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Update on Mineral Nutrition

N Demand and the Regulation of Nitrate Uptake[1]

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Uptake of nitrate by root cells followed by reduction and assimilation in plant tissues is the main route by which mineral N is converted into organic N by living organisms. Like photosynthesis, these are life-dependent processes that members of the animal kingdom are unable to perform for themselves. Nitrate and other mineral nutrients required for optimal plant growth and development frequently exist at relatively low concentrations in soil. To thrive on these dilute nutrients, plants have developed high-performance uptake systems in their root cells. To cope with wide variations in mineral concentrations in soil, plants have evolved mechanisms to regulate the activity of uptake systems so that net intake of a nutrient depends on the plant's need for this element rather than its concentration in the rooting medium. Indeed, uptake rates of most ions are seemingly controlled by specific demand-driven regulatory mechanisms. Such processes set the uptake rate of a given element to match the plant's current growth rate and developmental stage. Nitrate uptake is of special interest because nitrate is absorbed at a relatively high rate and because compounds that function as uptake sensors may have been identified. This paper focuses on whole-plant signaling processes involved in the regulation of nitrate uptake by N demand.

NITRATE UPTAKE LIMITS NITRATE ASSIMILATION

Nitrate-fed plants seldom accumulate an excess of either nitrite or ammonia, indicating that reduction of nitrate to nitrite usually is the rate-limiting step in nitrate assimilation. The rate of nitrate reduction in situ, however, is controlled primarily by the rate of nitrate uptake rather than by alterations in NR activity (Wilkinson and Crawford, 1993) or limitations in reducing power (Warner and Huffaker, 1989). Thus, nitrate uptake appears to control N assimilation in nitrate-fed plants.

WHAT LIMITS NITRATE UPTAKE?

Experiments in which both nitrate availability and plant growth rate are manipulated independently show that nitrate uptake rates are determined mainly by regulatory processes that coordinate nitrate uptake and biomass production. This generalization is seemingly independent of plant species. Legumes, however, are a particularly interesting family because they can obtain N via nitrate uptake, symbiotic N_2 fixation, or both. Hence, even when N concentrations of legumes are rather constant, their rates of nitrate uptake vary appreciably, depending on nitrate availability, the extent of nodulation, and plant developmental stage.

In higher plants, two types of variation in nitrate uptake rate have been identified: (a) temporal responses to modifications of environmental factors such as light intensity, temperature, or stress conditions; and (b) variations that occur during ontogeny. In the first case, when a discrepancy exists between internal N supply and growth rate, nitrate uptake varies so that the amount of N in the different internal pools remains relatively constant. During ontogeny, the rate of nitrate uptake varies dramatically. Nitrate uptake per unit of root weight is several times greater in 3-week-old hydroponically grown soybean plants (Touraine et al., 1992) than in 5-d-old seedlings (Muller and Touraine, 1992). Furthermore, rate of nitrate uptake by cowpea, green gram, and soybean increases throughout vegetative growth, peaks during the early reproductive stages, and then declines during pod and seed development (Imsande and Edwards, 1988). By midpod fill, the rate of nitrate uptake can be less than half of the maximum rate observed during the full-bloom stage. This decline in nitrate uptake is at least partly responsible for the apparent N deficiency that develops in soybean during pod fill and explains why well-nodulated, field-grown plants generally do not respond to added fertilizer N (Imsande, 1989).

At harvest, 75% of total plant N is contained in the soybean seed. Approximately half of the seed N is derived from foliar proteins. Thus, during seed development, foliar N concentrations decrease with an increasing rate from approximately 45 mg N g^{-1} leaf dry weight to less than 20 mg N g^{-1} (Hanway and Weber, 1971). The resulting amino acids are transported via the phloem to the developing pods and seeds. Thus, during reproductive growth, the rates of nitrate uptake and foliar protein degradation are negatively correlated. Is there

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Abbreviations: HATS, high-affinity NO_3^- transport system; LATS, low-affinity NO_3^- transport system; NR, nitrate reductase; VSP, vegetative storage proteins.

a mechanistic link between these two events? If so, does it involve a demand-driven control of nitrate uptake?

CARBOHYDRATE SUPPLY AND N DEMAND

It has been proposed that the supply of carbohydrate in the phloem sap may regulate nitrate uptake. The carbohydrate-supply:N-demand theory rests upon the fact that transport of an anion across a membrane polarized with the negative face inside is likely to be energy dependent. Indeed, calculated values suggest that the uptake of 1 mol of nitrate consumes 1 to 2 mol of ATP. Although not insignificant, this level of energy utilization accounts for only 5% of the total root carbon catabolism (Bloom et al., 1992) and perhaps 10% of the total N nutritional costs (Raven, 1985). Nevertheless, positive correlations can be observed between carbohydrate supply to the root and nitrate uptake. For example, oscillations in nitrate uptake can be synchronized with leaf emergence (Vessey et al., 1990). The observed rhythmicity in nitrate uptake was proposed to result from changes in the rate of carbohydrate translocation, which, in turn, resulted from the N-dependent development of new leaves (Lim et al., 1990). Such correlations, however, are not evidence that carbohydrate supply per se is a regulatory signal for nitrate uptake, and they provide no insight into molecular mechanisms controlling nitrate uptake. Furthermore, if carbohydrates per se were to play a regulatory role in ion uptake, this role could not be specific for nitrate because energy-dependent variations in ATPase-H^+ pump activity would change the activity of all carriers simultaneously.

NITRATE UPTAKE AND PHLOEM-TRANSLOCATED AMINO ACIDS

N demand of the plant may be defined as the difference between organic N derived from current assimilation and the amount of N required to sustain an optimal growth rate. The simplest scheme for demand-driven ionic uptake would be one where the concentration of that ion in the root cytosol controls either the synthesis or activity of the carrier responsible for the absorption of that ion. Indeed, using NR-deficient barley mutants, it has been shown that nitrate accumulation in root cells inhibits nitrate uptake (King et al., 1993). This scheme, however, cannot be functional at the whole-plant level, simply because nitrate ions are not translocated in the phloem, and hence, cannot inform roots of shoot N demand.

Internal signals for specific alterations in nitrate uptake must be molecules whose concentration, distribution, or compartmentation is dependent on the N demand of the plant. The presence, or at least the amount, of the signal(s) in the plant must thus be related to nitrate assimilation. More precisely, some product of ammonia assimilation must be involved in regulation of nitrate uptake because inhibitors of Gln synthetase, the major enzyme activity in ammonium assimilation, are reported to prevent the inhibitory effect of ammonium on nitrate uptake (Breteler and Siegerist, 1984).

When added to nutrient medium, several amino acids inhibit uptake of nitrate by roots of *Arabidopsis*, dwarf bean, and soybean (Muller and Touraine, 1992). This effect is unlikely to be caused by an interaction of exogenous amino acids with the outer face of the plasma membrane of root cells because a 3-h lag phase generally occurs before nitrate uptake is affected. Moreover, when seedlings are transferred to an amino acid-free solution, nitrate uptake rates are not restored for several hours (Muller and Touraine, 1992). These delays are inconsistent with an allosteric interaction between external amino acids and a nitrate transporter.

The role of amino acids in nitrate uptake also can be studied by immersing cotyledons of young seedlings in a concentrated solution of amino acids. With this technique, the phloem translocation rates of several amino acids were specifically enhanced (Muller and Touraine, 1992). Some amino acids (Arg, Ala, Asn, Gln) strongly inhibited nitrate uptake, whereas others (Glu, Met, Asp) were only weak inhibitors, and still others (His, Ile, Ser, Val, Phe, Leu) had no effect or slightly stimulated nitrate uptake. This pattern of inhibition was very similar to that found when amino acids were supplied directly to roots in nutrient medium (Muller and Touraine, 1992), suggesting that amino acids or peptides circulating in the phloem may control the rate of nitrate uptake by roots.

CONTROL OF NITRATE UPTAKE BY N DEMAND: THE SATIETY SIGNAL

The net rate of nitrate uptake usually is markedly lower than the uptake capacity of the roots. This suggests that the rate of nitrate uptake is normally under negative control and, thus, is more likely to be depressed by a satiety signal than to be stimulated by a positive demand signal. Under steady-state conditions, cycling of amino N between shoot and root provides a realistic scheme for the down-regulation of nitrate uptake.

Synthesis and degradation of VSP in soybean may provide additional support for the satiety model. During the vegetative phase, accumulation of VSP in leaf tissue correlates positively with the availability of NH_4NO_3 in the rooting medium (Staswick et al., 1991). As the rate of nitrate uptake declines during pod fill, the abundance of VSP drops from approximately 15% of the soluble leaf protein to 1%. Thus, nitrate uptake rates seem to parallel rates of synthesis and subsequent degradation of VSP. Also, both nodule development and nitrogenase activity may be regulated, at least in part, by nitrogenous compounds in the phloem sap (Oti-Boateng and Silsbury, 1993).

COORDINATION OF NITRATE UPTAKE AND NITRATE REDUCTION RATES

Intracellular pH control by organic acids is another possible mechanism for regulation of nitrate uptake. Alkaline ions formed during nitrate reduction (1 mol of hydroxide equivalent for every mol of nitrate reduced) cannot simply be expelled from the cells. Therefore, to maintain pH homeostasis, the plant synthesizes strong organic acids (mainly oxal-

oacetate from PEP and HCO_3^-) by PEP carboxylase in the leaf. Thus, when nitrate reduction occurs primarily in the leaves, as is the case for most *Phaseoleae*, considerable amounts of organic acids are formed. The number of negative charges generated by nitrate reduction in nitrate-fed plants exceeds by 3-fold the accumulated concentration of carboxylates (Touraine et al., 1988). This disparity results from the fact that the carboxylates are transported, mainly as K malate, by the phloem to the root, where they are decarboxylated. The HCO_3^- is excreted by the root, and the K^+ subsequently accompanies a newly acquired nitrate anion up the xylem to the leaf (Touraine et al., 1988). The translocation rate of K malate thus depends on the rate of nitrate reduction in the leaves and reflects the status of N metabolism in these organs. Hence, K malate may be responsible for the observed coordination between nitrate uptake and nitrate reduction. Nitrate uptake rates by roots of intact soybean plants are altered by malate, whether supplied directly at the root surface or internally via the phloem (Touraine et al., 1992). As would be predicted, stimulation or inhibition of nitrate reduction in the shoot induces or depresses, respectively, nitrate uptake in the roots (Touraine et al., 1992).

LEAF EXPORT PRODUCTS AND THE CONTROL OF NITRATE UPTAKE

Control of nitrate uptake both by amino acids and organic acids may, at first glance, seem contradictory because these two signals have opposite effects. However, the two classes of compounds generally are not exported in parallel. Compare, for example, the physiology of a legume during rapid vegetative growth with that during rapid pod fill. During rapid vegetative growth, the rates of nitrate reduction, carboxylate synthesis, and amino acid synthesis are high. Most of the amino acids will be used locally for the synthesis of Chl, Rubisco, VSP, etc., whereas the carboxylates will be exported rapidly to the root. Thus, the nitrate transport system encounters a high level of HCO_3^- and a low level of phloem-translocated amino acids (Fig. 1a).

During pod fill, on the other hand, nitrate reduction in leaves and the availability of HCO_3^- in the roots are decreased, whereas amino acid export from the leaves is increased because of leaf N remobilization. Most of the N translocated from the leaves is ultimately designated for the filling pods. Because the cycling rate of N in the xylem-phloem circuit is very rapid (Cooper and Clarkson, 1989), the phloem entering the root during pod fill is likely to be enriched with amino compounds. Thus, as depicted in Figure 1b, the rate of nitrate uptake should be lessened both by a slower rate of organic acid translocation and by a higher amino acid concentration in the phloem.

LEAF-GENERATED SIGNALS LEAVE UNANSWERED QUESTIONS

Clearly, the proposed mechanisms controlling the rate of nitrate uptake by amino acids and carboxylates are different. Whereas amino acids would act as a regulatory signal, carboxylates might act as substrates for the nitrate uptake system. Control of nitrate uptake by phloem-translocated organic acids poses two questions: (a) what determines the export rate of organic acids from the leaves, and, (b) by what mechanism do carboxylates function in the roots? It can be calculated that the shoot of a 16-d-old soybean plant releases about 1 mM OH^- min^{-1} (Touraine et al., 1988). Since the cytosolic buffer capacity in plant cells is below 20 mM H^+ per pH unit (Takeshige and Tazawa, 1989), this flux of alkaline charges would produce a pH shift of 0.5 units in less than 10 min. Although there is debate about whether or not the very small pH variations observed in vivo are sufficient to enhance PEP carboxylase activity (Kurkdjian and Guern, 1989), ultimately, nitrate reduction in shoots is obligatorily bound to organic acid synthesis. Accumulation of carboxylate anions and their accompanying mineral cations poses acute osmotic problems for leaf cells. Thus, it is likely that increased production of organic acids in shoots leads to increased export via the phloem.

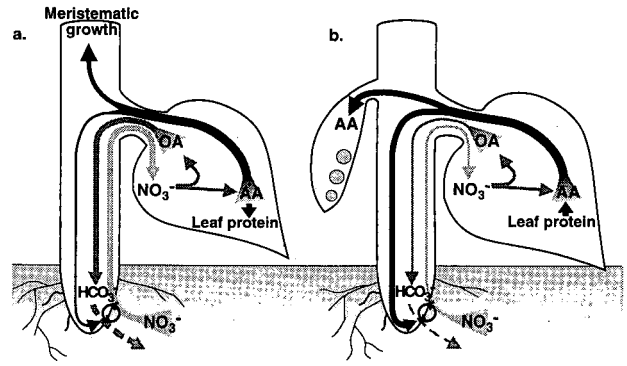

Figure 1. A model for the control of nitrate uptake by leaf-generated signals during rapid vegetative growth (a) and rapid pod fill (b). During vegetative growth (a), nitrate ions are rapidly absorbed by the root and transported via the xylem to the leaf. In the leaf, nitrate reduction produces organic acids (OA) and amino acids (AA). Most of the newly formed OA are translocated to the root where a carboxyl group is released in exchange for a nitrate ion, whereas the newly assimilated N is incorporated primarily into leaf N compounds. During rapid pod fill (b), leaf proteolysis occurs and much of the amino N in the leaf is exported to the filling pods. Consequently, the phloem is enriched with amino compounds, which repress nitrate uptake and consequently diminish the rate of nitrate reduction.

In the root, by what mechanism could organic acids stimulate nitrate uptake? Numerous electrophysiological data demonstrate that influx of NO_3^- ions into root cells is energetically coupled to a pH gradient across the plasma membrane. Thus, the nitrate transporter system has been viewed as a H^+:NO_3^- symport (Glass, 1988; Ruiz-Cristin and Briskin, 1991). However, convincing evidence for this model, as opposed to an OH^-:NO_3^- or a HCO_3^-:NO_3^- antiport model, has not been reported. The fact that nitrate uptake is stimulated by added malate (Touraine et al., 1992) suggests that uptake is limited by the availability of either malate or bicarbonate, produced by malate decarboxylation. Thus, ma-

late stimulation is consistent with the activity of the nitrate transporter being controlled by the efflux of bicarbonate ions via a HCO_3^-:NO_3^- antiport system. The validity of this hypothesis has not been thoroughly tested because of a dearth of data on the concentration of HCO_3^- in the cytoplasm of root cells. On the other hand, the hypothesis of a coupling between NO_3^- influx and HCO_3^- efflux is reinforced by the observation that enhancement of malate translocation to roots simultaneously stimulates NO_3^- uptake, C excretion, and alkalinization of the medium (Touraine et al., 1992).

AMINO EFFECTORS: PRIMARY OR SECONDARY SIGNALS

Regarding the putative control of nitrate uptake by cycling amino acids, it is clear that (a) a great pool of amino acids cycle between root and shoot and (b) increasing the concentration of certain amino acids in the phloem sap causes an inhibition of nitrate uptake. How the shoot controls the concentration of its emitted effector(s) and how that effector functions in the root is unknown. Although the export rate of total N varies with plant developmental phase, changes in the amino acid composition of the phloem sap during plant development have not been rigorously established. Furthermore, regulation of nitrate uptake may be a complex chain of events (Muller and Touraine, 1992), where certain amino acids are secondary signals and nitrate uptake per se is controlled by an unidentified primary signal in root cells.

The target site for the amino acid signal could be one or more of the nitrate transporters, or it could be the nitrate efflux system. This latter hypothesis is doubtful because N starvation, which is likely to decrease the concentration of cycling amino acids, stimulates nitrate influx, which can account for the increase in net nitrate uptake (Hole et al., 1990). However, the precise nature of the carrier system is still unknown. Indeed, it has been shown that at least three distinct systems, HATS, which includes both a constitutive and an inducible NO_3^- transporter, and LATS, are responsible for nitrate influx (Siddiqi et al., 1990; King et al., 1993).

Mechanistically, it seems quite certain that the signal amino acids do not act as external allosteric effectors. Furthermore, N starvation causes a change in the V_{max} of NO_3^- influx, suggesting that the amount of plasma membrane-carrier protein has been modified (Hole et al., 1990). Whether these effects are exerted at a transcriptional or a translational level could not be addressed previously because molecular probes of a nitrate transport system were not available.

CONCLUSIONS AND PERSPECTIVES

Elucidation of the relationships between (a) nitrate uptake rates and plant development, (b) variations in nitrate uptake rates produced by environmental perturbations, and (c) nitrate availability and N_2 fixation rates will require a detailed identification of the signaling mechanisms outlined above. The specific activities of the various putative phloem-translocated signals must be established, the nature of their target sites must be characterized, and the interactions among these components must be determined.

The identification and functional expression of an *Arabidopsis* gene, CHL1, encoding a nitrate transporter (Tsay et al., 1993) open new avenues for studying the effects of organic acids and amino acids on the regulation of nitrate uptake. This finding is especially encouraging because other higher plants (broccoli, cabbage, corn, spinach, squash, and tomato) have mRNAs that hybridize to the cloned gene. Although the CHL1 gene seems to encode a LATS and regulation is more likely to be exerted on HATS, these and other recent developments in plant molecular biology will provide opportunities to attack the details of the physiological questions posed by the demand-driven control of nitrate uptake.

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