The role of long-distance signalling in plant responses to nitrate and other nutrients

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Abstract

The phenotypic plasticity that plants display in response to changes in their nutrient supply requires the operation of both short- and long-range signalling pathways. Long-distance signals arising in the root can provide the shoot with an early warning of fluctuations in external nutrient concentrations, while signals in the reverse direction are needed to ensure that root physiology and development are integrated with the nutritional demands of the shoot. In this review, the focus is on recent advances in the understanding of these long-distance signalling pathways with an emphasis on nitrate nutrition, and a personal view of the key issues for future research is put forward.

Key words: Auxin, cytokinins, iron deficiency, lateral roots, nitrate transport, phosphorus deficiency, sulphur deficiency.

Introduction

Plants display a high degree of physiological and developmental plasticity in response to changing nutritional conditions (Clarkson and Lütte, 1990; Robinson, 1994; Forde and Lorenzo, 2001). Some nutritional responses are restricted to those roots directly exposed to the nutrient signal: for example, the localized proliferation of lateral roots that occurs in NO$_3^-$ or phosphate (Pi)-rich soil patches (Robinson, 1994), and the increased root hair production which is associated with Fe or P deprivation (Bates and Lynch, 1996; Schikora and Schmidt, 2001). However, many other nutritional responses are systemic and must involve the transmission of long-distance signals, usually between the root and the shoot.

Two types of systemic response can be distinguished. One depends on the nutrient status of the whole plant (or specifically the shoot), while the other results from short-term fluctuations in the nutrient supply to the root. An example of the former is the regulation of nutrient transport systems in the root by ‘demand’ from the shoot (Marschner, 1995). Systemic responses of the second kind can provide the shoot with an early warning of changes in nutrient availability. This is seen, for example, in the rapid decline in leaf expansion rates that follows withdrawal of NO$_3^-$ from the roots (McDonald and Davies, 1996).

This article examines the importance of long-distance signalling in plant nutrition and what is known of the processes involved, with an emphasis on NO$_3^-$ nutrition.

Recent advances

Shoot-to-root signalling in the feedback regulation of NO$_3^-$ uptake

The high-affinity NO$_3^-$ uptake system (HATS) in roots is both substrate-inducible and feedback-regulated according to the shoot’s demand for N (Clarkson and Lütte, 1990). Feedback regulation of the NRT2 genes that specify components of the HATS for NO$_3^-$ occurs at least partly at the mRNA level (Forde, 2000; Orsel et al., 2002), and recent studies with split roots of Arabidopsis have confirmed the involvement of long-range signals from the shoot in regulating expression of the AtNRT2-1 gene (Gansel et al., 2001).

It has been proposed that the recycling of amino acids that occurs between the shoot and the root could provide a mechanism for communicating changes in the N status of the shoot (Cooper and Clarkson, 1989; Imsande and Touraine, 1994). However, a study with split-roots of
Ricinus communis found that up-regulation of the NO$_3^-$ influx system was not correlated with changes in the amino acid composition of the phloem sap nor with the absolute rates of amino acid transport into the root (Tillard et al., 1998). Nevertheless, it still cannot be ruled out that there are subtle short-term fluctuations in phloem amino acid composition or fluxes to which the NRT2 genes are sensitive.

**Shoot-to-root signalling in the developmental response to plant N status**

Studies with tobacco have revealed a strong negative correlation between the shoot NO$_3^-$ content and the allocation of resources to root growth and branching (Scheible et al., 1997; Stitt and Feil, 1999). In Arabidopsis the negative effect of the plant's NO$_3^-$ status was specifically on the outgrowth of the laterals (Zhang et al., 1999). Since auxin transported from the shoot is a positive regulator of lateral root development (Reed et al., 1998), it has been tentatively suggested that NO$_3^-$ accumulation in the shoot might negatively regulate root branching by inhibiting auxin biosynthesis or its transport to the root (Forde, 2002).

**Sugars as long-distance signals interacting with NO$_3^-$-regulated processes in roots**

Sugars are important signals controlling many aspects of plant metabolism and development (Smeekens, 2000). Nitrate uptake systems are diurnally regulated, with activities generally being highest during the light period and lowest in the dark, and there is evidence that this regulation may be mediated at least in part by sugars (reviewed in Forde, 2002). Lateral root development is also stimulated by sugars, supplied either externally or through the phloem (Bingham et al., 1998; Crookshanks et al., 1998), and an increase in the sucrose concentration in the medium can overcome the inhibitory effect of high NO$_3^-$ concentrations (Zhang et al., 1999). Thus, sugars transported in the phloem may play a significant role as positive regulators of both physiological and developmental responses to the shoot NO$_3^-$ status. Operating in parallel with feedback regulation by the N status of the shoot, this can be seen as providing a mechanism for modulating these responses in accordance with the plant's N/C balance.

**The role of cytokinin as a long-distance signal modulating the shoot's response to changes in NO$_3^-$ supply**

Some genes expressed in shoots are NO$_3^-$-inducible only if the NO$_3^-$ is supplied through the roots. Examples are the C4Ppc1 gene for phosphoenolpyruvate (PEP) carboxylase in maize (Sugiharto et al., 1992) and a subgroup of genes belonging to the response regulator family in maize and Arabidopsis (the ZRR and ARR genes, respectively) (Taniguchi et al., 1998; Kiba et al., 1999). Although not inducible if NO$_3^-$ is supplied directly to detached leaves, these genes are rapidly induced by cytokinin treatment (Taniguchi et al., 1998; Kiba et al., 1999). When coupled with the evidence that NO$_3^-$ resupply to N-deprived roots rapidly stimulates cytokinin biosynthesis and transport to the shoot (Samuelson and Larsson, 1993; Walch-Liu et al., 2000; Takei et al., 2001), these observations suggest that cytokinins are long-distance signals mediating the molecular response to changes in NO$_3^-$ availability (Sakakibara et al., 2000; Takei et al., 2001).

Nitrate responses in the shoot also include rapid changes in the rate of leaf expansion: when roots are deprived of NO$_3^-$, leaf expansion rapidly slows down (McDonald and Davies, 1996). Given the known role of cytokinins as regulators of growth and cell division (D’Agostino and Kieber, 1999), the NO$_3^-$-dependent changes in cytokinin production in roots could provide a mechanism for regulating leaf expansion in response to short-term fluctuations in NO$_3^-$ availability. The finding that response regulator genes (the ARR/ZRR genes) are also up-regulated by this NO$_3^-$/cytokinin signalling pathway is of particular interest because response regulators are components of the His-to-Asp phospho-relay signal transduction pathways (Sakakibara et al., 2000). Thus, as discussed previously (Forde, 2002), it is possible that one or more of the cytokinin-inducible ARR/ZRR gene family are components of a signal transduction pathway linking the NO$_3^-$ signal in roots to increased rates of leaf expansion.

**Long-distance signals in the regulation of responses to other nutrients**

Like the NO$_3^-$ influx system, the uptake systems for other nutrients are regulated by demand from the shoot (Marxen, 1995). This feedback regulation also extends to enzymes involved in nutrient assimilation and the mobilization of nutrients from the rhizosphere (Leustek et al., 2000; Raghothama, 2000; Schmidt and Steinbach, 2000). The role of long-distance signals from the shoot in regulating gene expression in the root has been demonstrated for the responses to deficiencies in phosphorus (Burleigh and Harrison, 1999), iron (Grusak and Pezeshgi, 1996; Li et al., 2000) and sulphur (Lappartient et al., 1999). A remarkable feature of these systemic responses is that each appears to be specific to the nutrient in question, in as far as this has been tested (Martin et al., 2000). As is the case for the NO$_3^-$-regulated responses discussed above, clear evidence for how these long-distance signalling pathways operate is largely lacking. The S-metabolite glutathione, transported in the phloem,
has been suggested to act as a negative regulator of S starvation-inducible genes in Arabidopsis and Brassica napus (Lappartient et al., 1999). However, later studies in rice and poplar suggest that glutathione concentrations in the phloem sap are quite stable and that the SO₂⁻ to glutathione ratio is more closely correlated with the response to S deprivation (Herschbach et al., 2000; Kuzuhara et al., 2000).

Whereas auxin and sugars have both been discussed above as possible long-range regulators of the root’s developmental response to NO₃⁻, a recent study has found that the root architectural response to shoot Pi status was independent of the sucrose supply and auxin signalling (Williamson et al., 2001). The lack of correlation between root Pi concentrations and expression of P starvation-inducible genes in split roots and in a mutant (pho1) defective in Pi translocation to the shoot, has been taken as evidence that Pi itself is not the long-distance signal (Burleigh and Harrison, 1999). However, a different conclusion was reached in a later study, where a P starvation-inducible promoter was fused to the GUS reporter gene and its expression studied at the cell level in the wild-type and a pho1 mutant (Martin et al., 2000). Thus it remains plausible that Pi recycling between the shoot and the root (Drew et al., 1984) provides the signal that reports on the Pi status of the shoot.

In an exciting new development the Arabidopsis PHRI gene, which is part of the systemic response pathway for P starvation, has been shown to encode a MYB transcription factor that is homologous to a P regulatory gene (PSR1) in Chlamydomonas (Rubio et al., 2001). The putative riboregulators encoded by the P starvation-inducible At4/Mt4/AtIPS1 gene family (Burleigh and Harrison, 1999; Martin et al., 2000) may be upstream components of the same systemic response pathway.

Studies of the Fe starvation-induced expression of Fe(III) reductase in pea roots led to the conclusion that the shoot-derived signal is not Fe itself (Grusak and Pezeshgi, 1996). Shoot-derived auxin is a candidate for a positive regulatory signal for Fe(III) reductase in Phaseolus vulgaris L. (Li et al., 2000), but the same study failed to find evidence for auxin regulation of Fe(III) reductase in cucumber. In Arabidopsis, hormone signalling does not appear to be involved in the regulation of Fe(III) reductase (Schmidt et al., 2000).

### Issues for the future

Table 1 summarizes the current picture of long-distance signalling pathways in higher plants. One of the key questions about these long-distance signalling pathways concerns the nature of the inter-organ signals. From the discussion above it can be seen that the nutrients themselves, their assimilation products and phyto-hormones have all been implicated. Novel signal molecules transported in the phloem such as peptides and ribonucleoprotein complexes might also be involved (Lucas, 1997; Xoconostle-Cazares et al., 2000). One pre-requisite for further progress in this area will be improved techniques for accurately monitoring changes in phloem sap composition, possibly by extending the use of sap-feeding aphids beyond the few plant species in which this is currently possible, as discussed previously (Forde and Clarkson, 1999). This might be combined with improved microanalytical methods currently being applied to solutes sampled from individual plant cells (Tamos and Sharrock, 2001).

Novel screens to identify nutrient signalling mutants in Arabidopsis, similar to those that have already yielded lesions in the P starvation response (Chen et al., 2000; Rubio et al., 2001; Zakhleniuk et al., 2001) will be of enormous value in elucidating the signalling pathways. It is expected that the sequencing of the Arabidopsis genome and the use of microarrays to monitor global changes in gene expression, combined with reverse genetics, will further accelerate progress in this area. Given the extensive interactions and crosstalk known to occur

### Table 1. Summary of long-distance nutrient signalling pathways in higher plants

<table>
<thead>
<tr>
<th>Type of systemic response</th>
<th>Examples of target enzymes/processes</th>
<th>Possible long-distance signals involved</th>
<th>Possible regulatory genes implicated in the response</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Shoot-to-root</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fe status</td>
<td>Fe(III) reductase</td>
<td>Auxin</td>
<td>PHRI, At4/Mt4-related genes</td>
</tr>
<tr>
<td>P status</td>
<td>Pi transporters, alkaline phosphatase</td>
<td>Pi</td>
<td></td>
</tr>
<tr>
<td>N status</td>
<td>High-affinity NO₃⁻ transporters/lateral root development</td>
<td>Amino acids/auxin</td>
<td></td>
</tr>
<tr>
<td>S status</td>
<td>High-affinity SO₂⁻ transporters, ATP sulphurylase</td>
<td>Glutathione or glutathione: SO₂⁻ - ratio</td>
<td></td>
</tr>
<tr>
<td>(b) Root-to-shoot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃⁻ supply to the root</td>
<td>PEP carboxylase/leaf expansion</td>
<td>Cytokinins</td>
<td>ARR/ZRR response regulators</td>
</tr>
</tbody>
</table>

Further details and references can be found in the text.
between signalling pathways (Genoud and Métraux, 1999), it should also be profitable to test the nutrient-responsiveness of signalling mutants isolated in other screening programmes.

Concluding remarks

The homology between phosphate regulatory genes from higher plants and algae (Martin et al., 2000), and the conservation of the PII nitrogen regulatory gene between plants and prokaryotes (Hsieh et al., 1998), indicate that nutrient regulatory pathways in plants have ancient origins. However the need for long-distance signalling pathways of the kind considered here would presumably only have arisen with the evolution of vascular plants. The finding that a homologue of the Chlamydomonas PSR1 phosphate response gene is part of the systemic P response pathway in Arabidopsis (Martin et al., 2000) suggests that long-range signalling systems may have been superimposed on existing intracellular signal transduction pathways.

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