

REVIEW

Nitrogen Regulation of Root Branching

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• **Background** Many plant species can modify their root architecture to enable them to forage for heterogeneously distributed nutrients in the soil. The foraging response normally involves increased proliferation of lateral roots within nutrient-rich soil patches, but much remains to be understood about the signalling mechanisms that enable roots to sense variations in the external concentrations of different mineral nutrients and to modify their patterns of growth and development accordingly.

• **Scope** In this review we consider different aspects of the way in which the nitrogen supply can modify root branching, focusing on *Arabidopsis thaliana*. Our current understanding of the mechanism of nitrate stimulation of lateral root growth and the role of the *ANRI* gene are summarized. In addition, evidence supporting the possible role of auxin in regulating the systemic inhibition of early lateral root development by high rates of nitrate supply is presented. Finally, we examine recent evidence that an amino acid, L-glutamate, can act as an external signal to elicit complex changes in root growth and development.

• **Conclusions** It is clear that plants have evolved sophisticated pathways for sensing and responding to changes in different components of the external nitrogen supply as well as their own internal nitrogen status. We speculate on the possibility that the effects elicited by external L-glutamate represent a novel form of foraging response that could potentially enhance a plant's ability to compete with its neighbours and micro-organisms for localized sources of organic nitrogen.

Key words: *Arabidopsis thaliana*, auxin, dissolved organic nitrogen, foraging, glutamate, lateral roots, MADS box transcription factor, nitrate, nitrogen, root architecture, root development, roots, signalling, *Thlaspi caerulescens*.

INTRODUCTION

The growth and development of a root system is highly sensitive to modification by both intrinsic and extrinsic factors (Forde and Lorenzo, 2001; Bloom *et al.*, 2002; Porterfield, 2002). Intrinsic factors that influence root growth and development include the supply of photosynthate from the shoot and the nutrient status of the plant; extrinsic factors include the supply and distribution of nutrients in the soil, soil compaction and gradients of water potential. One important aspect of root plasticity is the proliferation of lateral roots that occurs within soil patches enriched in certain nutrients, including NH_4^+ , NO_3^- , Pi (Robinson, 1994; Forde and Lorenzo, 2001) and even Zn^{2+} (Haines, 2002). This form of plant behaviour is commonly referred to as foraging, as it represents the preferential utilization of habitat patches that are rich in essential resources (Hutchings and de Kroon, 1994).

Increased branching (of shoots or roots) in resource-rich conditions serves to enhance the precision with which the resource-capturing structures (leaves or roots) are placed within the environment (Sutherland and Stillman, 1988). Thus, an understanding of the mechanisms underlying root foraging is very much dependent on understanding how intrinsic and extrinsic nutritional factors influence

root branching. In this brief review we will consider recent progress towards elucidating the signalling mechanisms by which different forms of nitrogen regulate root branching in *Arabidopsis thaliana*.

EFFECT OF EXTERNAL NITRATE ON LATERAL ROOT GROWTH AND THE ROLE OF THE *ANRI* GENE

The ability to respond to localized nitrate supplies by proliferating lateral roots within the nitrate-rich zone is a property common to many species of plants (Robinson, 1994; Hodge, 2004). In barley, this ability is due to a combination of increased numbers of lateral roots and increased rates of lateral root elongation (Drew and Saker, 1975). In *Arabidopsis*, the primary effect of a localized nitrate treatment was to stimulate lateral root elongation (Zhang and Forde, 1998; Linkohr *et al.*, 2002), with one report indicating a small localized increase in lateral root numbers (Linkohr *et al.*, 2002). This stimulation of lateral root elongation appears to be attributable to a signalling effect from the NO_3^- ion itself rather than to a downstream metabolite (Zhang and Forde, 1998; Zhang *et al.*, 1999). Nitrate stimulates lateral root elongation by increasing rates of cell production in the root tips directly exposed to the signal (rather than through any effect on cell elongation) (Zhang *et al.*, 1999).

How the nitrate signal is converted into an increase in meristematic activity in the root tip is an intriguing question

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that as yet has no clear answer. One component of the NO_3^- signalling pathway has been identified in the form of the product of the *ANR1* gene, which is a member of the MADS box family of transcription factors (Zhang and Forde, 1998). Using a reverse genetic approach it was shown that lateral roots of *Arabidopsis* lines in which *ANR1* was down-regulated were defective in their response to a localized supply of NO_3^- . We have obtained additional evidence that *ANR1* is a positive regulator of lateral root growth using transgenic *Arabidopsis* lines in which *ANR1* can be rapidly post-translationally activated by a treatment with the synthetic steroid dexamethasone (DEX). These transgenic lines carry a construct constitutively expressing a translational fusion between ANR1 and the ligand-binding domain of the rat glucocorticoid receptor (rGR). The ANR1-rGR fusion protein is held inactive in a cytoplasmic complex with the HSP90 protein until addition of DEX, when it is released and is able to enter the nucleus where it can activate or repress its target genes (Picard *et al.*, 1988). When seedlings of these ANR1-rGR lines were treated with 1 μM DEX, lateral root growth was strongly stimulated (Filleur *et al.*, 2005). Remarkably, even though the *ANR1-rGR* gene was expressed under the control of a strong constitutive promoter (CaMV 35S), the effect of the DEX treatment was specific to lateral root growth, suggesting that one or more components of the regulatory pathway of which ANR1 is a part must be absent in the primary root tip.

Evidence has been obtained using transgenic lines constitutively overexpressing *ANR1* that there is an NO_3^- -dependent component of the ANR1 signalling pathway (Y. Gan and B.G. Forde, unpubl. obs.). These lines showed increased rates of lateral root growth, but the effect was dependent on the presence of NO_3^- in the medium. Thus, *ANR1* overexpression appears to be necessary but not sufficient for stimulating lateral root growth. One possible model to explain this observation is that ANR1 is post-translationally regulated and that an NO_3^- signal is required to convert it to its active form. Alternatively, NO_3^- could be required to induce another essential component of the signalling pathway (Walch-Liu *et al.*, 2005).

Initial studies using *Arabidopsis* root cultures had indicated that *ANR1* itself was NO_3^- -inducible (Zhang and Forde, 1998). However, recent results obtained with mature hydroponically grown *Arabidopsis* plants have established that *ANR1* expression in roots of intact plants is up-regulated approx. two-fold after 2.5 d of N starvation and rapidly down-regulated when NO_3^- or another N source is re-supplied (Gan *et al.*, 2005). Consistent with these data, an earlier study had shown that *ANR1* was down-regulated when NH_4^+ -grown *Arabidopsis* seedlings were treated with NO_3^- (Wang *et al.*, 2000).

Because ANR1 is a positive regulator of lateral root growth, its down-regulation under conditions of N sufficiency suggests a possible mechanism for feedback regulation of lateral root growth rates by the N status of the plant. It is well established that lateral root growth in one part of the root system is not only dependent on the immediate external NO_3^- concentration but also on the amount of NO_3^- supplied to the remainder of the root system (Drew *et al.*, 1973). The inhibition of early lateral root

development by high shoot NO_3^- concentrations has already been identified as one mechanism for feedback regulation of root branching (Stitt and Feil, 1999; Zhang *et al.*, 1999). It has therefore been suggested that the down-regulation of ANR1 under conditions of high N status (not necessarily NO_3^- status) could provide an effective means of feedback regulating elongation of the mature lateral root (Gan *et al.*, 2005). In this case, the inverse relationship between *ANR1* expression and N status can be viewed as a mechanism for modulating the intensity of the lateral root response to a localized NO_3^- supply to take into account the plant's demand for N.

There is evidence for widespread interactions between MADS box proteins at both the post-translational and the transcriptional levels. MADS box factors bind to DNA as dimers, which can be either homodimers or heterodimers with other MADS box proteins (Theissen *et al.*, 2000). There are also numerous cases in which one MADS box protein has been found to regulate the transcription of another MADS box gene, either directly or indirectly (Riechmann and Meyerowitz, 1997; Jack, 2004). With at least half the >100 members of the MADS box gene family in *Arabidopsis* shown to be transcribed in roots (Rounsley *et al.*, 1995; Alvarez-Buylla *et al.*, 2000; Burgeff *et al.*, 2002; Parenicova *et al.*, 2003), this gives ample scope for possible regulatory interactions between *ANR1* and other MADS box genes and also for functional redundancy between different members of the gene family, as is the case among some MADS box genes involved in flower development (Jack, 2004). It has been suggested that the *AGL21* gene, which is a member of the same clade as *ANR1* and appears to have a similar spatial pattern of expression in roots, may be functionally redundant with *ANR1* (Burgeff *et al.*, 2002).

In an attempt to throw light on these issues, a recent study has used quantitative real-time PCR (qRT-PCR) to compare the responsiveness of *ANR1* and 11 other root-expressed MADS box genes to fluctuations in the supply of N, P and S (Gan *et al.*, 2005). Four MADS box genes (*AGL12*, *AGL17*, *AGL18* and *AGL79*) were unresponsive to a 2.5-d period of N starvation or to a 3-h period of NO_3^- resupply. However, the other seven MADS box genes responded in a similar way to *ANR1*, although less strongly. These included two members of the *ANR1* clade, *AGL16* and *AGL21*, with only one member of this clade (*AGL17*) failing to respond. Three of the other N-regulated genes belong to the *SOC1*-like clade (*AGL14*, *AGL19* and *SOC1*), and the other two (*AGL26* and *AGL56*) belong to the poorly characterized type I lineage of MADS box genes. *SOC1* was the only gene of those tested that was responsive to changes in P and S supply. Any of the five type II MADS box genes, particularly the two members of the *ANR1* clade (*AGL16* and *AGL21*) could be candidates for having roles that at least partially overlap with those of *ANR1*.

In the same study, the expression of these 11 MADS box genes was examined in an *ANR1* knock-out mutant to test for possible regulatory interactions (Gan *et al.*, 2005). It was found that inactivation of *ANR1* had no discernible effect on the expression of any of the genes, indicating that these genes are not under either direct or indirect control of *ANR1*, at least at the transcriptional level.

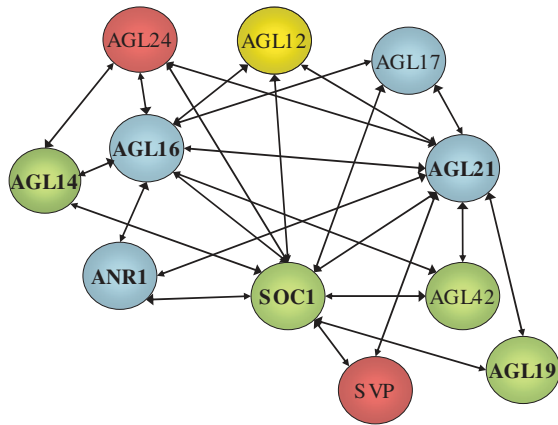


FIG. 1. Network of potential protein–protein interactions involving ANR1 and other root-expressed members of the type II lineage of MADS box proteins. Those proteins for which interactions were detected in the matrix-based yeast two-hybrid system (de Folter *et al.*, 2005) are connected by a line. Different clades are colour-coded: blue, ANR1-like; red, SVP-like; green, SOC1-like. AGL12 is an orphan MADS box protein. Those proteins encoded by genes that are co-regulated by changes in the N supply (Gan *et al.*, 2005) are indicated by bold type.

Recent evidence, however, has suggested the possibility of regulatory interactions between some of the root-expressed MADS box genes at the protein level. A comprehensive map of protein–protein interactions among the *Arabidopsis* MADS box family has been compiled using a matrix-based yeast two-hybrid screen (de Folter *et al.*, 2005). Figure 1 shows one part of this ‘interactome’ map, representing only those type II MADS box proteins whose expression has been reported in roots (Parenicova *et al.*, 2003) and which interacted either with ANR1 or with MADS box proteins that themselves interacted with ANR1. The complexity of the network of potential interactions is remarkably high, with each of the 11 proteins interacting with 2–10 other MADS box proteins. However, these findings must be treated with caution because the interactions have not been confirmed *in planta* and in any case can only be biologically meaningful if the relevant proteins are expressed in the same cells at the same time. Nevertheless, it is noteworthy that ANR1 was found to interact with only three other proteins (AGL16, AGL21 and SOC1), all of which are regulated by the N supply in a similar manner to ANR1 (Gan *et al.*, 2005).

SYSTEMIC INHIBITION OF LATERAL ROOT DEVELOPMENT AT HIGH RATES OF NITRATE SUPPLY

When *Arabidopsis* seedlings were grown at external NO_3^- concentrations ≥ 10 mM, early lateral root development was inhibited, resulting in the accumulation of short laterals that were blocked just after emergence from the primary root (Zhang *et al.*, 1999). The finding that this effect was enhanced in a nitrate reductase-deficient mutant suggested that the internal NO_3^- concentration rather than accumulation of the products of NO_3^- assimilation was the key factor triggering the developmental response (Zhang *et al.*, 1999). It has

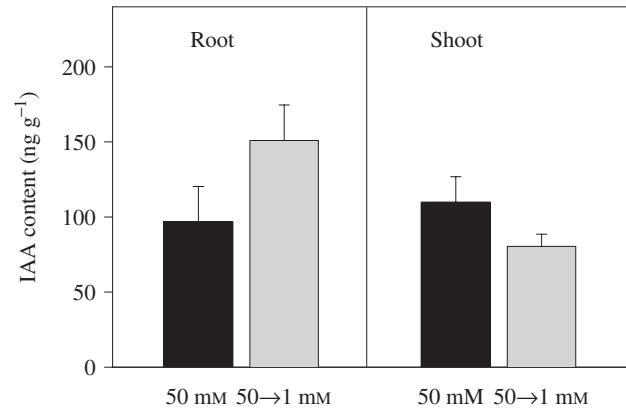


FIG. 2. Effect of short-term changes in the external NO_3^- supply on the IAA (indole 3-acetic acid) content of *Arabidopsis* roots and shoots. *Arabidopsis* seedlings were germinated and grown at 25 °C on vertical agar plates containing 50 mM KNO_3 . The composition of the medium and the growth conditions were as previously described (Zhang *et al.*, 1999). After 10 d, when lateral roots had initiated but had arrested at around the time of emergence, half of the seedlings were transferred to fresh plates containing 50 mM NO_3^- as controls and the other half were transferred to plates containing 1 mM KNO_3 . In other experiments, lateral root out-growth could be observed during the second day after transfer in seedlings transferred to 1 mM KNO_3 , but not in controls maintained on 50 mM KNO_3 (data not shown). Twenty-four hours after transfer the roots and shoots were harvested separately and their IAA contents determined immunologically using an enzyme-linked immunosorbent assay (ELISA) (Veselov *et al.*, 1992). Data are expressed per g fresh weight and are the means obtained from two independent experiments each having two replicates (\pm standard error; $n = 4$).

been proposed that the accumulation of high tissue concentrations of NO_3^- in the leaf are responsible for generating a long-distance signal that regulates lateral root development (Scheible *et al.*, 1997; Zhang *et al.*, 1999). The nature of this long-distance signal is unknown, but an obvious candidate is auxin because it has been shown that shoot-derived auxin is important for stimulating lateral root emergence (but not initiation) in *Arabidopsis* seedlings (Bhalerao *et al.*, 2002). It has been hypothesized that NO_3^- accumulation in the shoot may inhibit the flux of auxin to the root, leading to a failure of the lateral roots to pass an auxin-requiring checkpoint in lateral root development (Forde, 2002).

We have tested this hypothesis by measuring tissue auxin concentrations in the root 24 h after transferring *Arabidopsis* seedlings from 50 mM NO_3^- to 1 mM NO_3^- . It has been found that lateral roots whose development is arrested by growth on 50 mM NO_3^- begin to develop normally 24–48 h after removal of the high NO_3^- concentration (unpublished results). If auxin is involved in regulating this process, its concentration in the root might therefore be expected to increase in the period preceding the release of the lateral roots from their inhibition. In agreement with this prediction, we observed a 50% increase in the IAA (indole 3-acetic acid) content of the roots of seedlings transferred to 1 mM NO_3^- compared with those that were maintained on 50 mM NO_3^- (Fig. 2). A small decrease seen in shoot IAA content was not statistically significant. In soybean it has similarly been found that plants grown on 8 mM NO_3^- had a four-fold lower concentration of IAA in their roots than those grown on 1 mM NO_3^- (Caba *et al.*, 2000), suggesting that down-regulation of the root auxin

content by high rates of NO_3^- supply may be a widespread phenomenon in plants. The mechanism by which this effect of NO_3^- is achieved remains to be established, but could involve, for example, an inhibition of IAA biosynthesis in the shoot or some form of restriction of IAA transport from the shoot to the root.

There is also evidence that a second plant hormone, abscisic acid (ABA), may be involved in regulating the systemic effect of endogenous NO_3^- pools on lateral root development. Three ABA-insensitive mutants (*abi4-1*, *abi4-2* and *abi5-1*) were insensitive to the inhibitory effect of NO_3^- and four ABA synthesis mutants (*aba1-1*, *aba2-3*, *aba2-4* and *aba3-2*) showed reduced sensitivity (Signora *et al.*, 2001). To explain these data the authors suggested that there are two regulatory pathways mediating the inhibitory effects of NO_3^- , one that is ABA-dependent and involves *ABI4* and *ABI5*, and another that is ABA-independent. One possibility is that auxin is involved in long-distance signalling from shoot to root, while the ABA-dependent signalling pathway operates within the developing lateral root primordium.

EFFECT OF EXTERNAL L-GLUTAMATE ON PRIMARY ROOT GROWTH AND ROOT BRANCHING

While investigating the effect of different N sources on plant development we observed that primary root growth in aseptically grown seedlings of *Arabidopsis* was markedly inhibited in the presence of even low concentrations of L-glutamate (0.05–0.5 mM) (Filleur *et al.*, 2005; P.W.-L. *et al.*, submitted for publication). This response was highly specific for L-glutamate as similar concentrations of the related amino acids aspartate, glutamine, γ -aminobutyric acid (GABA) and D-glutamate had no effect. The only other amino acid to affect root growth was tryptophan, which at 50 μM inhibited primary root growth by about 25% (P. Walch-Liu and B. G. Forde, unpublished results). Tryptophan is a precursor of IAA (Muller *et al.*, 1998) and its overall effects on root architecture (including stimulation of lateral root initiation) differed from those of L-glutamate but resembled those classically seen when *Arabidopsis* roots are treated with IAA (Evans *et al.*, 1994; Celenza *et al.*, 1995). Although these experiments were performed in the presence of 0.5 mM glutamine as background N source, L-glutamate had the same effect in the absence of any other form of N.

The effects of L-glutamate on root architecture are complex because, although inhibition of primary root growth and reduction in mitotic activity are detectable within 24 h of transfer to L-glutamate, lateral roots only acquire sensitivity to L-glutamate some time after emergence. It appears that L-glutamate sensitivity in lateral roots is developmentally regulated, with the result that they continue growth on glutamate until they reach a fairly uniform average length of 5–7 mm. A third effect, which is probably a consequence of the inhibition of primary root growth, is that lateral root outgrowth behind the primary root tip is stimulated. The net result is that L-glutamate-treated seedlings have a shorter, more branched root system not dissimilar to the phenotype

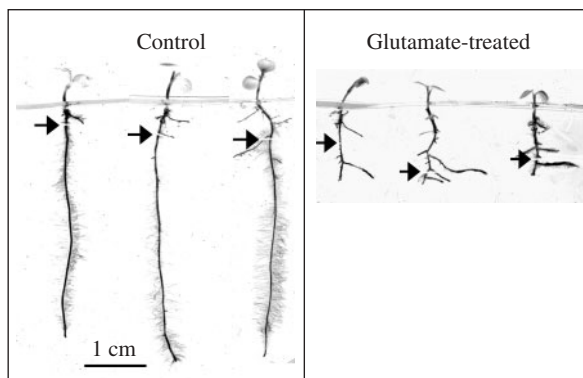


FIG. 3. Effect of L-glutamate on root architecture in *Thlaspi caerulescens*. Seed of *T. caerulescens* was surface-sterilized with Na hypochlorite and germinated on vertical agar plates as previously described for *Arabidopsis* (Zhang *et al.*, 1999) except that Phytigel™ (1%, w/v) was used instead of agar and the medium contained 1 mM MgCl_2 and 1 mM CaCl_2 to aid solidification. The background N source was 0.5 mM glutamine. The temperature was 18–23 °C and the photon irradiance was approx. 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a 16:8-h light–dark regime. After 6 d growth, and before lateral roots had appeared, the seedlings were transferred to fresh plates containing the same medium plus either 1 mM KCl (control) or 1 mM potassium L-glutamate (treated) and incubated for a further 9 d before image capture. Arrows indicate the locations of the primary root tips at the time of transfer.

seen when *Arabidopsis* seedlings were grown on a limiting supply of P (Williamson *et al.*, 2001).

The effect of L-glutamate on *Arabidopsis* root growth was unexpected, but it is a phenomenon not restricted to this genus. Figure 3 shows the effect of L-glutamate on seedlings of *Thlaspi caerulescens*, a metal hyperaccumulator related to *Arabidopsis*, illustrating how the L-glutamate treatment dramatically alters root architecture in a similar way to that seen in *Arabidopsis* (Filleur *et al.*, 2005). Other taxa found to be sensitive to 1 mM glutamate were tomato, Icelandic poppy and another *Arabidopsis* relative, *Thellungiella halophila* (P. Walch-Liu and B. G. Forde, unpublished results). However, a survey of 19 different ecotypes of *Arabidopsis* has shown that within a single species a high degree of natural variation in L-glutamate sensitivity can exist. Among these ecotypes, from diverse geographical locations and habitats, the inhibition of primary root growth by 50 μM L-glutamate, measured over a 6-d period of treatment, varied from 0 to 80% (P.W.-L. *et al.*, submitted for publication). Nevertheless, even the least sensitive ecotype (RLD1) was sensitive to 1 mM L-glutamate, being inhibited by 26%.

Arabidopsis roots have a high-affinity uptake system for L-glutamate which has a K_m for L-glutamate of 14–15 μM (W. Koch and W. Frommer, personal communication). However, it seems unlikely that the effect of L-glutamate on root growth arises from its contribution to N metabolism. We have found that 50 μM L-glutamate has the same inhibitory effect on root growth whether it is applied with or without a ten-fold excess of glutamine, which is supplied as a N source. Furthermore, if 1 mM L-glutamate is supplied to the root system while avoiding contact with the primary root tip, primary root growth is unaffected. By contrast, if 50 μM L-glutamate is applied only to the root tip it has the same

effect on primary root growth as 50 μM L-glutamate applied to the whole root system. We conclude that L-glutamate is detected specifically at the root tip and that the localized nature of this effect is most consistent with the L-glutamate signal being sensed extracellularly, presumably in the apoplast close to the root apex.

What is the mechanism of L-glutamate sensing? A study by Sivaguru and colleagues found that millimolar concentrations of L-glutamate inhibited *Arabidopsis* root growth and replicated the effects of Al^{3+} in rapidly depolymerizing cortical microtubules and depolarizing the plasma membrane (Sivaguru *et al.*, 2003). Because this effect could be blocked with AP-5, an antagonist of mammalian ionotropic glutamate receptors (iGluRs), the authors concluded that plant homologues of these iGluRs may be involved in sensing the glutamate signal. The existence in plants of genes encoding homologues of mammalian iGluRs was first reported by Lam *et al.* (1998). It has since been established that *Arabidopsis* has 20 genes (*AtGLR* genes) belonging to the family of glutamate receptor-like proteins (Lacombe *et al.*, 2001; Chiu *et al.*, 2002). However, although there is physiological evidence for the existence of glutamate-gated cation channels in roots (Dennison and Spalding, 2000; Dubos *et al.*, 2003; Demidchik *et al.*, 2004), the ion transport properties of the *AtGLR* gene products and the identity of their ligand(s) are still unclear (Davenport, 2002; Demidchik *et al.*, 2004).

We were unable to confirm the ability of AP-5 or other antagonists of mammalian iGluRs (DNQX or MK801) to block the inhibitory effect of L-glutamate on root growth. However, the phenomenon we observe appears to differ from that studied by Sivaguru and colleagues. They obtained a 60% inhibition of root growth within 2.5 min of L-glutamate treatment, indicating rapid effect on cell elongation. By contrast, we find that the onset of inhibition of root growth is delayed, with only a 20% decline measurable over the first 24 h, and that a decrease in mitotic activity, not cell elongation, accounts for this decline. Therefore, there may be a rapid, transient effect of L-glutamate on cell elongation that is mechanistically distinct from the longer term effect we observe. Nevertheless, the *AtGLR* genes at present are the likeliest candidates for a role in glutamate sensing. Expression studies have shown that 15 of the 20 *AtGLR* genes are expressed most strongly in roots, and five of these are root-specific (Chiu *et al.*, 2002). We are currently screening T-DNA insertion mutants in individual members of this gene family to establish whether any are altered in the L-glutamate sensitivity of their root growth.

Our observations have led us to speculate on the possible physiological and ecological significance of the L-glutamate effect. Amino acids are present in soils as the largest component of the low-molecular-weight fraction of dissolved organic N (DON) (Lipson *et al.*, 2001; Jones *et al.*, 2005a, b). Traditionally it has been thought that microbial competition for this highly labile pool of organic N would be too intense for it to represent a significant source of N for plants. However, this view is now changing and there is strong evidence to suggest that both mycorrhizal and non-mycorrhizal plants in a variety of ecosystems directly

absorb amino acids from the soil (Lipson and Nasholm, 2001; Neff *et al.*, 2003). It has been pointed out that plants that were able to access the organic N pool directly would be able to release themselves from reliance on microbial mineralization to produce inorganic N, which is generally considered to be a bottleneck in the N cycle in soils (Neff *et al.*, 2003). On this basis, there would have been strong selective pressure for plants to acquire mechanisms that enhanced their ability to compete with other plants and micro-organisms for organic N. One physiological adaptation that could have emerged as a consequence of this selective pressure is the range of high-affinity amino acid uptake systems with differing substrate specificities that exists in the roots of many plant species (Fischer *et al.*, 1998; Lipson and Nasholm, 2001).

We propose that changes in root architecture in response to the presence of significant accumulations of L-glutamate in the soil may represent a second (morphological) adaptation that enhances a plant's ability to compete for organic N. It has been suggested that plants are most likely to be able to compete effectively with micro-organisms for soil amino acids within organic N-rich soil patches where the concentrations of these amino acids are highest (Raab *et al.*, 1996; Jones *et al.*, 2005b). The slowing of primary root growth, the increased root branching behind the root tip and the developmentally delayed inhibition of lateral root elongation, which are the responses observed when an *Arabidopsis* root system encounters a source of L-glutamate, can be seen as a potential foraging mechanism because they would serve to increase the precision of root placement within the soil (Sutherland and Stillman, 1988). Although the concentrations of L-glutamate normally found in the bulk soil solution may be too low to affect root growth (Jones *et al.*, 2005b), within regions of decomposing organic matter its concentration can be expected to frequently exceed that needed to elicit a growth response in roots of sensitive genotypes. Plant and animal tissues contain free glutamate at millimolar concentrations (Joy *et al.*, 1992; Young and Ajami, 2000) and an even larger pool of glutamate is available for proteolytic release in the protein fraction (Tapiero *et al.*, 2002).

Glutamate appears to be widely used by both unicellular and multicellular organisms as a chemoattractant and foraging signal. It has previously been identified as an important cue for foraging behaviour in organisms as diverse as bacteria (Brown and Berg, 1974), protozoa (Van Houten *et al.*, 2000), cnidarians (Bellis *et al.*, 1991) and crustaceans (Trott *et al.*, 1997). Future studies should be directed towards examining the relationship between the ability of a plant species or genotype to modify its root architecture in response to L-glutamate signals and its ability to compete for soil organic N, particularly when the organic N supply is spatially heterogeneous.

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