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PLANT FACTORS AND OPPORTUNITIES FOR THE IMPROVEMENT OF ROOT FUNCTIONING

by

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ABSTRACT

This paper describes the movement of water and nutrients through the soil to the root surface, their uptake across the root into the xylem and their flux through the xylem to the leaves. Limitations to nutrient and water uptake and distribution are described and some opportunities for the manipulation of uptake are identified. We consider the possibility of optimising the water and nutrient use efficiency of the plant and enhancing plant growth in water limited environments.

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Keywords: Water and nutrient use efficiency, Plasmamembrane, transporters, Nitrogen sensing, Signalling, Root morphology.

1. INTRODUCTION.

Plants get most of their nutrient and water requirements from the soil and although there are still some surprising gaps in our understanding, we have a good general appreciation of how this is achieved. It is now possible to use new genetic tools to identify the molecular and cellular basis of the regulation of water and ion uptake by roots. The identification of genes involved in ion and water uptake at the membrane level and in the modification of root growth and morphology in response to water and nutrient stress will provide us with new ways of maximising water and nutrient use efficiency in agriculture. We highlight recent developments in root cell biology and recent increases in understanding of root functioning under stress that might be exploited in the future for the benefit of agriculture and the environment.

2. THE MOVEMENT OF NUTRIENTS AND WATER THROUGH THE SOIL AND THE ROOT TO THE XYLEM.

2.1. Movement to the root surface.

In order for roots to take up water and nutrients, they must reach the root surface. Roots can only absorb nutrients from the soil solution and, in moist soil, water and some nutrients will move by mass flow to the roots (driven by a water potential gradient). This mechanism is most important for those nutrients needed by the plant in both large and very small amounts. In drying soil this can become a problem as water flow to roots will be greatly reduced and in loose soil the root soil interface can become particularly dry, greatly limiting the supply of water and nutrients to the root surface. Under these circumstances, the continued growth of roots is important and there is good evidence that root growth can be sustained when water and nutrient availability is low. Restricted shoot growth under these conditions will enhance root shoot ratios (see below).

In moist soil, ions will diffuse down a concentration gradient through the soil to the surface of the roots. Rates

of diffusion will vary enormously with diffusion slowed by the influence of clay particles which act as cation exchangers. Some ions are very immobile because of their low solubility (e.g. phosphate, PO_4^{3-}). Again here the continued growth of roots or the proliferation of roots into nutrient rich patches can be important to sustain uptake of ions that can only move slowly through the soil. The cation exchange capacity of the plant cell wall can also be an issue for ion uptake. Pectins act as cation exchangers so that many multivalent cations accumulate in the cell wall. Walls of different plant species differ in cation exchange capacity.

The movements of nutrients and water to the root surface are important and it is necessary to give active consideration to this component of the transport pathway. There is little point in manipulating root uptake capacity if the supply of nutrients or water is limiting. For example in drying soil, water uptake is limited substantially by water supply to the root surface, however low the root resistance to water uptake.

2.2. Movement from the root surface to the xylem.

Water and ions can move across the root surface to the xylem through an apoplastic or a symplastic path. Once in the symplasm, ions will move from cell to cell via plasmodesmata. Apoplastic movement is constrained by the Casperian band in the walls of the endodermis. Similar bands can also develop in the hypodermis of some roots.

Freundl *et al.* (1998) have recently highlighted species differences in the proportion of water moving into plants along a pathway that is exclusively apoplastic (i.e. no membranes transport is involved in the pathway). These authors have identified a water transport pathway that contains a so-called apoplastic bypass in *Zea mays* while they have noted that in *Helianthus* a greater proportion of the transpiration stream moves through the symplast. Apoplastic water flow raises the possibility that nutrients and other soil-sourced chemicals will gain free access to the transpiration stream and Hartung and co-workers have reported concentrations of ABA in the soil of up to 10 nM (nano molar, 10^{-9}). It seems likely that the importance of this ABA is not as a potential root signal but rather to prevent the ABA in the plant from flooding out into the soil (Slovik *et al.*, 1995). Nevertheless, the micro-organisms in the rhizosphere can also produce other plant hormones in the vicinity of the root and it seems likely therefore that root sourced chemicals may have a role to play in signalling, particularly in plants with an apoplastic bypass. This bypass will also have a dramatic influence on the effect of transpiration flux on the concentration of nutrients in the xylem stream and the delivery of these nutrients to the shoot.

There are not many data in the literature which allow us to test these hypotheses but we have investigated the effects of sap flow rate on the flux of nutrients to the shoots of cherry trees from different rootstocks. This has been done using the root pressure vessel which allows calculation of nutrient delivery with varying sap flow rates that compare with those shown by intact plants. This is important if we are to avoid artefacts induced by concentrating effects of root exudation. Variation between rootstocks in delivery with sap flow (Figure 1, overleaf) suggests some difference in the extent to which concentrations are diluted by increasing transpiration flux and therefore perhaps, some variation in the extent of apoplastic bypass. Differences of this kind presumably contribute to the well-defined dwarfing and stress resistance properties of rootstocks.

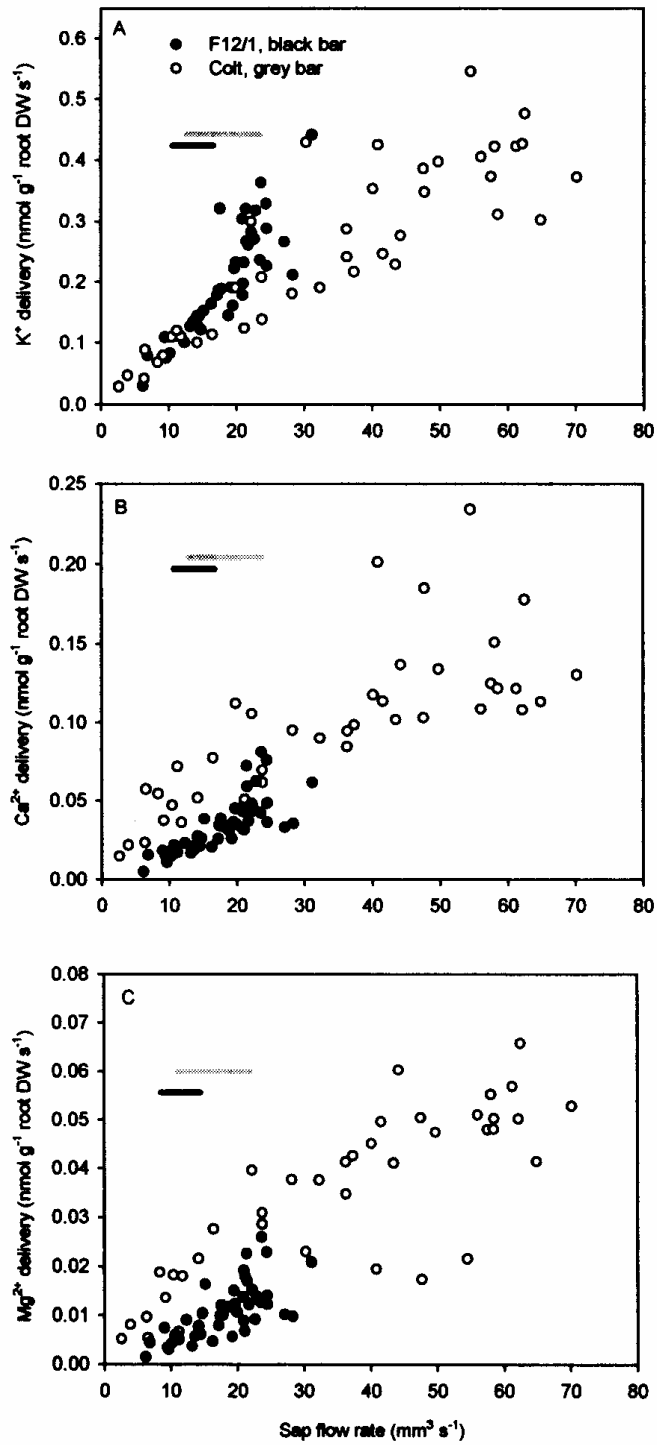


FIGURE 1: : The effect of xylem sap flow rate on delivery of K⁺ (A), Ca²⁺ (B) and Mg²⁺ (C) from sweet cherry rootstocks Colt and F12/1. Deliveries are expressed per unit of root dry weight. Horizontal bars indicate the range of transpiration rates measured in intact plants.

3. THE PLASMA MEMBRANE AND MEMBRANE TRANSPORTERS FOR WATER AND NUTRIENTS.

Ions and water moving symplastically through the root will need to cross the plasma membrane. This is a barrier about 8 nm thick which controls chemical traffic into and out of the cell. The membrane is made up of a lipid bi-layer and it is well known that such bi-layers are selectively permeable to different types of molecule. The smaller the molecule and the less polar it is, the more rapidly it diffuses across the bi-layer. This means that the bi-layer will be a barrier to inorganic ions, amino acids and sugars but will be relatively permeable to water, ethanol and CO₂ and oxygen. Biological membranes have rather different properties to synthetic membranes and this is because biological membranes also contain proteins, many of which have specific functions in facilitating and regulating the movements of solutes (and water!).

There are several different kinds of membrane transporters:

3.1. Channels.

These allow movement of small ions through a hydrophilic pore. Movement of ions is generally passive down an electrochemical gradient and can be very rapid. Channels can be opened or closed by physical or chemical factors. Water channels or aquaporins in plant plasma membranes have been identified relatively recently.

3.2. Carriers - uniporters.

These allow facilitated diffusion (no hydrophilic core) where the ion binds to the protein causing conformational change. Ion movement is slow.

3.3. Carriers – co-transporters.

These catalyse active transport against an electrochemical gradient with ion movement driven by a proton gradient. Again, substrate binding will cause conformational change. There are two types of co-transporters, namely antiporters, where solute is moved in the opposite direction to protons and symporters where solute is moved in the same direction as protons.

3.4. Pumps.

These directly use the energy from ATP to transport solutes across membranes (e.g. protons and calcium) The plasma membrane proton pump creates a proton gradient and an electrical difference (membrane potential) across the plasma membrane and this proton gradient drives the symporters and antiporters. Cations will also move down the electrical gradient (membrane potential) across the membrane. Figure 2 shows the roles of different classes of plasma membrane transporters involved in nutrient and water uptake. Different types of transporters can be associated with different phases of the kinetics of nutrient uptake. Nutrient uptake systems are complex and are therefore not so easy to manipulate using molecular genetics. Nitrate uptake in *Arabidopsis* involves 5 to 8 genes.

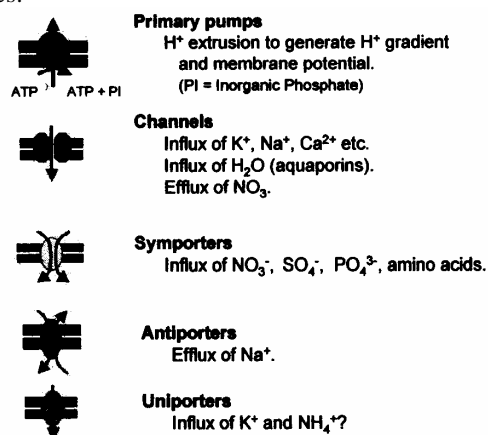


Figure 2: The roles of different classes of plasma membrane transporters involved in nutrient and water uptake.

Aquaporins may have a role in the fine tuning of water relations between different cell types of the plant rather than in modifying the hydraulic properties of roots or whole root systems (Tyerman *et al.*, 1999) (but see below). Down regulation of aquaporins does seem to influence the water relations of plants. For example, Kaldenhoff (pers. comm.) has reported that disappearance of turgor-driven diurnal leaf movements in anti-sense plants (plants in which gene activity has been suppressed).

3.5. Case study: K⁺ transport across roots – the involvement of transporters.

Tracer flux studies have identified two distinct systems of potassium uptake by roots, a high affinity system operating at external concentrations of 1 mM or less and a low affinity pathway that operates at higher concentrations. These systems are excellent targets for molecular analysis and some years ago now a cDNA (HKT1) was isolated from wheat which after heterologous expression in *Xenopus* oocytes was identified as a high affinity K⁺ symporter (Schachtman and Schroeder, 1994). Expression has been localised to the cortex of the root. More recently other genes coding for high affinity K⁺ uptake have been identified. One of these, HvHAK1 is expressed in roots and its expression is enhanced in K⁺-depleted conditions but inhibited by NH₄⁺ (Santa-Maria *et al.*, 1997).

Potassium channels will contribute to K⁺ uptake when the membrane potential is more negative than the Nernst potential for K⁺. Two cDNAs (KAT1 and AKT1) code for K⁺ channels in *Arabidopsis* (Sentenac *et al.*, 1992; Anderson *et al.*, 1992).

After entering the root symplasm, K⁺ can diffuse via plasmodesmata to the cells within the stele. Ion release to the apoplast and the xylem is highly regulated and likely to involve ion channels. Patch clamping has identified outwardly rectifying K⁺ dependent currents (K_{OUT}) in stelar cells from *Arabidopsis*, maize and barley. The channels that underlie these currents are highly selective for K⁺ among the monovalent cations (Roberts and Tester, 1995). A new gene (SKOR) coding for a K_{OUT} channel has recently been identified in *Arabidopsis* roots (Gaymard *et al.*, 1998) and SKOR knock out mutants had a 50% reduction in the shoot K⁺ content, showing that K_{OUT} channels mediate K⁺ release to the xylem.

4. REGULATION OF XYLEM LOADING AS A FUNCTION OF WATER AND NUTRIENT AVAILABILITY.

It is clear that changes in the water status of the roots and/or the accumulation of abscisic acid in roots will modify the activity of ion channels in the plasma membranes of the roots. For example, Roberts (1998) (see also Roberts and Snowman, 2000) has shown that ABA accumulation and water stress reduce potassium transport to the xylem and that potassium accumulates in the roots as a result. Measurements of membrane potentials of ABA treated steles of maize roots show that hormone treatment induces substantial increases in membrane potential and the observation that the magnitude of this increase depends on extracellular [K⁺] indicates that the major conductance across the plasma membrane results from K⁺ transport. Patch clamp techniques demonstrate significant inhibition of K_{OUT} channel activity by ABA and water stress treatment, while K⁺ activity is enhanced (Roberts and Snowman, 2000).

Potassium accumulation allows osmo -regulation and therefore continued root growth in drying soil (Sharp and Davies, 1979) but the importance of reduced potassium transport has not been fully considered. Extracellular potassium is likely to influence both stomatal behaviour and growth (Van Volkenburgh and Davies, 1983) and it therefore seems likely that modified transport of this ion can act as a 'measure' of soil drying. Gollan *et al.* (1992) have shown significant variation in K⁺ transport through the xylem of sunflower plants in drying soil.

Very interestingly, Holbrook and co-workers have shown that the concentration of potassium ions moving through the xylem can influence the hydraulic conductivity of the transport pathway, perhaps by affecting the nature of the pit membranes (Holbrook, pers. comm.). This means that a root- sourced chemical signal can influence the properties of the water transport pathways through the root and therefore influence the hydraulic signalling between the roots and shoots.

5. REGULATION OF WATER MOVEMENT THROUGH AQUAPORINS BY PLANT NUTRIENT STATUS.

Nitrogen, phosphorus and sulphur deficiencies result in major reductions in the hydraulic conductivity (L_p) of plants with conductivity decreasing in the early stages of ionic deprivation. These effects can be reversed within hours of re-supply of the nutrients. Clarkson *et al.* (2000) have shown that the L_p of nutrient-sufficient wheat plants is sensitive to treatment with mercury salts while this is not the case with nutrient deficient plants where the L_p is already low. Diurnal fluctuations in L_p are also apparent and there is a very marked diurnal cycle in the abundance of mRNAs of aquaporin gene homologues in the roots of *Lupinus japonicus* (*PIP1* and *PIP2*). The maxima and minima appear to anticipate the diurnal fluctuations in L_p by between 2 and 4 hours. The lack of Hg-sensitivity in roots from N-deprived plants is circumstantial evidence that lowered root L_p may be due to either a decrease in the activity of water channels or their density in the plasma membrane. Clarkson *et al.* (2000) have suggested that roots are capable of monitoring the nutrient concentration of the solution in the apoplast and initiating appropriate responses. It is the incoming nutrient concentration that is monitored, not the plant's nutrient concentration.

There may be some advantage in modifying the plant's sensitivity to nutrient deprivation in terms of modified root hydraulic properties. It is possible that a nutrient-induced limitation in shoot growth is actually a function of modified shoot water relations.

6. NUTRIENTS, WATER AND PLANT SIGNALLING.

There are now many other examples of situations where plants can 'measure' the water or nutrient availability in the soil and initiate appropriate physiological or developmental responses. One of the most important of these responses is the observation that the induction of nutrient transporters under conditions of nutrient deficiency (e.g. P deficiency induces PO_4^{3-} transporters, S deficiency induces SO_4^{2-} transporters). Nitrate uptake is also feedback-regulated by the N-status of the plant. This observation implies the existence of a mechanism for sensing nutrient status in the shoot and the movement of long distance signals through the phloem to the shoot (e.g. Ismande and Touraine, 1994). We have demonstrated the sensitivity of nitrate uptake of some plants to the presence of amino acids around the roots (Figure 3). Interestingly some plants are relatively insensitive to amino acid application, raising the possibility that long distance signalling of N-sufficiency may be of considerable ecological significance. Some soils contain significant accumulations of amino acids which can be a nitrogen source in their own right (e.g. Streeter *et al.*, 2000) but more interesting in the current context is the possibility that accumulations of amino acids in the soil may influence the plant's capacity to compete for nitrate.

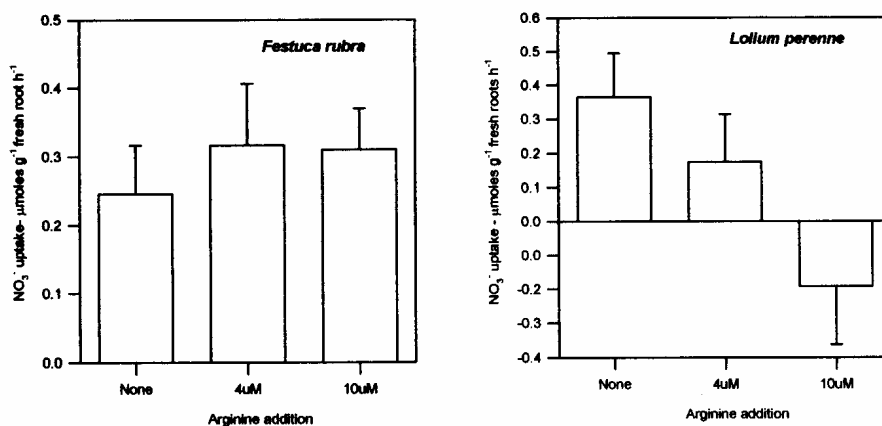


Figure 3: Uptake of nitrate nitrogen from a 0.5 mM solution by two plant species during a 24h period at the concentrations of arginine shown.

Roots contain enzymes and precursors that synthesise ABA, one of the plant's potent long distance hormonal signals. We still lack information on which enzymatic step of ABA biosynthesis is responsible for the measurement of reduced soil water availability or a change in nutrient availability but it seems likely that much of the plant's response to changes in nutrient and water availability in the soil is a function of changes in the long-distance signalling mechanisms in plants. These can involve hormones, water or nutrients themselves.

Besides a reduced water content, soils in arid environments exhibit high salt concentrations, high soil strength, alkaline pH values and low amounts of nutrients. All these factors will influence ABA accumulation. Reduced soil water availability, increased salt concentrations (NaCl and CaCl₂) ammonium supply, phosphate deficiency and high mechanical impedance stimulate ABA biosynthesis in roots. Nitrate deficiency, flooding and alkaline conditions reduce it Jeschke *et al.*, 1997; Wolf *et al.*, 1990; Hartung and Jeschke, 1999). Under conditions of soil drying and salt stress large amounts of ABA are deposited in root tissues and loaded into the xylem and this ABA signal can have dramatic effects on the growth development and functioning of the plant (e.g. Davies and Zhang, 1991; Davies *et al.*, 2000) with increased ABA concentrations usually limiting leaf growth, closing stomata and possibly acting to sustain root growth. In circumstances where plants generate chemical signals which can regulate growth and functioning of plants in drying soils, the intensity of the signal seems to depend upon the availability of water to the root system. We now have some knowledge of the key steps in the ABA biosynthetic pathway and some of these genes have been isolated. It will be interesting to investigate the detection of soil water availability by transgenics where the activities of some of these genes are suppressed.

Uptake and transport of several ions can also be modified by soil drying and some of these changes can be quite sensitive to small changes in water availability. For example, small moisture deficit-related changes in the transport of nitrate ions can act as a sensitive regulator of growth (McDonald and Davies, 1996) and cell physiology and biochemistry (Shaner and Boyer, 1976). Concentrations of a range of ions in the xylem are also affected by soil drying (Gollan *et al.*, 1992) and these changes and changes in xylem pH can have important effects on plant growth and functioning (e.g. Sauter *et al.*, 2001).

7. ROOT LENGTH AND ROOT LENGTH DENSITY.

We have noted above that the sustained growth of roots and root systems can be important particularly to sustain uptake of ions that move to root surfaces by diffusion. This is because roots will generate depletion zones of nutrients which depend upon the rate of uptake and mobility of the nutrient. Rapid growth and proliferation of roots can minimise this effect until depletion zones start to overlap and roots start to compete with one another. Root hairs can greatly extend depletion zones and increase the availability of nutrients.

It is commonly observed that the root:shoot ratio of plants increases substantially when nitrogen is in short supply. This may be because shoot growth is restricted while root growth is sustained under these conditions but recent observations by Zhang and Forde (1998, 2000) are also pertinent. These authors described two distinct effects of nitrogen on root branching, both of which will result in a modification of root morphology. One effect is an inhibition of lateral root growth as the N status of the plant increases and the other is a stimulation of lateral root growth in response to localised increases of nitrate availability in the soil (a patch of nutrient). The combination of the two effects can be an integrated regulation of root growth as a function of both nitrate availability in the soil and the N status of the plant.

7.1. A systemic inhibitory effect plant nitrogen status.

Zhang and Forde (1998) have reported a systemic inhibitory effect of NO₃⁻ on lateral root development of *Arabidopsis*. The extent of the inhibition is related not only to the external supply of nitrate but also to how much of the root system is exposed to nitrate supply. This indicates that it depends on the total amount of nitrate absorbed by the root system, rather than the external nitrate concentration *per se*. The inhibitory effect acts only on immature lateral roots during a discrete phase just after their emergence from the primary root. The numbers of lateral roots initiated are not affected by nitrate supply but when the N status of the plant is high, they only develop to a stage where they are a few tenths of a mm in length. Zhang *et al.* (1999) have reported that high concentrations of glutamine in the root will also inhibit the extension of lateral roots,

suggesting that N compounds generally may contribute to the inhibitory signal in the plant. We have considered above the idea that physiological processes in roots are regulated by the N status of the shoot, although the exact nature of the signal is still a matter for debate (Stitt and Scheible, 1998).

7.2. Localised root proliferation.

We have known for many years that development of plant root systems will respond to an uneven distribution of nutrients in the soil (e.g. Drew and Saker, 1975). The theory is that a response of this kind enables the plant to use resources optimally and concentrate growth in regions where most benefit will be gained. The response is particularly important for immobile nutrients such as phosphate and ammonium but can also be important for nitrate when plants are in competition.

Zhang and Forde (1998) have applied localised nitrate treatments to *Arabidopsis* roots and shown an increased production of lateral roots specifically within the nitrate rich zone. This was due to a 2-3 fold increase in the rate of lateral root elongation, there being no increase in the numbers of first order laterals. Even nitrate concentrations as low as 50pM in the enriched zone were able to stimulate elongation rates by 50%.

Two pieces of evidence suggest that the increased rate of lateral root elongation seen in nitrate enriched zones is a response to the nitrate ion alone and does not involve its role as an N source. Zhang *et al.* (1999) found that N sources other than nitrate failed to stimulate the root response. In addition, a nitrate reductase double mutant responded to nitrate in a similar fashion to the wild type, indicating that the response is not dependent on the plant's ability to assimilate nitrate. There is good evidence from a range of studies that roots are able to sense the presence of nitrate ions in the environment and use the signal to modulate gene expression. e.g. nitrate reductase genes are inducible within minutes and their induction is not dependent on a ability to synthesise new proteins (Gowri *et al.* 1992).

Zhang and Forde (1998) have identified a number of cDNA clones representing novel nitrate inducible genes. One of these, *ANRI*, was shown to be expressed mainly in roots and to be rapidly induced when N-starved roots were treated with nitrate. The role of this gene was investigated by generating a number of antisense and co-suppressed *Arabidopsis* lines in which the gene was down regulated to various degrees (Zhang and Forde, 1998). In the most strongly down regulated lines, lateral root growth no longer responded to the stimulatory effect of localised nitrate supply.

8. CONCLUSIONS.

We now have some understanding of the molecular genetics behind the sensing of water availability and nutrient availability in the soil and of some of the key plant responses to perturbations of this kind. This knowledge will allow us to manipulate many of these properties although modification of membrane properties affecting the uptake of ions and water will be of little benefit in circumstances where the supply of substrates to the root surface is restricted. Manipulation of root morphology will allow increased foraging for water and nutrients and Zhang and Forde's work on the molecular basis of nitrate sensing and the growth response of lateral roots seemingly provides an excellent opportunity for increasing the scavenging capacity of root systems for nitrate. It may also be possible to do this by relatively simple agronomical manipulation. Partial root drying techniques are now being used to increase the efficiency of water use (Davies *et al.*, 2000). These depend on a knowledge of the plant's stress signalling systems and one of the results of the use of controlled drought stress is the deeper growth of roots in soil with a consequent increase in water and nutrient availability. Whether we use molecular or agronomical techniques, an understanding of the physiology of the plant is of undeniable benefit in sculpting the appropriate manipulation of plant functioning.

9. ACKNOWLEDGEMENTS.

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