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Electrical signalling and cytokinins mediate effects of light and root cutting on ion uptake in intact plants

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ABSTRACT

Nutrient acquisition in the mature root zone is under systemic control by the shoot and the root tip. In maize, exposure of the shoot to light induces short-term (within 1–2 min) effects on net K^+ and H^+ transport at the root surface. H^+ efflux decreased (from –18 to –12 nmol m^{-2} s^{-1}) and K^+ uptake (~2 nmol m^{-2} s^{-1}) reverted to efflux (~–3 nmol m^{-2} s^{-1}). Xylem probing revealed that the trans-root (electrical) potential drop between xylem vessels and an external electrode responded within seconds to a stepwise increase in light intensity; xylem pressure started to decrease after a ~3 min delay, favouring electrical as opposed to hydraulic signalling. Cutting of maize and barley roots at the base reduced H^+ efflux and stopped K^+ influx in low-salt medium; xylem pressure rapidly increased to atmospheric levels. With 100 mM NaCl added to the bath, the pressure jump upon cutting was more dramatic, but fluxes remained unaffected, providing further evidence against hydraulic regulation of ion uptake. Following excision of the apical part of barley roots, influx changed to large efflux (–50 nmol m^{-2} s^{-1}). Kinetin (2–4 μM), a synthetic cytokinin, reversed this effect. Regulation of ion transport by root-tip-synthesized cytokinins is discussed.

Key-words: barley; kinetin; long-distance signalling; maize; potassium; trans-root potential; xylem pressure.

Abbreviations: BSM, basic salt media; BTP, Bis-Tris-propane; DHP, 1,4-dihydropyridine; MES, 2-(N-Morpholino) ethanesulfonic acid; MIFE, microelectrode ion flux estimation; P_x, xylem pressure; TEA, tetraethylammonium; TRP, trans-root potential.

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INTRODUCTION

In higher plants, ion uptake by roots is a crucial step in maintaining the cellular nutrient balance. This, in turn, is critically important for basic physiological processes such as photosynthesis and growth (Marschner 1995). Therefore, it is no surprise that the entire organism is involved in the control of transport processes at the root epidermis and cortex. The shoot was previously shown to have a regulatory function with respect to nutrient acquisition. Strong evidence has been presented that K^+ loading into the xylem, and, in turn, K^+ uptake by the root, are tightly adjusted to the demands of the shoot by feedback regulation (Tester & Leigh 2001), and it has been speculated that K^+ recirculation via the phloem is involved in long distance signalling to maintain cellular K^+ homeostasis (Wegner & De Boer 1997; White 1997). Nitrate transport to the shoot is also dependent on nitrate reduction and synthesis of amino acids; sugars translocated by the phloem have been identified as signalling molecules that regulate root nitrate uptake (Forde 2002; Lejay *et al.* 2003; Walch-Liu *et al.* 2005). It has also been shown that the exposure of the shoot to different light regimes has a strong impact on ion uptake mechanisms (Macduff & Wild 1988); one link appears to be the requirement of metabolic energy to maintain nutrient acquisition (Graham & Bowling 1977; Bowling, Watson & Ehwald 1985). The depletion of root tissue with respect to sugars and amino acids has been identified as a major factor that strongly affects the activity of the H^+ pump and related transport processes in the dark; when the sugar pool is replenished following an increase in light intensity, proton pumping activity is restored (Kennedy 1977). From these findings, it appears that the light regime interacts with nutrient acquisition, mainly via the energy status of the root tissue. This opinion was questioned by other authors (e.g. Schubert & Mengel 1986; Casadesus, Tapia & Lambers 1995), and recently, sugars were shown to affect H^+ ATPase activity in maize roots by a pathway that is independent of ATP synthesis (Camoni *et al.* 2006). Despite remaining uncertainties about the mechanism, it is clear from the available data that photosynthesis is involved in the control of root ion transport (Rao *et al.* 2002).

However, these well-established examples of shoot-to-root 'communication' operate in a timescale of hours or even days. Very little is known about rapid effects of light on ion transport in the root operating over a timescale of seconds to minutes. The lack of any information on regulatory functions by the shoot exerted on this timescale has shaped the opinion shared by many researchers in this field: that removal of the shoot has little effect on root ion and water transport processes, as long as the excision took place roughly within one hour after severing (Huang *et al.* 1992). Indeed, the composition of the exudate that is sequestered at the cut surface during this time is thought to reflect the composition of the xylem sap in the intact plant (e.g. Siebrecht & Tischner 1999) before ageing phenomena (e.g. due to altered gene expression) become manifest (Jacobson & Young 1975).

Another important issue is the role of root integrity and, specifically, of the root apex in regulating ion uptake at the mature root zone. Some reports that included measurements of transport processes after removing the root tip are available from the literature (Hong & Sucoff 1976; Smith & Majeed 1981), but again, changes only became apparent after a delay of more than an hour. No information on short-term effects is available. As a result, many researchers routinely use excised plant roots or root segments in transport experiments, assuming these are representative for the intact plant (BassiriRad & Radin 1992; Huang *et al.* 1992; Cohen *et al.* 1998, Herschbach *et al.* 2000, Osawa & Matsumoto 2001; Tournaire-Roux *et al.* 2003; Ye & Steudle 2006; Personeni *et al.* 2007).

Our lack of knowledge with respect to short-term systemic regulation of transport processes in the root (and related parameters) is, in part, due to limitations of available methods to study these processes. The peak of the interest in this topic occurred somewhere in the late 1970s to the early 1980s, when experimental techniques to study plant ion and water relations were not as advanced as today. The subsequent rapid progress in molecular biology has switched the attention of researchers to other topics, leaving many questions unanswered. In this work, we have revisited the problem. We combine, for the first time, two advanced biophysical techniques: the non-invasive MIFE technology for the measurement of net ion fluxes at the root surface at a high time resolution (ca 5 s) and the minimal-invasive xylem probe for recording xylem pressure (P_x) and trans-root potential (TRP; the electrical potential difference between a xylem vessel and a bath electrode) also on a timescale of seconds. Using this approach, the response of net ion fluxes and related parameters (electrical and hydraulic gradients) to a changing light regime were measured at an adequate timescale on roots of intact maize and barley seedlings. An alternative approach to examining systemic regulation of transport processes is to cut the root at various positions. Aside from testing the importance of plant integrity for studies on root transport, these experiments could potentially contribute to our understanding of physiological responses related to wounding. In the first set of experiments, the shoot was exposed to an increased light intensity. Under these conditions, net root K^+ uptake and H^+ release, membrane potential of cortical cells, TRP and P_x was recorded in order to test whether changing environmental conditions experienced by the shoot will have *rapid* (within seconds to few minutes) effects on root ion transport. These experiments were followed by investigating the mechanisms by which severing the shoot or cutting the root at various sites affects these properties. Our results imply that both rapid electric signalling and cytokinins are instrumental in mediating the above effects on root nutrient acquisition.

MATERIALS AND METHODS

Plant material and growth conditions

Maize (*Zea mays* L. cv. Zelltic, Bangui or Sweet Corn) and barley (*Hordeum vulgare* L. cv. CM72 or Gairdner) seedlings were grown hydroponically on a floating mesh in plastic containers above an aerated nutrient solution essentially as described in our previous publications (Wegner & Zimmermann 1998; Chen *et al.* 2007; Pang *et al.* 2007). Plants were grown under laboratory conditions (temperature 20–24 °C; relative humidity 20–50%; 16 h photoperiod; PAR 400–450 μmol m^{-2} s^{-1}). A Li-COR radiation sensor (Li-COR, Lincoln, NE, USA) and a humidity sensor (Testo 615, Testo, Lenzkirch, Germany) were used to control the photon flux density and relative humidity, respectively. The light source used was a medium pressure Hg lamp (Osram Floraset HQLR 80W, Osram, Munich, Germany). In most measurements, 10- to 17-day-old plants were used. In MIFE experiments on excised barley root segments (Fig. 1b), 4-day-old seedlings (root length between 60 and 80 mm) were employed.

Solutions

For probing the xylem, roots were immersed in a standard medium containing: 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM KCl or KNO_3, 10 mM MES, pH 5.5 (adjusted with BTP). When measurements were performed on 4-day-old barley roots using the MIFE technique (see below), a more simple basic [basic salt media (BSM)] solution (0.1 mM $CaCl_2$ and 0.5 mM KCl, pH 5.5 unbuffered) was used. This latter solution was also used as a bath medium during all ion flux measurements. The use of the BSM solution allowed us to maximize the signal to noise ratio and to avoid effects of the buffer on measured ion fluxes. Preliminary experiments had shown that P_x and TRP responses were qualitatively similar between plants exposed to the standard medium or BSM solution, suggesting that small variations in the bath ion composition had no significant impact on plant responses to light and excision.

In experiments with kinetin (6-Furfurylaminopurine; catalogue No. 48130; Sigma-Aldrich, St. Louis, MO, USA), a 20 μM stock was made in the BSM solution, and the appropriate amount was added to the measuring chamber to achieve the required concentration. Solution pH was

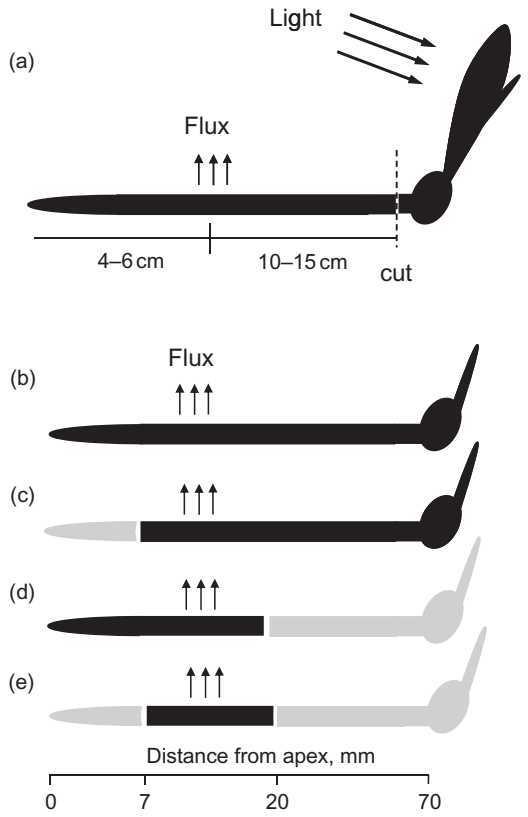

Figure 1. Specific details of experimental protocols on excised maize (a) and barley (b–e) roots. In maize, net ion fluxes were measured from mature root epidermis of 10- to 14-day-old plants, 4–6 cm from the root tip (drawing not to scale). The root excision was performed at the very base, several millimetres from the seed. In experiments on 4-day-old barley seedlings, ion fluxes were measured always at the same position, 12–15 mm from the root tip, regardless of the site of excision that was varied among experiments as indicated in the drawings (not to scale; positions of the cuts as indicated at the bar below e).

adjusted to pH 5.5 with NaOH. This resulted in ~10 mM Na^+ being added to the bath solution at highest (8 μM) kinetin concentration. Accordingly, an appropriate amount of NaCl was added to the control bath prior to each measurement.

P_x probe and TRP measurements

A detailed description of the xylem pressure-potential probe and the experimental setup, including schematic drawings thereof, has been given in our previous publications (e.g. Wegner & Zimmermann 1998, 2002, 2008; Wegner, Schneider & Zimmermann 2007). Briefly, the probe represented a four-ported Perspex chamber. The pressure-potential sensing barrel is attached to one of these ports by means of a tight rubber seal. The high-resolution pressure transducer (KPY-16, Siemens, Munich, Germany) is mounted at another port. Two remaining ports accommodate an Ag/AgCl electrode and a movable metal rod required for applying pressure (volume) pulses, respectively. The pressure transducer is galvanically isolated from the probe interior containing the electrode by a flexible membrane that does not interfere with pressure recording.

Plant roots were immobilized by fixing the tap root to a Teflon rod with a longitudinal notch to receive the root. The rod was supported by two rotary arms that were attached to a vertical stand. After mounting the seedling, the rod was lowered into a plastic cuvette filled with standard medium until the root was just covered by bath medium. Small holes (diameter 0.8 mm) drilled into the Teflon rod at a distance of 5 mm along the notch remained air-filled when the rod was submersed due to the hydrophobicity of the material, thus serving as an oxygen reservoir. The assembled probe was mounted on the three-dimensional mechanical manipulator and impaled into the root xylem under a binocular microscope (magnification up to 100×). During measurements, P_x and TRP signals were converted by the A/D converter (DAS 1601, Keithley, Taunton, MA, USA) and recorded on a PC by using Testpoint™ software (Keithley), at a sampling rate of 10 Hz. TRP data was filtered at 0.1 Hz to improve the signal-to-noise ratio. P_x values are given with respect to vacuum (corresponding to 0 MPa), following the usual convention (Balling & Zimmermann 1990).

Ion flux measurements

Net K^+ and H^+ fluxes were measured using the non-invasive microelectrode MIFE system (UTas Innovation Ltd, Hobart, Australia). Specific details on fabrication and calibration of H^+ and K^+ ion selective microelectrodes are available elsewhere (Shabala & Newman 1997; Shabala, Babourina & Newman 2000). Briefly, pulled and silanized microelectrodes with tip diameters of about 3 μm were back-filled with the appropriate solution, then the electrode tips front filled with ionophore cocktails (95297 for H^+; 60031 for K^+; both from Fluka). Electrodes were mounted on a three-dimensional electrode holder (MMT-5, Narishige, Tokyo, Japan) and positioned 50 μm from the root surface, with their tips spaced 2–3 μm apart. Electrodes were calibrated in an appropriate set of standards before and after use (pH from 4.4 to 7.8; K^+ from 0.1 to 1 mM). During measurements, electrodes were moved by a computer-driven hydraulic manipulator (MHW-3, Narishige, Tokyo, Japan) in a square-wave manner with a 10-s cycle so that the electrode tips moved between two points, 50 and 100 μm, respectively, from the tissue. The measured electrochemical gradient between these two positions was converted into the net flux of the ion on the root surface by the MIFEFLUX software, assuming cylindrical diffusion geometry (Shabala, Newman & Morris 1997; Newman 2001). Previous experiments have shown that the light regime does not interfere with MIFE measurements (Shabala & Newman 1999). Direct effects of light on ion transport processes at the root surface (that was not shaded) can also be excluded.

Experimental protocols used for ion flux measurements

Plant roots were securely immobilized in the measuring chambers to avoid any potential root movement in response to excision. When ion fluxes were measured from maize seedlings, the root was immobilized in at least four positions (~3, 7, 12 and 15 cm from the tip). Ion fluxes were measured from the mature root zone between 4 and 6 cm from the tip (Fig. 1a). Plants were left to adapt to low light conditions (about 10 μmol m^{-2} s^{-1}), until no evidence of any transient changes were present (often 40–50 min after immobilization and placement into Faraday cage). Net K^+ and H^+ fluxes were measured for 10–20 min and the light treatment (250–300 μmol m^{-2} s^{-1}) was then given, followed by a further 40–60 min of recording. Roots were excised (if needed) by gently cutting the root base using fine surgical scissors at a position close (5–7 mm) to the seed (Fig. 1a) under bright light conditions.

In experiments on barley roots, four different treatments were compared (Fig. 1b–e). Net K^+ fluxes were measured from: (1) intact roots of 4-day-old seedlings (Fig. 1b); or (2) roots in which the apical 7 mm segment was removed (Fig. 1c); or (3) roots where the basal part was removed (leaving the first 20 mm from the tip; Fig. 1d); or (4) from a root segment where both the apical (first 7 mm) and basal (beyond 20 mm) parts were removed (Fig. 1e). In all these cases, K^+ flux measurements were performed at exactly the same position, between 10 and 15 mm from the root tip. To avoid any confounding effects of wounding, root excision took place at least 3 h prior to measurements. No such evidence for wounding was found at any occasion, as judged by the steady K^+ flux values measured in all treatments.

Membrane potential measurements

Conventional KCl-filled Ag/AgCl microelectrodes (Shabala & Lew 2002) with a tip diameter of ~0.5 μm were used to measure the membrane potential of epidermal cells from the mature root zone of either intact roots or isolated root segments. Measured specimens were immobilized in the Perspex chamber as described for ion flux measurements and left to equilibrate in the appropriate solution for 50–60 min. Steady-state membrane potentials were measured from at least five individual roots (or root segments), with three to four individual epidermal cells recorded from each of these. Membrane potentials were normally recorded for 1.5–2 min after the potential had stabilized following cell penetration, before the electrode was removed and reinserted into another cell. Electrodes were replaced when any evidence of tip clogging became apparent.

Transient changes in membrane potential of root epidermal cells were measured from maize roots following the excision of the basal part (Fig. 1a). Measurements were made in the mature root zone, 4–6 cm from the root tip, on 10–12-day-old plants, with the shoots being exposed to bright (250–300 μmol m^{-2} s^{-1}) light for at least 30 min. Once impaled, cell membrane potential was recorded for several minutes to ensure seal stability, then membrane potential kinetics were recorded for another 30–40 min in response to root excision (root base cut; Fig. 1a).

Statistics

Significance of difference between data sets was evaluated by the Student's *t*-test.

RESULTS

Shoot illumination affects root P_x and electrical gradients in the root

The effect of light intensity on root P_x and TRP was investigated by impaling roots of intact maize seedlings with the xylem pressure-potential probe (Wegner & Zimmermann 1998). Part of an experiment, showing the typical response of these parameters to light, is depicted in Fig. 2. At a low photon flux density (about 10 μmol m^{-2} s^{-1}), P_x was below atmospheric (0.055 MPa; 0 MPa = vacuum), and the TRP was –19 mV. When irradiation was increased to 250 μmol m^{-2} s^{-1} (here at $t = 402$ min), the result was a hyperpolarization of the xylem (relative to bath) and a decrease in the P_x. Interestingly, the response of the TRP started within seconds of the increase in the photon flux density, while light-induced changes in the P_x were observed only after a ~3 min delay (Fig. 2, inset). After passing through a minimum of –22 mV, the electrical potential fluctuated somewhat, until slow depolarization commenced. It should be noted that in other experiments (not shown here), a more persistent hyperpolarization was observed.

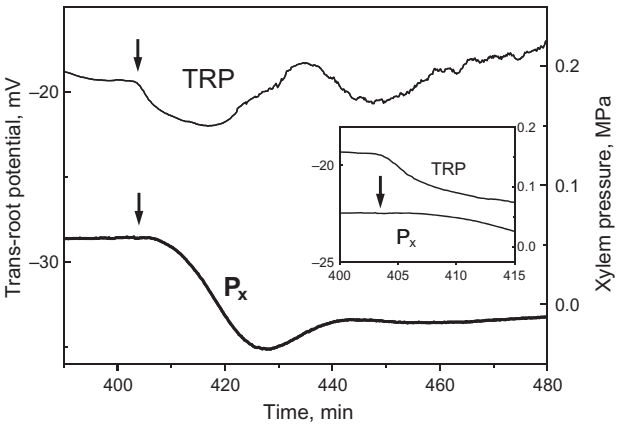

Figure 2. Part of an experiment showing light-induced changes in the xylem pressure (P_x) and trans-root potential (TRP) measured in the root of a 17-day-old maize seedling (*Zea mays* cv. Zelltic). The root was impaled 26 mm below the seed (total root length was 226 mm). Increase of photon flux density from about 10 to 250 μmol m^{-2} s^{-1} is marked by arrows. The temperature and relative humidity were 18 °C and 35 %, respectively. The inset shows the kinetics of parameters around the increase of light irradiation at an enlarged scale.

The light-induced drop in P_x was on average 0.14 ± 0.07 MPa (mean value ± standard deviation for $n = 11$; ~0.09 MPa in the example shown in Fig. 2); subsequently, P_x partly relaxed back to a new steady state (–0.012 MPa in Fig. 2). Except for the onset of the light-induced P_x and TRP changes, the time course of both parameters was quite variable. No correlation between the amplitudes of P_x and TRP changes was observed.

Net ion fluxes at the root surface are rapidly affected by shoot illumination

The effect of changing light regimes on ion uptake by roots and release was studied in MIFE experiments by measuring light-induced kinetics of net K^+ and H^+ fluxes from the mature zone of maize roots. Onset of illumination (at time zero in Fig. 3a) caused rapid (within 1–2 min) changes in net ion fluxes, reducing net H^+ efflux (from –18 to –12 nmol m^{-2} s^{-1}; Fig. 3a) and reversing K^+ from net uptake (about 2 nmol m^{-2} s^{-1}) to efflux (about –3 nmol m^{-2} s^{-1}; Fig. 3b). A very strong (significant at $P < 0.01$) correlation between the kinetics of H^+ and K^+ fluxes was observed (Fig. 3c). After long-term exposure to light (>1 h), H^+ efflux recovered back to its original values; K^+ uptake was significantly higher (32 ± 2.7 nmol m^{-2} s^{-1}; $n = 6$) than in darkness, indicating that long-term effects of light overruled the initial response of net K^+ flux.

Root cutting affects P_x, TRP, and cortical membrane potential

In another set of experiments, TRP and root P_x kinetics were measured in response to cutting. After the root of an intact maize seedling had been impaled with the xylem pressure-potential probe close to the root base (1.1–3.5 cm away from the seed), the root was cut either below (i.e. towards the apex) or above the site of impalement at distances varying from 0.7 to 3 cm. A typical example is shown in Fig. 4. Insertion of the probe tip in a vessel is again indicated by a rapid drop in pressure to values below vacuum. After about 5 min, a constant value of –0.074 MPa (with respect to vacuum) had been obtained. The electrical potential recorded by the probe relaxed from –40 mV (as measured during penetration of root tissue) to a less negative value (–20 mV) during this time. At t = 12 min, the root was excised 1.5 cm above the site of impalement; resulting in a rapid equilibration of pressure with the atmosphere, since the impaled xylem conduit made direct contact with the ambient medium via the cut root surface. Cutting also induced a biphasic response of the TRP; a transient hyperpolarization of the vessel by about 21 mV was observed – followed by a slower depolarization – in this experiment, more positive of the TRP value measured before cutting. After about 10 min, a new stable value of +12 mV was established. When the probe was subsequently removed from the root tissue, the electrical potential dropped to 0 mV. The amplitude of the rapid hyperpolarization varied somewhat among individual seedlings, but was independent

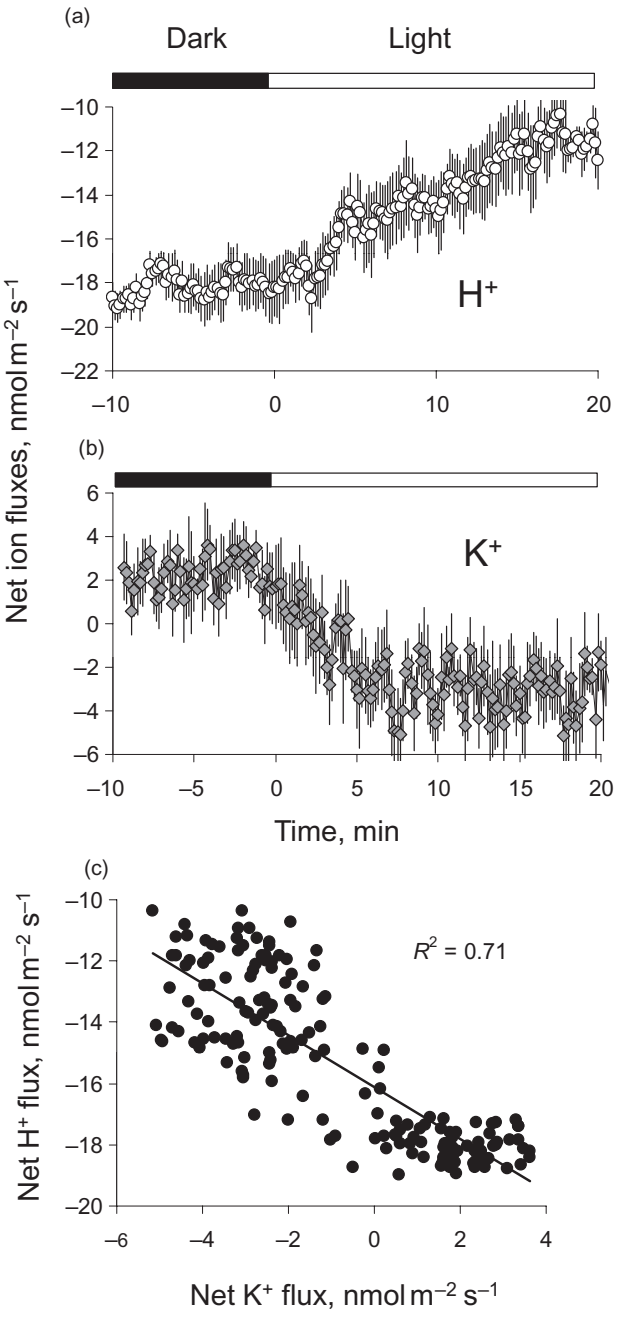

Figure 3. Effect of shoot illumination on net H^+ (a) and K^+ (b) fluxes from 10- to 14-day-old maize roots. Mean ± standard error ($n = 8$). For all ion flux measurements, the sign convention is 'influx positive'. (c) Correlation between light-induced changes in root K^+ and H^+ fluxes shown in panels (a) and (b).

of the position of the cut with respect to the site of impalement (Fig. 4b). Some variability was also observed with respect to the extent of the subsequent depolarization (again, not related to the position of the cut). In five experiments, the steady-state TRP in the cut root was more positive than before cutting (i.e. depolarization exceeded the preceding hyperpolarization); in seven experiments, it was more negative, and in three experiments, the TRP returned to the value measured before cutting. In the majority of the

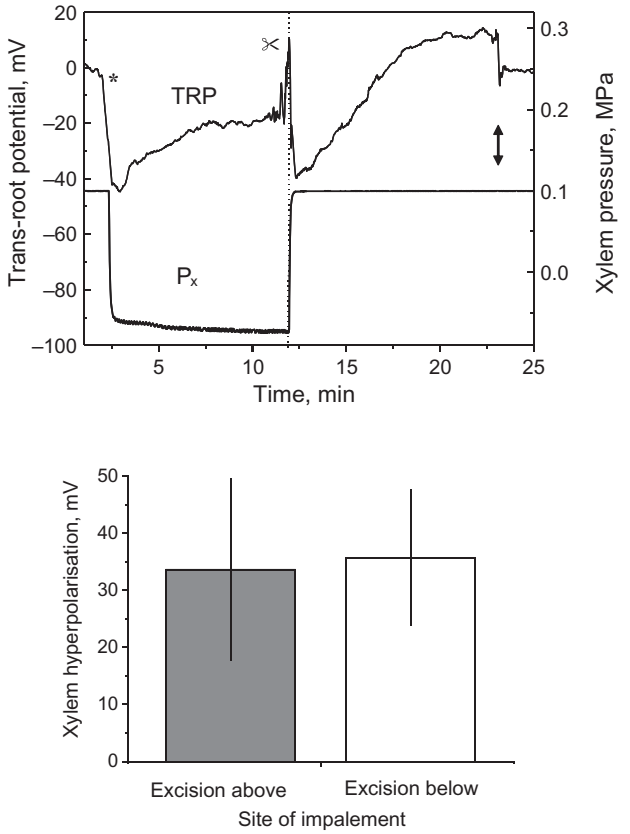

Figure 4. Effect of root cutting on root xylem pressure (P_x) and trans-root potential (TRP). A typical experiment performed on a 15-day-old maize seedling (*Zea mays* cv. Bangui; photon flux density 300 μmol m^{-2} s^{-1}, temperature 26 °C, relative humidity 37%) is shown in (a). The root was impaled 23 mm below the seed (total root length: 258 mm). During penetration of the root tissue, the electrical potential registered by the probe dropped to –40 mV. Insertion of the probe tip into a xylem vessel (asterisk) was registered by a pressure drop from atmospheric level (0.1 MPa) to –0.06 MPa: P_x decreased slightly further to a new steady state value of –0.074 MPa. The corresponding constant TRP value was –20 mV. When the root was cut (see corresponding symbol and dotted vertical line; TRP fluctuations result from handling of the scissors within the Faraday cage) 8 mm below the seed (i.e. above the site of insertion) pressure increased rapidly to atmospheric level. Concomitantly, a transient hyperpolarization of the xylem by about 21 mV was observed, followed by a steady depolarization to about +12 mV. When the probe tip was removed from the root (double-headed arrow), the voltage returned to 0 mV. (b) The amplitude of the initial hyperpolarization registered by the probe (mean ± SD) was independent of whether the root was excised above ($n = 7$) or below ($n = 8$) the site of impalement (i.e. towards the seed or the root tip, respectively). Cutting was performed at a distance of 7–30 mm away from the position of the probe tip, close to the root base.

experiments, a stable positive offset value with respect to the bath electrode prevailed after excision. Interestingly, this value was more positive in the stump that remained attached to the shoot (23 ± 7 mV, $n = 8$) than in the excised root below the cut surface (4 ± 9 mV, $n = 7$; significant at $P < 0.001$). It should also be mentioned that the characteristic response of P_x and TRP was restricted to the first cut. A second or third cut closer to the site of impalement did not elicit a similar response (data not shown).

The TRP is an integrative parameter that reflects changes in membrane potential of both stelar and cortical cells (De Boer, Prins & Zanstra 1983; Wegner *et al.* 1999). Conventional microelectrode experiments were performed to elucidate the potential contribution of changes in cortical and root epidermal membrane potential to the response of the TRP to root excision. When maize roots were cut at the base (refer to Fig. 1a), an instantaneous depolarization of about 10 mV was observed (Fig. 5a). The membrane potential values peaked at about 5 min after the cutting and then gradually recovered (although not to initial values) within the next 20–30 min.

Membrane depolarization upon root cutting was also observed in barley seedlings. In Fig. 5b, steady-state membrane potential values of cortical cells are compared between intact roots (refer to Fig. 1b) and root segments (refer to Fig. 1e) isolated about 2 h prior to measurements. As evident from this figure, epidermal cells in isolated root

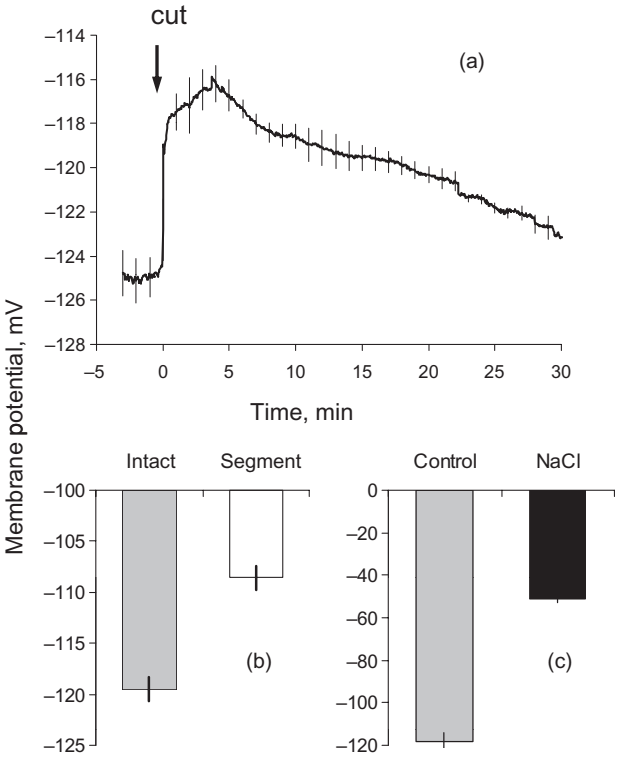

Figure 5. (a) Effect of basal root cutting on membrane potential of epidermal root cells in maize plants. Mean ± standard error ($n = 4$). (b) Steady-state membrane potential values of epidermal root cells of 4-day-old barley (cv. Gairdner) seedlings measured in intact plants (shaded bar) and excised root segments (open bar; refer to Fig. 1e). Mean ± SE ($n = 29$ and 24, respectively). (c) Steady-state membrane potential values of epidermal root cells of 4-day-old intact barley (cv. Gairdner) seedlings measured under control conditions (grey bar) and after 30 min of treatment with 100 mM NaCl (black bar). Mean ± SE ($n = 10$).

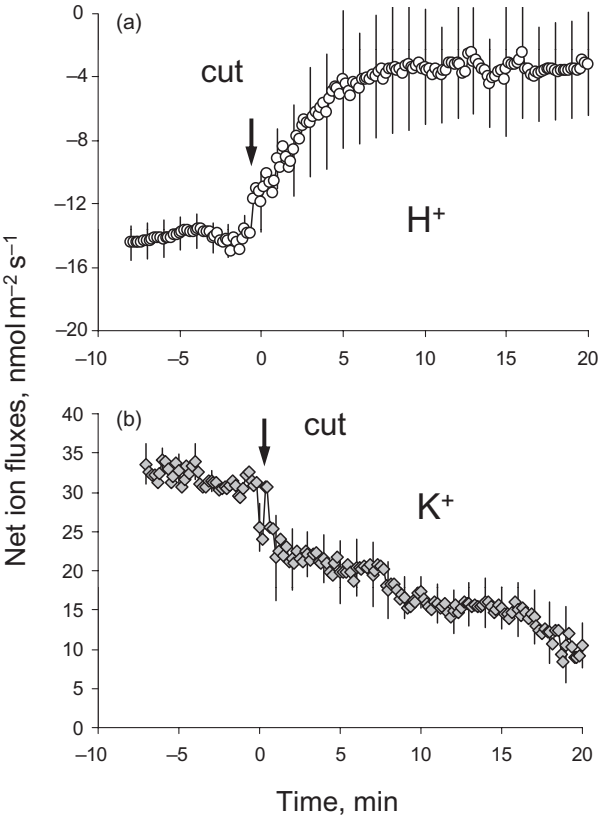

Figure 6. Effect of basal root cutting (arrows) on net H^+ (a) and K^+ (b) fluxes from roots of 10- to 14-day-old maize plants. Mean ± standard error ($n = 5$). Plants were incubated in BSM solution before and during measurements.

segments were depolarized by ~10 mV at the time of measurement (–108 ± 1.2 versus –119 ± 1.3 mV; $n = 24$–29; significant at $P < 0.01$). As salinity treatment apparently interfered with the effect of cutting on ion transport at the root surface (as shown below in Fig. 7), the effect of high external NaCl concentrations on the cortical membrane potential was studied. Salinity-induced depolarization was much more pronounced than the depolarization induced by root cutting, with ~70 mV depolarization measured after a 30 min treatment with 100 mM NaCl (Fig. 5c). A similar response was also reported for sunflower roots, indicating that this phenomenon is more widespread among higher plants (Cakirlar & Bowling 1981).

Root excision reduces proton efflux and net K^+ uptake under low-salt conditions

In addition to its strong impact on electrical and hydraulic driving forces, root excision also affected ion fluxes at the root surface, both in barley and maize seedlings. In maize, cutting the root close to the base resulted in an almost immediate (within the time resolution of the MIFE system) reduction in the magnitude of H^+ efflux (from –14.5 ± 1.2 to –3.5 ± 2.9 nmol m^{-2} s^{-1}; $n = 5$; $P < 0.01$; Fig. 6a) and a rapid decline in net K^+ uptake (from 32 ± 2.3 to 10 ± 3.8 nmol m^{-2} s^{-1} within 20 min; Fig. 6b). These experiments were conducted after prolonged (>1 h) shoot illumination. Similar results were obtained for barley. Here, cutting even reversed the direction of K^+ fluxes from uptake to release (Fig. 8). Results were similar for the two different genotypes investigated. H^+ flux was not measured.

Interestingly, the observed effects of root excision were absent when maize roots were exposed to high (100 mM NaCl) salinity levels. This is further illustrated in Fig. 7. Maize root pretreatment in saline solution for 2–2.5 h resulted in a much higher rate of H^+ efflux (presumably the result of NaCl-induced activation of H^+ pumping; Bageshwar *et al.* 2005; Janicka-Russak & Klobus 2007) (Fig. 7a) and K^+ efflux at a low rate (Fig. 7b). However, cutting off the root base had no effect on either K^+ or H^+ fluxes when measured at high light irradiance (Fig. 7).

Root segmentation experiments on barley suggest a role of the apex in maintaining ion fluxes at the root surface

It remained to be answered whether the changes in ion fluxes elicited by root cutting at the base in standard medium were specific to the position of the cut, or if any violation of root integrity might have similar detrimental

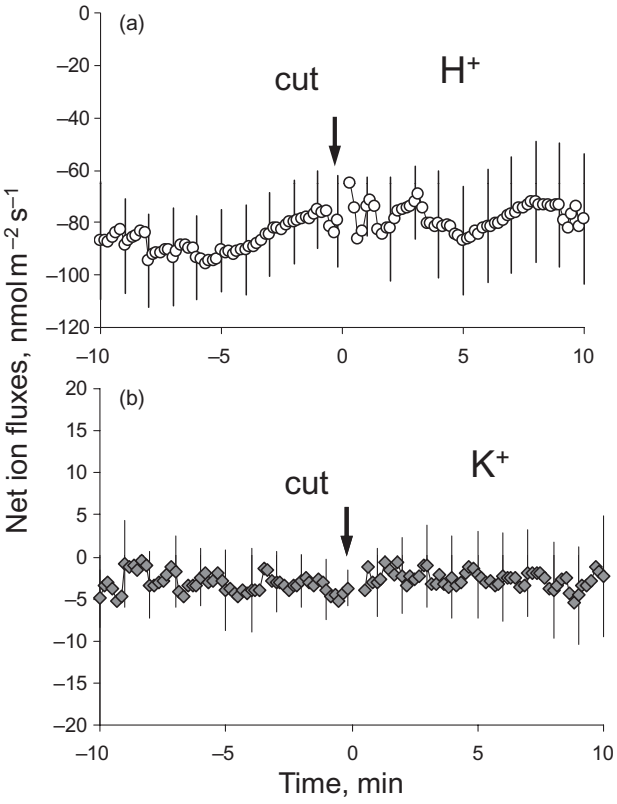

Figure 7. Effect of root cutting (arrows) is absent when maize plants were pre-incubated in BSM solution containing 100 mM NaCl for 2–3 h. Mean ± standard error ($n = 5$). All other details are as in Fig. 6.

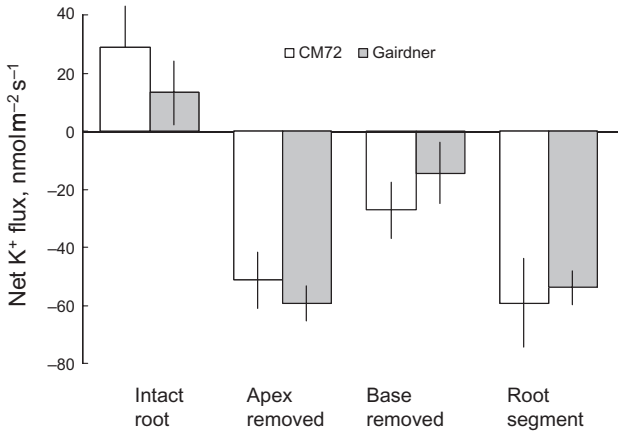

Figure 8. Steady-state net K^+ fluxes measured from intact and excised 4-day-old barley roots (refer to Fig. 1b–e for details). Mean ± standard error ($n = 6$–9). Data for two different genotypes, cv. CM72 (open bars) and Gairdner (shaded bars), are shown.

effects on root nutrient acquisition. Accordingly, experiments were conducted on excised barley root segments, comparing the effects of basal and apical root removal (and their combination; compare Fig. 1) with intact roots. These results are summarized in Fig. 8.

When fluxes were measured from intact roots (refer to Fig. 1b), both genotypes showed net K^+ uptake of between 15 and 30 nmol m^{-2} s^{-1} (Fig. 8), that is, values were in the same order of magnitude as those measured on maize roots after prolonged illumination (Fig. 6). Removal of the root apex (a 7 mm segment from the root tip; refer to Fig. 1c) resulted in a significant ($P < 0.01$) net K^+ efflux of around 50 nmol m^{-2} s^{-1} measured several hours after excision; it was significantly larger (by more than 20 nmol m^{-2} s^{-1}; $P < 0.05$) than the efflux elicited by the excision of the root base. It should be emphasized here that the observed efflux induced by removal of the apex was measured far away (>5 mm) from the site of excision. Moreover, it was steady, so not caused by the transient wounding response. Additional support for the above statement may be found in the fact that the observed K^+ efflux was strongly (>80%) suppressed after 60 min exposure to 20 mM TEA, an inhibitor of K^+-selective plasma membrane channels (data not shown).

Interestingly, net K^+ efflux measured from isolated root segments of both genotypes (refer to Fig. 1e) closely equalled that measured after excision of the root apex only.

Exogenous application of kinetin rescues K^+ uptake in excised root segments

Keeping in mind the fact that the root apex is an important source of cytokinins and previous reports of interactions between cytokinins and plant K^+ nutrition (Green & Muir 1979; Abutalybov *et al.* 1980; Abutalybov & Akhundova 1982; Alizade, Akhundova & Alieva 1988; Alizade *et al.* 1990), effects of exogenous cytokinin application on root K^+ fluxes were studied. Isolated root segments (lacking the apex and hence the source of endogenous cytokinins) were used. Net K^+ fluxes were measured from isolated barley (cv. Gairdner) root segments (as depicted in Fig. 1e) in response to μM concentrations of kinetin (a synthetic cytokinin). These results are summarized in Fig. 9. Adding 2 μM kinetin to isolated segments resulted in an immediate increase in net K^+ uptake, not only preventing initial K^+ efflux, but also resulting in a substantial (~250 nmol m^{-2} s^{-1}) K^+ uptake, 15–20 min after the treatment (Fig. 9a). The effect of kinetin on net K^+ fluxes showed a clear dose-dependency (Fig. 9b), with the optimal concentrations between 2 and 4 μM. Higher kinetin concentrations appear to be adverse (or even toxic), and concentrations below 0.5 μM were not capable of preventing the initial K^+ efflux (Fig. 9b).

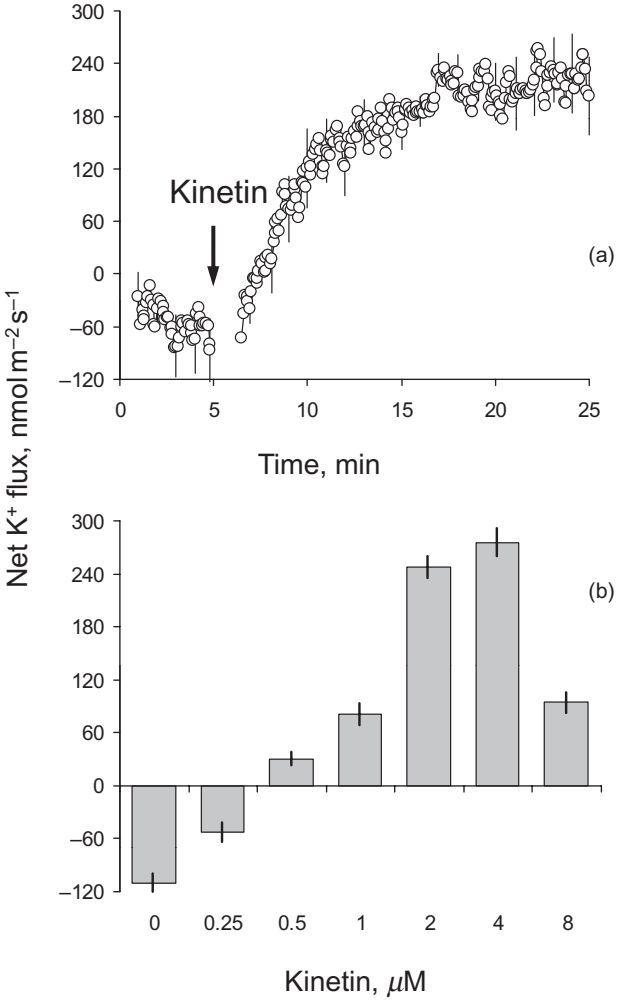

Figure 9. Effects of exogenously supplied kinetin on net K^+ fluxes from excised barley root segments (refer to Fig. 1e). (a) Transient ion flux responses to addition of 2 μM kinetin (as indicated by an arrow). Mean ± standard error ($n = 5$). (b) Dose-dependence of kinetin effects on net K^+ fluxes from excised barley root segments.

DISCUSSION

Using intact plants, we have demonstrated that the photon flux density to which the shoot is exposed has a strong impact on root ion transport as well as on the electrical and hydraulic properties of the root. These parameters respond within a range of seconds to minutes to abrupt changes in the light regime. This light effect demonstrates that the shoot tightly controls ion transport in the root almost instantaneously. Consistently, the removal of the shoot by cutting the root at its base had a rapid (and lasting) effect on the measured parameters (including net K^+ and H^+ fluxes). The effect of cutting was even more dramatic when the root apex was severed. Taken together, these findings strongly argue against the use of root segments or excised roots as a model system to study transport processes and related physiological phenomena occurring in the intact plant. Notably, our results do not discredit experiments on excised roots and root segments *per se*; however, transfer of results to the intact plant have to be performed with extreme care. Thus, working with intact plants should be preferential whenever possible.

Rapid regulation of root ion transport by light in intact seedlings may be mediated by an electrical signal transmitted from the shoot to the root

Exposure of the shoot to a stepwise increase in the photon flux density resulted in a rapid, transient decrease in net proton efflux from the root tissue, while K^+ flux reversed from influx to efflux (Fig. 3). After about 1 h, the proton efflux had recovered, and K^+ flux had reverted back to influx again at a level significantly higher than under the low light regime. A likely explanation for the observed short-term effects of light is that a decrease in proton pump activity, the primary event, leads to the activation of voltage-gated, outward-rectifying cation channels in root epidermal and cortex cells. Previous studies dealing with the effect of the light regime on ion exchange by the root with its environment reported slow responses on a time scale of several hours. Schubert & Mengel (1986) found that acidification of a bath medium by maize roots, mostly reflecting H^+ ATPase activity, almost ceased when maize seedlings were shaded for 7.5 h. Correspondingly, shading the shoot resulted in a depolarization of maize root cortex cells (Schubert & Mengel 1986; see also Graham & Bowling 1977). Interestingly, the energy charge of the root tissue did not decrease significantly within this timescale, suggesting that the decreased proton pump activity was not the result of a reduced ATP level. No significant long-term effect of the light regime on H^+ efflux was observed in this study, possibly because the dark and light periods were not long enough to observe this effect (up to 2.5 h as shown in their experiments). However, we measured an increase in K^+ uptake after long-term exposure to light (>1 h) with respect to low-light conditions. A dependency of nutrient acquisition on the light regime has also been reported for other species (Clement *et al.* 1978; Rufty, Volk & MacKnown 1987; Rufty, MacKnown & Volk 1989; Le Bot & Kirkby 1992; Macduff & Jackson 1992).

Although our results do not allow us to postulate a detailed mechanism of rapid light-induced shoot-to-root signalling, some important conclusion can be drawn. K^+ efflux started to respond to switching on the additional light source within 1 min, indicating that the signal is transmitted over a timescale of seconds. This is probably too fast to be transmitted by any chemical signal (e.g. phytohormones, as suggested by Schubert & Mengel 1986). The signal is also not likely to be of a hydraulic nature, as P_x responds with a delay of about 3 min with respect to an increase in light intensity (i.e. *after* changes in ion fluxes manifest themselves; Fig. 2, insert), and P_x responds synchronously in both the root and the shoot (Wegner & Zimmermann 1998). As shown in Fig. 2 (insert), however, the TRP became more negative within seconds after changing the light regime. The electrical response was well ahead of the response in P_x, indicating that an electrical signal is passing from the shoot to the root. A similar effect was reported earlier by Wegner & Zimmermann (1998), who attributed it to the shift in the electrical charges from the xylem to the photosynthetic tissue in the leaf. However, it was found later that the electrical resistance of the xylem conduit is much higher than initially thought (due to low electrolyte concentrations; Wegner & Zimmermann 2002 and data not shown). Another possible alternative is that such a signal is passed through the phloem (van Bel 2003; Volkov *et al.* 2004). This issue should be addressed in a separate study.

Cutting of the root initiates a complex pattern of electrical (and related hydraulic) responses that may serve as a signal for regulation of proton extrusion and K^+ transport

Interestingly, cutting the root at the base had the same effect on net H^+ flux as an increase in light intensity: H^+ efflux decreased almost immediately upon root excision. Simultaneously, net K^+ uptake turned to net K^+ release, both in maize and barley seedlings. This response pattern is likely to involve a reduction of proton pump activity as suggested previously by Davidian, Soler & Grignon (1984) for barley and Gronewald & Hanson (1980) and Chastain & Hanson (1982) for maize. In the maize studies, it was shown that the effect of root cutting on H^+ fluxes was overcome by adding fusicoccin to the medium, supporting the idea that proton pump activity is involved. Consistent with this interpretation, we recorded a cutting-induced depolarization of cortical cells, several centimetres away from the cut surface. Our results are, at the first glance, at variance with those obtained by Huang *et al.* (1992) on wheat seedlings; these authors did not observe any effect of cutting when uptake of ^{86}Rb (used as a tracer for potassium) was measured. This can be explained by the fact that the ^{86}Rb technique measures only unidirectional ion uptake, while *net* K^+ flux was measured in our experiments. Earlier, we reported that depolarization-activated outward-rectifying K^+ channels

(KOR) were responsible for the K^+ efflux in plant roots in response to a variety of stress factors such as salinity (Shabala *et al.* 2006; Shabala & Cuin 2008), waterlogging (Pang *et al.* 2007) and oxidative stress (Cuin & Shabala 2007). Thus, the absence of any effect of root cutting on ^{86}Rb uptake in the experiments of Huang *et al.* (1992), combined with measured net K^+ efflux in MIFE experiments, may be interpreted as evidence for the involvement of KOR channels in response to root severing, provided that wheat and maize behave in the same way to root cutting.

Again, measurements with the xylem probe technique provided some clues concerning the signal transduction pathway from cutting to changes in membrane transport observed in maize and barley. Cutting resulted in a sudden, rapid release of xylem tension, and pressure relaxed from below-atmospheric values to the atmospheric level. It is tempting to speculate that this dramatic pressure increase (or rather, the concomitant decrease in radial water flow; Wegner & Zimmermann 2008), serves as a primary signal in the transduction chain that leads to a modulation of membrane transport in root cortical and epidermal cells. Nonetheless, it has to be kept in mind that an increase in light intensity and root cutting affects root H^+ and K^+ fluxes in the same way (at least in the short-term range), but have an *opposite* impact on P_x (a light-induced pressure decrease versus an increase in pressure due to cutting). Moreover, P_x was even more negative (hence, the pressure jump more dramatic) in the presence of 100 mM NaCl (unpublished data), but no change in H^+ and K^+ fluxes occurred upon cutting under these conditions. These observations argue against a 'simple' hydraulic mechanism. Cutting of the root induces complex, highly coupled hydraulic and electrical responses that follow a distinct pattern, as revealed by xylem probe measurements and recording of the cortical membrane potential. Interestingly, both light and cutting not only induced similar changes in net H^+ and K^+ ion fluxes, but also elicited a rapid, transient hyperpolarization inside the stele. Further work is required to investigate whether there is a causal link between these phenomena. The response of the TRP to cutting reflected changes in membrane potential of cortical and stelar cells; note that a depolarization of the latter cell type would result in a hyperpolarization of the TRP (De Boer *et al.* 1983). In addition, electro-kinetic phenomena associated with water being swept into the open vessels after a release of tension may contribute to TRP changes induced by cutting. The offset potential measured in the root stumps that remained attached to the shoot after cutting may be explained by transpiration-driven water uptake across the cut surface; the lower offset potentials recorded on excised roots are in accordance with this explanation.

Flux measurements on maize roots reported here were performed at a distance from the cut that was sufficient to avoid interference of ion leakage with the flux measurements, but still within reach of physiological wounding responses. However, at least for barley, it could be shown that the effect of cutting on K^+ fluxes (influx turning to efflux) was steady and not transient, as expected for a typical wounding response (Stahlberg & Cosgrove 1992, 1994). These authors found that cutting through the base of a pea epicotyl immersed in water resulted in a rapid depolarization near the cut, in agreement with our observations. Importantly, in pea this depolarization induced by cutting was found to propagate acropetally with a speed of about 1 mm/sec, so exhibiting classical 'slow-wave potential' characteristics (Stahlberg & Cosgrove 1995, Stahlberg, Cleland & van Volkenburg 2006). Concomittantly, a transient increase in the growth rate was observed. The absence of any propagating depolarization when cutting was performed in air suggested that the signal was transmitted by a hydraulic mechanism that was associated with a surge of water into the vessels when xylem tension was released. Consistently, when the experiment was repeated on cucumber seedlings that build up root pressure, cutting of the hypocotyl elicited a local depolarization close to the cut, but no propagating electrical signal (Stahlberg & Cosgrove 1994). In this case, cutting was associated with the release of an overpressure in xylem vessels. Slow-wave potentials could also be triggered in cucumber after lowering P_x by incubating roots with metabolic inhibitors or mannitol (Stahlberg & Cosgrove 1995). Evidence was obtained that (both local and propagating) depolarization was induced by a transient shut-down of the plasma membrane H^+ pump (Stahlberg & Cosgrove 1992); this is in accordance with a reduction of H^+ efflux reported here for maize roots. It is not clear whether such a wave of depolarization also occurs in maize, and, if so, whether the rate of its propagation is about the same as in cucumber and pea. The observation that the maximum membrane depolarization in response to base cutting occurred 4–5 min after excision (Fig. 5a), about 10–12 cm away from the cut, is in accordance with this scenario.

However, regulation of ion fluxes in maize roots appears not to be affected by hydraulic signals (at least not as the main factor), indicating that the mechanism of signal transduction is different from the one described for pea and cucumber seedlings. The absence of any (transient) decrease in H^+ ATPase activity when the root was excised after addition of 100 mM NaCl to the bath (i.e. at elevated xylem tensions) is also at variance with the signalling processes described by Stahlberg and Cosgrove. In maize roots, saline conditions had even stimulated net proton efflux, but the proton pump was apparently short-circuited by an increase in membrane conductance (Shabala & Cuin 2008); hence, the membrane was in a depolarized state (compare Fig. 5c for barley; see also Cakirlar & Bowling 1981). This may have compromised the sensitivity of the cells to propagating electrical or to chemical signals.

Cytokinins are involved in control of ion uptake by roots

The results of this study suggest that not only cutting the root at the base, but also removal of the root tip, has a pronounced impact on root ion fluxes (Fig. 8). Moreover,

experiments on barley demonstrate that removing the root apex (the first 7 mm) has a much stronger negative implication on K^+ uptake them the removal of the root base (Fig. 8). Cutting in basal or apical direction with respect to the site of measurement had no effect on the hydraulic and electrical response (Fig. 4). This suggests that, in addition to electrical (maybe coupled to hydraulic) signals, some other (most likely, chemical) factors are involved in the control of uptake and radial transport in excised roots. Accordingly, it was tested whether one of these factors could be a cytokinin.

Cytokinins have long been implicated in the regulation of K^+ transport in plants (Green & Muir 1979; Abutalybov *et al.* 1980; Abutalybov & Akhundova 1982; Alizade *et al.* 1988, 1990). However, reported results are often somewhat controversial. For example, it has been shown that exogenously applied cytokinins increase K^+ content in stomatal guard cells (Lechowski 1997). At the same time, other reports have suggested that KAT1, an inward-rectifying K^+ channel expressed in guard cells (providing the main route of K^+ influx into the guard cell) is not activated by kinetin (Mori, Uozumi & Muto 2000). Furthermore, while some reports suggest that kinetin causes an increase in the total ionic concentration of excised roots (Waisel, Neumann & Eshel 1965), there are numerous papers demonstrating that K^+ release into the xylem (as measured by ^{86}Rb release into root exudate) is inhibited by micromolar concentrations of kinetin (Rains 1969; Collins & Kerrigan 1974; Hong & Sucoff 1976). To the best of our knowledge, the only reported evidence for ion channel regulation by cytokinins at the cellular level is a paper by Schumaker & Gizinski (1993) showing the effects of 1,4-dihydropyridine on voltage-dependant Ca^{2+} channels in moss protoplasts. Here, we report that exogenously applied cytokinins (kinetin) stimulates K^+ uptake in plant roots (Fig. 9), most likely by controlling TEA-sensitive K^+ permeable channels in the root epidermis (Fig. 9).

Contrary to all previous reports, the beneficial effects of kinetin on root K^+ acquisition was resolved within a timescale of several minutes, not hours as in previous reports. It appears from our data that kinetin activation of K^+ uptake is a time-dependent process, with the plateau in K^+ uptake observed 5–7 min after exogenous application of kinetin (Fig. 9a). Such a delay suggests that the mode of kinetin action is not likely to be related to kinetin control of, or binding to, some K^+ permeable channel from the apoplastic side. More likely, kinetin is transported into the cell and controls K^+ uptake indirectly. It was suggested earlier that polystimulin K (a synthetic cytokinin) hyperpolarized root hair cells in aquatic plant *Trianea bogotenis*, most likely via stimulating H^+-ATPase activity (Allakhverdiev *et al.* 1992). Assuming that such a scenario is applicable to maize and barley roots, the presence of kinetin in the root apoplast is expected to activate K^+ uptake and/or reduce K^+ leak through hyperpolarization- and depolarization-activated K^+ channels, respectively (Shabala 2003; Very & Sentenac 2003). Another possibility is that cytokinins are competitive inhibitors of hexokinase and pyruvate kinase. These may also be involved in the regulation of K^+ transport (Tuli, Dilley & Wittwer 1964).

There appears to be also a possible connection between cytokinin control of K^+-permeable ion channels in root cells and the effects of light or root excision. It is reported that the amount of cytokinins in root exudates is dependent on day length and light conditions (Kinet 1984). Cytokinins are known to be transported to the shoot in the xylem flow (Beck & Wagner 1994; Beck 1996), and evidence for the basipetal transport of cytokinins has also been presented (Collier *et al.* 2003). All suggests that apoplastic (xylem) cytokinins are an important possible messenger, coordinating root K^+ uptake and adjusting it to the shoot's demands. The removal of the root apex will result in a decrease in the level of endogenous cytokinins, thus resulting in the observed K^+ leak from the root (Fig. 8), and a re-supply of cytokinins (by exogenous application) rescues K^+ uptake (Fig. 9). The specific control modes as well as the identity of the K^+-permeable channels mediating these affects are currently under further investigation.

CONCLUSION AND OUTLOOK

In this communication, we have provided evidence for rapid electrical and hydraulic coupling between the shoot and the root. For net K^+ and H^+ fluxes at the root surface of the mature root zone, we show regulation by the shoot and (in case of K^+ fluxes) by the apical part of the root. Changes in K^+ fluxes induced by severing the root tip can be reversed by adding kinetin, indicating that endogenous cytokinins synthesized in the root tip are transported basipetally and control ion fluxes in the mature root zone.

More work is required to show unequivocally that cytokinins form part of the signal transduction chain by which the root apex exerts control on root K^+ uptake, and to identify the other components of this signal transduction chain. With respect to shoot-to-root communication over such a short timescale, some clues for the (probably multiple) mechanisms by which the shoot intervenes in root ion transport have been provided. Further research will focus on electrical signalling triggered by an increase in light intensity, rather than on purely hydraulic or chemical (hormonal) signals.

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