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Abscisic acid and hydraulic conductivity of maize roots: a study using cell- and root-pressure probes

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Abstract. Using root- and cell-pressure probes, the effects of the stress hormone abscisic acid (ABA) on the water-transport properties of maize roots (*Zea mays* L.) were examined in order to work out dose and time responses for root hydraulic conductivity. Abscisic acid applied at concentrations of 100–1,000 nM increased the hydraulic conductivity of excised maize roots both at the organ (root Lp_r: factor of 3–4) and the root cell level (cell Lp: factor of 7–27). Effects on the root cortical cells were more pronounced than at the organ level. From the results it was concluded that ABA acts at the plasmalemma, presumably by an interaction with water channels. Abscisic acid therefore facilitated the cell-to-cell component of transport of water across the root cylinder. Effects on cell Lp were transient and highly specific for the undissociated (+)-*cis*-*trans*-ABA. The stress hormone ABA facilitates water uptake into roots as soils start drying, especially under non-transpiring conditions, when the apoplastic path of water transport is largely excluded.

Key words: Abscisic acid – Aquaporin – Hydraulic conductivity – Root – Water transport – *Zea*

Introduction

Investigations of the role of the stress hormone, abscisic acid (ABA), have been concerned principally with leaves and, in particular, with stomatal responses (Hetherington and Davies 1998). There is much less information documenting its role in roots, although it has been implicated in anatomical and morphological changes, such as the formation of lateral roots and root hairs, that facilitate water acquisition from drying and compacted soil (Trewavas and Jones 1991; Hartung et al. 1999). Abscisic acid and its conjugates are found in the soil solution itself, and their interactions with root function have been studied (Hartung et al. 1996; Sauter and Hartung 2000) as well as their transport pathways in roots. Their significance has been considered in relationship to long-distance signalling between the root surface and the leaves (Freundl et al. 1998, 2000; Sauter and Hartung 2000). Several authors have pointed to direct effects of ABA on root hydraulic conductivity (Lp_r). Jeschke et al. (1997) performed experiments with maize plants that were supplied with water by seminal roots only. The plants compensated for the limited root surface available for water uptake by an increased hydraulic conductivity of the root system. The authors concluded that ABA, synthesised in the leaves, was translocated to the roots where hydraulic conductivity was stimulated.

Earlier studies by Fiscus (1981) and Markhart et al. (1979) report different responses of root systems to ABA treatment, i.e. (1) a fast release of solutes from the root tissue into the xylem, (2) an increased volume (water) flow through roots, (3) an increased ion transport, and (4) a decreased Lp_r. The authors have used pressure chambers to induce steady water flows by applying hydrostatic pressure to the root system. Abscisic acid was added to root medium at fairly high concentrations (micromolar range). Karmoker and Van Steveninck (1978) and Van Steveninck et al. (1988) showed that there was a release of ions (Cl^-, K^+, Na^+) to the xylem of *Phaseolus vulgaris* induced by ABA. Increases in the osmotically driven J_{Vr} (water flow) were smaller than those of J_S (solute flow), resulting in a decreased Lp_r. Recently, Quintero et al. (1999) pointed out that Ca^{2+} might be involved in ABA-dependent regulation of hydraulic conductivity. An increase in Lp_r rather than a decrease was found by Glinka and Reinhold (1971), Glinka (1973, 1977), Ludewig et al. (1988), BassiriRad and Radin (1992),

Abbreviations: ABA = abscisic acid; J_{Vr} = water flow; Lp_r = hydraulic conductivity of root; Lp = hydraulic conductivity of cell

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Freundl et al. (1998, 2000) and Quintero et al. (1998) in response to ABA treatment.

In all previous work, measurements of root hydraulic properties have been made on intact root systems that may or may not have been excised. Most of the authors have reported effects of ABA on water flow through roots, induced by hydrostatic pressure gradients established in pressure chambers. Because this technique may cause artefacts, such as filling the intercellular spaces when pneumatic pressures are applied to the root medium, we have applied suction (vacuum) to the cut surfaces of excised roots of maize seedlings and examined interactions between root hydraulic properties and ABA added to the external medium (Freundl et al. 1998, 2000).

In the present paper, we have extended these observations to measurements of the hydraulic properties of the membranes of individual cortical cells. Measurements of the hydraulic conductivity of root cell membranes (Lp) were used as the estimate of the contribution of the cell-to-cell (transcellular plus symplastic) path for water transport across the root in addition to the apoplastic flow component (Steudle and Peterson 1998). The root-pressure probe has been employed for measuring the root pressure (root Lp_r).

Materials and methods

Plant material

Seeds of maize (*Zea mays* L. cv. Helix, Kleinwanzlebener Saatzucht AG, Einbeck, Germany) were germinated on filter paper soaked in 0.5 mm $CaSO_4$ for 4 d at 25 °C in the dark. Maize seedlings developed roots of a length of up to 110 mm and primary leaves of a length of up to 30 mm. Some of the seedlings were transferred to aerated hydroponic culture vessels as described by Freundl et al. (1998, 2000) containing the following nutrients (in mM) KH_2PO_4 1.5, KNO_3 2.0, $CaCl_2$ 1.0, $MgSO_4$ 1.0, and (in μM) FeNaEDTA 18, H_3BO_3 8.1, $MnCl_2$ 1.5 at a pH of 5.5. Others were grown in mist culture (aeroponics) the same nutrient solution being applied. Seven-day-old seedlings were used for experiments. Roots from aeroponic cultures developed a complete exodermal layer at about 30 mm from the root tip, but hydroponically grown roots lacked a complete exodermis. For more detailed information about anatomical and morphological differences between maize roots grown in aeroponics and hydroponics, the earlier papers of Freundl et al. (2000) and Zimmermann et al. (1998, 2000) should be consulted.

Cell-pressure-probe measurements

Plant culture

The cell-pressure probe was used on roots that had been excised from hydroponically grown plants. The tip of the cell-pressure probe was introduced into cells of the first and second cortical layers that were 40–60 mm from the root apex. Surface area and volume of the cells probed were estimated by measuring comparable cells viewed in longitudinal and radial freehand cross-sections from roots. Sections were stained with 0.05% (w/v) Toluidine blue O (Chroma, Stuttgart, Germany) for 1 min (O'Brien et al. 1964). Cell diameters (d) and cell lengths (l) in different cell layers were measured from bright-field microphotographs (Zeiss-photomicroscope; Zeiss, Oberkochen, Germany) by using a ruler. Cortical cells had an average diameter of 29 ± 6 μm (mean ± SD; $n = 100$ cells) and a length of 140 ± 50 μm ($n = 45$ cells).

Hydrostatic experiments

A cell-pressure probe was used to measure half-times of pressure relaxations, $T_{1/2}^W$, elastic moduli per μl cell volume (β) and the hydraulic conductivity of the cells (Lp) of individual cortical cells from maize primary roots (Azaizeh et al. 1992). The probe was filled with silicone oil (type AS4; Wacker, München, Germany). An oil-filled glass capillary (tip diameter: 5–7 μm) was attached to the probe. Roots were vertically fixed on a metal sledge by magnets. Nutrient solution flowed along the root during the experiment. The microcapillary of the cell-pressure probe was inserted into cortical cells using a micromanipulator. When a cell was punctured, cell sap formed a meniscus with the silicone oil inside the capillary. After the cell had become stable, a stationary cell turgor (P_0) could be measured. Then hydraulic parameters of the cell were determined. An electronic pressure transducer converted the pressure signal into a proportional voltage. Pressure versus time curves (relaxations) were produced and recorded on a chart recorder. Hydrostatic pressure relaxations were measured by rapidly moving the meniscus using a micrometer screw to a new position and keeping it there until a steady pressure was re-attained. For processing the data, recorder strips were digitised by using a digitising tablet (Kontron-Registriertechnik, Eching, Germany).

From half-times of pressure relaxations $T_{1/2}^W$, cell Lp was calculated according to Eq. 1 (Azaizeh et al. 1992; Henzler et al. 1999):

$$\mathrm{Lp} = \frac{V \cdot \ln(2)}{A \cdot T_{1/2}^W \cdot (\varepsilon + \pi_0^i)} \tag{1}$$

Here, A denotes the cell surface area ($A = \pi \cdot d \cdot l$; neglecting the top and bottom areas of cylindrical cells) and $\pi^i{}_0$ the osmotic pressure of the cells, which was estimated from steady-state turgor pressure, and the osmotic pressure of the root medium. Cell elastic moduli (ε in MPa) were evaluated from cell volumes and from changes in cell volumes (ΔV), which caused changes in cell turgor (ΔP) (Azaizeh et al. 1992), since:

$$\varepsilon = \beta \cdot V = V \cdot \frac{\Delta P}{\Delta V} \tag{2}$$

Usually it holds that $\varepsilon \gg \pi_0^i$. Under these conditions, Eq. 1 reduces to Eq. 3:

$$\mathrm{Lp} = \frac{\ln(2)}{A \cdot T_{1/2}^W \cdot \frac{\Delta P}{\Delta V}} \tag{3}$$

Treatment with ABA: effects of ABA concentration and time of exposure on cell Lp

Eleven-day-old maize plants were incubated in nutrient solution containing 10 nM, 100 nM and 1,000 nM ABA for time intervals of 10 min, 30 min, 1 h and 2 h. Nutrient solution was aerated. Five minutes before the end of each time interval, the youngest parts of primary roots were held in the apparatus and a cortical cell was immediately probed as described above. For a given root, a single cell only could be measured during the 5-min interval. Control roots were incubated for 1 h in nutrient solution without ABA. For testing the specificity of the ABA effect, roots were incubated 1 h in 1,000 nM ABA, kinetin or IAA at a pH of 5.5. Cell hydraulic conductivity was measured as described above.

Measurement of the dependence of the half-time of water exchange $T_{1/2}^W$ on the incubation time with ABA for single cells

In order to avoid variation between cells, cells from the first cortical layer were monitored over the entire time period of 2 h, after 1,000 nM ABA had been added to the external solution. In most cells, turgor (P_0) remained constant over the 2-h period. In cases

where turgor decreased, results were discarded since it was assumed that the insertion of the probe had caused a leakage of solutes from the cell.

Measurement of the dependence of cell hydraulic conductivity (Lp) in cortical maize root cells on the incubation time with a medium containing 100 nM ABA at a pH of 5.5 and 8.0

With some roots, single-cell experiments were performed at two different pH values. At a pH of 5.5, 17% of the ABA was in its undissociated form. At pH = 8.0, on the other hand, more than 99.9% of ABA was present in its charged form (anion). It is known that, in the latter case, the permeability of ABA through cell membranes is quite low (Bürner et al. 1993). Cell Lp was measured in 11-d-old roots that were incubated in nutrient solution containing 100 nM ABA at the two pH values 5.5 and 8.0 (10 mM Hepes-KOH) for 30 min, 1 h and 2 h. Control roots were incubated for 1 h in nutrient solution at a pH of 5.5 without ABA.

Root-pressure-probe measurements

Plant material used for the root-pressure-probe measurements was either grown aeroponically or hydroponically. Root surface areas (A_r) were determined as described earlier by Freundl et al. (1998). They ranged between 0.0025 m^2 and 0.0041 m^2 (aeroponics) and 0.0014 m^2 up to 0.0021 m^2 (hydroponics).

Prior to measurement, a root system was excised but the mesocotyl remained attached to it; the latter structure was connected to a root pressure probe (Steudle 1993). The mesocotyl was fixed to the probe with a silicone seal. The pot with the primary root contained nutrient solution (see above), which was continuously aerated. The seal was used to fix the root to the probe, and tightened in steps with the aid of a screw until root pressure began to increase after tightening. Root pressure (P_{0r}) became steady 3–4 h after attachment of the root pressure probe. After each experiment with a given root, the integrity of the seal was tested by cutting off the root at the seal and measuring the decrease in the time constants of pressure relaxations (Peterson et al. 1993). When the root xylem remained open, there was a drastic decrease in the half-times after the cut. If this was not the case, the experiment was discarded. Steady-state root pressures ranged between 0.08 and 0.28 MPa for aeroponically grown and 0.04 to 0.22 MPa for hydroponically grown roots. Root Lp_r was evaluated from root pressure relaxations. Water flows were induced by changing the root pressure with the aid of a movable metal rod in the equipment (for detailed experimental information of root pressure probes see Steudle 1993). Root pressures (P_r in MPa) were measured with a pressure transducer, which converted the signal into a proportional voltage. Pressure-time curves were recorded on a chart recorder. Hydrostatic relaxation curves were composed of two distinct regions brought about by different rates of changes of root pressure corresponding to time: the initial very rapid phase, immediately following the change in P_r (half-time $T^W_{1/2}$A: 0.2–2.3 s) and finally a slow component (half-time $T^W_{1/2}$ B: 1.0–9.0 s). For calculation of half-times $T^W_{1/2}$, chart recorder strips were digitised on digitising tablets. Curves were analysed using exponential fits. Root hydraulic conductivity (Lp_r) was calculated for $T^W_{1/2}$A and $T^W_{1/2}$B according to Eq. 4:

$$Lp_r = \frac{\ln(2)}{A_r \cdot T^W_{1/2} \cdot \frac{\Delta P}{\Delta V}} \quad (4)$$

Root hydraulic conductivity (Lp_r) of aeroponically and hydroponically grown maize roots dependent on the incubation time with 100 nM ABA

Eleven-day-old maize roots from aeroponic and hydroponic cultures were attached to the root pressure probe as described above. Roots were placed in 1,000-ml pots with aerated nutrient solution. When root pressure (P_{0r}) became steady, Lp_r was measured six times (control). Then ABA was added to the nutrient solution resulting in a final concentration of 100 nM ABA. Half-times, hydraulic conductivity, and root pressure ($T^W_{1/2}$A, $T^W_{1/2}$B, Lp_rA, Lp_rB and P_{0r}) were observed over a period of 140 min for roots of both aeroponically and hydroponically grown seedlings.

Statistical evaluation of Lp_r data

The statistical calculations were done using different procedures from the statistical software SAS release 6.12 (SAS Institute Inc., Cary, USA). The regression models and significance of the estimated model parameter were calculated using the REG procedure. The selection criterion was the adjusted R^2. For the decline in half-time (increase in root Lp_r) with time (t), a quadratic regression of the type $T^W_{1/2} = a + b \cdot t + c \cdot t^2$ had the best-adjusted R^2. Differences between hydro- and aeroponics were calculated using the GLM procedure. The homogeneity of slopes was tested in a linear model with interaction of the cultivation techniques aero- and hydroponics and the linear and quadratic time course of the model.

Specificity of ABA signal

This was tested with individual primary roots, from plant material grown hydroponically, using the suction technique (root level) and the cell pressure probe (membrane level) in the presence of different ABA isomers (see below).

Suction technique

Excised primary roots, with the mesocotyl still attached, were fixed to a capillary using a silicone seal that was made to fix tightly around the mesocotyl when it was compressed by a screw (Freundl et al. 1998). Roots were stored in a darkened 500-ml pot with aerated nutrient solution. Suction applied to the roots by a vacuum pump caused xylem sap flow into the calibrated capillary. Water flow through the root into the xylem (J_{Vr}) was determined by weighing harvested xylem sap fractions every 10 min. When a suction force of −0.06 MPa was applied for about 20 min, the flow of water across the root (J_{Vr}) became steady. Then J_{Vr} was measured for a time interval of 60 min to obtain a control value of Lp_r. Subsequently, different ABA isomers [(±)-*cis-trans*-ABA; (±)-*cis-trans*-ABA at pH = 8.0; (+)-*cis-trans*-ABA; (–)-*cis-trans*-ABA; (±)-*trans-trans*-ABA], ABA metabolites (ABA glucose-ester; ABA methyl-ester), a synthetic ABA analogue (LAB 173 711; Jung and Großmann, 1985), other phytohormones [gibberellic acid (GA_3), kinetin, IAA, zeatin] and acetic acid were added to the medium at a concentration of 100 nM. Subsequent changes in J_{Vr} (Lp_r) were observed for further 120–140 min.

Results

Transient effects of ABA on cell hydraulic conductivity (Lp)

Figure 1 summarises data of the cell hydraulic conductivity from 20 individual experiments (cells). Cortical cells from the first and second cell layer interior to the rhizodermis of control roots had an average $Lp = 6.8 \pm 6.4 \times 10^{-7}$ m s^{-1} MPa (mean ± SD, $n = 20$). After incubation of maize roots in a medium containing 100 and 1,000 nM ABA, cell Lp increased

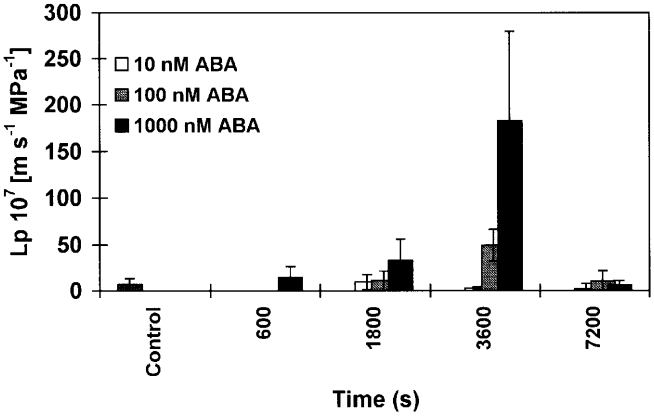

Fig. 1. Dependence of hydraulic conductivity (Lp) of cortical maize root cells incubated in 10, 100 and 1000 nM ABA on the incubation time. Control roots were incubated in nutrient solution without ABA. Data are means ± SD, $n = 4$–20; pH of the medium = 5.5

transiently to reach a maximum value after 1 h. Maximal Lp values were $Lp = 49 \times 10^{-7}$ ms^{-1} MPa and 180×10^{-7} ms^{-1}MPa, respectively, i.e they increased by a factor of between 7 and 27. After 2 h incubation, Lp values returned to the control level. Incubation in 10 nM ABA did not increase Lp (Fig. 1).

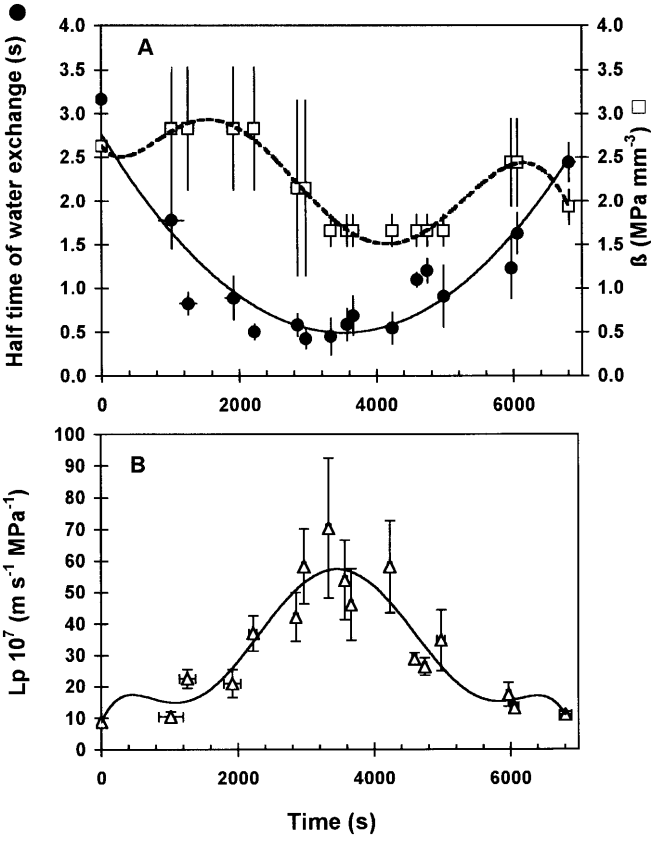

Fig. 2. **A** Time course of $T_{1/2}^W$ (half time of the water relaxation process) and β (cell elastic modulus per 1 mm^3 cell volume) from one single, cortical maize root cell. **B** Treatment with 1000 nM ABA started at time zero. Decreased $T_{1/2}^W$ and β resulted in an increased cell hydraulic conductivity (Lp). Data are means ± SD, $n = 3$; pH of the medium = 5.5

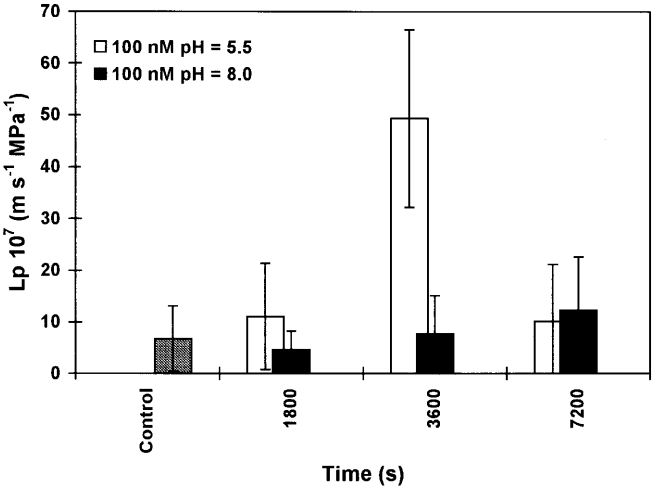

Fig. 3. Dependence of hydraulic conductivity (Lp) of cortical maize root cells incubated in 100 nM ABA at a pH of 5.5 and 8.0 on the incubation time. Control roots were incubated in a nutrient solution at pH 5.5 without ABA. Data are means ± SD, $n = 4$–20

In single-cell experiments lasting for 2 h, effects were similar. Figure 2A shows the response for half-time of water exchange of a cortical cell treated with 1,000 nM ABA. Within 1 h, $T_{1/2}^W$ decreased from 3 s to a minimum value of 0.5 s. The original half-time was re-gained 2 h after starting the ABA treatment. In parallel with $T_{1/2}^W$, the elastic coefficient (β) decreased from 2.5×10^4 to 1.7×10^4 MPa mm^{-3}. Overall, this resulted in a transient increase in cell Lp by a factor of 6 (Fig. 2B). When roots were treated with 100 nM ABA at slightly alkaline pH (pH = 8.0), cell Lp was not affected (Fig. 3) suggesting that the undissociated ABA rather than the anion was causing the effect. The anion does not penetrate membranes (Bürner et al. 1993).

Effect of ABA on Lp_r of maize roots from aeroponically and hydroponically grown seedlings

In these experiments, effects of ABA on root Lp_r were measured on primary roots with an exodermis (aeroponic culture) and those lacking it (hydroponics; Freundl et al. 2000). Measurement of relaxation curves for water transport across maize roots with a root pressure probe showed a biphasic response for water flow with two half-times of water exchange for each relaxation curve ($T_{1/2}^W$A and $T_{1/2}^W$B). The shorter one $T_{1/2}^W$A resulted in a mean root hydraulic conductivity Lp_rA of 2.0 ± 1.8×10^{-7} m s^{-1} MPa ($n = 201$ measurements on 5 roots) for aeroponically grown roots and 4.2 ± 1.7×10^{-7} m s^{-1} MPa ($n = 201$ measurements on 5 roots) for roots from hydroponics (Table 1). Root Lp_rA from aeroponically grown roots was significantly smaller (factor of 2) than Lp_rA from those grown in hydroponics (t-test; $P = 0.0001$). The half-time $T_{1/2}^W$A (Lp_rA) was not affected by ABA treatment of roots from both cultivation techniques (Fig. 4). Right after the treatment with ABA, the second, longer half-time ($T_{1/2}^W$B) resulted in a hydraulic conductivity (Lp_rB) that

Table 1. Hydraulic conductivities (Lp_r) of maize roots. Lp_rA was related to the apoplastic, Lp_rB to the symplastic conductivity. Lp_rA did not change during a treatment with 100 nM ABA while Lp_rB increased after 70–180 min. Values for Lp_r are means ± SD (n = number of measurements)

Aeroponic					Hydroponic				
Lp_rA									
Plant		$Lp_r \times 10^7$		n	Plant		$Lp_r \times 10^7$		n
		(ms^{-1} MPa^{-1})					(ms^{-1} MPa^{-1})		
A001		0.5 ± 0.2		44	H001		6.5 ± 1.8		11
A002		1.1 ± 0.9		43	H002		2.3 ± 0.9		50
A003		3.4 ± 1.5		42	H003		3.1 ± 1.6		53
A004		0.6 ± 0.5		31	H004		3.7 ± 1.8		49
A005		4.5 ± 1.7		41	H005		5.3 ± 1.8		38
Mean		2.0 ± 1.8			Mean		4.2 ± 1.7		
Lp_rB n = 3–7									
Plant	Time (min)	$Lp_r \times 10^7$ (ms^{-1} MPa^{-1})	Time (min)	$Lp_r \times 10^7$ (ms^{-1} MPa^{-1})	Plant	Time (min)	$Lp_r \times 10^7$ (ms^{-1} MPa^{-1})	Time (min)	$Lp_r \times 10^7$ (ms^{-1} MPa^{-1})
A001	0	0.02 ± 0.01	61–75	0.09 ± 0.03	H001	0	0.68 ± 0.21	73–74	1.90 ± 0.40
A002	0	0.10 ± 0.05	108–122	0.50 ± 0.20	H002	0	0.22 ± 0.05	121–147	0.60 ± 0.30
A003	0	0.30 ± 0.05	108–122	0.90 ± 0.30	H003	0	0.41 ± 0.06	116–138	0.76 ± 0.22
A004	0	0.10 ± 0.04	158–182	0.50 ± 0.17	H004	0	0.42 ± 0.15	152–166	1.10 ± 0.45
A005	0	0.30 ± 0.09	180–210	0.70 ± 0.20	H005	0	0.46 ± 0.12	106–123	1.50 ± 0.43
Mean		0.16 ± 0.13		0.54 ± 0.30	Mean		0.44 ± 0.16		1.18 ± 0.52

was smaller by one order of magnitude than Lp_rA (Table 1). In ABA-treated roots from both aero- and hydroponics, $T^W_{1/2}B$ was reduced during the treatment. In the presence of 100 nM ABA, minimum values of $T^W_{1/2}B$ were obtained 70–100 min after the treatment. This corresponded with an increase in root Lp_r by a factor of 4 for aeroponics and 3 for hydroponics (Table 1). Two hours after starting the experiment, a slight but not statistically significant tendency for a $T^W_{1/2}B$ increase could be observed. Transient changes in root Lp_rB ($T^W_{1/2}B$) resembled those found in cell Lp ($T^W_{1/2}$). The data suggest that for a given relaxation curve, most of the water was flowing apoplastically during the initial water flow ($T^W_{1/2}A$). During later stages a significant cell-to-cell component of water flow appeared. However, there are alternatives to this interpretation of the effect(s) (see *Discussion*). During the experiments, root pressure P_{0r} increased in mean by 16% for roots from aeroponic and by 32% from hydroponic culture (data not shown).

In Fig. 4 the decrease in $T^W_{1/2}B$ proved to be significantly different from zero ($P \leq 0.0001$), both for hydroponic and aeroponic cultures.

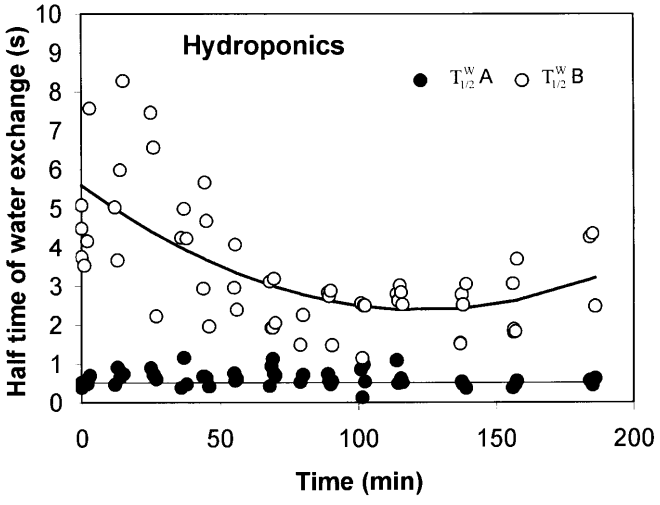

Fig. 4. Time course of $T^W_{1/2}B$ and $T^W_{1/2}A$ after 100 nM ABA treatment at time zero for roots from hydroponics. Data points originate from an individual, single root measurement. Quadratic regression model (*solid line*) is added

Specificity of ABA signal

The specificity of the ABA signal was determined by measuring the accumulation of xylem sap that results from water flow across roots; the suction technique used was described by Freundl et al. (1998). (±)-*cis-trans*-Abscisic acid caused an increase in radial water flow (J_{Vr}) of 250% (factor of 2.5 on average; Fig. 5). Radial water flow started to rise 10–20 min after addition of 100 nM (±)-*cis-trans*-ABA and tended to reach a steady state after 90–110 min (Fig. 6). The isomer (+)-*cis-trans*-ABA, LAB 173 711 (a synthetic ABA analogue) and ABA glucose ester also showed stimulated water flow (Fig. 5). Compared to the control, (−)-*cis-trans*-ABA caused an increase in J_{Vr} of 122%. However, this rise was significantly lower than that caused by (±)-*cis-trans*-ABA. All other ABA isomers and phytohormones tested proved to be ineffective (Fig. 5).

The specificity of the ABA effect was also tested at the cellular level using the cell pressure probe. Only in the presence of (±)-*cis-trans*-ABA was there a significant increase in cell hydraulic conductivity above control. The tested hormones kinetin and IAA

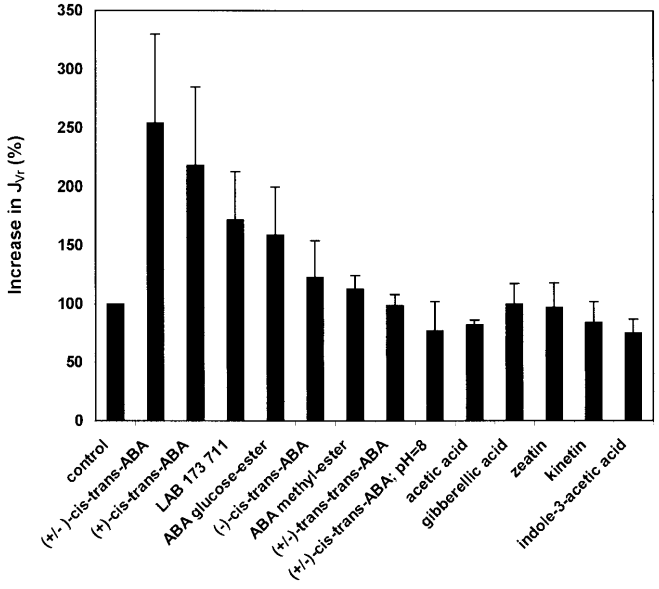

Fig. 5. Percentage increase in radial water flow J_{Vr} after application of 100 nM of different ABA isomers, -derivates, other phytohormones and the synthetic ABA analogue LAB 173 117. Data are means ± SD, $n = 3$–6

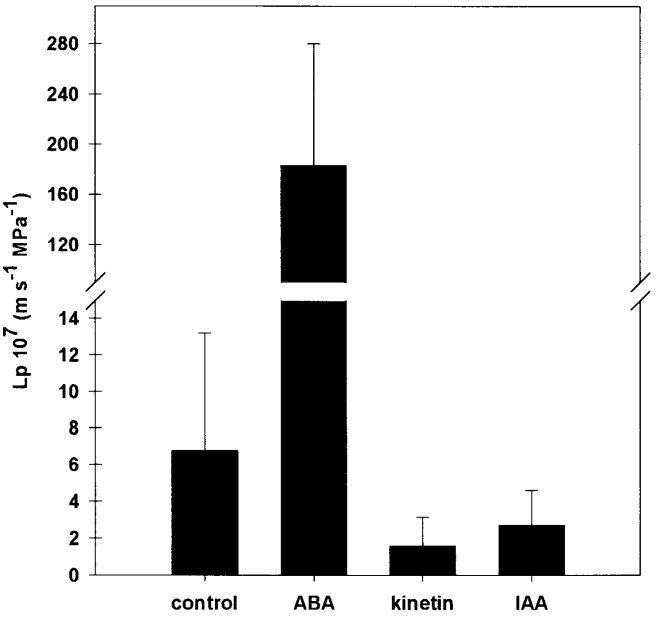

Fig. 7. Cell hydraulic conductivities (Lp) of cortical maize root cells after treatment with 1000 nM ABA, kinetin or IAA for 1 h. Control roots were incubated in nutrient solution without phytohormones. Data are means ± SD, $n = 6$–21

even reduced Lp by a factor of 4 and 3, respectively (Fig. 7).

Discussion

For the first time, this contribution presents detailed data of the effects of ABA on the hydraulic conductivity of root cell membranes. Abscisic acid has been applied at concentrations that might be expected to be in the apoplast of unstressed and stressed plant roots (10–1000 nM; Masia et al. 1994; Hartung et al. 1999). At 100 nM ABA and pH = 5.5, there was a transient increase in cell Lp by a factor of 7 over control level. An ABA concentration, typical for the apoplast of an unstressed maize root produced no effect (10 nM; Freundl et al. 1998). Maxima in the increase of Lp occurred after about 1 h. Thus, cell hydraulic conductivity was increased markedly only after the onset of the ABA accumulation within cells. This accumulation may send an intensified stress signal from roots to the shoot. One might expect that such a mechanism could result in increases in rates of water uptake by roots, i.e. in the overall root Lp_r. Although this has been found, the effects at the root level were less pronounced than those at the cell level. This is predictable because Lp_r is the composite of several pathways for water movement across the root tissues (cell-to-cell or apoplastic); the relative flows in these pathways may change in response to the conditions (Steudle and Peterson 1998, see below).

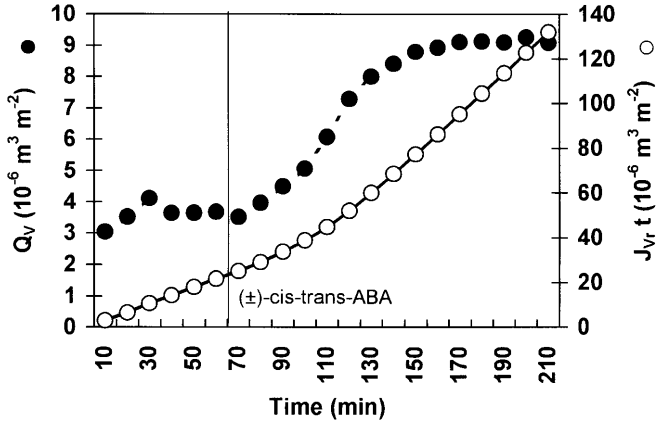

Fig. 6. Typical experiment for measuring the effect of ABA on J_{Vr} of a single maize root. Water flow density (Q_V) and the radial water flow are given. After treatment with (±)-*cis-trans*-ABA, J_{Vr} increases compared to the control flow to a new steady-state flow after 90 min

Since water flow across plant cell membranes is facilitated largely by aquaporins (water channels) present in the plasmalemma (Daniels et al. 1994; Kammerloher et al. 1994; Maurel 1997; Tyerman et al. 1999), effects at the cell level may be interpreted in terms of changes in the density or activity of aquaporins. Recent advances in cloning and functional characterisation of this family of major intrinsic proteins (MIPs) have provided important information about the biophysics of transport of water and small non-electrolytes across plant membranes (Steudle and Henzler 1995; Maurel 1997; Eckert et al. 1999). Although a decrease in root cell Lp in response to stress conditions has already been measured (Azaizeh et al. 1992; Zhang and Tyerman 1999), the effects of hormones on water channel activity have not yet been tested in intact tissue. Abscisic acid is one of the major hormonal stress signals responsible for regulating plant water status and it was suggested that it might influence aquaporins (Abe et al. 1997). A direct

relation between ABA and aquaporins of maize roots, responsible for this short-time effect, needs to be investigated. The signalling pathway of ABA is unknown. There is, however, evidence that ABA induces transcription factors involved in gene expression of aquaporins, which are also induced by water stress (Shinozaki et al. 1998). Kaldenhoff et al. (1993, 1996) found an activation of the promoter of the aquaporin PIP1b by ABA and gibberellic acid, but there were only minor effects with indole-3-acetic acid.

A simple interaction of ABA with the lipid-phase of the plasmalemma (Bürner et al. 1993) is very unlikely to be the reason for the increased Lp. Concentrations of ABA sufficient to enhance membrane permeability to cations, anions and neutral solutes are in the millimolar range (more references are cited in Bürner et al. 1993), which are higher by a factor of 1×10^3–1×10^4 of the ABA-concentrations used in this study.

A time course of the ABA effect has been demonstrated for the root hydraulic conductivity as well. Half times $T^W_{1/2}$B of water relaxation curves decreased up to 120 min after ABA application and then tended to increase again. The rapid phases of pressure relaxations $T^W_{1/2}$A were not affected by ABA. This is in accordance with findings for maize and other species which show that $T^W_{1/2}$A has to be largely related to an apoplastic water flow (e.g. Zhu and Steudle 1991; Azaizeh et al. 1992). This is because (i) cell Lp was similar to root Lp_r (as was also found in the present study) and because (ii) root Lp_r was substantially reduced in the presence of an exodermal Casparian band (Table 1; Zimmermann and Steudle 1998). In order to quantify the contribution of water flow through plant cells over the whole root radius, it would be necessary to know the Lp of cells from deeper layers of the cortex and from the stele. For geometrical reasons, the latter may contribute much more to the overall root Lp_r (Steudle and Brinckmann 1989). For example, in *Lotus*, the overall root Lp_r showed a marked diurnal rhythm (as did the messenger RNA encoding for water channels), although the Lp of cortical cells remained constant (Henzler et al. 1999; Clarkson et al. 2000). This has been explained by a limitation of root Lp_r by the endodermis and stelar tissue. For technical reasons, the contribution of the endodermis and stelar tissue is not known for maize (or for other roots). However, the role of cell membranes needs to be estimated during the regulation of radial water flow. The effect of ABA on the Lp of these cells may differ from those measured in the outer cortex. This, in turn, may cause the differences in the response of cell Lp and root Lp_rB presented in this paper.

Comparing root Lp_r and cell Lp it becomes evident that the fast component of pressure relaxations largely reflects apoplastic water flow (see above). This component was affected by the presence or absence of apoplastic barriers as shown by the comparison between roots from hydroponic and aeroponic culture (see above; Zimmermann et al. 1998). The Lp_r related to the slow component appears to be dominated by a flow of water from cell-to-cell and was, accordingly, smaller by an order of magnitude than the Lp_r referring to the rapid apoplastic component. Water must cross many membranes on its passage across the root. There is more than one reason that may explain the occurrence of such a component. One reason is that there might be concentration polarisation effects at the osmotic barriers in the root (endodermis), which result in additional osmotic driving forces in the presence of a radial water flow (Peterson and Steudle 1993). It might also be due to gradients in root water potential being built up across the root cylinder, i.e. there is a hydration or dehydration of root tissue as water is injected into or withdrawn from the root xylem with the aid of the probe. These gradients may equilibrate by a cell-to-cell transport of water. The fact that $T^W_{1/2}$B decreased (Lp_r increased) during ABA treatment parallel to the increase in cell Lp is in line with both of the above explanations. More detailed measurements will be necessary to work out the mechanism(s). Experimentally, the problem could be solved by subjecting roots to pressure clamping for different periods of time and measuring root pressure relaxations following the release of the clamp in the presence and absence of ABA. Results ought to be compared with those from osmotic pressure relaxations, i.e. from experiments in which an osmotic gradient is applied to induce relaxations of turgor (water potential). Published data show that the Lp_r measured in these experiments is smaller by about an order of magnitude ($T^W_{1/2}$ larger) than during the 'hydrostatic' relaxations (Steudle and Frensch 1989; Steudle and Peterson 1998). Up to now, there has not been any information about how ABA might affect the $T^W_{1/2}$ during osmotic relaxations. These experiments are underway.

It should be noted that, with the root pressure probe, root pressure (P_r) is measured along with root hydraulic conductivity Lp_r (Steudle 1993). Changes in the osmotic composition of xylem sap can be recorded as well as changes in P_r. As P_r increased during the first 60 min after treatment with 100 nM ABA by 16% up to 32% (data not shown), we conclude that ABA stimulated ion release into the xylem.

The results indicate that the effects of ABA on root water flow (cell Lp, root Lp_r) are quite specific. Increases have been only found with (+)-*cis*-*trans*-ABA. Other ABA isomers, -derivates and plant hormones have proved to be ineffective. The response of J_{Vr} after application of ABA glucose ester may be a result of a cleavage of this ABA conjugate by a β-glucosidase in the root cortex apoplast as demonstrated by Dietz et al. (2000) for leaves and Sauter and Hartung (2000) for root cortical tissues. (−)-*cis*-*trans*-Abscisic acid did not appear to be stable when dissolved in nutrient solution; up to 30% of it was converted into the (+)-isomer during the experiments (data not shown). The weak increase in J_{Vr} after treatment with (−)-*cis*-*trans*-ABA is very likely due to contamination with (+)-*cis*-*trans*-ABA. The effect of the synthetic ABA analogue LAB 173 117 (Jung and Großman 1985) is more difficult to explain. This preparation is a mixture of many isomers. In addition, we cannot exclude that some ABA has formed during storage and in solution. Auxin and kinetin slightly decreased J_{Vr}. The response in J_{Vr} due to

ABA treatment could not be related to the effect of a weak acid. Acetic acid and IAA at the same concentration as ABA did not influence J_{Vr}. Thus, a specific ABA interaction would seem to be involved in the regulation of root hydraulic conductivity; this might involve an ABA receptor.

When roots were treated with ABA under alkaline conditions (pH = 8), J_{Vr} was not changed. It is concluded that ABA has to move across cell membranes and be taken up before it acts from the inside of the cell. The permeability of the protonated ABA has been shown to be much larger than that of the anion (Slovik et al. 1995). Bürner et al. (1993) have shown that the deprotonated ABA anion, as it is present at pH = 8.0 nearly exclusively, cannot penetrate artificial phospholipid membranes.

In conclusion, the combination of measurements at the root (root pressure probe) and cellular level (cell pressure probe) suggest that ABA increased the hydraulic conductivity of whole maize roots by a stimulation of water channel activity of root cell membranes. In accordance with the composite transport model of water movement in roots, effects were more pronounced at the cell than at the root level. Depending on conditions, either the cell-to-cell or the apoplastic path dominated the overall flow of water. When the cell-to-cell path dominated, there was an effect of ABA on root Lp_r. When the water flow was largely apoplastic, no effect was found. In part, the effects of ABA were transient. They were highly specific for (+)-*cis-trans*-ABA. It is concluded that the stress hormone ABA facilitates water uptake into roots as the soil starts drying and ABA is accumulated in root tissue. ABA may thus contribute to the regulation of water uptake (root Lp_r) by affecting the cell-to-cell path of water transport. The apoplastic path is used mainly in the presence of a hydrostatic pressure gradient, it occurs in a transpiring plant. With this mechanism, plants are able to adapt to conditions of water shortage by varying the cell-to-cell component of water transport as stress develops. Whether or not aquaporins are the ABA-dependent 'switches' involved along the cell-to-cell path will be a matter of further research.

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