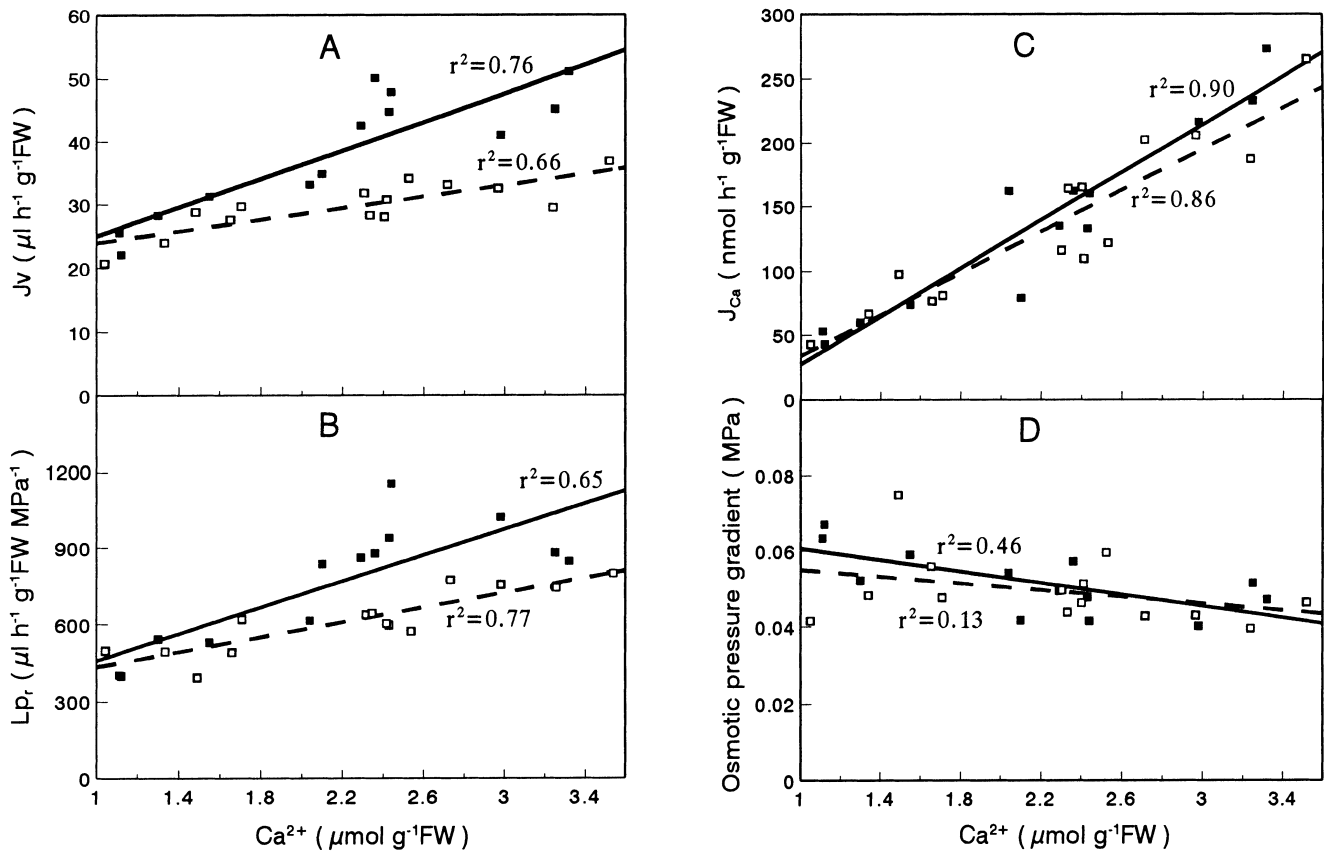
$$J_v = \sigma_{sr} Lp_r (\pi_x - \pi_o) \quad (1)$$

where  $J_v$  is the exudation rate;  $\pi_x$  and  $\pi_o$  are the osmotic pressures of the exudate and the external medium, respectively; and  $\sigma_{sr}$  is the overall reflection coefficient of the root, the value of which is assumed to be unity (BassiriRad *et al.*, 1991).

$K^+$  and  $Ca^{2+}$  were determined by atomic absorption spectrophotometry (Perkin Elmer 1100 B), either directly in the exudate or after extraction from the roots or shoots with a 10% acetic acid solution (Benlloch *et al.*, 1989).

### Experimental design and statistical analysis

A random experimental design was utilized in all cases. Linear regressions were established between the  $Ca^{2+}$  status of the root and every one of the hydraulic parameters (exudation rate, hydraulic conductivity and osmotic pressure gradient), and also with the  $Ca^{2+}$  flux in the xylem sap. In all cases, the analysis of linear regression was significant ( $P < 0.01$ ).



**Fig. 1.** Effect of the  $\text{Ca}^{2+}$  status of the root and of ABA in the root medium on the exudation rate (A), the hydraulic conductivity of the root (B), the  $\text{Ca}^{2+}$  flux of the xylem sap (C), and the osmotic pressure gradient between the xylem sap and the medium (D). Roots were exuding for 6 h in a solution containing  $\text{CaSO}_4$  (0.1 mM), glucose (10 mM) and with (■) or without (□) ABA (4  $\mu\text{M}$ ). In all cases, the analysis of linear regression was significant ( $P < 0.01$ ).

## Results

Although calcium starvation for 24 h neither modified plant growth (data not shown) nor altered the nutritive  $\text{K}^+$  status (Table 1), it reduced internal  $\text{Ca}^{2+}$  content, which was more marked in the root (Table 1). The  $\text{Ca}^{2+}$  level in the root affected the exudation process of isolated roots exhibiting root pressure (Table 2). In roots with low calcium levels, the exudation rate was inhibited ( $J_v$ ) and the stimulating effect of ABA on exudation disappeared (Table 2).

The  $\text{Ca}^{2+}$  status of the root was correlated with various

hydraulic parameters and with the  $\text{Ca}^{2+}$  flux in the xylem sap; results showed that as the  $\text{Ca}^{2+}$  level increased, the exudation rate ( $J_v$ ) also rose (Fig. 1A). Similarly, the hydraulic conductivity of the root ( $Lp_r$ ) and the flux of exuded  $\text{Ca}^{2+}$  ( $J_{\text{Ca}}$ ) rose in direct proportion to  $\text{Ca}^{2+}$  concentration in the root (Fig. 1B,C). In contrast, the osmotic pressure gradient, formed between the xylem and the external medium, decreased in direct proportion to  $\text{Ca}^{2+}$  concentration (Fig. 1D).

Addition of ABA (4  $\mu\text{M}$ ) at the onset of exudation produced various effects in the root (Fig. 1). The action

**Table 1.** Shoot and root  $\text{K}^+$  and  $\text{Ca}^{2+}$  levels (expressed in  $\mu\text{mol g}^{-1}\text{FW}$ )

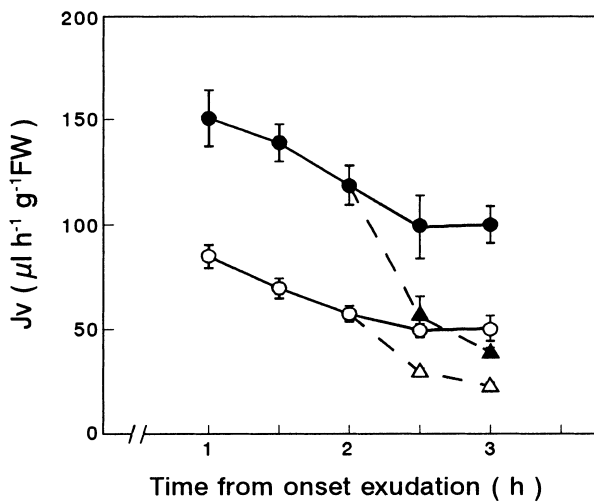
The plants were grown for 14 d in standard nutrient solution, and for 1 d in other solution with either  $\text{Ca}^{2+}$  (control plants) or without  $\text{Ca}^{2+}$  (low- $\text{Ca}^{2+}$  plants) (see Materials and methods). Values are means of four plants  $\pm$  SE of the mean.

Treatment	$\text{K}^+$		$\text{Ca}^{2+}$	
	Shoot	Root	Shoot	Root
Control	148.7 $\pm$ 1.4	118.4 $\pm$ 2.0	33.6 $\pm$ 0.9	2.4 $\pm$ 0.1
Low- $\text{Ca}^{2+}$	151.1 $\pm$ 2.4	115.1 $\pm$ 2.1	27.6 $\pm$ 1.3	1.4 $\pm$ 0.1

**Table 2.** Effect of the  $\text{Ca}^{2+}$  status of the root and of ABA in the root medium on the exudation rate ( $J_v$ ) (expressed in  $\mu\text{l g}^{-1}\text{FW h}^{-1}$ )

Roots were exuding for 6 h in a solution containing  $\text{CaSO}_4$  (0.1 mM), glucose (10 mM) and with (+) or without (-) ABA (4  $\mu\text{M}$ ). Values are means of four roots  $\pm$  SE of the mean.

$\text{Ca}^{2+}$	Treatment	Exudation rate ( $J_v$ )
Control	- ABA	33.1 $\pm$ 3.5
	+ ABA	47.6 $\pm$ 5.3
Low- $\text{Ca}^{2+}$	- ABA	25.3 $\pm$ 1.8
	+ ABA	26.8 $\pm$ 2.0



**Fig. 2.** Effect of  $\text{HgCl}_2$  and of ABA in the root medium on the exudation rate ( $J_v$ ). Roots were obtained from plants grown in standard nutrient solution for 15 d, and they were exuding for 3 h in fresh standard nutrient solution plus glucose (10 mM). ABA (4  $\mu\text{M}$ ) was added to the root medium at the onset of exudation and  $\text{HgCl}_2$  (1  $\mu\text{M}$ ) 2 h later. Control (○), ABA (●),  $\text{HgCl}_2$  (△), ABA plus  $\text{HgCl}_2$  (▲). Values are means of four roots  $\pm$  SE.

of ABA on the exudation rate ( $J_v$ ) and the hydraulic conductivity of the root ( $L_{p_r}$ ) also depended on the internal  $\text{Ca}^{2+}$  concentration: as that  $\text{Ca}^{2+}$  concentration increased, so did the effect of ABA, with the result that its action was greater in roots with higher  $\text{Ca}^{2+}$  concentrations. Significant differences were recorded with respect to controls for concentrations above 2  $\mu\text{mol g}^{-1}$  fr. wt. (Fig. 1A, B). On the other hand, ABA had no significant effect on either the flux of exuded  $\text{Ca}^{2+}$  ( $J_{\text{Ca}}$ ) or the osmotic pressure gradient between the xylem and the external medium (Fig. 1C, D).

In other experiments, the short-term effect (1 h) of  $\text{HgCl}_2$  on water transport in roots was studied with and without ABA in the external medium (Fig. 2). In the presence of ABA (4  $\mu\text{M}$ ), the exudation rate ( $J_v$ ) was greater than in the control roots (Fig. 2). The addition of  $\text{HgCl}_2$  (1  $\mu\text{M}$ ) to the external medium of the root 2 h after the onset of exudation had a rapid and dramatic effect on the exudation rate ( $J_v$ ) both in control and in ABA-treated roots. These results showed that the inhibitory effect of  $\text{HgCl}_2$  on the exudation rate ( $J_v$ ) was similar in the presence and in the absence of ABA (Fig. 2).

In other experiments, the long-term effect of  $\text{HgCl}_2$  was studied. Results showed that 2 h after its addition to the external medium of the roots,  $\text{HgCl}_2$  (1  $\mu\text{M}$ ) significantly inhibited the exudation rate ( $J_v$ ) and the hydraulic conductivity of the root ( $L_{p_r}$ ) (Table 3).

## Discussion

Our results suggest that  $\text{Ca}^{2+}$  and ABA are involved in the regulation of water flow across the root. The extent to which ABA enhanced water flow across the root depended on the concentration of  $\text{Ca}^{2+}$  in the root: higher concentrations of  $\text{Ca}^{2+}$  provided greater sensitivity to ABA (Table 2; Fig. 1A). The effect of  $\text{Ca}^{2+}$  and ABA on water flow in the root can be explained only by their promotive effect on root hydraulic conductivity and not by changes in the osmotic pressure gradient between the xylem and the surrounding medium (Fig. 1). The possibility that the effect of  $\text{Ca}^{2+}$  starvation on water flow in the root could be produced by injurious effects on root structure, have to be discarded. In these experiments,  $\text{Ca}^{2+}$  starvation was not severe for different reasons: (1) when the plants were transferred to nutrient solution without  $\text{Ca}^{2+}$ , the plant roots were not rinsed, retaining the apoplastic calcium; (2) the duration of starvation was only 24 h. Furthermore, the assays with detached root systems were always performed with  $\text{Ca}^{2+}$  in the surrounding medium ( $\text{CaSO}_4$  0.1 mM). In addition,  $\text{Ca}^{2+}$  starvation neither modified plant growth nor altered the nutritive  $\text{K}^+$  status (Table 1), which indicates that the plants were not affected by serious disorders.

The hydraulic conductivity of the root ( $L_{p_r}$ ) was calculated using equation 1. The value of  $\sigma_{\text{sr}}$  is controversial. Several authors have assumed that  $\sigma_{\text{sr}}=1$  (Fiscus, 1981; BassiriRad *et al.*, 1991; Quintero *et al.*, 1998). However, other authors have reported that  $\sigma_{\text{sr}}$  values are substantially smaller than unity (Steudle and Frensch, 1996; Steudle and Peterson, 1998). In bean root systems, Fiscus determined  $\sigma_{\text{sr}}=0.99$ , and ABA did not affect this value (Fiscus, 1981). In excised maize roots,  $\sigma_{\text{sr}}$  values between 0.64 and 0.73 have been calculated (Azaizeh and Steudle, 1991), and these values were not significantly different in roots with different calcium status. These findings indicate that  $\sigma_{\text{sr}}$  does not change under the different experimental

**Table 3.** Effect of the  $\text{HgCl}_2$  in the root medium on the exudation rate ( $J_v$ ), the hydraulic conductivity of the root ( $L_{p_r}$ ), and the osmotic pressure gradient between the xylem sap and the medium

Roots were obtained from plants grown in standard nutrient solution for 15 d, and they were exuding for 4 h in fresh solution plus glucose (10 mM).  $\text{HgCl}_2$  (1  $\mu\text{M}$ ) was added 2 h after the onset of exudation. Values correspond at 2 h after treatment and are means of four roots  $\pm$  SE of the mean.

Treatment	$J_v$ ( $\mu\text{l g}^{-1}$ FW $\text{h}^{-1}$ )	$L_{p_r}$ ( $\mu\text{l g}^{-1}$ FW $\text{h}^{-1}$ $\text{MPa}^{-1}$ )	Osmotic pressure gradient (MPa)
Control	51.9 $\pm$ 8.4	756.6 $\pm$ 99.2	0.047 $\pm$ 0.009
$\text{HgCl}_2$	30.7 $\pm$ 2.0	592.9 $\pm$ 8.6	0.053 $\pm$ 0.005

conditions used in this work. It has been assumed here that  $\sigma_{sr} = 1$  in order to calculate  $Lp_r$ , but even if  $\sigma_{sr}$  were smaller than unity, it would not affect the conclusions about the effect of different treatments on  $Lp_r$ . However, it is possible that the values of  $Lp_r$  could be underestimated.

Curiously, findings elsewhere have reported that ABA and  $Ca^{2+}$  are involved in regulating stomatal opening and closing. Under stress conditions, ABA inhibits the transpiration across the stomata (Kearns and Assmann, 1993); this action is preceded by an increase in cytosolic  $Ca^{2+}$  concentration in occlusive cells (McAinsh *et al.*, 1990). In the present study, ABA and  $Ca^{2+}$  promoted water flow across the root, seemingly contrasting with its effect on the transpiration across the stomata: in the root, high levels of  $Ca^{2+}$  enhanced water flow; in the shoot, high  $Ca^{2+}$  contents in occlusive cells contributed to stomatal closure. The apparently contradictory effects in the root and in the shoot have also been described for  $K^+$ : stomatal closure, and the subsequent inhibition of the transpiration across the stomata, was accompanied by low levels of  $K^+$  in occlusive cells (Kearns and Assmann, 1993); in the root, however,  $K^+$  starvation promoted water flow (Quintero *et al.*, 1998).

The role of ABA and  $Ca^{2+}$  in the regulation of water flow in the shoot (McAinsh *et al.*, 1997) and in the root (described here), and of  $K^+$  in both plant organs (Kearns and Assmann, 1993; Quintero *et al.*, 1998), suggest that the same mechanism is involved in the action of ABA,  $Ca^{2+}$  and  $K^+$  in the root and in the shoot; although they manifest themselves differently, both effects pertain to the same physiological function at the whole-plant level: that is, keeping the plant properly hydrated in situations of stress.

It has recently been suggested that aquaporins are involved in water transport over both long and short distances (Chaumont *et al.*, 1998). There is also evidence indicating that the mercurial sulphhydryl reagents inhibit aquaporins and reduce root hydraulic conductivity (Maggio and Joly, 1995; Carvajal *et al.*, 1996, 1999). The results reported here support this theory: the treatment with  $1 \mu\text{M}$   $\text{HgCl}_2$  swiftly inhibited water flow across the root (Fig. 2). The effect of  $\text{HgCl}_2$  on water transport may be explained by its inhibitory effect on root hydraulic conductivity and not by changes in the osmotic pressure gradient between the xylem and the surrounding medium (Table 3). It has been suggested that the regulation of sulphhydryl groups by a plasma membrane reductase system can alter the transport of osmotically important cations, specially  $K^+$ , across the root cell plasma membrane (Welch *et al.*, 1993). However, the treatment with  $1 \mu\text{M}$   $\text{Hg}^{2+}$  used in the present work, did not alter the flux of  $K^+$  into the xylem (data not shown). Others authors have obtained similar results even with higher  $\text{Hg}^{2+}$  concentrations (Maggio and Joly, 1995; Carvajal

*et al.*, 1999). Furthermore, in this work, at the end of the short-term experiments with  $\text{Hg}^{2+}$ , roots looked healthy and their  $K^+$  status was similar in both root types ( $124.8 \pm 3.3$  and  $129.3 \pm 1.6$  in treated and control roots, respectively). In long-term experiments, the results were similar. Therefore, it is considered that the  $\text{Hg}^{2+}$  concentration and exposure durations used here only seemed to affect water transport through roots.

These results suggest that aquaporins were present in sunflower root cell membranes and were involved in regulating water flow in the root when, as in this experiment, root pressure produced water flow in the radicle system (Quintero *et al.*, 1998). The inhibitory effect of  $\text{HgCl}_2$  was similar in both the presence and the absence of ABA (Fig. 2), suggesting that ABA and aquaporins are independently involved in the regulation of water flow in the root.

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