

# Root Nutrient Foraging<sup>1</sup>

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During a plant's lifecycle, the availability of nutrients in the soil is mostly heterogeneous in space and time. Plants are able to adapt to nutrient shortage or localized nutrient availability by altering their root system architecture to efficiently explore soil zones containing the limited nutrient. It has been shown that the deficiency of different nutrients induces root architectural and morphological changes that are, at least to some extent, nutrient specific. Here, we highlight what is known about the importance of individual root system components for nutrient acquisition and how developmental and physiological responses can be coupled to increase nutrient foraging by roots. In addition, we review prominent molecular mechanisms involved in altering the root system in response to local nutrient availability or to the plant's nutritional status.

In natural and agricultural soils, the ability of plants to quickly and efficiently acquire nutrients may determine their competitive success and productivity. Because mineral elements interact differently with themselves and other soil constituents or are carried by water out of the rooted soil volume, their availability to plants may decrease and lead to nutrient deficiency. Under these conditions, plants activate foraging responses that include morphological changes, such as the modulation of root system architecture (RSA) or root hair formation, and physiological changes, such as the release of nutrient-mobilizing root exudates or the expression of nutrient transporters (Gojon et al., 2009; Hinsinger et al., 2009; Gruber et al., 2013). These responses are often spatially coupled to increase the root-soil interaction zone and improve the ability of the plant to intercept immobile nutrients. Noteworthy, although not discussed herein, symbiosis or associative rhizosphere microorganisms can also alter the RSA and enhance the foraging capacity of the plant (Gutjahr and Paszkowski, 2013). Here, we provide an update on the morphological responses induced by plants to forage sparingly available nutrients and some of the underlying molecular mechanisms known to date to be involved in RSA adaptations to nutrient availabilities.

### NUTRIENT AVAILABILITY IN SOILS AND THE FORMATION OF NUTRIENT PATCHES

Except for soil nitrogen, which originates primarily from the fixation of atmospheric nitrogen, all nutrients present in the soil arise mainly from the geochemical process of mineral weathering of the parental bedrock material (Schlesinger, 1997; Schulten and Schnitzer, 1997). The main factors determining the size of nutrient

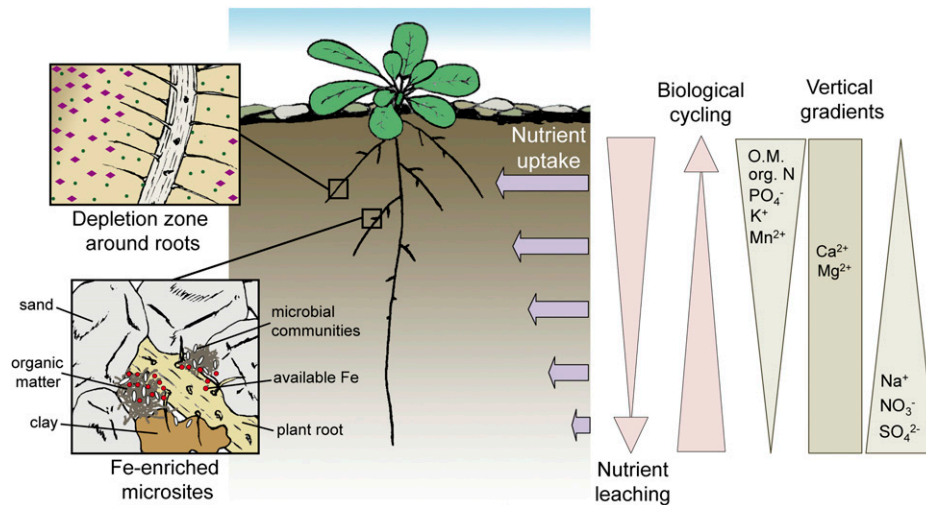
pools in a soil at a given time are, thus, the chemical composition of the parental material and the extent of weathering. Additional processes, like rainfall or the inflow of dust and in agricultural soils, the supply of manure and chemical fertilizers, bring in additional nutrients to the soil pool. In contrast, soils lose nutrients as a consequence of soil erosion, leaching, gaseous emission, or nutrient export by plant parts removed during harvest.

Some soils are naturally impoverished in specific nutrients, which is the case for acid mineral soils that often expose plants to proton or aluminum (Al) toxicity in association with magnesium (Mg) and calcium (Ca) deficiencies (Sanchez and Salinas, 1981). Apart from a substantial depletion of macronutrients from soils under intensive plant production, nutrient limitation is most commonly a problem of availability rather than presence. In fact, because of the complex chemistry of soils, the total amount of nutrients contained in a soil is not fully available to plants. Even in agricultural soils supplied with mineral fertilizers, nutrient availability still depends on the prevailing soil conditions, such as soil water content, soil pH, microbial activity, redox potential, and organic matter content.

Most soils are inherently heterogeneous, and consequently, nutrient availability is variable both in time and space (Jackson and Caldwell, 1993; Farley and Fitter, 1999; Lark et al., 2004). The constant interaction of processes, such as weathering, atmospheric deposition, nutrient leaching, and biological cycling, results in the formation of vertical and horizontal nutrient gradients within the soil (Fig. 1). Because organic matter is more concentrated in the topsoil, the availability of phosphorus (P) is increased in this soil strata (Jobbagy and Jackson, 2001; Lynch and Brown, 2001). In contrast, in soils not inherently poor in sulfur (S), the concentrations of the plant-available ion sulfate ( $\text{SO}_4^{2-}$ ) may increase in deeper soil profiles, because sulfate leaching predominates over plant cycling (Jobbagy and Jackson, 2001). Nutrient concentrations at the same soil depth can also vary significantly over short distances (Jackson and Caldwell, 1993; Lark et al., 2004). Along a linear transect across an

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**Figure 1.** Nutrient gradients and the formation of nutrient patches in soils. Many processes are involved in the formation of vertical gradients in soils, such as nutrient uptake, nutrient leaching, biological cycling, and movement of water. An increased nutrient uptake from the superficial soil strata decreases nutrient concentrations in this soil layer. In addition, concentrations of mobile nutrients may decrease as they move downward and become prone to leaching. Biological nutrient cycling acts in an opposite way to leaching, because it recovers nutrients from deeper soil profiles and brings them back to the surface in the form of litter deposits. In addition, the increased topsoil deposition of organic matter (O.M.) increases, for example, the availability of P and organic nitrogen (org. N) in this soil layer. Some nutrients, such as Ca and Mg, do not generally show strong vertical gradients in most soils. At a smaller scale, the intense uptake of immobile (purple) and mobile (green) nutrients can result in the formation of depletion zones for the immobile nutrient at the root-soil interface. Furthermore, localized organic matter depositions associated with intense microbial activity can result in increased availability of the immobile nutrient iron (Fe) within relatively localized patches in soils.  $\text{PO}_4^-$ , Phosphate;  $\text{SO}_4^{2-}$ , sulfate;  $\text{K}^+$ , potassium ion;  $\text{Mn}^{2+}$ , manganese ion;  $\text{Na}^+$ , sodium ion.

agricultural area, the concentrations of ammonium ( $\text{NH}_4^+$ ) varied over two orders of magnitude, whereas those of nitrate ( $\text{NO}_3^-$ ) varied over two orders of magnitude when the sampling positions were located only 4 m away from each other (Lark et al., 2004). In the same study, it was also found that soil pH showed a considerable variation within comparatively short linear distances, suggesting that the availability of those nutrients affected by pH may also vary considerably.

Nutrient availability can also be highly increased in relatively restricted soil portions. One reason for the formation of nutrient patches within the soil relates to the rather uneven distribution of organic matter (Van Noordwijk et al., 1993) and variable microbial activity (Nunan et al., 2002). For instance, the concentration of plant-available  $\text{Fe}^{2+}$  may rise substantially within microsities containing high organic matter deposition and reduced oxygen levels as a result of intense microbial activity (Fig. 1). Another factor that increases the heterogeneity of nutrient availability in soils is nutrient mobility. The degree of interaction of different ions with the charged surfaces of clays and organic matter determines their mobility in soils. Some nutrients, such as  $\text{NO}_3^-$ , move freely within the soil and reach the root surface by mass flow, which is driven by the nutrient concentration in the soil solution and the transpiration rate of plants. In contrast, nutrients like  $\text{K}^+$  and  $\text{NH}_4^+$  are readily adsorbed to clay minerals, whereas others, such as phosphate, tend to form sparingly soluble

complexes with  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$ , and  $\text{Ca}^{2+}$  (Tinker and Nye, 2000). As a result, adsorbed and precipitated minerals must be desorbed and transported to the root surface by diffusion, which depends mainly on their diffusion coefficient and the concentration gradient between the bulk soil and the root surface. Thus, an elevated uptake of a poorly mobile nutrient, such as phosphorus, can create depletion zones in the rhizosphere (Fig. 1). In this case, plants must adopt foraging strategies, such as increased root hair formation, to reach new phosphorus sources located outside this zone.

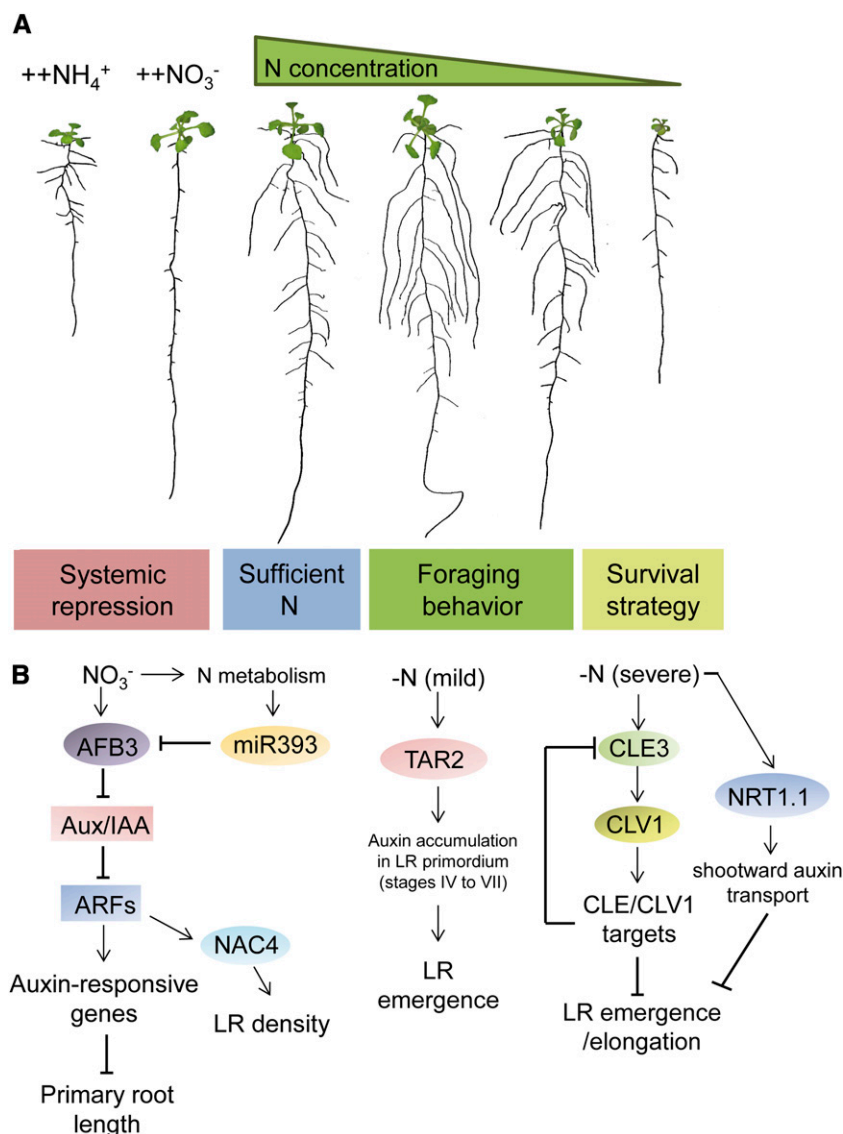
#### MECHANISMS UNDERLYING MORPHOLOGICAL ROOT RESPONSES TO NUTRIENTS AND THEIR IMPLICATIONS FOR NUTRIENT ACQUISITION

The three-dimensional arrangement of the root system of dicotyledonous plants, established by the length and thickness of the primary root (PR) and the density, length, thickness, and angles of lateral roots (LRs) of different orders, determines the soil volume that is explored by a plant. This also holds true for graminaceous roots, although the initial root system is dominated by seminal roots of embryonic origin and later complemented by crown roots originating from the stem base (Orman-Ligeza et al., 2013). Furthermore, the formation of root hairs allows plants to more intensively exploit root-colonized soil portions. The high degree of plasticity of root systems allows postembryonic alterations to

occur in response to environmental cues. Both the nutritional status of the plant and the external nutrient availability can induce changes in the overall root morphology (Giehl et al., 2014). Nutrient deficiencies can result in nutrient-specific alterations of RSA, which were recently shown by a systematic comparison of RSA plasticity changes in response to 12 nutrient deficiencies in *Arabidopsis* (*Arabidopsis thaliana*; Gruber et al., 2013). These responses reflect a systemic control of RSA by the plant nutritional status, which was manipulated by lowering the nutrient supply to plants. The same study also showed that different root traits can respond independently to nutrient deficiencies and exhibit a dose-dependent sensitivity to the imposed deficiency. In some cases, like for nitrogen, total root length is increased under mild nitrogen deficiency but decreased under severe nitrogen deficiency (Fig. 2A; Gruber et al., 2013). Such RSA alterations may reflect different strategies

used by plants in response to the amount of available nutrient. In contrast, plants exposed to most other nutrient deficiencies exhibited a progressive reduction of total root length as the supplied concentrations were decreased (Gruber et al., 2013).

In addition to systemic signals reporting the plant's nutritional status, plants also integrate information about local nutrient availabilities into RSA decisions. The local availability of nitrate and ammonium seems to have complementary effects on LR development, because ammonium stimulates branching, whereas nitrate stimulates LR elongation (Remans et al., 2006; Lima et al., 2010). Also, the micronutrient iron can stimulate LR emergence and elongation when locally available to plants (Giehl et al., 2012). Such responses to localized nutrient availabilities reveal that plants have evolved mechanisms to efficiently monitor and forage nutrient-rich patches in soils.



**Figure 2.** RSA responses to nitrogen availability. A, Excess supply of ammonium ( $++\text{NH}_4^+$ ) or nitrate ( $++\text{NO}_3^-$ ) leads to a systemic repression of root growth, where high ammonium inhibits mostly PR elongation and high nitrate represses mainly LR elongation. Compared with sufficient nitrogen supply, mild nitrogen deficiency ( $-N$ ) increases the lengths of PR and LRs, whereas severe nitrogen deficiency inhibits PR elongation as well as LR emergence and elongation. These distinct RSA responses likely reflect different strategies of the plants to cope with limited nitrogen availability. Figure based on Gruber et al. (2013). B, Examples of signaling pathways involved in modulating RSA responses to the supply of nitrate to otherwise nitrogen-deficient plants (Vidal et al., 2010, 2013) and mild (Ma et al., 2014) or severe (Krouk et al., 2010; Araya et al., 2014) nitrogen deficiency. Details are in the text. Aux/IAA, AUXIN RESISTANT/INDOLE-3-ACETIC ACID INDUCIBLE; miR393, microRNA393.

In the next sections, we review some of the currently known mechanisms that regulate discrete root morphological traits in response to nutrient availability.

## PR Length

### Role of the PR for Nutrient Acquisition

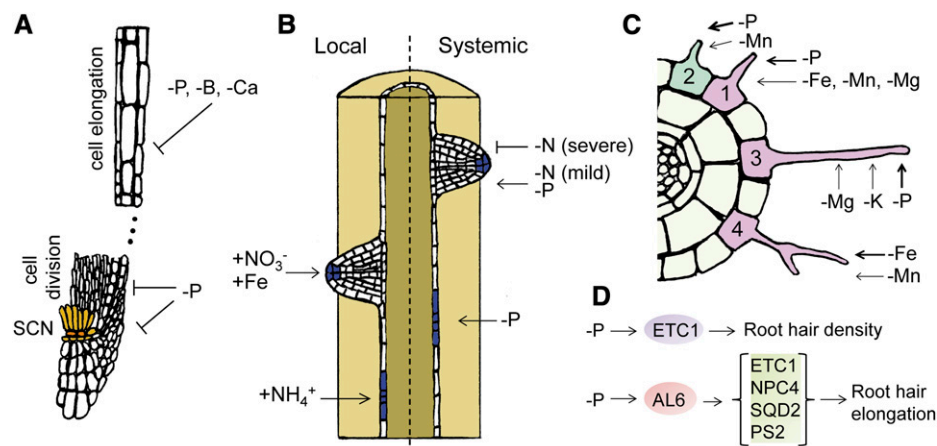
In dicotyledonous plants, the PR forms the main axis of the root system, from which LRs emerge. Because the growth direction of PRs is strongly guided by gravity, PR length mainly determines the depth of a particular root system and consequently, the vertical soil layers reached by the plant. In fact, it has been proposed that the uptake of nutrients, like nitrate and sulfate, that move to deeper soil profiles can be improved by breeding crops with steeper and deeper root systems comprised of a thick and deep PR with few and long LRs (Lynch, 2013).

### Nutrient-Dependent Mechanisms Regulating PR Length

Nutrient deficiencies can alter PRs by affecting different processes of PR elongation, such as cell division or elongation (Fig. 3A). There has been considerable progress in understanding the molecular and developmental mechanisms involved in modulating PR growth under nutrient deficiencies. Many of the nutrient-dependent root architectural alterations are likely controlled by hormones, although for most nutrients, the precise mechanism is not yet known. In the case of nitrogen, it has been shown that the supply of nitrate to nitrogen-deficient plants inhibits PR elongation and

stimulates LR growth by regulating auxin activity by a mechanism that involves the auxin receptor AUXIN SIGNALING F-BOX3 (*AFB3*; Vidal et al., 2010). Nitrate supply increases both auxin accumulation in PR tips and auxin sensitivity by transiently up-regulating *AFB3* expression. As a consequence, plants supplied with 5 mM nitrate exhibited shorter PRs than those grown on low nitrogen (Vidal et al., 2010). The small RNA microRNA393, which is responsive to metabolites produced by the reduction and/or assimilation of nitrate, is responsible for retuning *AFB3* expression back to basal levels (Fig. 2B).

One of the earliest changes in PRs of phosphorus-deficient plants is the occurrence of periclinal cell division in quiescent center cells, which is followed by a reduction of cell elongation and a progressive reduction of cell division (Sánchez-Calderón et al., 2005). It is not yet clear to what extent and at which step auxin is involved in the inhibition of PR elongation by low phosphorus (Nacry et al., 2005; Jain et al., 2007; Miura et al., 2011). However, there is evidence that the modulation of meristematic activity by phosphorus availability is independent of auxin (Jain et al., 2007). In fact, the decreased mitotic activity in the PR tip is most likely associated with a loss of quiescent center identity under low phosphorus (Sánchez-Calderón et al., 2005; Ticconi et al., 2009). The P5-type adenosine triphosphatase PHOSPHATE DEFICIENCY RESPONSE2 (*PDR2*) is involved in the regulation of PR growth in response to phosphorus starvation by maintaining the protein levels of the root patterning gene *SCARECROW* (Ticconi et al., 2009). Thereby, *PDR2* prevents stem cell differentiation and meristematic arrest. A multicopper oxidase encoded by *LOW PHOSPHATE ROOT1* functions together with



**Figure 3.** Effect of nutrient availabilities on root developmental processes. A, Effect of nutrients on stem cell niche (SCN) identity, meristematic activity, and cell elongation. B, Local and systemic regulation of LR emergence or LR initiation by a local availability (+) or the limitation (-) of the indicated nutrients. C, Effect of nutrients on rhizodermal development at hair (1) and nonhair (2) cell positions or on root hair elongation (3) or morphology (4). Hair and nonhair positions are represented in pink and green, respectively. Thicker arrows indicate that the respective nutrient deficiency has a comparatively stronger effect on the designated change in root hair development. D, In response to phosphorus deficiency, the expression of *ETC1* is up-regulated to increase root hair density (Savage et al., 2013). In addition, this nutrient deficiency also induces *AL6*, which, in turn, activates the indicated downstream targets that stimulate root hair elongation (Chandrika et al., 2013). Details are in the text. NPC4, NONSPECIFIC LIPASE4; SQD2, SULFOQUINOVOSYLDIACYLGLYCEROL2; PS2, PHOSPHATE STARVATION-INDUCED GENE2.

PDR2 to adjust the activity of the root apical meristem to external phosphorus and iron concentrations (Ticconi et al., 2009).

Different from phosphorus deficiency, a shortage of boron (B) inhibits mainly cell elongation (Miwa et al., 2013) without significantly altering cell division in the root apical meristem (Martín-Rejano et al., 2011). Boron plays a role in cell wall stability, because it is involved in the dimerization of the pectin polysaccharide rhamnogalacturonan II (O'Neill et al., 2001). The loss of the boron efflux transporter BORON TRANSPORTER2 (BOR2) results in defective cross linking of rhamnogalacturonan II and strong inhibition of cell elongation (Miwa et al., 2013). This transporter is preferentially expressed in epidermal cells within the elongation zone of roots. Thus, under boron deficiency, BOR2 may be responsible for exporting boron from cells to the apoplast to maintain cell wall stability. Because calcium is an important component of pectins in cell walls (Hepler, 2005), the most conspicuous effect of calcium deficiency in PRs is probably also related to cell elongation.

## LR Density and Length

### Importance of LRs for Nutrient Acquisition

The outgrowth of LRs from the PR increases significantly the soil volume explored by a particular root system. Apart from other functions, such as anchorage, LRs enhance the horizontal soil exploration and make a major contribution to the ability of a plant to outcompete neighboring plants when exploiting the same soil niche (Fitter et al., 2002). Different from PRs, gravitropic responses are partially suppressed in LRs, a mechanism that avoids that LRs compete with the parental root and other LRs for the same soil niches. LRs have a major importance for foraging immobile nutrients. The study of an LR-defective rice (*Oryza sativa*) mutant indicated that LRs contribute significantly to the acquisition of phosphorus, manganese, zinc, and copper, whereas the contribution was less significant for the mobile nutrients nitrogen and sulfur (Liu et al., 2013a). The fitness of the auxin mutant *axr4*, which has a reduced number of LRs, was significantly compromised when competing with wild-type plants for immobile phosphate but not mobile nitrate (Fitter et al., 2002). In addition, when the LR-defective *arf7arf19* mutant was challenged by restricting iron availability to a horizontal patch, it developed symptoms of iron deficiency in leaves, whereas LRs of the wild type secured iron acquisition and proper shoot development (Giehl et al., 2012). Likewise, the inability of NITRATE TRANSPORTER1.1 (NRT1.1)-defective mutants to increase LR proliferation into a nitrate-supplemented agar patch decreased their cumulative nitrate uptake and resulted in poor shoot growth (Remans et al., 2006). These observations emphasize the importance of LRs for the competitive acquisition of nutrients, especially immobile ones, by the spatial exploitation of localized nutrient patches.

### Nutrient-Dependent Mechanisms Regulating LR Development

LRs can respond to the extent of a nutrient deficiency, such as in the case of nitrogen (Fig. 2A). One of the developmental steps controlled by nitrogen is LR emergence. When wild-type plants are grown for a prolonged period under severe nitrogen limitation, less auxin accumulates in their LR primordia (Krouk et al., 2010). Interestingly, in the NRT1.1-defective mutant *chl1-1*, auxin levels remain high in LR primordia independent of the external nitrogen concentration. Evidence has been raised that NRT1.1 also transports auxin beside nitrate (Krouk et al., 2010). Thus, the up-regulation of *NRT1.1* in LR tips by severe nitrogen deficiency facilitates the shootward auxin movement, thereby maintaining low auxin levels in LR primordia and young LRs (Fig. 2B). Nitrate supply suppresses *NRT1.1* expression, and consequently, auxin levels are restored, allowing LR development to progress. Recently, a regulatory module consisting of CLAVATA3/ESR-related (CLE) signaling peptides and their receptor protein CLAVATA1 (CLV1) has been shown to regulate LR development under persisting nitrogen deficiency (Araya et al., 2014). The expressions of *CLE1*, *CLE3*, *CLE4*, and *CLE7* were induced after prolonged growth in low nitrogen, especially in root pericycle cells. The overexpression of any of these CLE peptides decreased LR density by inhibiting specifically LR emergence. To function, CLE peptides bind to the leucine-rich repeat receptor-like kinase CLV1 (Ogawa et al., 2008), which is expressed in companion cells of roots (Araya et al., 2014). In the *clv1-4* mutant, LR emergence is increased, irrespective of the nitrate levels supplied to plants. Thus, in nitrogen-deficient plants, CLE peptides move from the pericycle to phloem companion cells, where they interact with CLV1 to inhibit the outgrowth and emergence of LRs (Fig. 2B; Araya et al., 2014).

Both the NRT1.1-dependent and the CLE-CLV1-dependent mechanisms likely reflect a survival strategy, because plants suffering from severe nitrogen deficiency restrict the investment of additional plant resources in expanding their root system into a nitrogen-impooverished environment. However, depending on the extent of nitrogen deficiency, plants can also adopt a foraging strategy (Fig. 2A). Under relatively mild nitrogen deficiency, auxin accumulation was increased in LR primordia at developmental stages IV to VII (late) but not stages I to III (early; Ma et al., 2014). The expression of the auxin biosynthesis-related gene TRYPTOPHAN AMINOTRANSFERASE-RELATED2 (*TAR2*) has been found to be strongly up-regulated by low nitrogen in the root pericycle and vasculature. *TAR2* is involved in one of the tryptophan-dependent auxin biosynthesis pathways, in which *TAR2* converts L-tryptophan to indole-3-pyruvic acid (Stepanova et al., 2008). With decreased auxin accumulation in LR primordia at stages IV to VII, *tar2* mutants exhibited a reduced density of emerged LRs (Ma et al., 2014). Thus, *TAR2* is involved in promoting LR emergence in response to mild nitrogen deficiency (Fig. 2B).

As described above, both nitrate supply and the nitrogen status of the plant control PR and LR growth through the microRNA393/AFB3 module (Vidal et al., 2010). A study that combined genomics, systems biology, and molecular genetics approaches identified the NAM/ATAF1,2/CUC2 (NAC) transcription factor NAC4 as a downstream target of AFB3 (Vidal et al., 2013). Interestingly, *nac4* mutants exhibited reduced LR density in response to nitrate, whereas PR length remained similar to wild-type plants (Vidal et al., 2013), indicating that NAC4 specifically regulates LR density downstream of AFB3 (Fig. 2B).

LR initiation and emergence are induced by phosphorus deficiency (Pérez-Torres et al., 2008). This response has been shown to result mainly from an increased root sensitivity toward auxin (López-Bucio et al., 2002) in a mechanism that involves a phosphorus-dependent transcriptional modulation of the auxin receptor *TRANSPORT INHIBITOR RESPONSE1* (*TIR1*; Pérez-Torres et al., 2008). The increased *TIR1*-dependent auxin sensitivity of phosphorus-deficient plants induces the degradation of AUXIN RESISTANT/INDOLE-3-ACETIC ACID INDUCIBLE repressors and allows ARFs, such as ARF19, to up-regulate the expression of genes involved in LR initiation and emergence. Additional hormone signaling may also be implicated in this response, because it has been shown that the phosphorus-dependent up-regulation of *TIR1* is lost in the strigolactone signaling mutant *max2-1* (Mayzlish-Gati et al., 2012).

## Root Hairs

### *Role of Root Hairs for Nutrient Acquisition*

The formation and elongation of root hairs is controlled by genetic and environmental factors, including nutrient availability. Root hairs increase the root surface area to a much larger extent than LRs, although their longitudinal outreach is usually limited to less than 10 mm from the root surface. Studies with root hairless mutants in *Arabidopsis* have indicated that root hairs contribute significantly to phosphorus acquisition and offer plants a competitive advantage under limited phosphorus availability (Bates and Lynch, 1996, 2001; Brown et al., 2012). The significance of root hairs in nutrient acquisition was recently studied in the root hair-defective transgenic line NR23 of *Arabidopsis* (Tanaka et al., 2014). Compared with the wild type, NR23 plants exhibited decreased shoot biomass when grown under most nutrient deficiencies tested and accumulated less calcium, potassium, phosphorus, sulfur, boron, copper, and zinc in their shoots. These results further reinforce the importance of root hairs in the adaptation of plants to low nutrient availability.

### *Nutrient-Dependent Mechanisms Regulating Root Hair Development*

Root hairs are induced in response to many nutrient deficiencies (Fig. 3C), such as phosphorus, potassium,

magnesium, iron, and manganese (Bates and Lynch, 1996; Schmidt and Schikora, 2001; Yang et al., 2008; Jung et al., 2009; Niu et al., 2014). Although for most of these nutrients, the underlying mechanisms remain still largely elusive, significant progress has been made in understanding how phosphorus deficiency-induced root hair formation is regulated (Fig. 3D). Phosphorus deficiency stimulates root hair proliferation by increasing both root hair density and elongation (Bates and Lynch, 1996; Schmidt and Schikora, 2001). A higher root hair density under low phosphorus results from the inhibition of epidermal cell elongation (Sánchez-Calderón et al., 2006) and the ectopic differentiation of root epidermal cells into trichoblasts also in nonhair positions (Müller and Schmidt, 2004). Recently, it has been shown that phosphorus deficiency increases the expression of *ENHANCER OF TRY AND CPC1* (*ETC1*) in roots (Savage et al., 2013). Because *ETC1* overexpression induced root hair production (Kirik et al., 2004), *ETC1* seems to be a candidate to mediate the signaling between phosphorus deficiency and an increased number of epidermal cells entering the trichoblast cell fate (Fig. 3D). In addition to *ETC1*, the transcription factor BASIC HELIX-LOOP-HELIX32 (*BHLH32*) seems to be involved in the regulation of root hair formation in response to phosphorus limitation. *bhlh32* plants also exhibited a constitutively increased root hair density when grown under sufficient phosphorus, a condition that reduced root hair production in wild-type plants (Chen et al., 2007). Interestingly, *BHLH32* interacted *in vitro* with *TRANSPARENT TESTA GLABRA1* and *GLABRA3*, which are involved in the specification of nonhair versus hair cell fate (Ishida et al., 2008).

Root hair elongation is modulated by phosphorus availability in a dose-dependent manner, because phosphorus alters the rate and duration of elongation (Bates and Lynch, 1996). Recently, forward genetic screens isolated mutants exhibiting phosphorus-specific defects in root hair elongation. The first mutant, *Pi deficiency root hair defective1* (*per1*), showed inhibited root hair elongation when grown under low phosphorus (Li et al., 2010). The abundance of UBIQUITIN-SPECIFIC PROTEASE14 was decreased in *per1* mutants, most likely because of reduced translation efficiency. The insensitivity of *per1* root hairs to phosphorus deficiency could be reverted by supplying the metabolically inert phosphate analog phosphite (Li et al., 2010). This result suggested that UBIQUITIN-SPECIFIC PROTEASE14 is involved in phosphorus signaling. A second *per* mutant, *per2*, also failed to elongate root hairs when grown on limited phosphorus but still responded to iron and manganese deficiencies (Chandrika et al., 2013). In *per2*, a transfer DNA insertion disrupts the expression of the homeodomain protein *ALFIN-LIKE 6* (*AL6*). Transcriptome analysis of *per2* identified putative *AL6* targets, including *ETC1*, *NONSPECIFIC LIPASE4*, *SULFOQUINOVOSYLDIACYLGLYCEROL2*, and *PHOSPHATE STARVATION-INDUCED GENE2*, which are also necessary for phosphorus deficiency-induced root hair elongation (Fig. 3D; Chandrika et al., 2013).

### Coupling of Morphological and Physiological Mechanisms for Nutrient Foraging

To effectively increase nutrient uptake, it is assumed that morphological changes in response to nutrients must be accompanied by the activation of physiological responses, such as the up-regulation of genes involved in nutrient mobilization and high-affinity nutrient uptake. The most dramatic example of a close coupling of morphological and physiological adaptations to nutrient deficiency is the formation of cluster roots in a diverse range of plant species, including white lupine (*Lupinus albus*). These specialized structures, induced specially under low phosphorus availability, consist of very dense and short rootlets that typically arrange in a bottle brush-like manner and exhibit abundant root hairs (Lambers et al., 2006). Cluster roots do not only significantly increase the root surface area but at the same time, produce and release large amounts of carboxylates (Shane et al., 2004) that help mobilize phosphorus from sparingly soluble pools (Veneklaas et al., 2003). An RNA-sequencing analysis of cluster roots has revealed that genes involved in metabolic responses to phosphorus starvation, such as the biosynthesis of citrate, phenolics, and acid phosphatases, as well as putative phosphate uptake transporters are strongly up-regulated in mature cluster roots compared with juvenile ones (Wang et al., 2014). Thus, cluster-rooted plants mine intensively the root-colonized soil volume, thereby quickly depleting the phosphorus within this soil portion. Perhaps, therefore, cluster roots remain metabolically active for only 1 to 3 d (Neumann and Martinoia, 2002).

Although most studies on plants that do not form cluster roots focus on physiological and morphological responses separately, there is also evidence for an efficient coupling of such responses in these plants. The protein amounts of six root-expressed PHOSPHATE TRANSPORTER genes (*PHT1;1-PHT1;6*) in the root hair-less transgenic line NR23 were about one-half of that detected in wild-type roots (Tanaka et al., 2014). Phosphorus-deficient NR23 plants also showed a reduced secretion of acid phosphatases, malate, and citrate, indicating that these physiological responses are associated with the phosphorus deficiency-induced root hair formation. The association between root hair formation and the cell type-specific up-regulation of genes involved in iron acquisition was studied in cucumber (*Cucumis sativus*) by capturing iron deficiency-induced root hair cells and nonhair epidermal cells by laser ablation (Santi and Schmidt, 2008). It was found that the increased formation of root hairs in iron-deficient plants was associated with a marked up-regulation of cucumber *FERRIC REDUCTION OXIDASE1* (*CsFRO1*), cucumber *IRON-REGULATED TRANSPORTER1* (*CsIRT1*), and cucumber *H<sup>+</sup>-ATPASE1* (*CsHA1*). Noteworthy, the extent of rhizosphere acidification correlated more strongly to root hair formation than the activity of the ferric iron-chelate reductase (Santi and Schmidt, 2008). Recently, gene and protein expression levels were assessed in root hair cells obtained by sorting protoplasts

expressing GFP under the control of *EXPANSIN7*, a gene with expression restricted to trichoblasts (Lan et al., 2013). Among the genes expressed more strongly in *EXPANSIN7*-GFP-positive cells were transporters for nitrate (*NRT1.1*, *NRT2.1*, and *NRT6.2*), phosphate (*PHT1;1*), sulfate (*SULFATE TRANSPORTER1;1*), and the *H<sup>-</sup>-ATPASE7* (*AHA7*). Because this study was not carried out under nutritional conditions that induce root hair formation, it is likely that additional nutrient transporters may be associated to root hairs formed in response to nutrient limitation. Apart from root hairs, other root morphological responses may also be accompanied by physiological adaptations. As one example, not only was the elongation of LR's growing into an iron-containing patch stimulated, but also, the expression of the *Fe<sup>2+</sup>* transporter *IRT1* was more significantly up-regulated in these LR's (Giehl et al., 2012).

### CONCLUDING REMARKS

Current experimental evidence indicates that root hairs are more extensively formed under deficiencies of those nutrients that are transported to the root surface by diffusion. However, in the case of PR's and LR's, the mobility and the type of transport pathway taken in soils by a particular nutrient are mostly not related to changes of these RSA components. As far as differences in the developmental stage of PR's and LR's permit a comparison, they even show a differential sensitivity in their growth response to the availability of nutrients. For instance, PR elongation is strongly inhibited by systemic calcium deficiency, whereas LR elongation is even slightly induced (Gruber et al., 2013). Likewise, *NH<sub>4</sub><sup>+</sup>* concentrations that stimulate LR formation concomitantly suppress meristem size of the parental root (Lima et al., 2010; Liu et al., 2013b). As suggested by the specific effect of mild and prolonged nitrogen deficiency in *TAR2*-regulated or *CLE-CLV1*-regulated LR elongation (Araya et al., 2014; Ma et al., 2014), a dose-dependent or pronounced morphological response in LR's may rely on not only the size of a signal but also, the cell type-specific expression pattern of receptors translating these systemic signals into developmental changes. Noteworthy, there is a significant natural genetic variation for the RSA response to nutrients that is to be explored in future studies (for review, see Ristova and Busch, 2014).

The differential impact of systemic or localized nutrient-dependent signals on RSA clearly indicates that most nutrients interfere with the root developmental program at different checkpoints. Although we currently still have a rather scattered picture of these pathways, it seems that auxin takes in a central role, whereas nutrient-induced changes in other hormones may confer more specific developmental changes in RSA. Such combinatorial effects may also contribute to the high divergence of nutrient-dependent changes in the transcriptional regulation of root development-related genes (Giehl et al., 2014).

Uncovering these checkpoints in the future may also bear interesting opportunities in fertilizer management practices in plant production. A targeted exploitation of nutrient-dependent changes in RSA traits may pave the way to guide roots into those soil zones where limiting resources are localized. Moreover, uncovering nutrient-dependent RSA changes may also provide the opportunity for conventional or molecular breeding approaches to uncouple defined RSA responses from nutrient signals. For instance, uncoupling TAR2-dependent LR elongation from suppression by high nitrogen doses may allow for growing crops with more expanded root systems at high nitrogen supply for the sake of enhanced nitrogen fertilizer use efficiency.

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