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Linkohr, B I, Williamson, L C, Fitter, A H et al. (1 more author) (2002) Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. Plant Journal. pp. 751-760. ISSN 1365-313X

https://doi.org/10.1046/j.1365-313X.2002.01251.x

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The Plant Journal (2002) **29**(6), 751–760

Nitrate and phosphate availability and distribution have different effects on root system architecture of *Arabidopsis*

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Summary

Plant root systems can respond to nutrient availability and distribution by changing the three-dimensional deployment of their roots: their root system architecture (RSA). We have compared RSA in homogeneous and heterogeneous nitrate and phosphate supply in *Arabidopsis*. Changes in nitrate and phosphate availability were found to have contrasting effects on primary root length and lateral root density, but similar effects on lateral root length. Relative to shoot dry weight (DW), primary root length decreased with increasing nitrate availability, while it increased with increasing phosphate supply. Lateral root density remained constant across a range of nitrate supplies, but decreased with increasing phosphate supply. In contrast, lateral root elongation was suppressed both by high nitrate and high phosphate supplies. Local supplies of high nitrate or phosphate in a patch also had different effects. Primary root growth was not affected by a high nitrate patch, but growth through a high phosphate patch reduced primary root growth after the root left the patch. A high nitrate patch induced an increase in lateral root density in the patch, whereas lateral root density was unaffected by a high phosphate patch. However, both phosphate- and nitrate-rich patches induced lateral root elongation in the patch and suppressed it outside the patch. This co-ordinated response of lateral roots also occurs in soil-grown plants exposed to a nutrient-rich patch. The auxin-resistant mutants *axr1*, *axr4* and *aux1* all showed the wild-type lateral root elongation responses to a nitrate-rich patch, suggesting that auxin is not required for this response.

Keywords: Root system architecture (RSA), nitrate, phosphate, homogeneity, heterogeneity, auxin

Introduction

Plants can respond to the environment by altering their development. For example, the root system is highly responsive to nutrient availability and distribution within the soil. This is particularly true of phosphate and nitrate availability, which are the major growth-limiting nutrients in natural environments. Relative to shoot growth, root growth is generally favoured in nutrient-poor soils (Reynolds and D'Antonio, 1996). However, effective nutrient acquisition is not only dependent on the amount of root but also on the three-dimensional deployment of roots, the root system architecture (RSA). There is a large body of evidence demonstrating that RSA can be greatly influenced by nutrient availability, the nutrient status of the plant (Fitter and Stickland, 1992; Williamson *et al*., 2001; Zhang and Forde, 1998), and the heterogeneity of nutrient supply (Drew, 1975; Zhang and Forde, 1998). However, the mechanisms mediating these changes and their ecological significance are still poorly understood.

RSA in uniform nutrient supply

Shoot nitrate and phosphate status has been shown to play a role in the regulation of the root – shoot allocation of resources and the control of RSA. Scheible *et al*. (1997) found a highly significant inverse correlation between shoot nitrate levels and total root growth. In *Arabidopsis thaliana*, lateral root elongation was suppressed by growth on uniformly high nitrate concentrations (Zhang and Forde, 1998), even though other RSA parameters such as primary root growth and lateral root number remained the same under a wide range of nitrate concentrations (Zhang and Forde, 1998).

Phosphate availability had very different effects on RSA in *Arabidopsis* (Williamson *et al*., 2001). Uniformly low phosphate availability was found to favour lateral root growth over primary root growth, by dramatically reducing primary root length and increasing lateral root

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elongation and lateral root density (Williamson *et al.*, 2001). The *pho2* mutant, which over-accumulates phosphate in the shoot (Delhaize and Randall, 1995) had an extra long primary root on high phosphate, providing evidence that, as with nitrate, the internal nutritional status of a plant plays a role in the regulation of root growth (Williamson *et al.*, 2001).

RSA responses to local changes in nutrient supply

In many plant species nutrient-rich regions or patches stimulate local lateral root proliferation (Farley and Fitter, 1999). This proliferation response was found to be nutrient-specific in the classical work with barley (*Hordeum vulgare*) (Drew, 1975; Drew and Saker, 1975, 1978; Drew *et al.*, 1973). Drew and co-workers demonstrated that locally applied nitrate, ammonium, and phosphate, but not potassium, stimulated lateral root proliferation within a nutrient-rich zone and suppressed it elsewhere. Localised root proliferation was suggested to be a direct or indirect consequence of changes in metabolic activity in that particular part of the root system.

Zhang and Forde (1998) provided further insight into how a localised supply of nitrate stimulates lateral root development in otherwise nitrate-starved *Arabidopsis* seedlings. They established that heterogeneous nitrate availability specifically stimulates lateral root elongation in the area of the nitrate-rich patch but found no effect on lateral root initiation, primary root length or lateral root growth outside the patch (Zhang and Forde, 1998; Zhang *et al.*, 1999). Two lines of evidence suggest that localised lateral root elongation is controlled by the nitrate ion itself (Zhang and Forde, 1998). First, the metabolism of nitrate was not required for this architectural change, since a double mutant severely deficient in nitrate reductase activity was still able to respond. Second, localised ammonium and glutamine failed to elicit lateral root elongation in *Arabidopsis*. The hypothesis was further supported by the isolation of a MADS box transcription factor called *ANR1* suggested to be involved in nitrate signalling. Plants in which *ANR1* was down regulated by antisense mRNA expression or by co-suppression were unable to elongate lateral roots in response to a localised patch of high nitrate (Zhang and Forde, 1998). Zhang and Forde (1998) therefore proposed a model in which lateral root elongation is inhibited by a high internal N status, but when N is limiting, localised nitrate acts as a signal to the lateral root tip to increase elongation.

The influence of soil nutrient mobility on RSA responses

Given the contrasting mobility of nitrate and phosphate ions in soil, one might expect nitrate and phosphate limitations to affect RSA differently. Nitrate diffusion is three to four orders of magnitude faster than that of phosphate (Tinker and Nye, 2000) and the high mobility of nitrate means that roots 1 cm apart might compete for nitrate after only 1 day (Tinker and Nye, 2000). Therefore, a low root density is in theory sufficient to capture all the nitrate in a large volume of soil, raising the question as to why proliferation occurs (Robinson, 1996). However, when two plants grow close together the ability to proliferate roots in nitrogen-rich patches confers a competitive advantage because nitrate uptake is proportional to relative root length density, the root length in a given volume of soil (Hodge *et al.*, 1999; Robinson *et al.*, 1999). In contrast, because of the immobility of phosphate ions, spatial exploitation of soil is particularly important for phosphate acquisition by plant roots. In low phosphate, many plants, including *Arabidopsis*, increase their root hair length and density (Bates and Lynch, 1996; Ma *et al.*, 2001). Bean plants change the growth angle of basal roots towards the horizontal resulting in a shallower broader root system (Bonser *et al.*, 1996). A similar effect is achieved in *Arabidopsis* by increases in lateral root growth relative to primary root growth (Williamson *et al.*, 2001). These morphological and architectural changes of the root system maximise spatial exploitation of the topsoil where phosphorus is more available.

RSA control and auxin

There is considerable evidence that auxin is involved in lateral root development. Depending on concentration, lateral root initiation and elongation can be stimulated or inhibited by exogenous auxin application (Evans *et al.*, 1994). Consistent with a role in lateral root initiation, mutants with elevated endogenous auxin have increased numbers of adventitious and lateral roots (Boerjan *et al.*, 1995; Celenza *et al.*, 1995; King *et al.*, 1995) and auxin-resistant mutants such as *axr1*, *aux1* and *axr4,* have reduced numbers of lateral roots (Hobbie and Estelle, 1995; Timpte *et al.*, 1995). Looking for possible interactions between the signal transduction pathways of auxin and nitrate, Zhang *et al.* (1999) tested the response of several auxin-resistant mutants to a localised increase in nitrate supply. They found that *axr4* mutants lacked nitrate-induced lateral root elongation and saw this as evidence for an overlap between the nitrate and auxin response pathways. In contrast, Williamson *et al.* (2001) reported wild-type RSA changes in response to phosphate availability in the *axr1*, *aux1* and *axr4* mutants.

In order to compare more directly the effects of uniformly or locally varying phosphate and nitrate availability, we have studied the RSA response of *Arabidopsis* plants grown to a variety of nutrient regimes. Our data suggest that nitrate and phosphate have very different effects on primary root growth and lateral root density, but that they

have quite similar effects on lateral root elongation. Furthermore, we find no evidence that wild-type auxin signalling is required to mediate these lateral root elongation responses.

Results

RSA changes in response to homogeneous nitrate and phosphate supply

Arabidopsis thaliana plants were grown with a range of uniformly supplied nitrate and phosphate concentrations on vertically oriented agar plates to examine their root system architecture. All experiments on Petri dishes were conducted on agar medium containing 1% sucrose. Exogenous carbon supply has been shown to stimulate total root growth relative to shoot growth in low N supply in tobacco (Paul and Stitt, 1993) but in a pilot study we found similar RSA changes in homogeneous and heterogeneous nitrate supply with 1% sucrose and without (data not shown). Carbon availability had no effect on phosphate-dependent root – shoot allocation of resources (Paul and Stitt, 1993) and RSA (Williamson *et al.*, 2001).

Nitrate and phosphate supply had contrasting effects on primary root growth and lateral root density, but similar effects on total lateral root length (Figure 1). Relative to shoot DW, primary root length was shorter with increasing nitrate supply (Figure 1a) but it was longer with increasing phosphate supply, above a threshold of 0.164 mM [P] (Figure 1b). Below this threshold, primary root length was almost independent of phosphate concentration (Figure 1b) and the primary root was approximately 40% shorter than on high phosphate. Lateral root density was unaffected by nitrate supply (Figure 1c), but lateral root density decreased with increasing phosphate supply (Figure 1d). Relative to shoot DW, total lateral root length decreased on both high nitrate and high phosphate, with a threshold above 0.164 mM (Figure 1e,f).

RSA changes in response to heterogeneous nitrate and phosphate supply

To test the effects of nitrate- and phosphate-rich patches on RSA, *Arabidopsis* plants were first grown on agar plates with low nitrate or phosphate to produce nutrient-starved plants. The plants were then transferred to plates with the agar divided into three segments, separated by narrow air gaps, such that the primary root just bridged the first air gap and made contact with the middle segment (Zhang and Forde, 1998). For each patch experiment the upper segment and lower segment contained low nutrient levels (0.01 mM nitrate or no added phosphate), while the middle segment contained high nutrient levels (1 mM nitrate or 1 mM phosphate). In addition three different controls were used: uniformly low nutrient, uniformly high nutrient, and a uniform supply of nutrient amounting to approximately the same total nutrient availability as in the patch treatment plates (0.164 mM).

Growth on a segmented plate did not itself affect RSA response because segmented plates in which all segments included the same level of nitrate or phosphate showed the same concentration-dependent RSA responses described above (data not shown). Absolute primary root length was not significantly different on any of the nitrate nutrient regimes, except for the uniformly low nitrate treatment, which eventually resulted in reduced absolute primary root lengths (Figure 2a). In contrast, primary root length was reduced when the root grew through a phosphate-rich patch, compared to roots grown through uniformly supplied 0.164 mM [P], the control with the same P availability (Figure 2b). The primary root grew as fast as on the 0.164 mM [P] control while in the middle, phosphate-rich segment (Figure 2b, day 14), but it grew more slowly when the primary root tip reached the bottom segment at approximately day 15 ($P < 0.05$). The sensitivity of primary root growth to P supply can also be seen in the large difference in growth on uniformly high versus low P, apparent even after 14 days growth.

Lateral root density in the nitrate-rich patch increased by approximately one lateral root per cm of primary root compared to the homogenous nitrate controls ($P < 0.05$; Figure 3a). There were no significant density effects above the nitrate-rich patch. Lateral root initiation was suppressed in a nitrate-poor middle segment after the primary root had experienced a nitrate-rich patch in the top segment above ($P < 0.05$ when two experiments were analysed together by blocking). Heterogeneously supplied phosphate had no significant effects on lateral root density (Figure 3b).

Lateral root length decreased dramatically outside the nitrate-rich patch and increased slightly in the nitrate-rich patch compared to the 0.164 mM [N] control ($P > 0.05$) (Figure 4a). Lateral root growth on low nitrate was presumably limited by starvation and in high nitrate by inhibition (Figure 4a). Lateral root length responded in a very similar way to a heterogeneous phosphate supply. It was greatly reduced outside the phosphate-rich patch and slightly increased in the patch ($P > 0.05$) compared to the 0.164 mM [P] control (Figure 4b). Lateral root elongation was surprisingly vigorous in the plants grown with uniformly low phosphate (Figure 4b). This is most likely because the transfer of seedlings to the segmented agar plates during the experiment resulted in a small phosphate re-supply. At 1 mM [P], where primary root growth was stimulated, total lateral root growth was not suppressed, as in 2.5 mM [P] (Figure 1f).

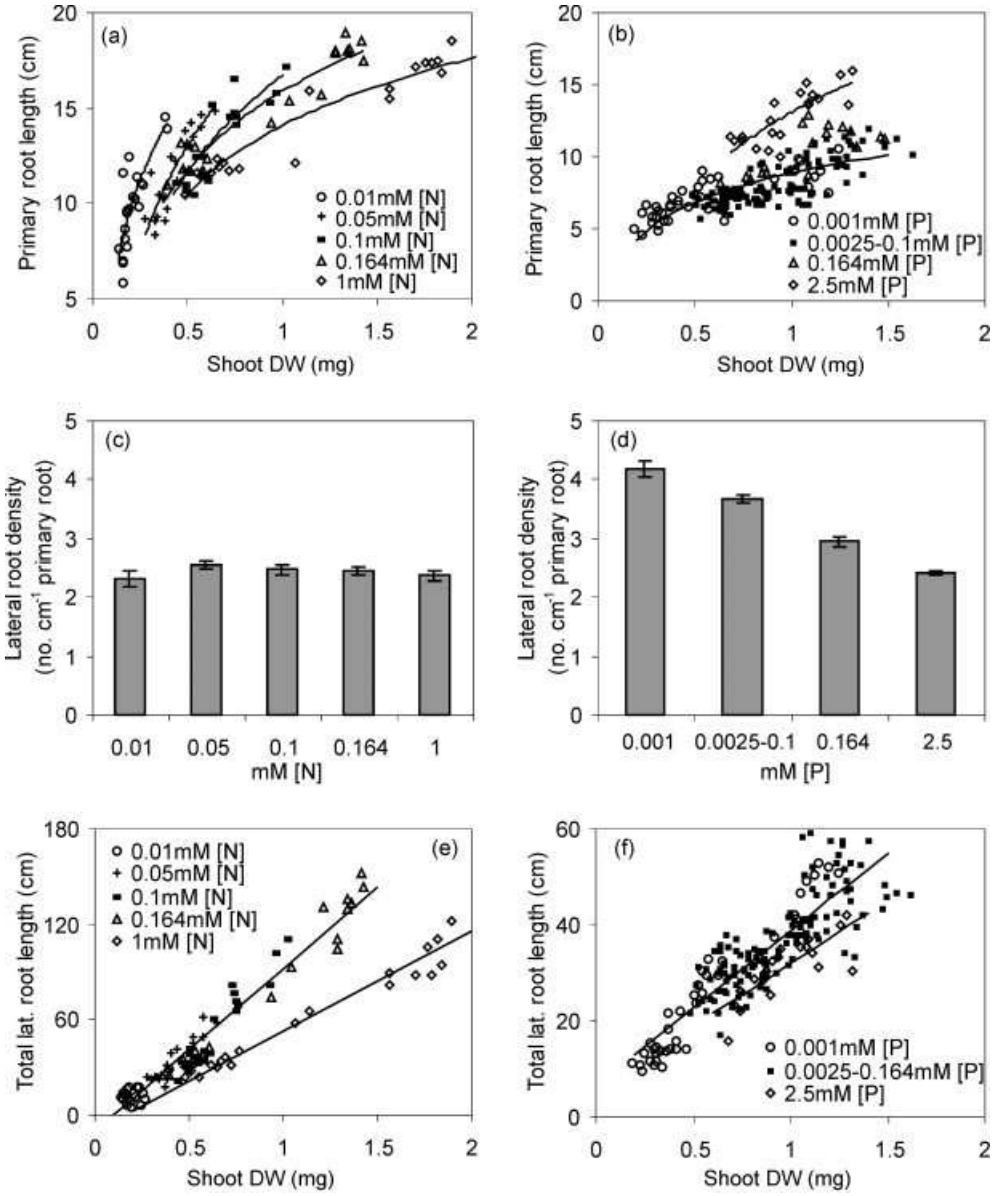

Figure 1. The effect of nitrate and phosphate availability on root system architecture.

Plots are presented for the effect of: (a) nitrate availability; and (b) phosphate availability on the relationship between shoot DW and primary root length; the effect of (c) nitrate availability; (d) phosphate availability on lateral root density (the number of lateral roots per cm primary root); and the effect of (e) nitrate availability and (f) phosphate availability on the relationship between shoot DW and total lateral root length. *Arabidopsis* seedlings were grown either for 17 or 18 days on 0.01, 0.05, 0.1, 0.2 and 1.0 mM [N] with 1 : 10 ATS, or for 14 or 18 days on 0.0, 0.0025, 0.01, 0.025, 0.1, 0.164 and 2.5 mM [P] with ATS in vertically oriented agar plates. Analysis of covariance shows that each RSA parameter varies significantly with ln shoot DW (d.f. 1,98; $P < 0.001$ for nitrate and d.f. 1154; $P < 0.001$ for phosphate).

(a) Nitrate concentration has a significant effect on primary root length relative to shoot DW: 0.01 mM [N]: $y = 6.74\ln(x) + 20.53$, $R^2 = 0.72$; 0.05 mM [N]: $y = 8.01\ln(x) + 18.59$, $R^2 = 0.79$; 0.1 mM [N]: $y = 7.31\ln(x) + 16.72$, $R^2 = 0.75$; 0.2 mM [N]: $y = 6.64\ln(x) + 15.60$, $R^2 = 0.91$; and 1 mM [N]: $y = 5.93\ln(x) + 13.60$, $R^2 = 0.89$.

(b) Phosphate concentration has a significant effect on primary root length relative to shoot DW: 0.001–0.164 mM [P]: $y = 2.94\ln(x) + 8.89$, $R^2 = 0.56$ and 2.5 mM [P]: $y = 7.53\ln(x) + 13.1$, $R^2 = 0.0.65$; $F_{1,189} = 128.69$, $P < 0.001$.

(e) Nitrate concentration has a significant effect on relative lateral root length: 0.01–0.2 mM [N]: $y = 102.9(x) - 11.29$, $R^2 = 0.94$ and 1 mM [N]: $y = 62.54(x) - 9.66$, $R^2 = 0.97$; $F_{1,98} = 4.77$; $P < 0.05$.

(f) Phosphate concentration has a significant effect on relative lateral root length: 0.001–0.164 mM [P]: $y = 32.11(x) + 6.68$, $R^2 = 0.78$ and 2.5 mM [P]: $y = 26.35(x) + 5.65$, $R^2 = 0.63$; $F_{1,154} = 8.97$; $P < 0.01$.

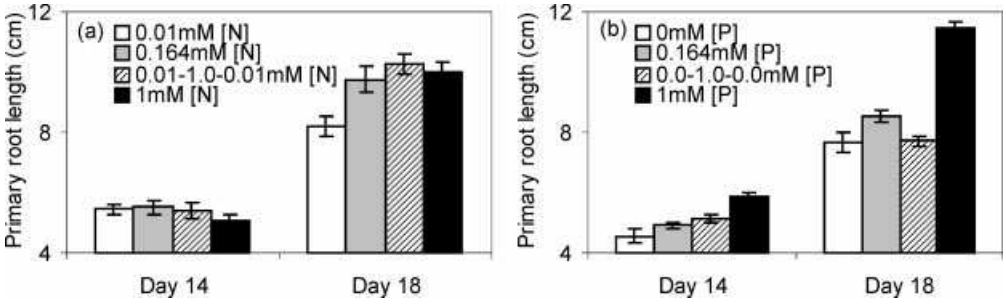

Figure 2. The effects of heterogeneous nitrate and phosphate supply on primary root length.
Data are presented for primary root length in (a)heterogeneous nitrate supply, and (b)heterogeneous phosphate supply, for 14- and 18-day-old plants. Seedlings were nitrate- or phosphate-deprived before the transfer to segmented agar plates, which consisted of solidified-agar medium divided horizontally by air gaps into a top (2 cm), middle (2 cm) and bottom (8 cm) segment to allow the localised application of nutrients. Nitrate- and phosphate-rich patch treatments received 1 mM [N] or [P] in the middle segment, the top and bottom segments contained 0.01 mM [N] or 0.0 mM [P]. Homogeneous supplies of [N] and [P] served as controls. The nutrient availability in homogeneous 0.164 mM [N] or [P] and the respective patch treatments were similar and represent the most relevant controls (grey bars). Primary root tips grew into the bottom segment 15 days after planting. Values represent the mean length of 9–10 primary roots ± SE.

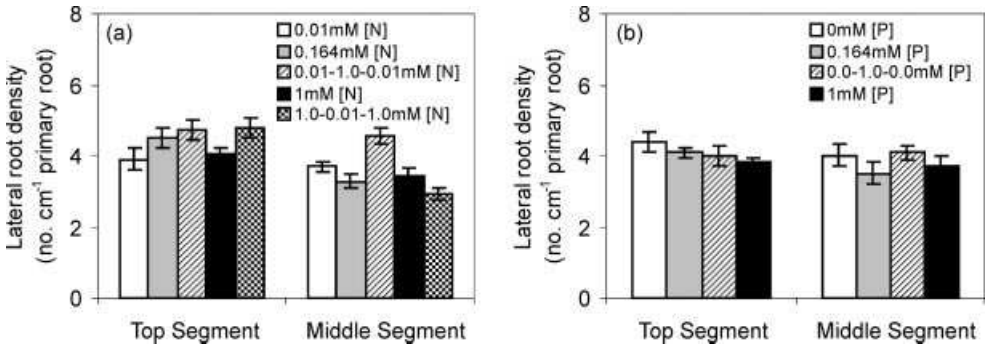

Figure 3. The effects of heterogeneous nitrate and phosphate supply on lateral root density.
Plots represent lateral root density (the number of lateral roots per cm primary root) for (a)heterogeneous nitrate supply, and (b)heterogeneous phosphate supply. Seedlings were nitrate- or phosphate-deprived before transfer to segmented agar plates. The treatments included 1 mM [N] or [P] patches in the middle segment with 0.01 mM [N] or 0.0 mM [P] in the top and bottom segment (and an additional top and bottom high [N] patch treatment with 0.01 mM [N] in the middle segment). Homogeneous supplies of [N] and [P] served as controls. The nutrient availability in homogeneous 0.164 mM [N] or [P] and the respective patch treatments were similar and represent the most relevant controls (grey bars). Values represent the mean of 8–10 plants ± SE, 18 days after planting.

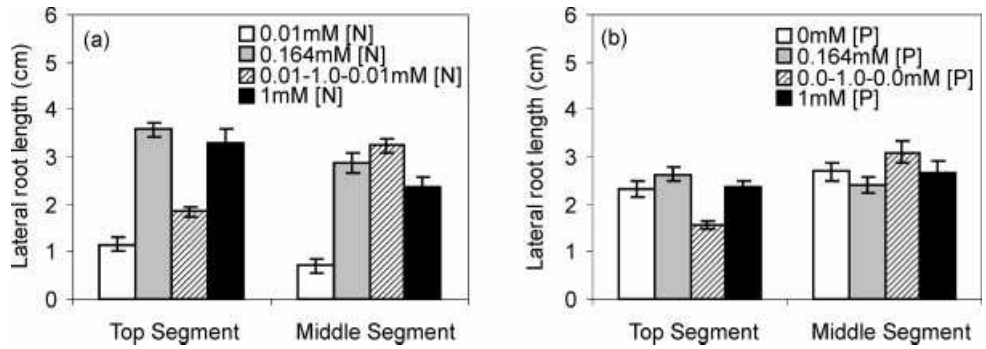

Figure 4. The effects of heterogeneous nitrate and phosphate supply on lateral root length.
Data are presented for the length of lateral roots of 18-day-old plants in (a)heterogeneous nitrate supply, and (b)heterogeneous phosphate supply. Seedlings were nitrate- or phosphate-deprived before transfer to segmented agar plates with homogeneous or heterogeneous supplies of [N] or [P]. The treatments included 1 mM [N] or [P] patches in the middle segment with 0.01 mM [N] or 0.0 mM [P] in the top and bottom segment. Homogeneous supplies of [N] and [P] served as controls. The nutrient availability in homogeneous 0.164 mM [N] or [P] and the respective patch treatments were similar and represent the most relevant controls (grey bars). Values represent the mean length of 8–10 lateral roots ± SE.

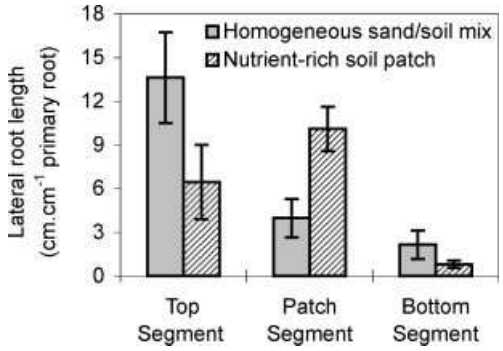

Figure 5. The effect of a nutrient-rich soil patch on lateral root proliferation in soil-grown *Arabidopsis*.
Total lateral root length per cm primary root is given for the top 3 cm, middle 1 cm and remaining lower part of the root system. *Arabidopsis* plants were grown either through a homogenous sand/soil mixture or with a 1-cm nutrient-rich soil patch layered between sand/soil mix and sand in glass plates for 23 days. Each bar represents the mean of 5–6 plants ± SE.

Co-ordinated lateral root proliferation in a nutrient-rich soil patch

To investigate the effects of a nutrient-rich soil patch on RSA in a more realistic microbial and physical environment, *Arabidopsis* plants were grown in a thin layer of sand/soil mix sandwiched between two glass plates. RSA was compared after 23 days growth through either a homogeneous sand/soil mix, or with a nutrient-rich soil patch. Total lateral root length was increased in the soil patch and decreased above and below it, compared to the homogeneous sand/soil control ($F = 19.345$, $P < 0.001$, Figure 5). Plants that experienced a nutrient-rich soil patch produced 50% of their total lateral root length from the patch area compared to less than 10% from the equivalent area in the homogeneous sand/soil controls. Average lateral root length in the patch was longer (1.8 ± 0.22 cm in the patch, 0.8 ± 0.19 cm in the control), while lateral root density was not significantly affected. Above the nutrient-rich patch, there was a tendency to fewer and shorter lateral roots. There was no difference in primary root length, total lateral root length and shoot DW between the treatments (data not shown).

Auxin response mutants in nitrate-rich patches

To test whether auxin is involved in mediating the RSA changes in a nitrate-rich patch, the auxin-resistant mutants *axr1*, *axr4* and *aux1* and the double mutant *axr1 axr4* were grown with a nitrate-rich patch. All the single mutants showed the characteristic reduction in lateral root elongation above the patch (Figure 6a) and the more modest increase in lateral root elongation in the patch (Figure 6b). A significant increase in lateral root density in the patch was also apparent in the *aux1* and *axr4* mutants

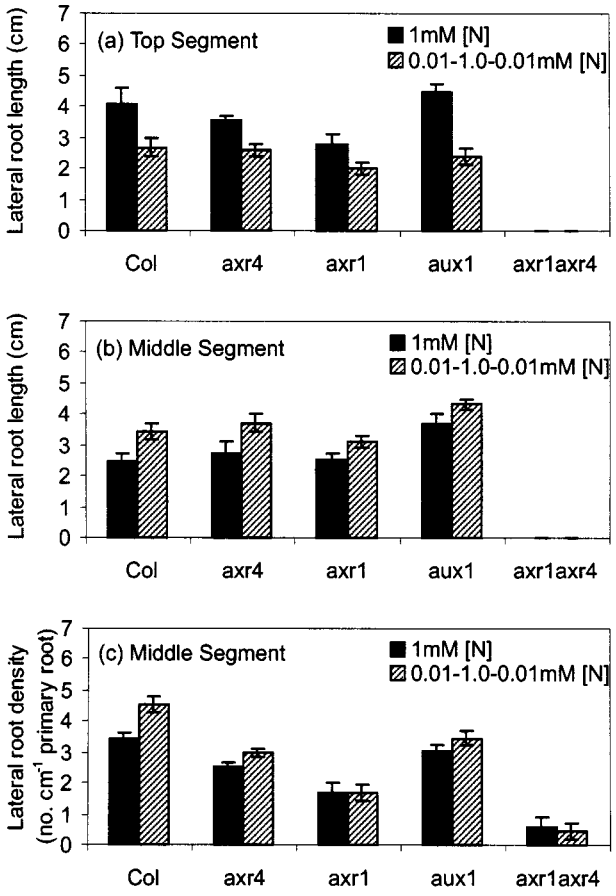

Figure 6. Comparison between Columbia wild-type (Col) and the auxin-resistant mutants *axr1*–3, *axr4*–2, *aux1*–7 and the double mutant *axr1*–3 *axr4*–2 in localised nitrate supply.
Data are presented for (a) lateral root length in the top segment, (b) lateral root length in the middle segment and (c) lateral root density in the middle segment (the number of lateral roots per cm primary root), in 18-day-old plants. Seedlings were nitrate-deprived before they were transferred to segmented agar plates with homogeneous 1 mM [N] (control) or with a nitrate-rich patch in the middle segment (0.01–1.0–0.01 mM [N]). Values represent the mean of 8–10 plants ± SE.

(Figure 6c). The double mutant *axr1 axr4* produced hardly any lateral roots at all in the top and middle segments. Some laterals were produced in the bottom segment, but only after the primary root reached the bottom of the dish. Deliberate damage to the primary root tip of *axr1 axr4*, e.g. by cutting, resulted in the emergence of lateral roots up to 1 cm above the cut (data not shown).

Discussion

Nutrient effects on the primary root

Uniformly high nitrate supply suppressed primary root growth while uniformly high phosphate supply stimulated primary root growth relative to shoot growth. In contrast

to our results, Zhang and Forde (1998) did not observe changes in primary root length in a range of nitrate concentrations from 0.01 mM to 100 mM. The difference in primary root length increases with the age of the plants, being reproducibly statistically significant in 18-day-old-plants (Figure 2a), several days after harvest in Zhang and Forde's experiments. The phosphate-dependent changes are in accordance with the results presented in Williamson *et al*. (2001) where low phosphate availability (0.1 mM) also reduced primary root growth. In response to a further decrease in phosphate supply, primary root growth was not reduced much more. The phosphate concentration of 0.164 mM appears to represent a threshold above which primary root length starts to increase, while lateral root length is only suppressed at very high phosphate levels (2.5 mM).

Heterogeneous nitrate supply did not affect primary root length in *Arabidopsis*, which in consistent with observations on barley (Drew *et al*., 1973), maize (Granato and Raper, 1989) and *Arabidopsis* (Zhang and Forde, 1998). Primary root growth was suppressed in heterogeneous phosphate supply, once the primary root tip had grown out of a phosphate-rich patch. Here, uniform nitrate and phosphate supply and localised phosphate, but not nitrate supply, can influence primary root growth.

Nutrient effects on lateral root initiation

Lateral root density was unaffected by nitrate availability, while nitrate distribution stimulated lateral root initiation in a nitrate-rich area and suppressed lateral root initiation in a nitrate-poor area. This result is in contrast to the findings of Zhang and Forde (1998) who did not observe a change in lateral root density in their nitrate-rich patch experiments. Differences in growth conditions and age of the plants could explain the discrepancy. Phosphate has the opposite effect on lateral root density as compared to nitrate. High phosphate availability decreases lateral root density (Williamson *et al*., 2001) but phosphate distribution had no effect on lateral root initiation. The contrasting effects of nitrate and phosphate availability on primary root growth and lateral root density suggest different regulatory mechanisms in the control of nitrate- and phosphate- dependent RSA in *Arabidopsis*.

Nutrient effects on lateral root elongation

Only lateral root length was regulated in a similar way by nitrate and phosphate availability and distribution. High uniform nitrate and phosphate availability suppressed lateral root length as reported before by Zhang and Forde (1998) and Williamson *et al*. (2001). In heterogeneous nutrient supply, lateral root elongation was greatly suppressed outside both nitrate- and phosphate-rich patches compared to the control treatments. In contrast, lateral roots growing in the nitrate- and phosphate-rich patches were only marginally longer than in the equivalent portion of root systems grown with similar amounts of the respective nutrient, uniformly distributed. This co-ordinated effect on lateral root length was by far the major effect observed in the sand/soil-grown plants, indicating its importance in more natural situations. Co-ordinated lateral root proliferation inside, and suppression outside nutrient-rich patches has also been noted by earlier workers (Drew, 1975; Drew and Saker, 1978; Drew *et al*., 1973; Granato and Raper, 1989; Sattelmacher *et al*., 1993). The ability to suppress lateral root elongation in less-optimal areas of the root system seems an efficient way of optimal resource allocation for nutrient capture.

Zhang and Forde (1998) reported no suppression of lateral root growth outside the nitrate-rich patch and a much larger increase in the nitrate-rich patch than observed in our experiments. The control plants used for their comparison were grown on uniformly low nitrate (0.01 mM [N]), and hence were nitrate-starved compared to the patch-treated plants. This might have led to a reduced absolute lateral root length in both the middle and top segments effectively enhancing differences in lateral root length between the treatment and the control within the patch, and reducing differences above the patch (see Figure 4a).

The mechanism mediating the co-ordinated root elongation response in and outside the patch is unclear. Zhang and Forde 1998 and Zhang *et al*., 1999 suggested a model in which lateral root elongation is systemically suppressed by a high internal nitrate status. When nitrate is limiting, they propose that locally high nitrate can directly stimulate elongation of lateral root tips. This model cannot account for the co-ordinated response that we observe since it does not explain suppression of lateral root elongation outside the patch, compared to the same portion of the root systems of plants of similar internal nitrate status. A similar co-ordination response was observed by Scheible *et al*. (1997), when they grew seminal roots of transformed tobacco plants (*Nicotiana tabacum*) with decreased expression of nitrate reductase in a split root experiment with high and low nitrate supply. Their data show that shoot nitrate levels in plants with split roots were comparable to the high nitrate control, yet root growth decreased in the low nitrate compartment and increased in the high nitrate compartment compared to the uniformly high nitrate control. Information about the relative uptake of nitrate by different portions of the root must somehow be relayed across the whole root system. *ANR1* seems to be required for lateral root elongation in response to a localised patch of high nitrate (Zhang and Forde, 1998). Given that nitrate and phosphate availability have similar effects on lateral root elongation it would be

interesting to know whether *ANR1* is also involved in the response to localised phosphate.

The role of auxin

There is considerable evidence that auxin is involved in lateral root development. Zhang *et al*. (1999) suggested an overlap between the auxin and nitrate response pathway, because in their experiments, although the *aux1* and *axr2* mutants showed wild-type responses, the auxin-resistant mutant *axr4* lacked the nitrate-induced lateral root elongation response in a nitrate-rich patch. In contrast, we show here that the auxin-resistant mutant *axr4*, as well as *axr1* and *aux1* all have wild-type responses inside and outside nitrate-rich patches. The exception to this is in the genotypes where lateral root initiation is severely compromised. In the *axr1 axr4* double mutant, there were too few lateral roots to determine effects on lateral root elongation. In both the double mutant and the *axr1* single mutant, the increase in lateral root density within the patch was not observed. This could be because these genes are required for the patch-induced lateral root density increase, or it could be that they undergo a proportional increase in lateral root density, but that the resulting increase in absolute density is below the level of detection of our assay. Furthermore, when *axr4* was grown with a nutrient-rich soil patch between glass plates it responded by co-ordinated lateral root proliferation like wild-type (data not shown). Williamson *et al*. (2001) recently reported wild-type RSA changes to phosphate availability in *axr1*, *aux1* and *axr4*. We therefore conclude that auxin does not appear to be directly involved in nitrate- and phosphate-induced RSA changes.

Conclusions

We have shown here that in *Arabidopsis thaliana*, changes in nitrate and phosphate availability and distribution have different, even contrasting effects on primary root length and lateral root density, despite there being remarkably similar effects on lateral root length. These changes in RSA in response to nitrate and phosphate supply can be easily reconciled with theoretical models for optimal nitrate and phosphate acquisition in natural environments. The nitrate-dependent changes are reminiscent of a herringbone structure (long primary root with low lateral root density) that optimise resource allocation and nitrate capture in a volume of soil (Fitter, 1991; Robinson, 1996). The response to patchy nitrate distribution is less intuitive since root proliferation of a single plant into a patch of highly mobile ions has little theoretical effect on nitrate acquisition. However, such proliferation does result in greater nitrate acquisition when in competition with other plants (Hodge *et al*., 1999; Robinson *et al*., 1999). Low phosphate in both homogeneous and heterogeneous supply favours lateral over primary root growth in *Arabidopsis.* This response produces a RSA that will maximise the exploitation of the invariably more phosphorus-rich surface soil layers or of local phosphate-rich patches. All these responses, however, maintain lateral root length in low nutrient supply, which is of fundamental importance since it allows higher order lateral root production and therefore further plasticity to a constantly changing soil environment.

Experimental procedures

Seed

The auxin-resistant mutant lines *axr1*–3, *axr4*–2, *aux1*–7 and double mutant *axr1*–3 *axr4*–2 are in the Columbia genetic background. The auxin-resistant double-mutant *axr1*–3 *axr4*–2 (Hobbie and Estelle, 1995) was obtained from the *Arabidopsis* Biological Resource Centre, Ohio, USA.

Agar growth conditions

Arabidopsis thaliana ecotype Columbia (Col) seed was surface-sterilised for 15 min in a 10% (v/v) Chloros bleach (Beveridge, Edinburgh, UK), 0.01% (v/v) Triton-X100 solution, followed by a brief wash with 70% (v/v) ethanol and five rinses with sterile distilled water. Prior to planting, seed was cold treated at 4°C for 48 h. Glassware in the phosphate experiments was washed with phosphate free detergent (Decon90, Decon Laboratories Limited, UK). Plants were grown in 14 cm diameter Petri dishes (NUNC™ Brand Products, Denmark), oriented vertically, in a growth room at 22°C under a 16-h light/8-h dark regime and with a light intensity of approximately 65 μmol m^{-2} s^{-1}. The growth medium contained 75 ml modified ATS (*Arabidopsis thaliana salts*) nutrient solution (Wilson *et al*., 1990), with 1% sucrose and 0.8% (w/v) agar (A-1296, plant cell culture tested, Sigma, USA). The agar contained 65 μg P/g agar (1.3 μmol [P] per 14 cm diameter plate with 75 ml of 0.8% (w/v) agar or a concentration of 16.8 μM [P]) as determined by the ascorbic acid-molybdenum method described in Allen (1974). ATS contains 5 mM KNO_3, 2.5 mM KH_2PO_4 buffered with K_2HPO_4 to pH 5.5, 2 mM $MgSO_4$, 2 mM $Ca(NO_3)_2$, 50 μM FeEDTA, and the following micronutrients 70 μM H_3BO_3, 14 μM $MnCl_2$, 0.5 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.2 μM $NaMoO_4$, 10 μM $NaCl_2$ and 0.01 μM $CoCl_2$.

To provide similar growth conditions to those used by Zhang and Forde (1998) in the homogeneous and heterogeneous nitrate experiments, ATS was used at a final dilution of 1 : 10, with 0.25 mM Na_2HPO_4/Na_2HPO_4 (pH 5.5) and 0.2 mM $CaCl_2$ replacing 0.25 mM KH_2PO_4/K_2HPO_4 and 0.2 mM $Ca(NO_3)_2$, respectively; and with 1 mM KCl added to maintain as far as possible the concentration of ions other than nitrate. In the nitrate-rich patch experiment, seedlings were first nitrate-starved on medium containing 10 μM KNO_3 and 1 : 100 ATS, in which 0.02 mM $Ca(NO_3)_2$ was replaced by 0.02 mM $CaCl_2$.

Removal of all phosphate from 1 : 10 ATS had comparatively little effect on growth, suggesting that some other nutrient is limiting in these conditions. However, against the background of more vigorous growth on full strength ATS, removal of P significantly affected growth. Hence full strength ATS with or without added P, was used in the homogeneous and heterogeneous phosphate experiments, with KH_2PO_4/K_2HPO_4 being

replaced with KCl in treatments with less than 2.5 mM KH_2PO_4/K_2HPO_4, in order to maintain potassium at 2.5 mM.

RSA changes in homogeneous nitrate and phosphate supply

Root system architecture was examined across a range of uniform nitrate and phosphate supplies. Two seedlings were grown in 14 cm diameter Petri dishes with a range of [N] from 0.01 to 1 mM on 1 : 10 ATS. Plants were harvested after 17 days in the first experiment and after 18 days in a repeat. Five seedlings were grown with a range of [P] from 0.001 to 2.5 mM on ATS. Plants were harvested after 14 days in the first experiment after 18 days in the repeat. Experiments were repeated with different harvest times to increase the range of plant sizes and results were combined. Five replicate agar plates were used per treatment. At harvest, shoots were cut off at the root-shoot junction, dried at 80°C for 3 days and weighed on an electronic microbalance (Sartorius M2P). Lateral roots were spread out and agar plates were scanned at 300 dpi on a HP DeskScan II scanner (HEWLETT PACKARD C6261A, Palo Alto, CA, USA). RSA was analysed using the WinRHIZO® image analysis system (V 4.1c Régent Instruments, Quebec, Canada).

RSA changes in heterogeneous nitrate and phosphate supply

The effects of nitrate and phosphate distribution on RSA were tested in segmented agar plates, modified from Zhang and Forde's (1998) technique. The application of localised nutrient treatments is achieved by separating agar medium by air gaps into discrete segments. To test for a root proliferation response in a nitrate- or phosphate- rich patch, seedlings had to be starved of the respective nutrient (Robinson, 1994). Eight to 10 seedlings were grown on 25 ml medium in 9 cm diameter Petri dishes on 10 μM KNO_3 or without phosphate for eight or 11 days, respectively, for the nitrate- or phosphate- rich patch experiment. The segmented agar plates were prepared as follows: agar medium was poured into round 14 cm Petri dishes and allowed to solidify for 30 min. The agar medium was cut into 3 segments, top (2 cm), middle (2 cm) and bottom (8.4 cm) and dried off for a further 30 min. Middle, or top and bottom patches were created by spreading 12.5 μl (middle), 8.9 μl (top) or 44 μl (bottom) of 1 mM [N] or [P] over the respective segments giving a final concentration of 1 mM in each segment. All plates, with and without patches, were dried off for a further 5 min, sealed with strips of micropore tape on either side of the plate and kept at 22°C for 24 h to allow the nutrient to diffuse into the agar segment. Hence, nitrate- and phosphate-rich patch treatments contained in the top-middle-bottom segment 0.01–1.0–0.01 mM [N], 1.0–0.01–1.0 mM [N] or 0.0–1.0–0.0 mM [P], respectively. Homogeneous 0.01 mM [N] or 0.0 mM [P], 0.164 mM [N] or [P] and 1 mM [N] or [P] served as control treatments. The total nutrient availability in 0.164 mM [N] and 0.01–1.0–0.01 mM [N] and 0.164 mM [P] and 0.0–1.0–0.0 mM [P] are similar. Two seedlings with 2.5–3 cm long primary roots were transplanted per plate by placing them across the top segment and the upper air gap. In order to measure individual lateral root growth on a daily basis it was necessary to 'direct' lateral roots away from the primary root or other laterals with the help of a blunt sterile needle. Root hairs were damaged but laterals in all plants in all treatments were prodded in this way. Lateral and primary root length was measured daily from day 13 after planting using LUCIA G software (version 3.52a, 1991, Laboratory Imaging, Nikon UK Limited, Kingston, UK). The primary root of *axr1* and *axr1 axr4* had to be 'directed' away from the plate rim with a blunt sterile needle due to their faster growth rate (Hobbie and Estelle, 1995; Timpte *et al.*, 1995). Plants were harvested 18 days after planting and shoot DW and RSA was determined as described above.

Lateral root proliferation in a nutrient-rich soil patch

To assess RSA in soil grown plants, *Arabidopsis* was grown in a thin layer of soil and sand, sandwiched between two glass plates. One glass plate unit consisted of two plates (8 cm × 23 cm) with 1 cm wide, 0.2 cm deep Perspex® strips attached to the 2 long edges and one short edge of one plate. 0.1 cm drainage gaps were incorporated at the bottom to prevent waterlogging. The internal volume of the glass plate unit was filled with 38 g coarse dried silica sand (pH 7.2, Hepworth Minerals and Chemicals, UK) and 3 g sieved (< 2 mm) loamy garden soil (Walled Garden, University of York, UK; 23% water content). Homogeneous sand/soil mix control plates contained sand in the bottom 12.5 cm and a homogeneous mixture of 3 g soil and 16 g sand in the upper 9 cm. Nutrient-rich soil patch plates contained sand in the bottom 17.5 cm, a 1-cm nutrient-rich soil patch (2 g) and a homogeneous mixture of 1 g soil and 5.5 g sand in the top 3 cm. Hence, both treatments provided similar growth conditions during seedling establishment in the top segment. The filled plates were sprayed with dH_2O to carrying capacity. The second plate was clamped on top to the sandwich and the glass plate unit was wrapped with black plastic to prevent light penetration. Plants were grown in a glass house under natural light conditions for 23 days and watered with dH_2O every 2 days by pipette. At harvest primary roots were cut above and below the patch or the equivalent control area, roots were extracted and carefully spread onto acetate sheets. Roots were traced onto new acetate sheets with a marker pen and RSA was determined as described.

Statistical analysis

All data was analysed statistically in SPSS 10. Analysis of covariance was used for the overall data in the homogenous nitrate and phosphate experiments and linear regressions of different nutrient concentrations were compared by the F-ratio method. One-way ANOVA with a Bonferroni Post Hoc test was used for testing primary and lateral root length in heterogeneous nitrate and phosphate supply and lateral root density was analysed by blocking two experiments in the General Linear Model, Repeated Measures with Bonferroni Post Hoc tests. Blocking experiments takes away environmental variation from treatment effects. The General Linear Model, Repeated Measures was used for overall effects in the glass plate experiment. The effects of localised nitrate supply on auxin-resistant mutants in the individual segments were tested with independent–sample *t*-tests. Means are always given with the standard error of the mean (SE) and a *P*-value of 0.05 or less was considered statistically significant.

Acknowledgements

We would like to thank Angela Hodge for advice on the glass plate experiment, the horticultural team for expert plant care, Sebastien Ribrioux for helpful discussions on *Arabidopsis* growth in low phosphate concentrations and Birger Lammering for critical

reading of the manuscript. The Biotechnology and Biological Sciences Research Council, UK, funded the work of Birgit Linkohr as part of the RASP initiative.

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