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: 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 undisassociated (+)-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). 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 Ficus (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. 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 Jₓ (water flow) were smaller than those of Jₛ (solute flow), resulting in a decreased Lp. Recently, Quinto et al. (1999) pointed out that Ca²⁺ might be involved in ABA-dependent regulation of hydraulic conductivity. An increase in Lp, rather than a decrease was found by Glinka and Reinhold (1971), Glinka (1973, 1977), Ludewig et al. (1988), BassiriRad and Radin (1992),

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).

Materials and methods

Plant material

Seeds of maize (Zea mays L. cv. Helix, Kleinwanzlebener Samzucht AG, Einbeck, Germany) were germinated on filter paper soaked in 0.5 mm CaSO₄ 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) K₂HPO₄ 1.5, KNO₃ 2.0, CaCl₂ 1.0, MgSO₄ 1.0, and (in μM) FeNaEDTA 18. H₂BO₃ 8.1, MnCl₂ 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, τ₁/₂, elastic moduli per μl cell volume (β) and the hydraulic conductivity of the cells (Lp) of individual cortical cells from maize primary roots (Azaiżieh 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 slide 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 (Pₒ) 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-established. For processing the data, recorder strips were digitised by using a digitising tablet (Kontron-Registriertechnik, Eching, Germany).

From half-times of pressure relaxations τ₁/₂, cell Lp was calculated according to Eq. 1 (Azaiżieh et al. 1992; Henzler et al. 1999):

\[ Lp = \frac{V \cdot \ln(2)}{A \cdot \frac{\tau}{\tau_{1/2}} \left(e + \pi_0\right)} \]  

(1)

Here, \( A \) denotes the cell surface area (\( A = \pi \cdot d \cdot l \); neglecting the top and bottom areas of cylindrical cells) and \( \pi_0 \) 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 (\( e \) in MPa) were evaluated from cell volumes and from changes in cell volumes (\( \Delta V \)), which caused changes in cell turgor (\( \Delta P \)) (Azaižieh et al. 1992), since:

\[ e = \beta \cdot V = V \cdot \frac{\Delta P}{\Delta V} \]  

(2)

Usually it holds that \( e \gg \pi_0 \). Under these conditions, Eq. 1 reduces to Eq. 3:

\[ Lp = \frac{\ln(2)}{A \cdot \frac{\tau}{\tau_{1/2}} \cdot \frac{e}{\pi_0}} \]  

(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 \( \tau_{1/2} \) 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 (Pₒ) remained constant over the 2-h period. In cases