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Distinct roles of electric and hydraulic signals on the reaction of leaf gas exchange upon re-irrigation in *Zea mays* L.

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ABSTRACT

The hypothesis that electric and hydraulic long-distance signals modify photosynthesis and stomatal aperture upon re-irrigation in intact drought-stressed plants was examined. Maize plants (*Zea mays* L.) were exposed to drought conditions by decreasing the soil water content to 40–50% of field capacity. The decrease in water content resulted in a decline in stomatal conductance to 50–60% of the level in well-watered plants. Re-irrigation of the plants initiated both hydraulic and electric signals, followed by a two-phase response of the net CO_2 uptake rate and stomatal conductance of leaves. The transitional first phase (phase 1) is characterized by a rapid decrease in both levels. In the second phase (phase 2), both parameters gradually increase to levels above those of drought-stressed plants. Elimination of either the hydraulic signal by compensatory pressure application to the root system, or of the electric signal by cooling of the leaf blade gave evidence that the two signals (1) propagated independently from each other and (2) triggered the two-phase response in leaf gas exchange. The results provided evidence that the hydraulic signal initiated a hydropassive decrease in stomatal aperture and for the involvement of electric signals in the regulation of photosynthesis of drought-stressed plants.

Key-words: *Zea mays*; drought stress; electrical signals; photosynthesis; root-shoot communication; stomatal aperture.

INTRODUCTION

The CO_2 uptake of plants occurs through the stomata of the leaves and is accompanied by water loss. Light, carbon dioxide, air humidity and soil moisture affect stomatal aperture (Cowan 1977; Raschke 1979; Schulze *et al*. 1982) in concert with the pH of the xylem sap, inorganic and organic ions as well as phytohormones [e.g. abscisic acid (ABA), Schulze 1994; Wilkinson & Davies 1997]. Drought acts through increases in xylem water tension and pH, which is accompanied by a rise in activated ABA (Zhang & Davies 1990; Davies & Zhang 1991; Felle & Hanstein 2002).

In parallel with biochemical processes, non-chemical signals ensure physiological communication and integration within and between plant organs. Electrical signals, for example, can affect respiration (Dziubinska, Trebacz & Zawadzki 1989), water uptake (Davies, Zawadzki & Witters 1991), activation of proteinase inhibitor genes (Wildon *et al*. 1992), and the gas exchange of leaves (Fromm & Eschrich 1993; Koziolek *et al*. 2004; Kaiser & Grams 2006). Within short distances, such signals mediate pollination (Fromm, Hajirezaei & Wilke 1995) or can trigger rapid movement processes in carnivorous plants (Pickard 1973). Apart from impacts of electrical signals on diverse processes in plants, it has been shown recently that plants also synthesize numerous neuronal molecules and fulfil some criteria for intelligent behaviour (Baluska, Volkmann & Menzel 2005).

The propagation of action potentials depends on ion channels. In willow, channel blockers like tetraethylammonium chloride (TEACl) as well as $LaCl_3$ inhibit the propagation of action potentials induced by electric pulses (Fromm & Spanswick 1993). In guard cells, the sensitivity to electric stimuli, which is mediated through ion channels in the plasma membrane, may provide, therefore, a component involved into stomatal regulation (cf. Fromm & Eschrich 1993; Kaiser & Grams 2006). In drought-stressed maize plants, re-irrigation initiates electrical signals that precede the increase in CO_2 uptake and transpiration (Fromm & Fei 1998). In addition, hydraulic signals have been documented in signalling upon wounding at the leaf level (Malone & Stankovic 1991) or in affecting stomatal width as a function of cell turgor (Raschke 1970b; Cowan 1977; Wei, Tyree & Steudle 1999), for example, by manipulations of root pressure (Gollan, Passioura & Munns 1986) or of the water relations within the root system through 'split-root' experiments (e.g. Yao, Moreshet & Aloni 2001). As shown by Wegner & Zimmermann (1998), both electric and hydraulic signals are generated by changes in irradiance. In intact plants, electric and hydraulic signals, therefore, may be complementary to each other in stomatal regulation.

The present study examined the hypothesis that in intact plants, both electric and hydraulic signals that serve in

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long-distance communication as stimuli in stomatal movement and photosynthesis are initiated. For this purpose, drought-stressed maize plants (*Zea mays* L.) were re-irrigated while examining cell turgor, electric potential, leaf gas exchange and chlorophyll fluorescence. By alternately eliminating each of these signals during experimentation, the extent of their interdependence was examined along with their differential impact on stomatal conductance and photosynthesis.

MATERIALS AND METHODS

Plant material

Plants of *Zea mays* L. var. *Mozart* were grown in a greenhouse, from seeds in pots (3 l; Fruhstorfer Erde, Typ P; Archut, Lauterbach, Germany; photosynthetic photon flux density (PPFD) > 200 μmol m^{-2} s^{-1}, 14/10 h light/dark period; air temperature > 22 °C; relative air humidity fluctuating with outside conditions) under non-limiting water supply. One week prior to experimentation, maize plants of 120–140 cm in height were transferred into a climate-controlled phytotron (York, Mannheim, Germany; temperature 22 °C, relative air humidity of 60%, PPFD 200–300 μmol m^{-2} s^{-1}, 14/10 h light/dark period) for the remainder of the investigations. Measurements were performed on mature, 3–4-week-old leaves.

Leaf gas exchange measurements

Leaf gas exchange was measured using a mini-cuvette system (open gas exchange system with CO_2/H_2O infrared gas analyzer; Walz, Effeltrich, Germany; cf. Matyssek *et al.* 1991a) at a constant CO_2 concentration of 360 μL L^{-1}, relative air humidity of 60%, air temperature of c. 25 °C and PPFD of 300–400 μmol m^{-2} s^{-1}.

Chlorophyll fluorescence

Assessment of chlorophyll fluorescence was performed on fully light-adapted leaves using a Mini-PAM system (Walz). The photochemical quantum yield of photosystem II (PSII) was calculated as $(F'm - F)/F'_m$ (Genty, Briantais & Baker 1989).

Electric potential measurements

Experiments were performed at constant temperature inside a Faraday cage mounted on a vibration-stabilized table in a laboratory. As shown in Fig. 1a, a microelectrode, filled with 100 mM KCl, was inserted into the phloem of a leaf. For identification of the cells measured, Lucifer yellow was loaded into the cell after measurement (Fig. 1b). At 20–25 cm distance, a surface electrode was attached to the shoot and moistened with 100 mM KCl agar to provide an appropriate contact (= ground, Fig. 1a). KCl concentrations > 100 mM resulted in damage of the epidermal cells. Prior to each experiment, both Ag/AgCl electrodes had been calibrated (0 mV) in 100 mM KCl agar and were connected

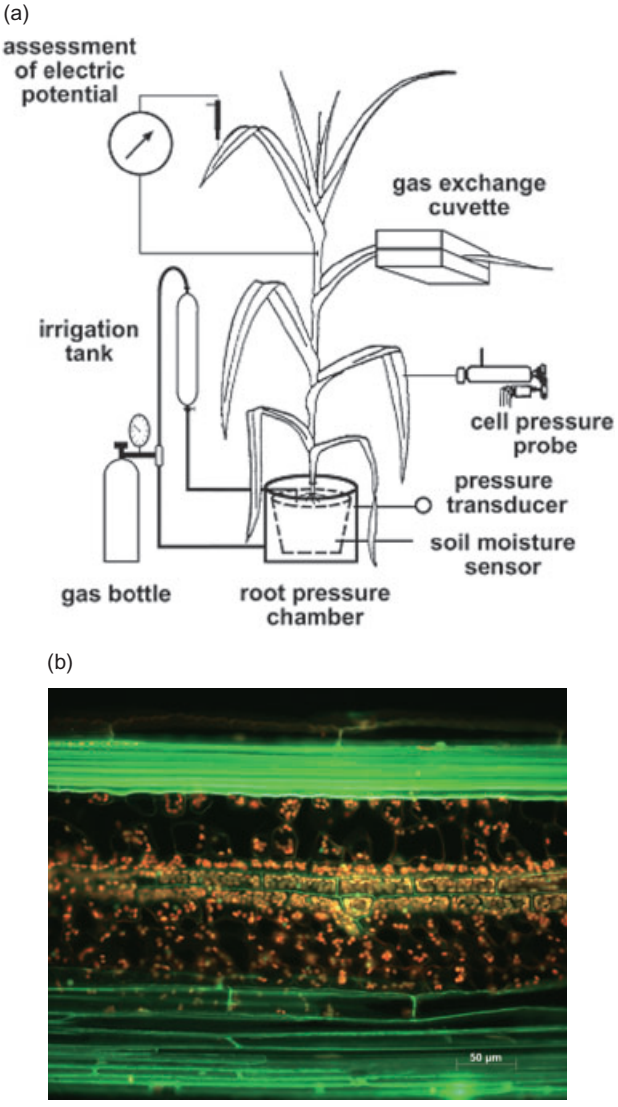

Figure 1. Experimental arrangement of (a) electric potential, turgor and gas exchange assessments on one intact plant. The system allowed re-irrigation under pressure. Phloem cells of a leaf bundle, electrophoretically loaded with Lucifer yellow (b). Dye concentration was 0.1 mM.

to a differential amplifier (model 750; WPI, Sarasota, FL, USA). Data were recorded in parallel by a chart recorder and computer.

Measurement of cell turgor

The turgor of single epidermis cells was measured on abaxial leaf sides at 5–8 cm distance from the leaf tip, using a cell pressure probe (Steudle 1993). Data were recorded in parallel by a chart recorder and a computer. The pressure within the probe was manipulated once a minute before re-irrigation to ensure hydraulic continuity between cell and probe as reflected by induced movements in the cell fluid/oil interface meniscus inside the probe capillary. Re-irrigation of the root resulted in water influx into cells and, therefore, shifts in meniscus position. By displacement

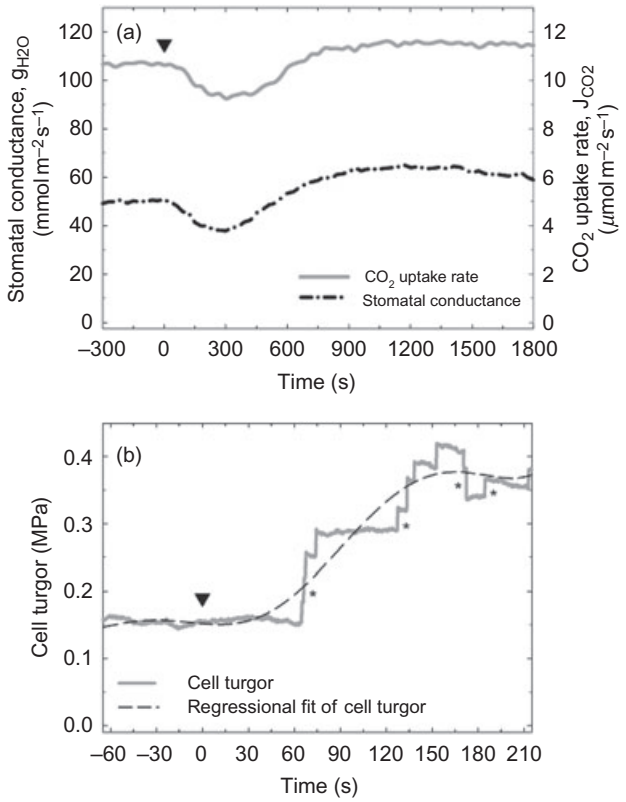

Figure 2. Representative response of (a) the stomatal conductance (g_{H2O}, dotted line) and the net CO_2 uptake rate (J_{CO2}, solid line) and (b) the cell turgor in epidermal cells (MPa, solid line; regression fit, dashed line) of a drought-stressed maize plant to re-irrigation. Arrows denote the instant of re-irrigation at time zero. Asterisks (*) denote stabilization of the meniscus at its initial position.

of the metal rod within the probe, compensatory pressure was applied and, by this, repositioning of the meniscus enabled the assessment of turgor increase (experimental pressure manipulations marked as asterisks, cf. Fig. 2b; procedures according to Steudle 1993). For clarity, the response of the cell turgor was fitted with a regressional function.

Elimination of the hydraulic signal

The root system of each experimental plant was sealed into a root pressure chamber (Fig. 1a; cf. Gollan *et al*. 1986; Passioura 1988). In order to compensate for the hydraulic signal upon re-irrigation, pressure was applied to the root system of a drought-stressed maize plant 1 h prior to re-irrigation to balance the tension of xylem water to zero. For root pressurization, the balancing pressure was determined by removing a leaf from the lower stem section underneath the range of measured leaves prior to the experiments. In the stump of a removed leaf, the xylem meniscus was observable during soil–root pressure manipulation, serving as orientation for the balancing pressure.

Elimination of the electric signal

In order to disrupt the propagation of the electric signal upon re-irrigation, part of the leaf was cooled using two aluminium blocks that were cooled by flushing with a mixture of glysanthin/water (cryostate: Haake K and F3; Haake, Berlin, Germany). In this experiment, both measuring and reference electrode were positioned at the mid part of the leaf while it was cooled at its base. The temperature of the leaf surface was measured with a thermosensor (type: M 4011, ∅: 2 mm; Metrawatt, Mannheim, Germany). The temperature at the leaf surface was cooled to 0.1–0.5 °C, as higher temperatures turned out to be insufficient for disrupting the signal propagation. Care was taken to prevent cooling to below 0 °C, as frost injury manifested as an irreversible breakdown of the gas exchange in the distal section of the leaf blade above the cooling position.

Assessment of soil and leaf water content (lwc)

Soil water content in the pots was measured with an FDR sensor (Theta Probe; UMS, Munich, Germany). For assessing lwc, the three upper mature leaves were harvested in the following way from the same plant: The first leaf prior to the drought period from non-stressed plants, the second leaf after applying drought stress and the third leaf 1 h after re-irrigation of stressed plants. The lwc (in per cent) was calculated according to the equation

$$\text{lwc} = \frac{\text{fresh mass} - \text{dry mass}}{\text{dry mass}} \cdot 100$$

We could not detect any change in dry mass of the leaf within 1 h after re-irrigation.

Statistics

Student's *t*-test was performed using the program Excel, version 1997 (Microsoft, Unterschleißheim, Germany). Experiments were repeated 3 to 11 times. Responses depicted in figures represented a characteristic response of the measured parameters.

RESULTS

Irrigation of one set of plants was suspended until stomatal conductance had decreased substantially in comparison to non-stressed plants (control). Stomatal conductance for water vapour of the non-stressed plants was about 120 mmol m^{-2} s^{-1}, and net CO_2 uptake rate was about 20 μmol m^{-2} s^{-1}. The latter declined, in parallel to stomatal conductance to about 13 μmol m^{-2} s^{-1} in water-deficient plants during progressive drought so that a substantial range in performance resulted between stressed and non-stressed plants. The PPFD of 300–400 mmol m^{-2} s^{-1} as employed during measurements was in the 80–90% range of photosynthetic light saturation, as the cultivation of the plants had occurred in the greenhouse in the absence of high light conditions. In parallel, the turgor of epidermal

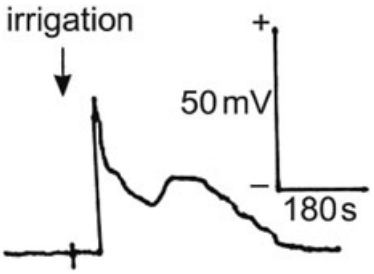

Figure 3. Action potential measured with a microelectrode in the phloem of a mature leaf, induced by re-irrigation of a drought-stressed plant. The arrow denotes irrigation.

cells declined to 0.15–0.2 MPa relative to 0.4–0.6 MPa in the non-stressed control. In parallel, the soil water content had dropped to 40–50% of its field capacity in the drought treatment. When the leaf gas exchange, electric potential and cell turgor had stabilized, the drought-stressed plants were re-irrigated. The electric, hydraulic and leaf gas exchange responses were recorded in parallel with each other prior to and during the first hour after re-irrigation (Fig. 1a). In order to detect electric signals in maize leaves, a microelectrode filled with 100 mM KCl solution was inserted into the phloem of a leaf at 20–25 cm distance from the reference electrode, which was attached to the shoot surface. Both electrodes were connected to a conventional microelectrode amplifier (model 750, WPI). After watering the roots (Fig. 3, arrow), an action potential with an average amplitude of 50 mV and a velocity of 1 cm s^{-1} was evoked and measured in the phloem. By loading with Lucifer yellow, it could be shown that phloem cells were measured (Fig. 1b).

Approximately 60 s after re-irrigation, the CO_2 uptake rate (J_{CO2}) and stomatal conductance (g_{H2O}) displayed a transient decrease that preceded a gradual increase (Fig. 2a) up to enhanced levels relative to those of the initial drought conditions. Nevertheless, the levels of the non-stressed control plants were not reached during the first hour after re-irrigation. In contrast, the photochemical quantum yield of PSII (as derived from the analysis of chlorophyll fluorescence) did not significantly respond to re-irrigation (data not shown). A distinct increase, however, was observed in the turgor of epidermal cells upon re-irrigation (Fig. 2b). The hydraulic and electric signals appeared significantly earlier in the leaf than the initiation of the responses in gas exchange.

In comparison with experiments under atmospheric pressure, re-irrigation experiments were conducted while either eliminating the hydraulic signal through compensatory pressure application to the root system (Fig. 1a) or disrupting the propagation of the action potential through cooling of the measured leaf. A pressure of 0.4–0.5 MPa was applied to the root system to compensate for the hydraulic signal upon re-irrigation. Pressure enhancement by itself caused a transitory decrease of J_{CO2} and g_{H2O} that was followed by a minor increase in g_{H2O} (data not shown). Cell turgor reflected an increase by 0.2–0.4 MPa upon pressure application that resulted from water influx into cells, similar to re-irrigation under atmospheric pressure, although the soil water content was not yet changed. After J_{CO2} and g_{H2O} of drought-stressed plants had stabilized at a new, elevated level at about 1 h after pressurizing the roots, the plants were re-irrigated. When the applied root pressure completely balanced the hydraulic xylem tension, g_{H2O} and J_{CO2} only displayed an increase (Fig. 4a), and no change in cell turgor was observed (data not shown). If the applied root pressure was insufficient to compensate for the hydraulic xylem tension, a small increase in the cell turgor was observed. This effect was followed by a slight, transitory decrease in J_{CO2} and g_{H2O} prior to eventual increase (data not shown). Re-irrigation induced an action potential in the leaf phloem similar to the effect of irrigation under atmospheric pressure.

To eliminate the action potential, the leaf was cooled 10 cm basipetally to the position of the microelectrode and the reference electrode, which was also attached to the leaf in this experiment. Cooling the surface of the leaf per se did not affect J_{CO2} and g_{H2O}. Remarkably, a small but still significant transitory decrease in g_{H2O} was observed upon re-irrigation ($n = 10$, $P = 0.014$, Fig. 4b), whereas in J_{CO2}, no apparent response was detected. Stomatal conductance restabilized at its initial level. While cooling disrupted the propagation of the action potential, the hydraulic signal was not affected and the turgor began to increase upon

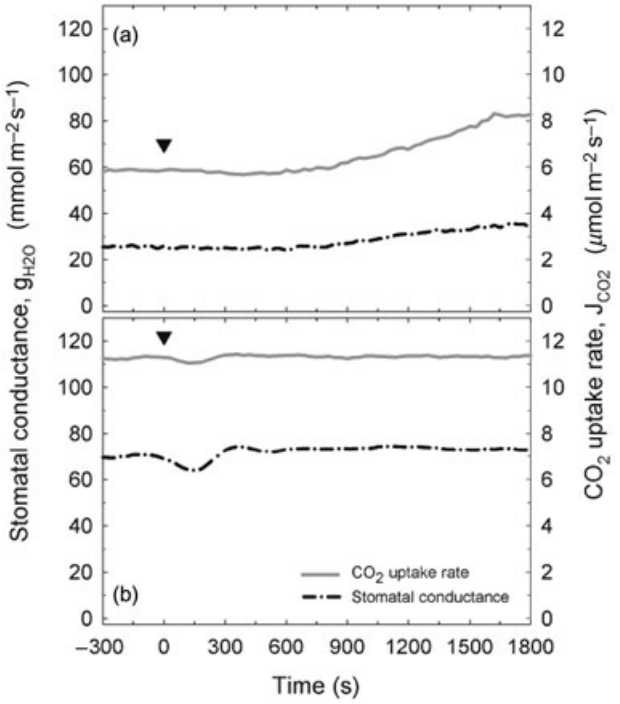

Figure 4. Response of the net CO_2 uptake rate (J_{CO2}, solid line) and the stomatal conductance (g_{H2O}, dotted line) to re-irrigation under enhanced pressure in the root pressure chamber (a) and to re-irrigation during leaf cooling. (b) Transient decline in stomatal conductance upon re-irrigation was statistically significant ($P = 0.014$). Arrows denote the instant of irrigation at time zero.

re-irrigation (data not shown). Hence, the two signals were propagated independently of each other. The elimination of the hydraulic signal did not affect the phase 2 response of the gas exchange (prolonged increase of both g_{H2O} and J_{CO2}); neither did the elimination of the electric signal affect the instance of the phase 1 response (transitory decrease of both g_{H2O} and J_{CO2}).

In addition, the lwc was detected before and after re-irrigation. It displayed a significant decrease upon drought stress. Non-stressed plants showed an lwc of 471 +/– 43%, while drought-stressed plants had only an lwc of 298 +/– 35% ($n = 3$, $P = 0.01$). One hour after re-irrigation, a significant increase in the lwc to 439 +/– 43% was observed ($n = 3$, $P < 0.05$).

DISCUSSION

The hypothesis posed at the beginning was confirmed in that, in intact maize plants, both electric and hydraulic signals were initiated and did serve in long-distance root–shoot communication as stimuli with two distinct roles in photosynthesis and stomatal aperture. The two signals were initiated in the root upon re-irrigation after drought and propagated independently of each other, the hydraulic one through the xylem (being compensated for by pressure application to the root system; cf. Munns *et al*. 2000), and the electric one through the phloem (being eliminated by cooling of the leaf). Both signals arrived within less than 40 s in the leaves, significantly prior to the initiation of the stomatal response (after about 60 s).

Upon re-irrigation, a two-phase response in the leaf gas exchange was observed: a transitory, rapid decrease of J_{CO2} and g_{H2O} (phase 1) that was followed immediately by a gradual increase to levels higher than those achieved under drought (phase 2). Phase 1 was ascribed to the impact of the hydraulic signal on the leaf epidermis, where, because of the specific tissue mechanics ('mechanical advantage of subsidiary cells'; cf. Cowan 1977), a turgor increase in both epidermal and guard cells will induce a temporary, hydropassive movement, that is, partial closure of the stomata ('inverse Iwanoff Effect'; Raschke 1970a). Hydraulic signals have the capacity of rapidly spreading through plants and interfering with the cell metabolism, for example, as reflected by intermittent disruption of elongation growth (Matyssek, Maruyama & Boyer 1991b; Tang & Boyer 2003) or sudden upward movement of the water column in the trunks of tall, severely drought-stressed trees upon irrigation (Cermak, Matyssek & Kucera 1993). Consistently, the re-irrigation of maize plants released a pressure pulse through the xylem vessels upon water influx into the roots that was responsible for the turgor increase of the epidermal and guard cells. The 'phase 1' response in the leaf gas exchange upon hydraulic impact through re-irrigation appears to be mediated via the apoplast, which can proliferate water flux rather rapidly (as opposed to rehydration processes in the leaf via the symplast: Westgate & Steudle 1985). Relative to the time of re-irrigation, the hydropassive closure of stomata was delayed c. 60 s, which may be explained by (1) a time lag in water uptake by the roots and (2) a damping of the xylem turgor increase in the leaf lamina. The 'phase 2' response of the gas exchange of maize leaves upon re-irrigation was initiated through an action potential, which was propagated through the phloem and in the absence of a hydraulic pulse. The action potential was required to induce the gradual recovery of J_{CO2} and g_{H2O}, probably through the involvement of physiological processes (as opposed to the phase 1 response). A link between electric signals and photosynthetic responses was also demonstrated in recent studies on signal transmission in *Mimosa pudica* (Koziolek *et al*. 2004; Kaiser & Grams 2006) and poplar (Lautner *et al*. 2005).

In summary, electric and hydraulic signals between root and shoot turned out to have distinct roles on photosynthesis and stomatal aperture in intact maize plants. The hydraulic signal initiated the hydropassive decrease in stomatal aperture, while electric signals provoked the subsequent physiological control of net CO_2 uptake upon re-irrigation under drought stress. The rapid, independent generation and propagation of electric and hydraulic signals in the root system enables maize leaves to respond rapidly to increasing soil moisture. This appears to be ecologically meaningful for C_4 plants (such as maize) that had their evolutionary origin in water-limited habitats.

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