

Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress

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Abstract

It has been shown that N-, P- and S-deficiencies result in major reductions of root hydraulic conductivity (Lp_r) which may lead to lowered stomatal conductance, but the relationship between the two conductance changes is not understood. In a variety of species, Lp_r decreases in the early stages of NO_3^- , $\text{H}_2\text{PO}_4^{2-}$ and SO_4^{2-} deprivation. These effects can be reversed in 4–24 h after the deficient nutrient is re-supplied. Diurnal fluctuations of root Lp_r have also been found in some species, and an example of this is given for *Lotus japonicus*. In nutrient-sufficient wheat plants, root Lp_r is extremely sensitive to brief treatments with HgCl_2 ; these effects are completely reversible when Hg is removed. The low values of Lp_r in N- or P-deprived roots of wheat are not affected by Hg treatments. The properties of plasma membrane (PM) vesicles from wheat roots are also affected by NO_3^- -deprivation of the intact plants. The osmotic permeability of vesicles from N-deprived roots is much lower than that of roots adequately supplied with NO_3^- , and is insensitive to Hg treatment. In roots of *L. japonicus*, gene transcripts are found which have a strong homology to those encoding the *PIP1* and *PIP2* aquaporins of *Arabidopsis*. There is a very marked diurnal cycle in the abundance of mRNAs of aquaporin gene homologues in roots of *L. japonicus*. The maxima and minima appear to anticipate the diurnal fluctuations in Lp_r by 2–4 h. The temporal similarity between the cycles of the abundance of the mRNAs and root Lp_r is most striking. The aquaporin encoded by *AtPIP1* is known

to have its water permeation blocked by Hg binding. The lack of Hg-sensitivity in roots and PMs from N-deprived roots provides circumstantial evidence that lowered root Lp_r may be due to a decrease in either the activity of water channels or their density in the PM. It is concluded that roots are capable, by means completely unknown, of monitoring the nutrient content of the solution in the root apoplasm and of initiating responses that anticipate by hours or days any metabolic disturbances caused by nutrient deficiencies. It is the incoming nutrient supply that is registered as deficient, not the plant's nutrient status. At some point, close to the initiation of these responses, changes in water channel activity may be involved, but the manner in which monitoring of nutrient stress is transduced into an hydraulic response is also unknown.

Key words: *Lotus japonicus*, hydraulic conductivity, diurnal cycle, aquaporin, root, plasma membrane.

Introduction

A plant can have its transpiration, stomatal conductance (G_s) and root hydraulic conductivity (Lp_r) influenced strongly by its supply of certain mineral nutrients. This much has been recognized for many years (Desai, 1937), but while it is agreed that these responses occur without there being any gross perturbation in leaf water status, they are not completely explained. When they are deficient in the growth medium, three plant nutrients, nitrate,

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phosphate and sulphate, which are transported, metabolized and utilized in different ways, all produce similar effects on G_s and Lp_r . It seems improbable that these effects are caused by completely separate sequences of events. Thus, one should not, for instance, expect to explain stomatal closure in P-deficient plants solely by reference to P-metabolism or tissue distribution of P; when there is no P-limitation, G_s is equally sensitive to N-deficiency and S-deficiency. Similarly, root Lp_r is strongly responsive to the N supply, but it may also be affected by P and S deficiency (Radin and Eidenbock, 1984; Karmoker *et al.*, 1991). One might ask, therefore, whether stomatal closure and diminished Lp_r are central objectives of the nutrient-stress response, elicited by some common sequence of events. The idea that there may be a centralized stress response system had already been advanced (Chapin, 1990).

It is important to consider the timing of events and the order in which they unfold. In Fig. 1 the sequence has been divided arbitrarily into two branches: the first embraces nutrient-specific responses of transport systems, while the second, more general branch, is concerned with hydraulic and morphological events. It must be stressed that this scheme of things, particularly in the general branch, is speculative. The response of plants to potassium deficiency seems to be restricted to the specific branch. As with other nutrient deficiencies, K-deprivation de-represses K transport systems in roots, appearing to

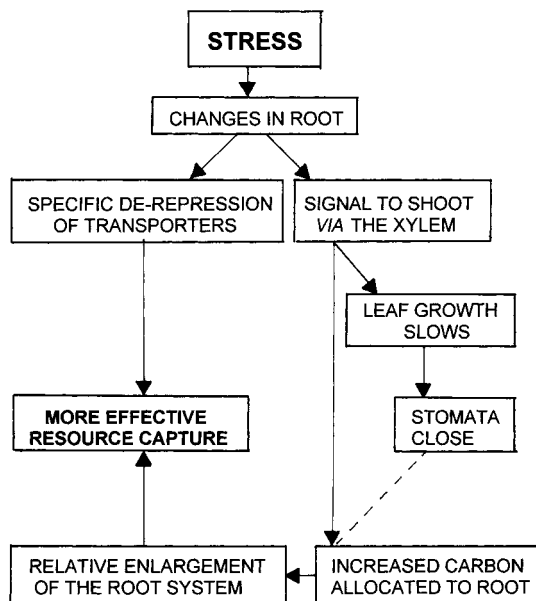


Fig. 1. Hypothetical sequence of events leading from the initial perception of nutrient stress to the more effective capture of resources by the root system. The left hand branch involves increased rates of transcription of high affinity nutrient transporter genes; these effects are specific to the nutrient which is perceived as limiting. The right hand branch represents a more general sequence of events which is set in motion by a variety of nutrient, hydric and oxygen stresses. The linkages between the steps are poorly understood.

make the root a more avid absorber of K from dilute solutions. But, unlike N, P or S deficiency, K does not appear to influence the events in the general branch. In itself, this is a most interesting issue but not one which can be treated in this short paper.

In this paper the hypothesis is advanced that anion fluxes (in which energized transport dominates the overall process) are linked with the hydraulic conductivity (Lp) of root plasma membranes (PM), possibly through the activity of water channels. The essence of this idea is that nutritional information is transduced into an hydraulic response.

Nutrient deficiencies and water relations of crop plants

There were a number of early reports that certain nutrient deficiencies resulted in partial or complete stomatal closure in plants adequately supplied with water (Desai, 1937; Wallace and Frohlich, 1965). In more recent times, the work of John Radin and his colleagues has given the clearest insights into the relationships between N and P nutrition, stomatal conductance and leaf expansion. Work with cotton plants, grown in a controlled environment, revealed that both N- and P-deficient conditions restricted leaf expansion, reduced transpiration and decreased the hydraulic conductivity of the plants, without there being any effect on leaf water potential (Radin and Ackerson, 1981; Radin and Eidenbock, 1984). With P-deficient plants it was established that the root was the site of diminished hydraulic conductance and that this could be detected well in advance of measurable effects on leaf expansion (Radin and Eidenbock, 1984), but that the effect on Lp_r diminished if the temperature of the roots was increased to values $>30^\circ\text{C}$ (Radin, 1990). A cell pressure probe, inserted into cortical cells of cotton roots, showed the Lp of the PM was smaller, than in nutrient-sufficient controls, by approximately 60% and 85% in P- and N-stressed plants, respectively. The effects could be picked up within 1 d of N-deprivation (Radin and Matthews, 1989).

N-deficiency in barley and tomato plants lowered Lp_r in advance of effects on G_s and photosynthesis (Chapin *et al.*, 1988). In barley, it was found that S-deprivation diminished root Lp_r progressively over 4 d to a value $<20\%$ of S-replete controls (Karmoker *et al.*, 1991); these effects preceded reductions in transpiration and net assimilation rate (Gilbert *et al.*, 1997). In wheat plants, effects on root Lp_r , caused by N- and P-deprivation, were quickly reversed when nutrient supplies were resumed (Carvajal *et al.*, 1996). Rapid reversibility of effects on Lp_r have been reported in *Zea mays* when N-starved roots were supplied with either NO_3^- or NH_4^+ ; the effect being dependent on NO_3^- reduction (Barthes *et al.*, 1996).

In summary it can be said that depriving plants of

adequate supplies of the three major nutrient anions results in a prompt diminution of cell and root hydraulic conductivity which is fully reversible when the nutrient supply is restored. Effects can be detected before those on photosynthesis, but may be concomitant with the slowing of leaf expansion. The authors are not aware of any report that K-deficiency affects root Lp_r .

Nutrient deficiencies and root growth

It has been observed frequently that plants which are N- or P-limited in their growth allocate a greater proportion of their total assimilated carbon to root growth (Robinson, 1994). Not all species respond in this way and examples can be found of closely related ones which behave differently, for example, *Plantago* spp. (Lambers *et al.*, 1981a, b) and *Agropyron* spp. (Jackson and Caldwell, 1989). The relative expansion of the root system when plants experience scarcity of an essential nutrient conforms to a commonsense notion that this is a 'sensible' strategy to maximize resource capture below ground. It has been questioned, however, whether or not this is as sensible as it appears at first, especially in the case of N-deficiency (Robinson, 1996). Nitrate is free to diffuse towards root surfaces and will do so more rapidly if influx at the root surface is increased. Following this logic there is no 'need' to expand the root surface, an increase in absorption rate should increase the rate at which NO_3^- diffuses to the root surface, and yet the effect is very widely seen. When conditions of N-supply are shifted from a sufficient to a sub-sufficient level there is usually a period during which the root:shoot weight ratio increases, but, after some time, it reaches a new steady value (for example, in young plants of *Betula pendula*, McDonald *et al.*, 1986).

Local sources of N (both as NO_3^- or NH_4^+) or P, in media which are generally nutrient deficient, elicit local root proliferation (Drew and Saker, 1975, 1978). The nutrient absorption that occurs in the zone where roots proliferate can compensate, to some extent, for deficiencies in other root zones. Much work of this kind has been reviewed (Robinson, 1994). Interestingly, neither the root:shoot ratio response nor the localized proliferation of roots is elicited by K-deficiency. It should be noted, however, that S-deprivation in barley, while de-repressing sulphate transporter genes (Smith *et al.*, 1998) and causing a major diminution of Lp_r , had relatively little effect on the root:shoot ratio (Karmoker *et al.*, 1991). There is, therefore, no invariable linkage between the various effects in the general branch of Fig 1.

Root Lp_r and transpiration

The apparent value of root Lp_r has been reported to increase with transpiration rate (Mees and Weatherley,

1957; Passioura and Tanner, 1985). This phenomenon has been interpreted in several ways, but all of them fall short of describing, in molecular terms, a resistance which decreases as flow increases. Explanations in terms of additive effects of osmotic and hydrostatic driving forces (Fiscus, 1975), or of changes in the dominant pathways for the lateral movement of water across the root cylinder (Steudle, 1994; Steudle and Peterson, 1998) have been advanced as alternatives or have been incorporated into models in which effects of different pathways are compensatory (Steudle and Peterson, 1998). In these models, however, the role of water transport across root cell membranes remains to be quantified, although there have been attempts in which water relations have been worked out in great detail at the cell and root level (Zhu and Steudle, 1991; Azaizeh *et al.*, 1992). In these studies, cell Lp appeared to be quite variable. The discovery of aquaporins in tonoplast and plasma membranes (Daniels *et al.*, 1994; Kammerloher *et al.*, 1994) and the demonstration that some of them at least, do function as PM water channels (Kammerloher *et al.*, 1994; Kaldenhoff *et al.*, 1998; Tyerman *et al.*, 1999) provides an opportunity to see if they play a role in the apparent variability of Lp_r .

Both transpiration rate and Lp_r have been found to vary diurnally, but a simple cause and effect was seemingly ruled out in a classic experiment (Parsons and Kramer, 1974) which showed that the diurnal rhythm continued for several cycles after root systems of cotton had been excised; the phases of the rhythm seemed to be set by the onset of light. In some of the experiments summarized below, it is shown that there are also diurnal cycles in the expression of mRNAs which are homologous to those encoding *Arabidopsis* aquaporins, but that their abundance is not influenced by concurrent transpiration rate.

Behaviour of wheat roots during N- and P-deprivation

Both N- and P-deprivation decreased the apparent value of Lp_r in excised wheat roots (Carvajal *et al.*, 1996); in these experiments the flow of water was driven by osmotic pressure differences between the xylem sap and the solution in the root apoplasm. The measurements were made, therefore, at low rates of water flow, equivalent to, or less than those normally occurring during the dark period. A marked diurnal fluctuation in the value of root Lp_r was found in plants sampled at different times during day and night; the amplitude of the cycle was much reduced by N- or P-deprivation, but remained significant (Table 1). If the roots of wheat plants were divided between solutions containing, or lacking NO_3^- , those in the $-\text{NO}_3^-$ half had lower root Lp_r than those in the $+\text{NO}_3^-$ half (Carvajal *et al.*, 1996).

Root Lp_r was extremely sensitive to brief exposure to $50 \mu\text{M}$ HgCl_2 . The inhibition was removed when roots

Table 1. Effect of nitrogen and phosphate deprivation on the hydraulic conductance (L_o), of wheat roots exuding sap under root pressure, sampled in night and day

Treatment	Hydraulic conductance (L_o) (mg g^{-1} root FW h^{-1} MPa^{-1} \pm SE ($n=10$))	
	Dark	Light
Control	350 \pm 44.7	796 \pm 52.4
-N (5 d)	83 \pm 9.7	121 \pm 11.8
-P (7 d)	109 \pm 21.6	189 \pm 17.0

Wheat plants grown on rafts in solution culture; individuals taken for measurements of xylem exudation in the middle of the dark and photoperiods. Sap osmolarity determined by freezing point osmometry (data from Carvajal *et al.*, 1996).

were rinsed with the mercury scavenging reagent dithiothreitol (Fig. 2). The value of L_{p_r} in N- and P-deprived roots was unaffected by the Hg treatment; evidently, some Hg-sensitive component had been lost during nutrient deprivation. While it is tempting to suggest that the lack of Hg inhibition corresponds with a lack of PM water channels of the PIP1 type, which have been shown to be Hg-sensitive (Kammerloher *et al.*, 1994; Kaldenhoff *et al.*, 1998), some caution is necessary. When expressed in *Xenopus* oocytes, PIP1 type aquaporins were inhibited only when concentrations of HgCl_2 in the mM range were used (R Kaldenhoff, personal communication). However, in *Chara* internode cells, a 50 μM HgCl_2 treatment was effective in apparently blocking water channels (Henzler

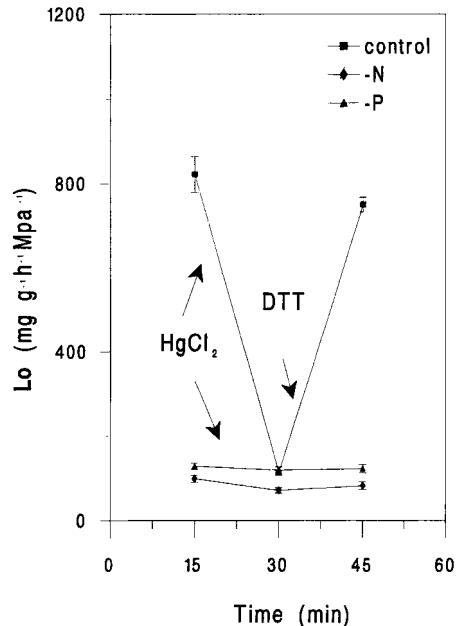


Fig. 2. The response of root hydraulic conductance (L_o) in wheat plants to 15 min treatment with 50 μM HgCl_2 . Conductance is decreased in nutrient-sufficient controls, but is rapidly reversed when Hg is removed from roots by the thiol reagent dithiothreitol (DTT). Roots which had been N- or P-deprived had much lower conductances and were unresponsive to treatment with Hg (from Carvajal *et al.*, 1996).

and Steudle, 1995). Mercury can hardly be thought of as a very specific inhibitor, especially when high concentrations are applied to tissues, but, if L_{p_r} decreased because of a general metabolic blockade by Hg, rather than because of a direct interaction of Hg with water channels, one would expect to see a similar proportional collapse in L_{p_r} in control and nutrient-deprived roots. This did not happen, even though, for example, Hg would be expected to have some effect on membrane potential. There is a report in the literature that water-flow through roots of tomato, driven by an applied external pressure, was severely inhibited by 500 μM HgCl_2 (Maggio and Joly, 1995). Where relatively great rates of water flow are measured, interactions of Hg with ion transport to the xylem may be less important.

In an attempt to resolve this question of whether or not there can be a direct interaction between Hg and water conductance, PM was prepared from wheat roots grown for 4 d with or without NO_3^- in the culture medium, and their rate of shrinkage during exposure to hypertonic solutions was observed. Changes in volume, monitored by changes in light scattering at 500 nm in suspensions of PM vesicles, are very rapid and can be observed only with stopped-flow spectrophotometry. This technique has been explored rigorously by other researchers (Maurel *et al.*, 1997; Niemietz and Tyerman, 1997). Figure 3a and b show the time-course of vesicle shrinking in PM vesicles from control and N-deprived roots. The initial rate, determined graphically, was much slower in the PM from N-deprived roots. This difference was maintained over a temperature range of 15–30 $^{\circ}\text{C}$ (data not shown). The initial rate of shrinkage was slowed down by approximately 50% by the treatment of control PM with 50 μM HgCl_2 for 5 min prior to osmotic challenge. There was no effect on the slower shrinking rate of the PM vesicles from N-deprived roots (Table 2). PM and tonoplast vesicles from wheat roots have been studied (Niemietz and Tyerman, 1997), but, in this case, the water permeability of PM vesicles was not Hg-sensitive; the difference between their results and the results of this study may be related to the nitrate-free medium used to grow their plant material. The cultured tobacco cells used by Maurel *et al.* (Maurel *et al.*, 1997) to prepare membranes were exposed to high concentrations of nitrate in Murashige and Skoog medium, but were obviously habituated to conditions where water fluxes across the PM would have been low; these too showed little or no response to Hg, suggesting the absence of sensitive water channels.

Studies of root L_{p_r} in *Lotus japonicus*

Lotus japonicus can be used to study both diurnal and nutritional responses of L_{p_r} . A well-marked diurnal cycle of L_{p_r} can be observed when root systems are enclosed

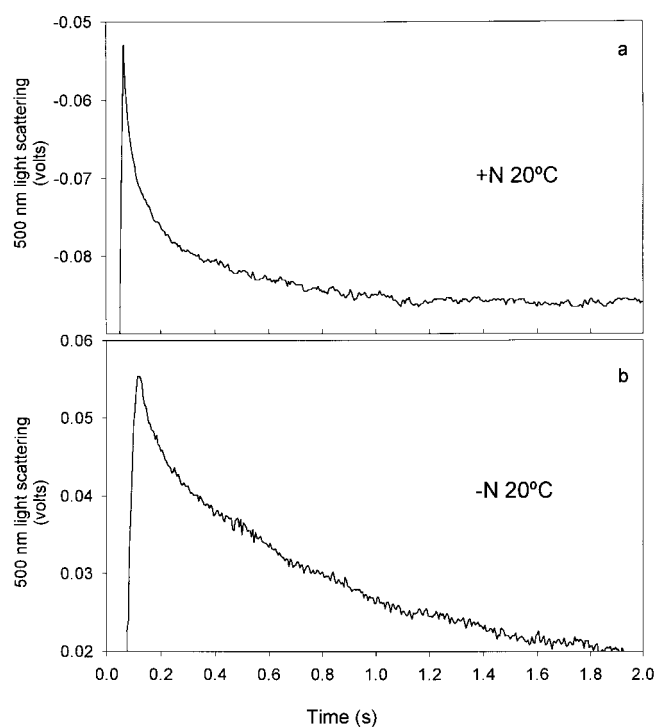


Fig. 3. Changes in light scattering, measured at 500 nm, when PM vesicles from wheat are placed in hypertonic medium and undergo shrinkage. The initial velocity of shrinkage is far greater in vesicles derived from roots grown in nitrate-containing medium. Measurements made using stopped-flow spectrophotometry.

Table 2. Rate of shrinkage of plasma membrane vesicles from wheat roots when placed in hypertonic medium, the effects of N-deprivation (4 d) and brief exposure (5 min) of vesicles to 50 μM HgCl_2

Treatment	Initial shrinking rate (arbitrary units s^{-1})	
	-Hg	+Hg
Control (+N)	1.43	0.45
-N (4 d)	0.81	0.87

LSD ($P=0.05$)=0.287 based on 8 d.f.

Plasma membranes purified by aqueous two-phase separation (Larsson *et al.*, 1987) and kept frozen at -25°C until used. Changes in light scattering as vesicles decreased in diameter were recorded as absorbance changes in a stopped flow spectrophotometer (data from Carvajal, unpublished results).

in pressure chambers and water driven through them at rates comparable to those found in transpiring plants (T Henzler, DT Cooke, DT Clarkson, unpublished results). The same cycle was found in plants transpiring in ambient conditions and where transpiration was greatly reduced (Table 3). The effect of withdrawing nitrate from the culture medium (Fig. 4) was to decrease root L_{p_r} by 80% over 4 d; there was a recovery in when NO_3^- was resupplied. The initial delay in the response is probably explained by the fact that the roots had been supplied with 5 mM NO_3^- and the root tissues contained more

than 60 mM NO_3^- . For some time, the vacuolar store of NO_3^- would have supported both export to the shoot and efflux into the root apoplast (Van der Leij *et al.*, 1998). After 24 h the vacuolar NO_3^- in *Lotus* roots would have been largely dissipated (A Massonneau, unpublished data).

When mRNA from *L. japonicus* roots was probed with cDNAs to *AtPIP1* and *AtPIP2*, strongly hybridizing transcripts were found (Clarkson *et al.*, 1996; RN Waterhouse and AJ Smyth, unpublished results) whose abundance varied diurnally (Fig. 5). Transcript abundances increased to a maximum in the first part of the photoperiod, then declined to a minimum value at the beginning of darkness. The transcript abundance increased at the end of the night, thus anticipating dawn and the daytime increase in transpiration. Figure 5 also shows Northern blots of transcripts of the root-expressed cytosolic form of *L. japonicus* glutamine synthetase and a homologue of the barley high affinity nitrate transporter HvNRT2 (Trueman *et al.*, 1996); these show little diurnal cycling. As might be expected from the results in Table 3, current rates of transpiration had no effect on the abundance of the *PIP1*-homologous mRNA (Fig. 6).

Nitrate-deprivation decreased strongly the abundance of *PIP1*-homologous mRNAs in half of the experiments, but had weaker effects in others. At present there is no explanation for this variable response which contrasts so markedly with the reproducible diurnal pattern.

Western blots of proteins isolated from the PM of *L. japonicus* reveal a single highly abundant band which cross reacts with anti-*AtPIP1a* antiserum. The antibody was raised against 42 N-terminal amino acid residues which distinguish *PIP1*-type aquaporins from others (Clarkson *et al.*, 1996). The apparent MW of this band, 27–29 kDa, is characteristic of aquaporins.

Water channels and variable root L_{p_r}

In *Lotus japonicus* there is strong evidence that the PM contains one or more aquaporins which are homologous to the *PIP1* and *PIP2* types of *A. thaliana*. It has been proved that *AtPIP1b* in that species is an Hg-sensitive water channel; if the expression of the *PIP1b* gene is down-regulated by antisense in *A. thaliana*, the water permeability of the PM is lowered to between 20–30% of that in wild type roots. Water permeability was inhibited by >90% by treatment of protoplast membranes with HgCl_2 (Kaldenhoff *et al.*, 1998). The evidence from wheat roots (Carvajal *et al.*, 1996) and from *Chara* (Henzler and Steudle, 1995) also indicates that root L_{p_r} and L_p of the PM, respectively, are extremely sensitive to brief Hg exposures. However, sensitivity of water permeation to HgCl_2 may not be a reliable guide to the involvement of aquaporins in water fluxes. There is at least one major class of aquaporin which lacks mercury sensitivity (for

Table 3. Diurnal variation of L_p in roots of *Lotus japonicus* transpiring at ambient or reduced rates

Measured under an applied pressure to root systems of 0.4 MPa.

Point in cycle	Root hydraulic conductance ($\text{mg g}^{-1} \text{root FW h}^{-1} \text{MPa}^{-1}$)		(log _e transformed)	
	Ambient transpiration		Reduced transpiration	
Noon	2089	7.664	2579	7.859
Mid-afternoon	1800	7.496	2323	7.751
End of day	484	6.182	885	6.786
Pre-dawn	710	6.565	1052	6.958
Mid-morning	1374	7.225	2365	7.769

LSD ($P=0.05$) between log_e transformed means = 0.447 (based on 34 d.f.): four plants from each treatment were used for measurements at each time of day. Plants were grown in mist culture (Waterhouse *et al.*, 1996); transpiration was reduced by placing transparent covers over the plants. Excised root systems were placed in a pressure chamber and measurements of water flow made for two 30 min periods (data from DT Clarkson unpublished results).

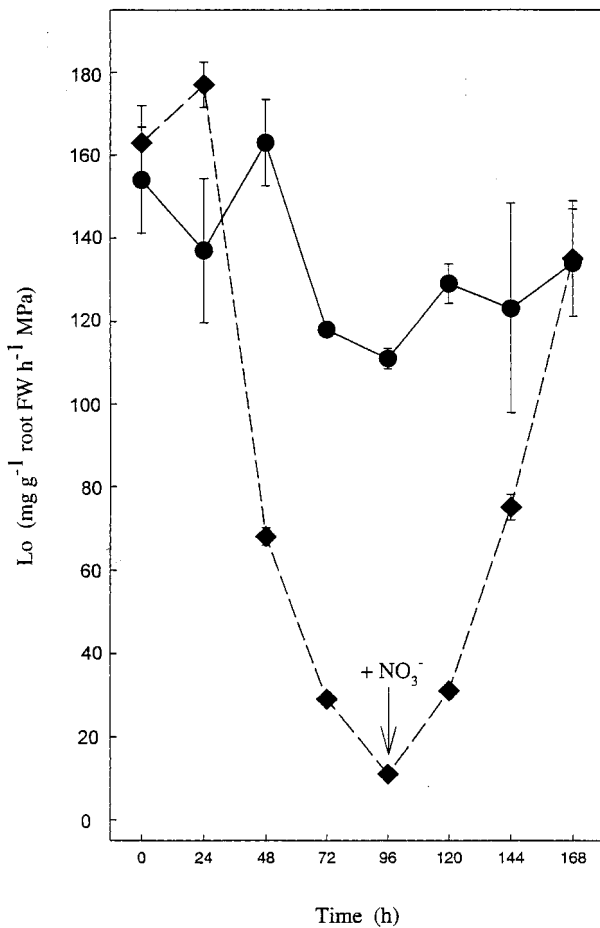


Fig. 4. Time-course of the effects of N-deprivation and re-supply on the hydraulic conductance (L_o) of exuding roots of *Lotus japonicus*. Standard errors of the mean are shown where they are greater than the extent of the symbols. Roots were grown in aeroponic (mist) culture at 18 °C. Shoots were excised, but the roots were undisturbed during attachment of a glass tube to collect xylem sap from the severed stem base. Osmotic potentials of xylem sap and the external medium were estimated by freezing point depression osmometry. (M Carvajal, unpublished results.)

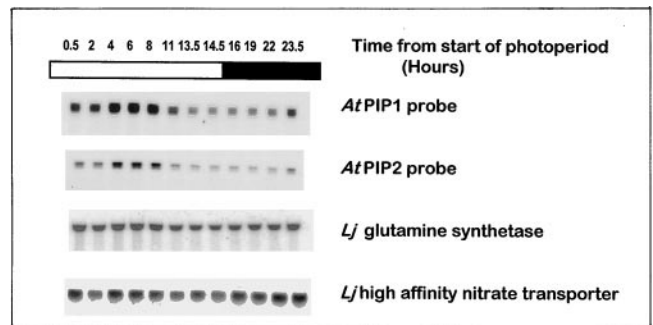


Fig. 5. Northern blots of mRNA from *Lotus japonicus* hybridized to cDNAs from two types of *Arabidopsis* aquaporin genes. Samples taken at different times of day and night. Results from hybridizations to cytosolic glutamine synthetase and a *Lotus* homologue of the barley high affinity nitrate transporter are shown for comparison; they show that diurnal cycling is not characteristic of all mRNAs. (Adapted from T Henzler *et al.*, unpublished results.)

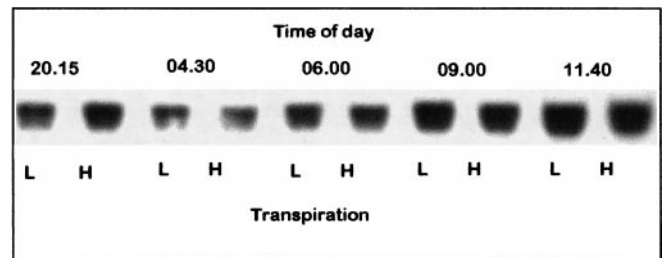


Fig. 6. Northern blots of mRNA from roots of *Lotus japonicus* sampled at different times of day. Low transpiration was achieved by covering the shoots with transparent covers; H refers to plants transpiring in ambient conditions in a greenhouse. Blots were probed with cDNA to *AtPIP1*. (From T Henzler *et al.*, unpublished results.)

example, RD28, Daniels *et al.*, 1994). Clearly, in a membrane where such an aquaporin is the dominant water channel, there would be no Hg-sensitivity. It was found that water permeability of PM vesicles prepared from cultured tobacco cells was not Hg sensitive (Maurel *et al.*, 1997). Moreover, these authors observed that the temperature dependence and activation energy of water transport in these preparations were not significantly different from values to be expected by simple diffusion

of water across the lipid bilayer. From this they concluded that no case could be made for water channels having an important role in PM water permeability in their preparations. In *A. thaliana*, it has been pointed out that if there are Hg-insensitive aquaporins present in root PMs from NO_3^- -fed plants, they, and the diffusive permeability of the PM to water account for no more than 15% of the total permeability (Kaldenhoff *et al.*, 1998). It has also been shown that Hg has no effect on the diffusive water permeation of tobacco PM (Maurel *et al.*, 1997). This study's results with NO_3^- -grown roots of wheat are more in agreement with those of Kaldenhoff *et al.* (Kaldenhoff *et al.*, 1998). The loss of Hg-sensitivity of root L_{p_r} after N- or P-deprivation suggests that either the activity or the density of water channels in the root cell PMs is diminished during nutrient deficiency. This idea has also been advanced to explain changes in the hydraulic properties of roots of *Zea mays* with various levels and types of N nutrition (Barthes *et al.*, 1995, 1996; Hoarau *et al.*, 1996).

It should be borne in mind that changes in the contribution of the apoplasmic pathway to the overall water conduction by the root may contribute to the observed fluctuation in root L_{p_r} (Zimmermann and Steudle, 1998). In particular, water flowing through apoplasmic leaks in younger parts of the root, or at the points of lateral root insertion, may increase root L_{p_r} at higher flow rates. Nevertheless, it is suggested that the opening and closing of water channels will modify the relative rates of flow in the cell-to-cell and apoplasmic pathways (Zimmermann and Steudle, 1998).

During P-deficiency observations on cytosolic Pi levels by ^{31}P -NMR show that it is strongly buffered by vacuolar reserves (Lee and Ratcliffe, 1993). Similar homeostasis in cytosolic NO_3^- has been observed in the first few days of N-deprivation in barley (van der Leij *et al.*, 1998). If there is little change in the concentration of these anions on the cytosolic side of the PM during the early stage of nutrient-deprivation, it is possible that the responses seen are due to changes in the composition of the solution in the apoplasm, i.e. at the extracellular face of the PM. It is by no means clear how such a response might be brought about; there might be some regulatory interaction, perhaps by phosphorylation (Johansson *et al.*, 1996), between ion movement through the anion transporters and either the activity or turnover of water channel proteins. In short, it may be that ion currents through these anion transporters is the message to which the system responds. Against this idea must be set the observations of Barthes *et al.* (Barthes *et al.*, 1996) that the increased L_{p_r} in roots of *Z. mays*, seen when they are moved from an N-deficient medium to one containing nitrate, depended on nitrate reduction. One must conclude that the signal, in this instance, came from the cytoplasm where nitrate reduction occurs. A

signal derived from nitrate or ammonium assimilation cannot explain, however, the strikingly similar effects of P-, S- and N-deficiencies on L_{p_r} . Perhaps there are, indeed, parallel pathways leading to the effect on L_{p_r} .

Downstream effects of changing L_{p_r}

Much evidence is against the view that the stress-induced changes in L_{p_r} grossly or permanently perturb plant water relations. Some of the earliest observations showed that stomata closed without there being any reduction in leaf water status (Chapin *et al.*, 1988). These results call to mind similar effects on stomata in plants where soil begins to dry; where some form of chemical signalling between roots and leaves has been advanced to explain these effects (Davies and Zhang, 1991; Davies *et al.*, 1994; Passioura, 1988; Schurr and Schultze, 1996). Both abscisic acid (Davies and Zhang, 1991) and unknown components of the xylem sap (Munns and King, 1988; Munns *et al.*, 1993) have been shown to increase in concentration in the transpiration stream of plants with roots in drying soil. The stomata close without there being a perturbation in leaf water potential. The signal for the increased release of stomata-closing substances from the root may be some change in root water potential or root turgor pressure. In the case of N-deficiency there are changes in the flux of two plant growth regulators from roots to leaves, namely abscisic acid, the concentration of which has been reported to go up in some species (Krauss, 1978; Clarkson and Touraine, 1994), but not in others (Peuke *et al.*, 1994) and cytokinin, the concentration of which goes down (Beck and Wagner, 1994). At present there is no basis upon which these changes in flux might be related to decreased L_{p_r} . It is well known, however, that ABA can increase L_{p_r} if applied exogenously to roots (Hose and Hartung, 1999).

As mentioned earlier, another frequent response to N- and P-deprivation is increased allocation of dry matter to root growth. This response is also seen during drought stress in *Lolium perenne* (Jupp and Newman, 1987), *Zea mays* (Schmidhalter *et al.*, 1998), *Glycine max* (Huck *et al.*, 1983) among many other species of economic interest. In experiments with *A. thaliana*, lines carrying antisense constructs to *PIP1b* were found to have their root systems, both relatively and absolutely enlarged (Kaldenhoff *et al.*, 1998); there was no evidence of alteration in transpiration rate or shoot growth. The L_{p_r} of the roots of *Arabidopsis*, and of the protoplasts derived from them, was diminished to about the same extent as found for N- and P-deprivation in wheat roots. In all lines, this decline was associated with more extensive root systems; in some cases five times as extensive as those of wild-type plants without there having been a major reduction in shoot size (R Kaldenhoff, personal communication). It seems likely

that the net assimilation rate of the leaves was increased in the antisensed lines to cope with the additional demands created by the growth and maintenance of such a large root system. It has been argued that the carbon costs of extensive root systems are negligible when set against nutritional gains or increased competitiveness (Thomas, 1994).

The intrinsic size of the root system of wheat genotypes may be genetically linked to drought and salinity tolerance; this was indicated by QTL analysis of a mapping population of doubled haploid lines arising for a cross of cv. Chinese spring \times SQ1 (Chinoy *et al.*, 1998). Lines tolerant of these stresses may also have more efficient nitrogen acquisition than those with relatively smaller, or shorter root systems (DT Clarkson and S Quarrie, unpublished results). The enlargement of the root system in 24 independently transformed lines of *A. thaliana* carrying antisense to *PIP1b* suggests a relatively simple manipulation for increasing stress tolerance if other species of economic interest are found to behave similarly. The *Arabidopsis* plants in earlier experiments (Kaldenhoff *et al.*, 1998) behaved as if they had detected hydric or nutrient stress without developing any adverse symptoms.

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