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Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function

Micaela Carvajal, David T. Cooke, David T. Clarkson

IACR-Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol BS18 9AF, UK

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Abstract. The sap flow (Jv) and the osmotic hydraulic conductance (L_0) of detached, exuding root systems from wheat (*Triticum aestivum* L. cv. Chinese Spring) plants deprived of nitrogen for 5 d (− N) or of phosphorus for 7 d (− P), were measured and compared with controls receiving a complete nutrient supply. In the roots of − N and − P plants, Jv and L_0 decreased markedly, but between 4 and 24 h after resupplying N to − N plants (NRS plants) and P to − P plants (PRS plants), Jv and Lo recovered to values similar to those of control plants. Values of Jv and L_0 were always greater during the light period than during the dark, due to the diurnal variation of these parameters. Reducing transpiration in the light had no effect on Jv and L_0 of − N and − P plants. Sap flow and L_0 were also determined using individual axes from plants which had been grown with their roots divided between nutrient-deficient (− N or − P) solution and a complete nutrient solution. Differences were observed in Jv and L_0 between axes of the same plant, but stomatal conductance (Gs), which was also measured, was not affected in these split-root experiments. In control plants, Jv and L_0 declined sharply to values similar to those of roots from − N and − P plants after $HgCl_2$ treatment (50 μM), but were restored by treating with 5 mM dithiothreitol. In plasma membranes from − N and − P roots, the amount of stigmasterol increased relative to sitosterol compared with control roots. The degree of unsaturation of bound fatty acids also increased, compared with controls, as a result of a decline in the relative amounts of 16 : 0 and 18 : 0 and an increase in 18 : 2. Plasma-membrane fluidity, estimated by steady-state fluorescence polarisation using 1,6-diphenyl hexatriene, showed that the plasma membranes from nutrient-deprived plants were less fluid than those from control plants, measured during both the light and dark periods and in split-root experiments. In NRS plants, the relative abundance of sitosterol increased, so that the stigmasterol/sitosterol ratio returned to a value similar to that of controls. However, in PRS plants, the difference in stigmasterol/sitosterol ratio was maintained, compared with controls. The degree of unsaturation of bound fatty acids, membrane fluidity and the hydraulic conductivity of root systems also recovered in NRS and PRS plants to values similar to those of control plant plasma membranes. The results obtained suggested that − N and − P treatment decreased L_0, by reducing either the activity or the abundance of Hg-sensitive water channels. Also, there may be an interaction between the increase in membrane lipid ordering and the decrease in L_0.

Key words: *Triticum* – Hydraulic conductance – Membrane fluidity – Mercury sensitivity – Nutrient deficiency (N, P) – Water channel

Introduction

It has been observed that nutrient deficiences decrease the osmotic hydraulic conductance (L_0) of roots. For example, the L_0 of barley roots, which had been deprived of SO_4^{2-} for 4 d, was only 20% of that of a sulphate-replete control (Karmoker et al. 1991). In cotton roots, after 10 d of N deficiency, L_0 declined to 6% of the initial value (Radin and Matthews 1989); similar gross effects on L_0 were seen in cotton roots during the development of phosphate deficiency (Radin and Eidenbock 1984). The effects of nutrient deficiency on root L_0 have also been observed on the plasma membrane of individual cells of the root cortex of cotton (Radin and Matthews 1989).

The hydraulic conductivity of roots is generally regarded as being a composite of water movement along several pathways (Steudle 1992), one of which comprises two or more plasma membranes in series (Steudle and Jeschke 1983). Water fluxes across the plasma membrane may also have several components. Recent observations

Abbreviations: DTT = dithiothreitol; Gs = stomatal conductance; Js = solute flow into the sap; Jv = sap flow; L_0 = hydraulic conductance; − N = nitrogen-deprived for 5 d; NRS = nitrogen resupplied after 5 d deprivation; − P = phosphorus-deprived for 7 d; PRS = phosphorus resupplied after 7 d deprivation

Correspondence to: D.T. Cooke; FAX: 44 (1275) 394281

have identified proteins which form water channels in the membranes of plants (Preston et al. 1992a; Fushimi et al. 1993). These proteins, which have been given the name 'aquaporins' (Agre et al. 1993), form channels which allow water to pass freely across cellular membranes, following osmotic or hydrostatic pressure gradients (Chrispeels and Maurel 1994). Although water channels have never been considered in simple biophysical models of water movement in plant cells, they may introduce a new dynamic aspect to water permeation of membranes in which short-term and environmentally responsive regulation of membrane conductance is possible. Water-channel proteins have been identified in the tonoplast (Höfte et al. 1992) and in the plasma membrane of *Arabidopsis* (Kammerloher et al. 1994). In this species, plasma-membrane water channels are encoded by a gene family of at least 11 members.

The water channels in mammalian tissues are known to be blocked by sulphydryl reagents, such as mercurials, e.g. $HgCl_2$, resulting in decreased water permeability of the membranes. These changes are reversed when the mercury is removed from the membrane by thiol reagents (Preston et al. 1992b). The hydraulic conductivity of the plasma membrane in the alga, *Chara*, was greatly reduced by brief exposure to mercury and was restored by treatment with dithiothreitol (Henzler and Steudle 1995). Expression of cDNAs encoding water channels in *Xenopus* oocytes, greatly increased the water permeability of the plasma membrane (Kammerloher et al. 1994). In *Arabidopsis*, PIP1 aquaporin proteins can be extremely adundant in the plasma membranes of some cells, comprising more than 1% of the membrane protein (Robinson et al. 1996). Thus, evidence is accumulating to suggest that a significant proportion of the water moving across the plasma membranes of some plant cells may occur via water channels. In addition, water permeation of membranes will occur by non-Stokesian diffusion through the bilayer lipids (Stein 1986).

In the present study, we have set out to compare the effects of two nutrient-deprivation treatments on water transport via putative water channels and on the physical ordering and lipid composition of the plasma membrane, which may be relevant to water movement by non-Stokesian diffusion.

Materials and methods

Plant culture. Seeds of wheat, *Triticum aestivum* L. (cv. Chinese Spring) were surface-sterilised in 5% sodium-hypochlorite solution for 10 min, washed with deionised water and grown on 'nutrient rafts' in a controlled environment with a 16-h photoperiod at 20 °C, relative humidity (RH) 70% and photosynthetically active radiation (PAR) 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$, as previously described (Cooke et al. 1983). At sowing, the seeds were supplied with a complete nutrient regime containing (mM): N, 5; P, 0.65; K, 4; Ca, 1; Mg, 0.75; Na, 0.65; Cl, 1; S, 0.38; Fe, 0.1; Mn, 0.005; B, 0.015; Zn, 0.0005; Cu, 0.0005; Mo, 0.0001. After 15 d, nutrient solutions were replenished in half of the plants (controls) and in the other half, nitrate (replaced with chloride) or phosphate was withdrawn. Five days later (nitrogen-deprived plants; − N plants) or 7 d later (phosphorus-deprived plants; − P plants), sap flow, hydraulic and stomatal conductance measurements were made and roots harvested for plasma-membrane isolation. In some experiments, NO_3^- or PO_4^{3-} was added back to − N or − P plants, respectively (NRS and PRS plants) and 4 d later, hydraulic-conductance measurements made and roots harvested for plasma-membrane isolation, to determine whether the effects of nutrient deprivation were reversible. Plants were also grown under transparent covers for 2 d in order to reduce the transpiration and the effect on hydraulic conductance measured.

'Split root' experiments. Seeds were germinated on wet filter paper in the dark at 20 °C. On the third day, the main axes were cut and seedlings transferred to square containers divided into two parts. They were placed across the dividing wall, with the roots split into two axes, one in each compartment. They were grown under the same conditions and with the same nutrient solution as described above, but − N or − P nutrient solutions were applied to only one side of the containers, with control nutrient on the other. Sap flow (Jv) and hydraulic conductivity (L_0) were measured and roots harvested for plasma-membrane isolation from individual root axes.

Measurement of Jv. The method was based on volume flow through detached root systems. The aerial parts of the plant were removed, leaving a cylinder of leaf bases, which were sealed with silicone grease into tapered glass tubes. After 2 h, the exuded xylem sap was collected using a finely drawn-out Pasteur pipette and transfered to Eppendorf tubes, the amount of sap determined by weight and the roots removed and weighed. Sap flow was expressed in $mg \cdot (g\ root\ FW)^{-1} \cdot h^{-1}$. Sap flow from individual axes in the split-root experiments was obtained by placing a 50-µl-capacity 'microcap' over the cut end of the root and sealing it with silicone grease.

Measurement of osmolarity of exuded sap. Samples of sap (50 µl) were placed in Eppendorf tubes and the osmotic potential of the sap and the root bathing medium were used to measure osmotic pressure using an osmometer (Digital Osmometer, Roebling, Berlin, FRG). This was calibrated using a standard solution of KNO_3. Osmotic pressure difference between the xylem sap and the external solution, $\Delta\Psi_\pi$, was calculated from the osmolarity values. The solute flux into the sap, Js, was estimated as the product of osmolarity and Jv.

Hydraulic conductance. The driving force $\Delta\Psi_\pi$ used to estimate root hydraulic conductance, L_0, which has the units, $mg \cdot (g\ root\ FW)^{-1} \cdot h^{-1} \cdot MPa^{-1}$, was:-

$$L_0 = \frac{Jv}{\Delta\Psi_\pi} \qquad \text{(Eq. 1)}$$

Stomatal conductance. Measurements were made on intact leaves with a portable Delta-T porometer, type AP4.

Mercuric chloride treatment. Two series of experiments were done to examine the effects of $HgCl_2$ on the exudation of sap from the roots. In both cases, the leaves were cut from plants and the remaining cylinder of leaf bases sealed into a calibrated micro-capillary tube. In the first series of experiments, the roots were then treated for 5 min with $HgCl_2$ (50 µM), washed, returned to mercury-free culture solution and the rate of exudation into the micro-capillary measured over 1 h, using a travelling microscope. The exuded sap was sampled at the end of this period and osmotic potential and L_0 determined. In the second series of experiments, the mercury treatment was continued for 15 min while the rate of sap exudation into the micro-capillary tube was measured, again using a travelling microscope. In both series, the roots were subsequently treated with the thiol reagent dithiothreitol (DTT; $5\ mol \cdot m^{-3}$) and sap flow was observed for a further period of 1 h or 15 min, respectively.

Plasma membrane isolation. Plasma membranes were isolated using the two-phase aqueous polymer technique (Larsson et al. 1987), as follows. Approximately 15 g of root material was chopped finely and vacuum-infiltrated with 60 ml of 50 mM Hepes and 0.5 M sucrose, adjusted to pH 7.5 with NaOH, plus 1 mM DTT, 5 mM ascorbic acid and 0.6% (w/v) insoluble polyvinylpyrrolidone (PVP). The

buffer-saturated material was homogenised using a pestle and mortar, and filtered through a 240-μm-mesh nylon cloth. The filtrate was centrifuged at 10000·*g* for 15 min and the supernatant further centrifuged at 100000·*g* for 30 min to yield a microsomal pellet which was resuspended in 2 ml 0.33 M sucrose in 5 mM phosphate buffer (pH 7.8). The suspension (2 ml) was added to 6 g of an aqueous two-phase mixture producing an 8-g two-phase system with a final composition of 6.1% (w/w) Dextran T500 (Pharmacia), 6.1% (w/w) polyethyleneglycol 3350 (Sigma), 3 mM KCl, 5 mM phosphate buffer (pH 7.8) and 0.33 M sucrose. The phase-system was centrifuged for 3 min at 4000·*g*. The resulting plasma membranes (upper phase) were purified using a batch procedure (Larsson et al. 1987). The third upper phase was diluted with phosphate buffer (pH 7.8), centrifuged at 100000·*g* for 30 min, the resulting pellet resuspended in 0.9 ml 5 mM Mes-Tris, 0.33 M sucrose at pH 6.5 and stored at −20 °C.

Fluidity measurements. Plasma-membrane fluidity was determined by steady-state fluorescence polarisation using 1,6-diphenyl hexatriene as the probe (Cooke et al. 1991). Determinations were made at 25 °C using resuspended plasma-membrane fractions, diluted in an assay medium of 40 mM Hepes/KOH (pH 7), 100 mM potassium chloride, 5 mM magnesium sulphate and 0.1 mM EGTA, to give a protein concentration of 150 μg·ml^{-1}. Samples contained 1 mM 1,6-diphenyl-1,3,5-hexatriene (DPH), 0.5 μl in tetrahydrofuran (THF) and were incubated in a water-bath for 1 h at 25 °C prior to measurement. Individual fluorescence intensities were corrected for light-scattering by scattering emission values obtained from the same membrane samples without the fluorescent probe. Measurements were made of the fluorescence emission of samples and blanks at 430 nm with excitation at 360 nm. The fluorescence polarisation (*P*, a dimensionless parameter, since it is a ratio) was calculated from the following equation:-

$$P = \frac{I_W - I_{VH}(G)}{I_W + I_{VH}(G)} \qquad \text{(Eq. 2)}$$

where, I = fluorescence intensity and G (the grating correction factor) = I_{HV}/I_{HH}. The subscripts, V (vertical) and H (horizontal), describe the positions of polarisers in the excitation or emission beams, respectively.

Lipid analysis. Sterols, bound fatty acids and phospholipids were determined as described by Mas et al. 1994. In an Eppendorf tube, $CHCl_3$-methanol (0.75 ml) was added to resuspended membranes (0.5 ml) along with β-cholestanol (20 μl, 0.1 mg·ml^{-1}), as an internal standard for sterol analysis. Chloroform (0.25 ml) was added, the mixture shaken and centrifuged at 10000·*g* for 6 min. The $CHCl_3$ layer was retained, evaporated to dryness under N_2 and made up to 100 μl with $CHCl_3$.

For sterol analysis, 20 μl of the $CHCl_3$ extract was placed in a glass vial (2 ml), evaporated to dryness under N_2 and acetylated using pyridine (50 μl) and Ac_2O (100 μl). After 2 h, solvents were evaporated under N_2, ethyl acetate (20 μl) added and the sterols analysed by GC using an SE52-bonded capillary column coupled to a flame ionisation detector (FID), with H_2 as carrier (1 ml·min^{-1}) and a temperature programme of 120–265 °C at 10 °C·min^{-1}. The injector and detector temperatures were 250 °C and 320 °C, respectively.

Bound fatty acids were determined by using 20-μl portions of the $CHCl_3$ extract, evaporating to dryness under N_2 and transmethylating with sodium methoxide (0.5 N) in methanol (0.5 ml) and heating at 30 °C for 7 min. The resultant fatty-acid methyl esters were extracted with hexane (1 ml), evaporated under N_2, dissolved in ethyl acetate (20 μl) and analysed by GC using an RSL 500-bonded capillary column with FID and He as carrier (1 ml·min^{-1}). The injector and detector temperatures were 250 °C and 300 °C, respectively.

Phospholipids were analysed by HPLC, using the method described by Christie (1985) with minor modifications. The $CHCl_3$ extract (20 μl) was injected into an Econosphere silica 3-μl column (150 mm long, 4.6 mm i.d.) and compounds detected with an evaporative light-scattering detector. The detector conditions were as follows: N_2 flow, 20 ml·min^{-1}; detector response, 900 V; temperature, 50 °C. A three-solvent gradient system was used, comprising: (A) hexane-dimethoxypropane (99:1, v/v), (B) *iso*propanol-$CHCl_3$ (4:1, v/v) and (C) *iso*propanol-H_2O (1:1, v/v); the flow rate was 2 ml·min^{-1} throughout. The solvent programme was as follows: 100% A at time 0, and 1 min. At 5 min, A was 80% and B 20%. A, B and C were 42, 52 and 6%, respectively, at 5.1 min and at 15 min they were 35, 49 and 16%. At 20 min A was 42%, B 52% and C 6% and at 25 min A was 30% and B 70%. Finally, A was 100% at 30 min. Phospholipids were quantified by comparing peak areas with those of known standards.

Protein determination. Protein was determined using the method of Bradford (1976) using the Bio-Rad reagent with thyroglobulin as standard.

Data analysis. All experiments were repeated at least three times, except where indicated, and data were analysed using unpaired *t*-tests or calculating least significant differences (LSD).

Results

Preliminary note. The true driving force for water movement is the osmotic pressure difference between the solution in the xylem vessels and that in the outer medium. While there can never be certainty that the osmotic pressure of exuded sap is the same as that in the xylem vessels at a given moment, the probability that it is a good estimate will increase as the volume of sap collected increases, assuming that the flow rate does not change. The volume of the xylem in our wheat roots is close to 1% of the root volume, or weight. Thus, the exudation of 10 mg of sap per g of root represents approximately one complete volume change of the xylem vessels. Hence, when a root is exuding at, say, 25 mg sap·(g root Fw)$^{-1}$·h^{-1}, there will have been approximately 2.5 complete volumes of xylem collected in an hour.

Diurnal variation of Jv and L_0. Every 4 h, over a 28-h period, Jv and the osmotic potential of the sap exuded over a 2-h period by of a group of replicate plants were measured; from these values, Js and L_0 were calculated. It was found that the parameters Jv and L_0 were greater in the light than in the dark (Fig. 1). Changes in the latter values were very rapid. The calculated value of Js was greatest in the period immediately after the end of darkness and then declined during the photoperiod. Thus, the increase in L_0 occurring during the day was supported by a smaller driving force than the lower L_0 at night. This gives a clear indication that changes in Jv are dependent on changes in hydraulic conductance (or resistance).

Influence of deprivation/restoration of N or P supply on Jv and L_0. The removal of NO_3^- and $H_2PO_4^-$ from the nutrient solution, resulted in a significant decrease in Jv and L_0, independently of whether the measurement was made in the dark or light period (Table 1). The −N plants were much more affected than −P plants. However, when measured in the light, the values of Jv and L_0 were greater for all treatments, particularly for controls.

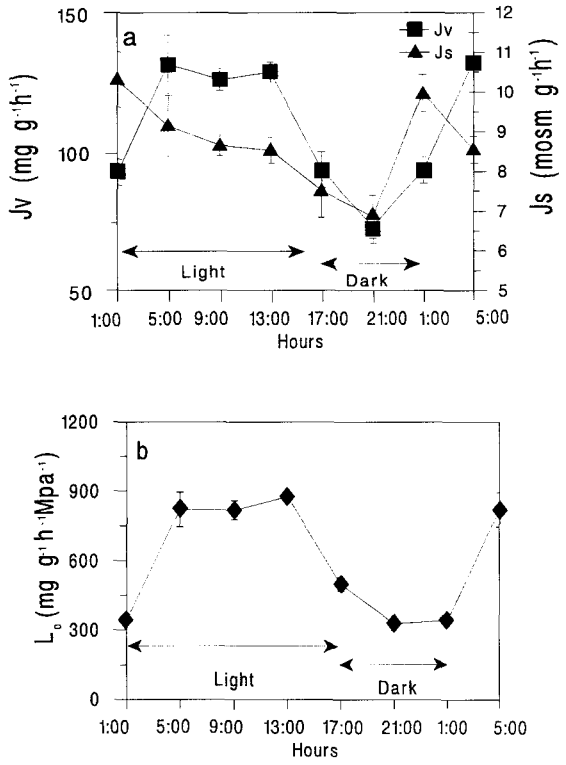

Fig. 1a, b. Diurnal variation of sap-flow (Jv) and solute flow into the sap (Js) (**a**) and hydraulic conductance (L_0) (**b**) of wheat roots measured every 4 h for 28 h. Data are means ± SE, shown only when greater than symbols ($n = s$)

Table 1. Values of Jv and L_0 (± SE; $n = 10$) for nitrogen (5 d)- or phosphorus (7 d)-deprived wheat plants, measured in the middle of the dark and light periods

	Jv ($mg \cdot g^{-1} \cdot h^{-1}$)	L_0 ($mg \cdot g^{-1} \cdot h^{-1} \cdot MPa^{-1}$)
DARK		
Control	79.0 ± 8.75	394.9 ± 43.77
− N	12.8 ± 3.67	83.2 ± 9.67
− P	27.7 ± 3.15	108.5 ± 21.63
LIGHT		
Control	154.5 ± 25.70	796.4 ± 52.43
− N	23.3 ± 2.11	121.4 ± 11.75
− P	38.0 ± 4.13	189.2 ± 17.02

Four days after resupplying NO_3^- and $H_2PO_4^-$ to plants which had been grown − N or − P nutrient solution, L_0 and Jv increased and had values similar to nutrient-replete controls (Table 2). The differences between the measurements made in the dark and in the light were maintained, with the light values remaining greater than those determined in the dark period.

In a more detailed study of the reversibility of the effect of nutrient deprivation, Jv was restored to values similar to that of the control after 24 h when N and P were re-supplied (Figs. 2a, 3a). In plants which had been N-deprived, an increase in L_0 could be detected within 1 h

Table 2. Values of Jv and L_0 (± SE; $n = 10$) for nitrogen- or phosphorus-deprived wheat plants, to which nitrogen (NRS) or phosphorus (PRS) had been re-supplied for 4 d, measured in the middle of the dark and light periods

	Jv ($mg \cdot g^{-1} \cdot h^{-1}$)	L_0 ($mg \cdot g^{-1} \cdot h^{-1} \cdot MPa^{-1}$)
DARK		
Control	43.4 ± 3.52	211.5 ± 17.19
NRS	47.2 ± 9.16	210.7 ± 85.54
PRS	43.3 ± 11.42	210.3 ± 53.80
LIGHT		
Control	55.9 ± 5.03	345.0 ± 29.17
NRS	49.3 ± 1.33	366.8 ± 24.92
PRS	40.1 ± 2.43	337.6 ± 13.51

and became significantly greater than the control until 4 h, before decreasing again to a value similar to that of the control (Fig. 2b). Over the same period, it is instructive to compare values for Js with the calculated value for L_0. In the first few hours after P was resupplied (Fig. 3a), Js (Fig. 3c) changed little in comparison with Jv and, hence, the osmotic driving force decreased at a time when water flow, Jv, was increasing. In plants re-supplied with N, Js (Fig. 2c) increased approximately twofold over the first 2 h, while Jv increased fivefold; again, the osmotic potential (a function of Js/Jv see Eq. 3) was smaller at the increased flow rate.

$$\pi_{xyl} = RT\ Js/Jv \qquad \text{(Eq. 3)}$$

where π_{xyl} is the osmotic potential of the xylem sap.

This gives an unequivocal indication of changing resistance to water flow into the xylem.

Influence of transpiration on Jv and L_0. To determine whether transpiration influenced, after subsequent excision, the values of the root Jv and L_0, plants (control, − N and − P) were covered with glass beakers, to reduce transpiration in the middle of the light period. Both Jv and L_0 were decreased by approximately 50% in covered control plants, but − N and − P plants were unaffected (Table 3). There were no significant differences in Jv and L_0 between N- and P-deprived plants, with or without transparent covers.

Stomatal conductance. There was a significant decrease in the stomatal conductance of N- and P-deprived plants, compared with controls. However, with NRS and PRS plants, values were similar to those of control plants (Fig. 4).

'Split root' experiments. Sap-flow measurements were made from individual root axes on both sides of the containers (i.e., from − N or − P and the corresponding controls). Values for Jv and L_0 were significantly lower in roots on the − P or − N side of a split root system than on the sides supplied with complete nutrient solution (Table 4). This result points to a localized response to

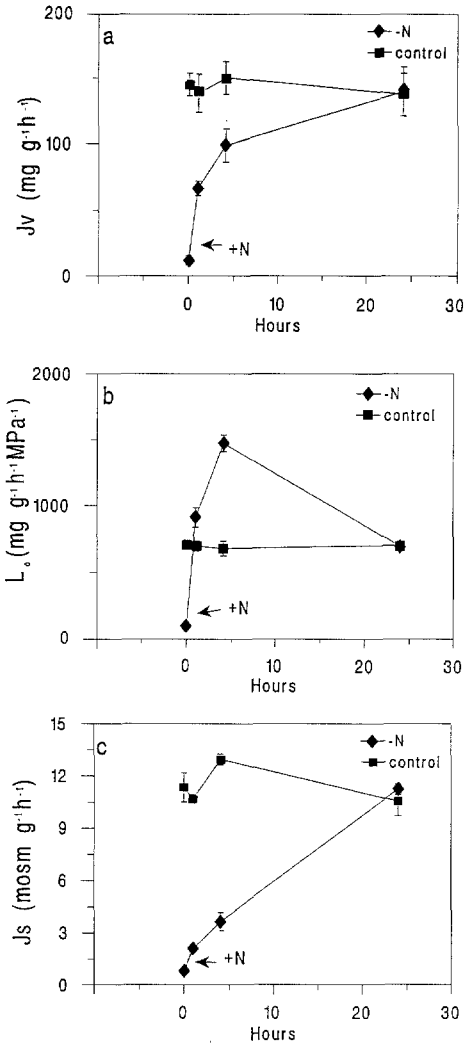

Fig. 2a–c. Sap-flow (**a**), hydraulic conductance (**b**) and solute flow into the sap (**c**) of wheat roots from 5-d nitrogen-deprived plants, to which nitrogen had been re-supplied and the respective controls. Data are means ± SE, shown only when greater than symbols ($n = 5$)

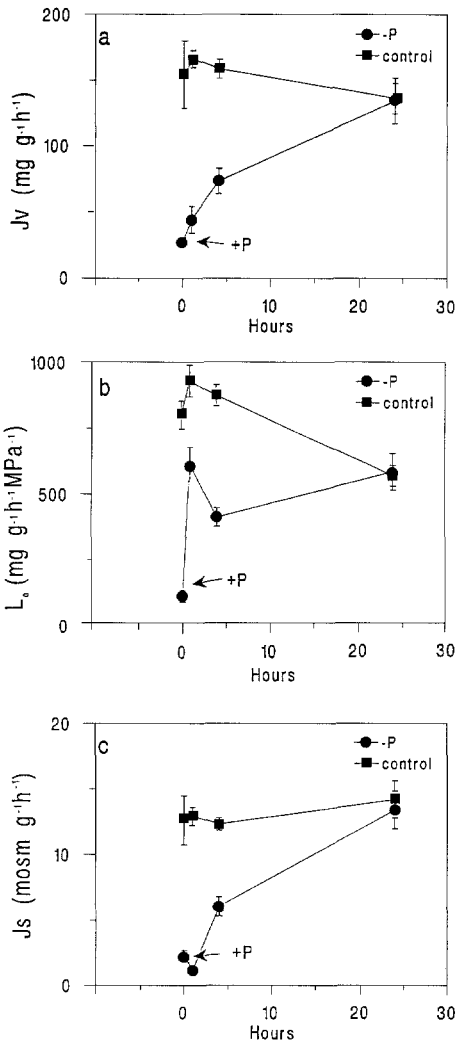

Fig. 3a–c. Sap-flow (**a**), hydraulic conductance (**b**) and solute flow into the sap (**c**) of wheat roots from 7-d phosphorus-deprived plants, to which phosphorus had been re-supplied and the respective controls. Data are means ± SE, shown only when greater than symbols ($n = 5$)

nutrient deprivation. There was no difference in the stomatal conductance of leaves from − N or − P split-root plants, compared with plants where both sets of roots were in control nutrient (Table 4). This result suggests that the observed declines in Jv and L_0 were not caused by diminished transpiration.

Sensitivity to $HgCl_2$. The Jv and the L_0 of control plant roots declined to values similar to those of − N and − P plants after 5 min treatment with 50 µl $HgCl_2$ (Table 5). During the second period (after Hg treatment) the estimated osmotic potential of the sap declined by 25%; thus, there would have been a decrease in Js. By contrast, exudation, sap osmotic potential and L_0 in N- and P-deprived plants were not strongly affected by the Hg treatment. Addition of DTT (5 mM) restored Jv and L_0 to initial values in control plants, without any increase in sap osmotic potential, but had no effect on these parameters in − N and − P plants. During each hour-long period of this experiment, there would have been > 2 xylem volumes of sap collected from the Hg-treated control plants.

Table 3. Values of Jv ($mg \cdot g^{-1} \cdot h^{-1}$) and L_0 ($mg \cdot g^{-1} \cdot h^{-1} \cdot MPa^{-1}$) (± SE; $n = 5$) for nitrogen (5 d)- or phosphorus (7 d)-deprived wheat plants with and without transparent covers to reduce transpiration, as measured in the middle of the light period

Jv	Without transparent cover	With transparent cover
Control	124.8 ± 11.03	68.8 ± 11.76
− N	19.1 ± 4.53	15.3 ± 2.35
− P	20.9 ± 2.34	24.4 ± 0.85
L_0	Without transparent cover	With transparent cover
Control	851.4 ± 71.43	425.7 ± 67.98
− N	119.3 ± 26.59	79.5 ± 8.53
− P	123.6 ± 10.09	136.4 ± 4.65

A second series of experiments confirmed the relative insensitivity of exudation of nutrient-deprived roots to

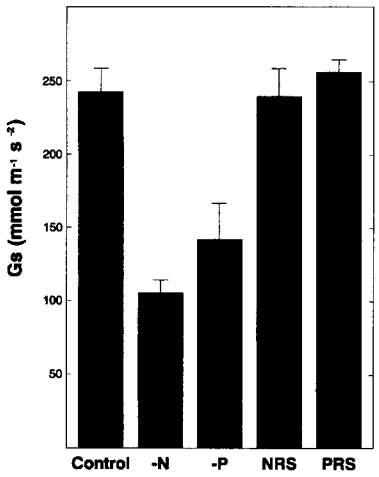

Fig. 4. Stomatal conductance (*Gs*) of intact wheat leaves from N- (5 d) and P-deprived (7 d) plants, 4-d N- and P-re-supplied (*NRS*, *PRS*) plants, and their respective controls. Data are means ± SE ($n = 10$)

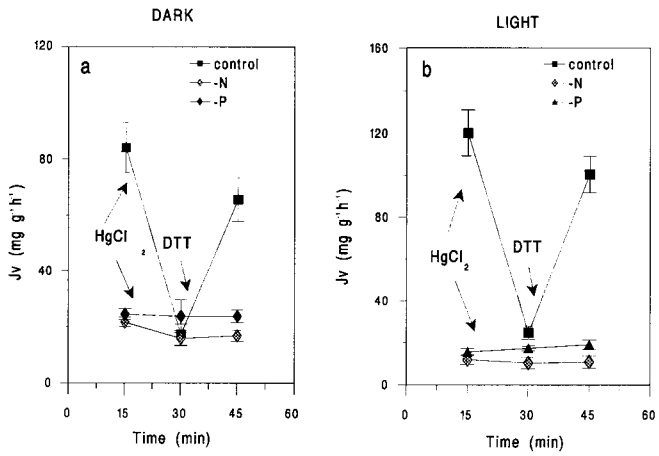

Fig. 5a, b. Sap-flow of roots from control, N- (5 d) and P-deprived (7 d) plants before and after the addition of $HgCl_2$ (50 μM) and DTT (5 mM), measured in the middle of the dark (**a**) and the middle of the light period (**b**). Data are means ± SE ($n = 5$)

Table 4. Values of Jv and L_0 from split wheat roots in control and − N (5 d) or control and − P (7 d) plants and Gs of intact, split-root plants, measured in the middle of the light period. Means ± SE ($n = 10$)

	Jv ($mg \cdot g^{-1} \cdot h^{-1}$)	L_0 ($mg \cdot g^{-1} \cdot h^{-1} \cdot MPa^{-1}$)	Gs ($mmol \cdot m^{-2} \cdot s^{-1}$)
Control	69.6 ± 2.93	478.7 ± 19.05	242.6 ± 18.29
− N	30.3 ± 4.10	249.0 ± 33.38	241.8 ± 18.38
− P	28.7 ± 0.56	356.8 ± 7.03	278.6 ± 26.10

mercury treatment (Figs. 5, 6). In control plants, $HgCl_2$ treatment had a greater impact on L_0 in plants treated in the light period than in the dark period (Fig. 6). In these experiments, in Hg- treated controls and in N- and P-roots, the volume of xylem sap collected over a 15-min period was only 50–60% of the xylem volume. This makes the estimate of the driving force, used to calculate L_0, most uncertain. For this reason, the values for L_0 are best regarded as semi-quantitative.

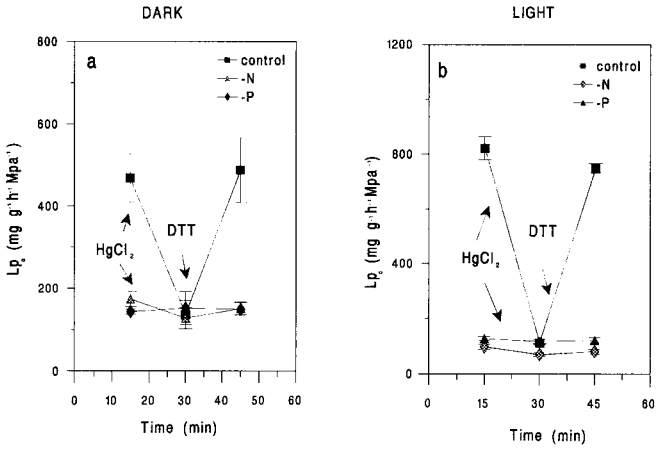

Fig. 6a, b. Hydraulic conductance of roots from control, N- (5 d) and P-deprived (7 d) plants, before and after the addition of $HgCl_2$ (50 μM) and DTT (5 mM), measured in the middle of the dark (**a**) and the middle of the light period (**b**). Data are means ± SE ($n = 5$)

Table 5. Values of Jv ($mg \cdot g^{-1} \cdot h^{-1}$), osmotic potential ($\Psi_\pi$; MPa) and L_0 ($mg \cdot g^{-1} \cdot h^{-1} \cdot MPa^{-1}$) (± SE; $n = 5$) of control, nitrogen (5 d)- or phosphorus (7 d)-deprived wheat plants, measured in the middle of the dark period. Sap was collected for 1 h before the addition of $HgCl_2$ (50 μM), 1 h after an $HgCl_2$ treatment of 5 min, and 1 h after DTT (5 mM) addition

	Control			− N			− P		
	Jv	Ψ_π	L_0	Jv	Ψ_π	L_0	Jv	Ψ_π	L_0
Before $HgCl_2$ addition	89.0 ± *6.60*	0.12 ± *0.000*	760.3 ± *56.48*	15.9 ± *1.05*	0.13 ± *0.007*	123.3 ± *2.51*	23.5 ± *4.58*	0.13 ± *0.008*	175.1 ± *56.48*
After $HgCl_2$ addition for 5 min	21.7 ± *2.83*	0.09 ± *0.006*	276.7 ± *65.83*	10.8 ± *1.76*	0.13 ± *0.007*	85.32 ± *15.83*	20.2 ± *3.02*	0.10 ± *0.008*	198.3 ± *65.83*
After DTT addition	68.1 ± *8.01*	0.09 ± *0.006*	816.32 ± *52.16*	17.81 ± *1.28*	0.12 ± *0.006*	148.59 ± *13.10*	23.1 ± *2.82*	0.15 ± *0.007*	155.2 ± *4.79*

In both series of experiments, the effects of Hg treatment on control roots were completely reversed by 15 min treatment with DTT.

Plasma-membrane order parameter. The fluorescence polarisation (P) values for plasma membranes from the roots of N- and P-deprived plants, sampled at the middle of the dark period, were greater than those from their respective controls (Fig. 7a, b). However, when nitrogen (NRS) and phosphorus (PRS) were re-supplied to previously deprived plants, P values steadily decreased with time, until it was no different from controls. This decrease was quicker for PRS plants (24 h) compared with NRS plants, which took 3 d to reach control values (Fig. 7a). In experiments using an alternative probe (1-anilinonaphthalene-8-sulphonic acid), to confirm the data obtained using 1,6-diphenyl-1,3,5-hexatriene, similar differences between treated and control plants were found (data not shown).

Although it has been found that L_0 of control roots differed between day and night (Table 1, Fig. 1b), there was no difference in the P values of membranes sampled in the middle of the light or dark periods (Table 6).

Plasma-membrane lipid composition. Extensive analyses were made of plasma-membrane lipid composition. However, these failed to provide any obvious explanation for the changes in the membrane ordering parameter, P. Our findings are briefly summarized in Table 7. The most pronounced effect was an increase in the double-bond index of the fatty acids in − N and − P root plasma membranes and a slight shift in the stigmasterol/sitosterol ratio, which was significantly different from the controls in − N root plasma membranes.

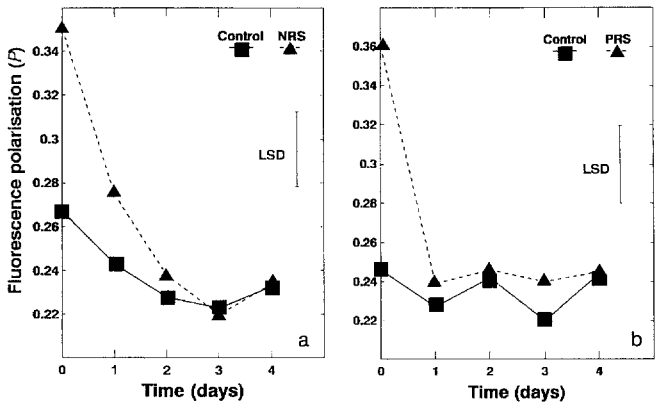

Fig. 7a, b. Fluorescence polarisation (P) of wheat root plasma membranes from N-deprived plants ($n = 4$) and 4 d N-re-supplied (NRS) plants ($n = 3$) (**a**) and from P-deprived plants ($n = 4$) and 4 d P-re-supplied (PRS) plants ($n = 3$) (**b**) with respect to the time after resupplying, and their respective controls. (LSD, $P = 95\%$)

Table 6. Membrane order parameter ($\pm$ SE; $n = 5$) of wheat root plasma membranes of nitrogen (5 d)- or phosphorus (7 d)-deprived plants measured at different times of day

	Middle dark period	Middle light period	
	Normal root	Normal root	Split root
Control	0.26 ± 0.005	0.26 ± 0.007	0.27 ± 0.019
− N	0.35 ± 0.009	0.36 ± 0.014	0.44 ± 0.046
− P	0.36 ± 0.005	0.35 ± 0.015	0.33 ± 0.001

Discussion

Driving forces for water movement in exuding roots. Although the data on sap flow, Jv, can be acquired very simply from de-topped, exuding roots, their interpretation is complicated by the fact that solute and water fluxes interact to determine the value of the osmotic driving force between the xylem and the outside solution. If a given treatment alters the solute flux, Js, it is to be expected that there will be an effect on Jv if the hydraulic conductance and the reflection coefficient remain constant (see Steudle, 1994 for more detailed discussion). The most unequivocal evidence for a change in conductance is where there is a change in Jv without any change in Js. In such circumstances, if conductance decreases, due to some experimental treatment, the osmotic potential of the sap will rise; if L_0 increases, the osmotic potential will fall. Examples of the latter type of behaviour were found in the present work. Jv increased during the photoperiod while Js decreased (Fig. 1a); thus, the osmotic driving force also decreased. Similarly, when P was re-supplied to P-deprived roots, there was a substantial increase in Jv over the first 4 h accompanied by a relatively small change in Js (Fig. 3a, c). The estimation of the driving force for water movement based on the osmotic potential of exuded sap is only valid if a steady state has been reached. Over long

Table 7. Summary of lipid analysis of control and − N and − P plasma membranes from wheat roots ($n = 10$)

	Control	− N	− P
Cholesterol (% total sterol)	0.5	0.5	0.6
Campesterol (% total sterol)	35.2	35.1	35.6
Stigmasterol (% total sterol)	6.5	7.6	6.7
Sitosterol (% total sterol)	57.8	56.4	57.2
Free sterols ($\mu g \cdot mg^{-1}$ protein)	120.6	152.1	130.9
Stigmasterol/sitosterol	0.11 ± 0.002	0.13 ± 0.003*	0.12 ± 0.003
Phospholipid/sterol	0.21	0.27	0.25
Double-bond index	117	132	146

* Indicates significantly different from control at the 95% level of confidence or better.

periods of time, where many xylem volumes of sap are collected, the integration of the estimated driving force with the observed flow can be made with increasing confidence. In most of our experiments, sap was collected for long enough for there to be several xylem volumes displaced into the collection tube. In the case of short-term experiments (Figs. 5, 6), this condition was not met, but in another type of experiment involving the use of $HgCl_2$ treatment, sap was collected for a longer period and was equivalent to at least one complete xylem volume displacement (Table 5).

Diurnal effects and transpiration. The decrease in L_0 in nutrient-deprived roots is a well-known phenomenon (Chapin et al. 1988; Radin and Ackerson 1981; Radin and Boyer 1982; Radin and Matthews 1989). In the present work, we showed that the lower values of L_0 in − N and − P plants, relative to the control, were maintained during light and dark phases of the photoperiod. In many studies, nutrient deprivation has been shown to lead to stomatal closure and reduced transpiration (Chapin et al. 1988; Clarkson and Scattergood 1982). Therefore, we considered the possibility that the lower values for L_0 were a consequence of reduced transpiration. If this were to be the case, the effect on L_0 would be a consequence of events causing stomatal closure and would be much less interesting as a possible signal indicating nutrient shortages in the root. Experiments on plants with split roots showed that there was no effect on Gs, when part of the root system was deprived of either nitrate or phosphate, and, yet, in the nutrient-deprived parts, L_0 declined significantly to below that of roots on the same plant fully supplied with nutrients. Thus, the effect on L_0 appears to reflect the local nutrient supply experienced by a root and is independent of transpiration.

Our results show that the effects on root L_0 were swiftly reversed when nutrients were re-supplied (Figs. 2, 3); evidently, no major anatomical change in the structure of the root is likely to have occurred during nutrient deprivation. In control plants, it was clear that there were marked diurnal variations in the value of L_0. This declined towards the end of the light period, and rose again at the end of the dark period (Fig. 1b). These changes resemble those reported in the classic experiment of Parsons and Kramer (1974) and which have been interpreted as showing that root-resistance to water-flow can decrease as the flow increases (Mees and Weatherley 1957; Passioura and Tanner 1985). This behaviour has been interpreted in terms of relative changes in the flows of water through several pathways in the root (Steudle 1992, 1994).

Chemical and physical properties of the plasma membranes. In wheat plants, plasma-membrane steady-state fluorescence polarisation (P) values were much greater from controls after 5 d of N-deprivation and 7 d of P-deprivation. The value of P is related to the membrane order parameter, which is the reciprocal of fluidity (Van Blitterswijk et al. 1981), i.e. a low value of the order parameter is associated with high membrane fluidity (Harwood 1989). The results obtained in the present work are consistent with the data from sulphur-deprived barley plants, i.e. in the short term, the membrane became more ordered in nutrient-stressed plants. In sulphur-stressed barley plants, there was no clear indication that changes in membrane lipid composition could account for the differences in fluidity (Cooke et al. 1993). However, in wheat plants, there was an increase in the stigmasterol/sitosterol ratio, brought about by an increase in the content of stigmasterol. The increase in the stigmasterol/sitosterol ratio seems to be a general feature of plasma membranes from stressed plants (Guye 1987), although it has not previously been linked to fluidity measurements. In work with liposomes, it has been suggested that sitosterol and stigmasterol probably play different roles in regulating plant membrane properties. Sitosterol is thought to increase the membrane acyl-chain order parameter and could be involved in the regulation of membrane permeability. In this context, the specific role of stigmasterol is as yet unknown and may be different (Schuler et al. 1991). Furthermore, changes in the intricate mixture of sterols and phospholipids that constitute native membranes may have complex effects on the membranes due to their differential solubilities and membrane-partitioning (Hennessey 1992).

The sphingolipid and phospholipid composition remained unchanged with − N and − P treatment, although the increase in the sterol/phospholipid ratio, which was the result of the increase in sterols and decline in phospholipids in the − N plasma membranes, may partially explain the increase in membrane ordering (i.e. decrease in fluidity).

Any functional connection between the reduction in fluidity of plasma membrane and the decreased L_0 seems to apply only to nutrient-deprived plants. In controls, substantial changes in L_0 during the diurnal cycle (Fig. 2) were not accompanied by changes in the order parameter (Table 7). Evidently, it is possible for L_0 to vary in membranes where the fluidity does not change. This makes it very hard to interpret the rather clear correlations between changes in the order parameter and changes in L_0 after nutrient deprivation and during the recovery (Fig. 7). The results of Radin (1990) encourage the idea that low L_0 in nutrient-deprived cotton plants was due to membrane fluidity because, at high temperature, differences between stressed roots and control were eliminated. The clear implication was that some change of state of all, or in a fraction of, the membrane lipids, induced by nutrient deprivation, could be reversed by increasing temperature. On the basis of the Hg-inhibition experiments reported in the present paper, the residual water flow across the root may represent the flux through the diffusive pathway across the bilayer. Of course, it is possible that there is an interaction between lipid fluidity and water-channel function, but such speculation cannot get very far in the almost total ignorance of the components of the system.

Sensitivity to $HgCl_2$. In experiments with $HgCl_2$, Jv and L_0 declined rapidly in control plants towards values similar to those of N- and P-deprived plants, which were, themselves, unaffected by Hg treatment (Figs. 5, 6). It is notable, in the nutrient-deprived roots, where Jv and L_0 were already low, that Hg treatment had little or no

effect on the value of Js. If Hg were to have had a catastrophic effect on, say the activity of membrane ATPases or high-affinity transporters in the control plants, it is hard to see why such an effect would not have been found in the nutrient-deprived plants as well. The continued vigorous growth of the N- and P-deprived roots suggests that their metabolism was not generally depressed. Although these experiments do not prove the point, it seems likely that the principal perturbation caused by Hg in the short term, may have been through an effect on hydraulic conductance. It has been shown that $HgCl_2$ blocks the flow of water through aquaporins (Chrispeels and Agre 1994; Knepper 1994; Van Os et al. 1994; Preston et al. 1992a) and that flow is restored with reducing agents (Chrispeels and Arge 1994; Preston et al. 1992a). Using DTT as a scavenger for $HgCl_2$, the L_0 of control roots was quickly restored to its pre-treatment value; this reagent had no effect on N- or P-deprived plants. These results suggest that the putative aquaporins in wheat root cells are Hg-sensitive and that the very weak response of the L_0 of nutrient-deprived roots to Hg treatment is because water channels are either greatly reduced in numbers or, if present, are non-functional. Nothing is known about the regulation of plasma-membrane water channels in plants; it may be via gene expression, as in amphibian tissues (Abrami et al. 1995) or via some kind of gating function. Nevertheless, the early perception by roots that the N or P supply has become sub-optimal may be via an effect on water channels. There was a small increase in Hg sensitivity during the light period in N- and P-deprived plants which may indicate some residual water-channel function. There are water channels in *Arabidopsis* which are not sensitive to inhibition by mercury (Daniels et al. 1994), but these seem to be expressed only under desiccation and loss of turgor. It is perhaps improbable that they would contribute much to water flows across the membranes of turgid root cells.

The residual water flow across roots where water channels were blocked by Hg was around 20% of normal, suggesting that simple diffusion through the lipid bilayer is unlikely to be the major pathway for water movement. Thus, in well-nourished plants, water uptake may be dominated by flows through water channels in the presence of osmotic driving forces. In the transpiring plant, where root conductance may increase substantially because of flow through apoplasmic bypasses, it remains to be seen what the quantitative effect of water channels may be. A recent study showed that the hydraulic conductance decreased very sharply to 43% of the control when $HgCl_2$ was added to the solution bathing tomato roots in a pressurized system where water fluxes were similar to those in an intact plant (Maggio and Joly 1995). The $HgCl_2$ concentration used was ten-times that in our work and exposure times were far longer; even so, the hydraulic conductance was restored to values close to the control when the Hg was scavenged by mercaptoethanol. This result suggests that some Hg-sensitive component is also important in hydrostatic-pressure-driven water flow across roots.

Perhaps the most compelling question raised by the present results is this: *What control mechanisms can be envisaged which would produce similar effects on both membrane ordering and putative water-channel activity, by withholding either of two nutrients whose transport and metabolism are quite distinct?* We may add that sulphate deprivation in barley produces similar effects on L_0 (Karmoker et al. 1991) and membrane fluidity (Cooke et al. 1993). Perhaps, the transport of the ions is itself the message to which the system responds. The availability of PIP cDNAs from *Arabidopsis* (Kammerloher et al. 1994) suggests that the impact of anion deprivation on water-channel gene transcription can be studied.

Another question relates to the broader significance of changes in L_0 as a response to nutrient deprivation. Given the rather general occurrence of decreased L_0 in a range of species, when one or several nutrient anions are deficient (Karmoker et al. 1991; Radin and Eidenbock 1984; Radin and Matthews 1989), it is not surprising to find that decreased stomatal conductance is frequently observed in nutrient-deficient plants. Especially in the case of N- and P-deprivation, there seems also to be good correlation with increased dry-matter partitioning to roots. These similarities have led Chapin et al. (1988) to suggest that there may be a general stress-response syndrome. Our results indicated that an intervention in the expression or in the activity of water channels, may be an important event in this syndrome.

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