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REVIEW

The genetic basis of nitrogen-dependent root system architecture in plants

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KEYWORDS

Nitrogen, nitrogen signals, phytohormones, prospectives, root system architecture

HIGHLIGHTS

- Root system architecture (RSA) of plants has the plasticity to fluctuations in nitrogen availability within their environment.
- N-dependent RSA is regulated by the intricate interplay between N and phytohormones.
- More attention should be given to deciphering the genetic basis of N-dependent RSA in crops.

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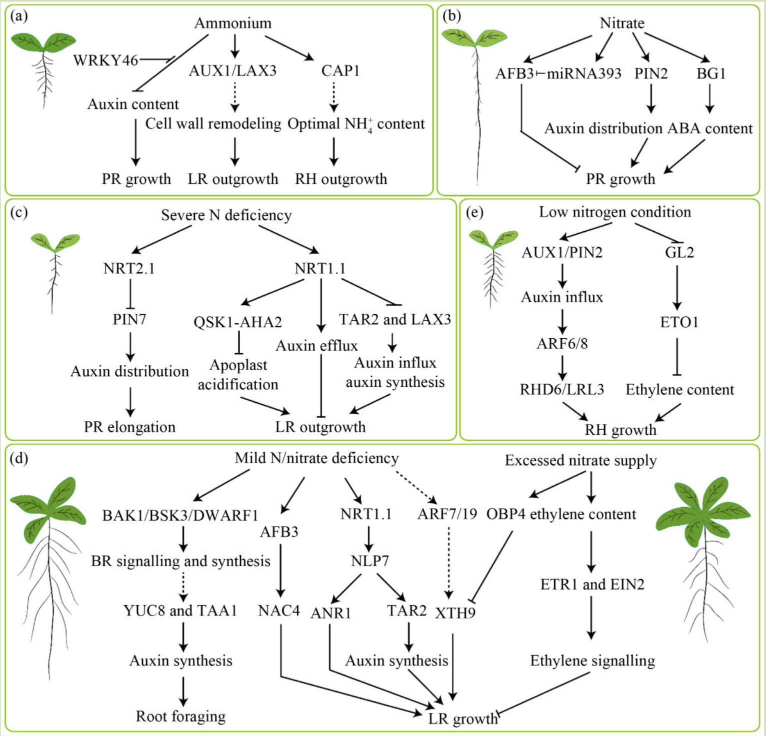
GRAPHICAL ABSTRACT



ABSTRACT

Plant roots are crucial for nitrogen uptake. To efficiently acquire N, root system architecture (RSA), which includes the length and quantity of primary roots, lateral roots and root hairs, is dynamically regulated by the surrounding N status. For crops, an ideotype RSA characterized by enhanced plasticity to meet various N demands under fluctuating N conditions is fundamental for high N utilization and subsequent yield. Therefore, exploring the genetic basis of N-dependent RSA, especially in crops, is of great significance. This review summarizes how plants sense both local and systemic N signals and transduce them to downstream pathways. Additionally, it presents the current understanding of genetic basis of N-dependent root plasticity in *Arabidopsis* and major crops. Also, to fully understand the mechanisms underlying N-dependent root morphogenesis and effectively identify loci associated with an ideotype RSA in crops, more attention should be paid to non-destructive, *in situ* phenotyping of root traits, cell-type-specific exploration of gene functions, and crosstalk between root architecture, environment and management in the future.

1 Introduction

Supply of nitrogen (N), an essential macronutrient for plant growth, is foundational for crop yield and global food security. The distribution of nitrogen in soil has significant spatial heterogeneity, promoting plants to develop complex regulatory mechanisms to adapt to varying environmental conditions. These mechanisms include the presence of nitrate transporters, which are categorized into high-affinity and low-affinity types. High-affinity transporters facilitate nitrate uptake in low-nitrate environments whereas their low-affinity counterparts operate under high-nitrate conditions[1]. Roots are pivotal in N acquisition, responding with physiologic and morphological plasticity under different N conditions. This plasticity is crucial for optimizing N use efficiency[2,3]. As a result, extensive research has been conducted to uncover the genetic basis for N-dependent root system architecture (RSA), with *Arabidopsis* serving as a primary model organism[4]. In this review, we summarize how plants perceive both local and systemic N signals and transduce these cues internally, how N signaling interacts with phytohormones to orchestrate RSA remodeling, enabling plants to thrive under diverse N conditions. Additionally, we highlight the emerging technologies to precisely and systematically identify the key genetic elements that govern nitrogen-responsive RSA modifications, enabling advancements in agricultural practices aimed at enhancing N use efficiency.

2 Root system architecture and its N-dependent plasticity

A fundamental structure distinctive of dicotyledonous plants is a prominent taproot, which consists of primary and lateral roots. The latter originating from the pericycle at the xylem poles of the primary roots and eventually penetrate the overlying cells through various developmental stages[5]. In contrast, monocotyledonous plants have a fibrous root system, primarily composed of seminal and adventitious roots that sprout from non-root organs, with lateral roots emerging from both of these kinds of root[6]. It is noteworthy that both dicots and monocots develop root hairs, which are specialized epidermal cells. The proliferation of lateral roots and root hairs significantly increases the root surface area, thereby greatly enhancing N acquisition from the surrounding environment.

As the primary organ for N acquisition, roots have remarkable architectural plasticity responding to the forms, quantities and distribution of N in the soil, and N status within plants. This adaptability is particularly pronounced given the heterogeneous distribution of N in soil. In general, ammonium stimulates root branching while inhibiting root elongation, whereas nitrate promotes root elongation (Fig. 1(a,b))[5,7]. When compared to conditions of N sufficiency, the growth of both primary and lateral roots is inhibited under severe N deficiency. Conversely, root length is increased to enhance the N uptake capacity under mild N deficiency (Fig. 1(c,d))[8,9]. Apart from general regulatory mechanisms, localized N starvation signals on one side of the root in low-N environments enable modified root growth on N-rich side through root-shoot-root mobilization, effectively capturing N[10]. Also, the length and density of root hairs are also regulated by N, further optimizing the ability of root system to forage for essential nutrients[11].

3 N signaling network in plants

N-regulated RSA depends on the integration of both local and systemic N signaling[12]. Local N signals are mainly received by receptor located in the plasma membrane or cytoplasm. Once received, N signals are gradually transmitted downstream, eventually reaching the nucleus to activate the expression of N responsive genes. The long-distance transduction of N signals, which is critical for adaptive responses to fluctuating N availability, heavily relies on root-to-shoot or shoot-to-root translocation of N-induced phytohormones or peptides by local N signaling[13].

3.1 Local N signaling pathway in plants

The plasma membrane-localized nitrate transceptor NRT1.1 functions as a crucial nitrate sensor across various organisms. NRT1.1 in *Arabidopsis* is the best known one that has received significant attention for its sensing function[14]. In the plasma membrane, NRT1.1 interacts with CNGC15 (Cyclic Nucleotide-Gated Channel 15), a Ca^{2+}-permeable channel, suppressing its channel activity. Stimulating by nitrate signals, the interaction between NRT1.1 and CNGC15 diminishes, enabling Ca^{2+} influx via CNGC15[15]. In cytoplasm, nitrate-induced Ca^{2+} signaling leads to phosphorylation of NIN-like protein 7 (NLP7), the central transcriptional factor of nitrate signaling, by calcium-dependent protein kinase CPK 10/30/32. Subsequently, phosphorylated NLP7 is imported into nucleus, activating the expression of numerous nitrate response genes[16]. It is noteworthy besides being regulated by extracellular nitrate, the transcriptional activation of NLP7 is also enhanced by direct nitrate binding[17]. In rice, the nitrate signaling cascade is mediated by NRT1.1B-NBIP1-SPX4 module. Upon binding with nitrate, NRT1.1B recruits an E3 ubiquitin ligase NBIP1 (NRT1.1B interacting protein 1) to promote the ubiquitination and degradation of SPX domain-containing protein 4 (SPX4). This leads to the release of the master transcription factor NLP3 from SPX4-NLP3 complex, facilitating its nuclear translocation[18]. Similarly, ZmNRT1.1B-ZmNLP3.1 module mediates the perception and transduction of nitrate signal in maize[19]. As described above, NRT1.1-NLP module represents a fundamental aspect of nitrate signaling pathway across various plants. However, the modulation of cytoplasm-to-nucleus shuttling of NLPs is divergent among various plants. In *Arabidopsis*, Ca^{2+} works as the second messenger that triggers the phosphorylation and nuclear translocation of NLP7; localization of NLP3 in nucleus in rice is facilitated by NBIP1-mediated degradation of SPX4. However, the molecular mechanism of ZmNLP3.1 cytoplasm-to-nucleus shuttling remains elusive.

Although ammonium is widely recognized as a potential signal for plant growth, the precise signaling pathway that mediates the response to ammonium is still unclear[20].

3.2 Perception and transduction of systemic N signaling

Phytohormones are essential for mediating the transduction of systemic N signaling. The NLP7-regulated nitrate signal facilitates the production of cytokinin in roots and its mobilization from root to shoot. In shoot, cytokinin response factors are induced by elevated cytokinin, resulting in the activation of auxin transporter genes. This regulation governs the strategic distribution of auxin, ultimately promoting plant growth[21]. Additionally, cytokinin response in shoot also triggers shoot-to-root signals that modulate root architecture and N acquisition in root, in conjunction with local nitrate signals[22].

Also, translocation of small peptides between root and shoot serves as the crucial mediator in the transduction of systemic N signaling. In root, the CLE (CLAVATA/ESR-related)-CLV1 (CLAVATA1) module is critical for sensing and transducing low N signaling. In response to N-deficiency, the expression of CLE peptides encoding gene, *CLE3*, is upregulated. Subsequently, elevated CLV3 in pericycle potentially diffuses into phloem companion cell and binds to the leucine-rich repeat receptor-like kinase CLV1. This interaction transduces the N-deficiency signals to downstream components, resulting in the inhibition of lateral roots[23,24]. Additionally, C-terminally encoded peptides (CEP) induced by N starvation in root are transported from root to shoot. Here, they are recognized by two CEP receptors, CEPR1 and CEPR2[25]. Next, the CEP DOWNSTREAM 1/2 are induced in shoot and translocated to root via phloem, activating the expression of high-affinity nitrate transporter gene *NRT2.1* in root surrounding with nitrate-rich rhizosphere[26]. When N content in shoot falls below a certain threshold, a polypeptide, CEPD-like 2 is activated and mobilized to root to promote N acquisition[27]. Additionally, a bZIP transcriptional factor ELONGATED HYPOCOTYL5 is also translocated from shoot to root, where it reinforces the activation of *NRT2.1* expression[28].

4 Genetic basis of N-depended root system architecture

N-dependent RSA is intricately regulated by complex network, in which the interplay of local and systemic signals, N signals and phytohormones creates a coordinated and fine-tuned system (Fig. 1). We summarize the modification of root architecture by N status in *Arabidopsis* and major crops (Table 1).

4.1 N-dependent primary root growth

The inhibition of primary root growth by ammonium can be attributed to the reduced auxin content within primary root elongation region. With ammonium treatment, auxin in this region is transformed into inactive forms, resulting from its

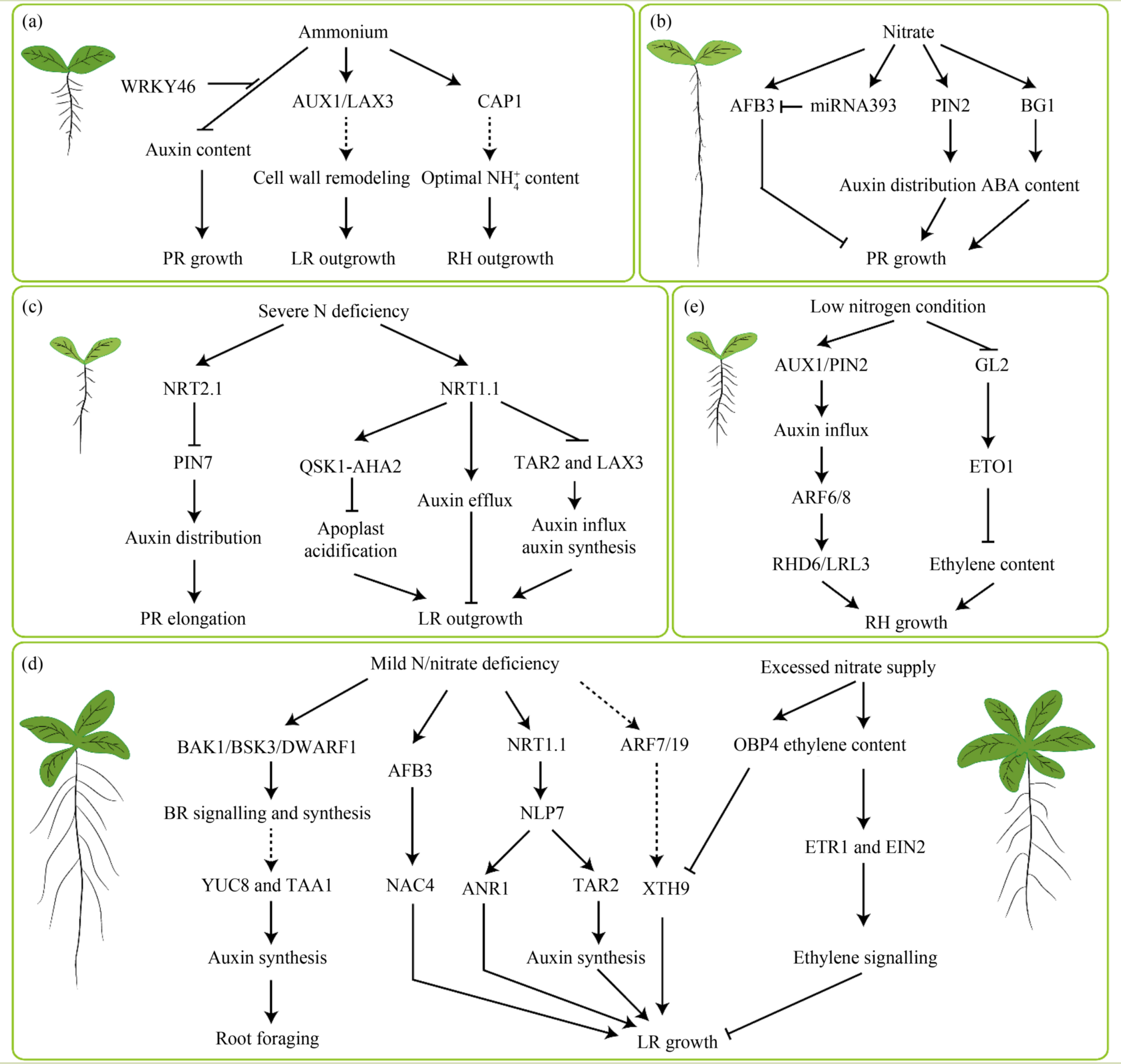


Fig. 1 Schematic of the progress of N-dependent root system architecture in *Arabidopsis*. (a) Ammonium-mediated root system architecture. AUX1, AUXIN RESISTANT 1; LAX3, LIKE AUX 3; CAP1, $[Ca^{2+}]_{cyt}$-associated protein kinase 1. (b) Nitrate-mediated primary root growth. AFB3, Auxin Signaling F-Box 3; PIN2, PIN-FORMED 2; BG1, β-GLUCOSIDASE1; ABA, Abscisic acid. (c) Severe N deficiency-mediated root architecture. QSK1, Qian Shou Kinase 1; AHA2, *Arabidopsis* plasma membrane H^+-ATPase isoform 2; TAR2, Tryptophan aminotransferase related 2. (d) Mild nitrate- and excessive nitrate-mediated root system architecture. BAK1, BRI1-ASSOCIATED RECEPTOR KINASE 1; BSK3, BRASSINOSTEROID SIGNALING KINASE 3; YUC8, YUCCA8; TAA1, Tryptophan aminotransferase of *Arabidopsis* 1; NAC4, NAM ATAF CUC 4; NLP7, NIN-like protein 7; ANR1, *ARABIDOPSIS* NITRATE REGULATED 1; ARF7/19, AUXIN RESPONSE FACTOR 7/19; XTH9, Xyloglucan endotransglucosylase/hydrolase 9; OBP4, OBF Binding Protein 4; ETR1, ETHYLENE RECEPTOR 1; EIN2, ETHYLENE INSENSITIVE 2. (e) Low nitrogen-mediated RH growth. RHD6, ROOT HAIR DEFECTIVE 6; LRL3, LOTUS JAPONICA ROOT HAIRLESS-LIKE 3; GL2, GLABRA 2; ETO1, ETHYLENE OVERPRODUCER 1. PR, primary root; LR, lateral root; RH, root hair.

conjugation with sugars or amino acids[72,73]. Notably, the deactivation of auxin could be disrupted by a WRKY transcription factor, WRKY46, which directly suppresses the expression of auxin-conjugating genes[29]. Additionally, GDP-mannose pyrophosphorylase enables the recovery of NH_4^+-inhibited primary root growth in some degree[30].

Table 1 Major genes or modules involved in nitrogen-dependent root system architecture of plants

Host plant	Gene or module	Effect to N-dependent root system architecture	Reference
Arabidopsis	*WRKY46*	Decreases ammonium-promoted inactivation of auxin and alleviates ammonium-inhibited primary root elongation	[29]
Arabidopsis	*GDP-mannose pyrophosphorylase*	Partially recovers ammonium-inhibited primary root growth	[30]
Arabidopsis	*PIN-FORMED 2*	Redirects auxin distribution and promotes root growth in nitrate-dependent manner	[31,32]
Arabidopsis	microRNA393/*Auxin Signaling F-Box 3*	Fine-tunes nitrate-dependent primary root growth	[33]
Arabidopsis	*β-GLUCOSIDASE1*	Promotes primary root growth by elevating abscisic acid content	[34]
Arabidopsis	*DWARF1*	Promotes root foraging by modifying brassinosteroid biosynthesis under mild nitrogen deficiency	[35]
Arabidopsis	*BRI1-ASSOCIATED RECEPTOR KINASE 1* and *BRASSINOSTEROID SIGNALING KINASE 3*	Promotes root foraging by modifying brassinosteroid signaling under mild nitrogen deficiency	[36]
Arabidopsis	*CALMODULIN-LIKE-38- PEP1 RECEPTOR 2*	Negatively regulates root growth by integrating low nitrogen and brassinosteroid signals	[37]
Arabidopsis	*Nitrate transporter protein 2.1-PIN-FORMED 7*	Inhibits primary root elongation under severe nitrate deficiency condition by suppressing auxin influx to root tip	[38]
Arabidopsis	*AUXIN RESISTANT 1* and *LIKE AUX 3*	Mediates ammonium stimulated distribution of auxin in root and promotes root branching	[39]
Arabidopsis	*Nitrate transporter protein 1.1*	Suppresses lateral root growth by mediating auxin efflux from primordia and young lateral root tips under free-nitrogen or low nitrate conditions, promotes lateral root elongation due to suppressed auxin transport activity under mild nitrate deficiency conditions	[40,41]
Arabidopsis	*Nitrate transporter protein 1.1-Qian Shou Kinase 1-Arabidopsis plasma membrane* H^+*-ATPase isoform 2*	Represses lateral root growth by inhibiting H^+ efflux from cytoplasm to apoplast	[42]
Arabidopsis	*Tryptophan aminotransferase related 2*	Promotes lateral root growth by enhancing auxin accumulation in lateral root primordia	[43]
Arabidopsis	*YUCCA8* and *Tryptophan aminotransferase of Arabidopsis 1*	Promotes lateral root elongation by enhancing auxin synthesis under mild nitrogen deficiency conditions	[44]
Arabidopsis	*NRT1.1-NIN-like protein 7-ARABIDOPSIS NITRATE REGULATED 1*	Stimulates nitrate-induced lateral root growth	[45,46]
Arabidopsis	*Auxin Signaling F-Box 3-NAM ATAF CUC 4*	Promotes nitrate induced lateral root emergence	[47]
Arabidopsis	*OBF Binding Protein 4-Xyloglucan endotransglucosylase/hydrolase 9*	Regulates nitrate-dependent lateral root growth	[48]
Arabidopsis	microRNA167/ *AUXIN RESPONSE FACTOR 8*	Fine-tunes nitrate suppressed lateral root initiation	[49]
Arabidopsis	*ETHYLENE RECEPTOR 1* and *ETHYLENE INSENSITIVE 2*	Regulate the nitrate-inhibited lateral root growth	[50]
Arabidopsis	$[Ca^{2+}]_{cyt}$*-associated protein kinase 1*	Regulates root hairs growth by balancing ammonium content in the cytoplasm	[51]
Arabidopsis	*AUXIN RESPONSE FACTOR 6/8- ROOT HAIR DEFECTIVE 6- LOTUS JAPONICA ROOT HAIRLESS-LIKE 3*	Promotes root hairs elongation under low nitrogen conditions	[52]
Arabidopsis	*GLABRA 2-ETHYLENE OVERPRODUCER1*	Fine-tunes ethylene-dependent root hair growth	[53]
Rice	*NAR2.1-nitrilase 1/2*	Regulates both primary root and lateral root growth	[54,55]
Rice	*REGULATOR OF N-RESPONSIVE RSA ON CHROMOSOME 10-DULL NITROGEN RESPONSE1*	Modulates root plasticity response to nitrogen	[56]
Maize	*NIN-like protein 3.2-AUXIN RESPONSE FACTOR 19- AUXIN/INDOLE-3-ACETIC ACID14*	Regulates root biomass under low nitrogen	[57]
Maize	*PIN-FORMED 1a*	Promotes primary root elongation and suppresses lateral root initiation under low nitrate supply conditions	[58]
Wheat	*Tryptophan aminotransferase related 2.1*	Regulates lateral root growth by manipulating auxin biosynthesis	[59]
Rice	*DWARF3*	Modulates root architecture under low nitrate level	[60]

(Continued)

Host plant	Gene or module	Effect to N-dependent root system architecture	Reference
Rice	*DWARF57- SQUAMOSA PROMOTER BINDING PROTEIN LIKE 14/17-PIN-FORMED 1b*	Regulates root growth with nitrate supply	[61]
Rice	microRNA444a-*MADS23/27a/27b/57*	Alters nitrate dependent root architecture	[62]
Rice	microRNA444-*MADSs-BR-deficient dwarf 1*	Inhibits root growth under ammonium conditions	[63]
Rice	*N-mediated heading date 1-Nitrate transporter 2.4/Ammonium transporter 1.3*	Promotes root growth	[64]
Rice	*argininosuccinate lyase1*	Promotes root elongation under ammonium condition by converting excessive glutamine to arginine	[65]
Maize	*TGA*	Regulates low nitrogen induced root elongation	[66]
Wheat	*Nitrate transporter 1/peptide transporter family 2.12*	Inhibits root length under low nitrogen condition	[67]
Rice	*bZIP1*	Inhibits root length under low nitrogen condition	[68]
Rice	*Cellulose synthase-like D1*	Involves in nitrogen-regulated root hair growth	[69]
Soybean	*NIN-like protein1/4-Nitrate-induced CLE peptide1a/b*	Mediates high nitrate-inhibited nodulation	[70]
Soybean	*Germin-like protein 20.4*	Promotes primary and lateral growth, increases root biomass	[71]

Nitrate-modulated primary root elongation is orchestrated by auxin, abscisic acid (ABA), and brassinosteroid (BR) in root. nitrate treatment triggers dephosphorylation and polarized localization of an auxin efflux carrier PIN2 (PIN-FORMED 2), thereby promoting root growth by redirecting auxin distribution[31,32]. Also, nitrate induces the expression of *AFB3* (*Auxin Signaling F-Box 3*), an auxin receptor gene in root tip, which in turn inhibits primary root elongation. However, *AFB3* expression is negatively regulated by microRNA393, which is induced by metabolites of nitrate utilization. The microRNA393/*AFB3* module might fine-tune nitrate-dependent primary root growth[33]. Additionally, nitrate induces the expression of *BG1*, encoding the ABA-glucose ester-deconjugating enzyme *β*-GLUCOSIDASE1 that converts inactive ABA-glucose ester to bioactive ABA, leading to elevated ABA content, and thus promoted primary root growth[34]. Modification of BR signaling and BR content in root by mild N deficiency induced expression of BR co-receptor gene *BRI1-ASSOCIATED RECPTOR KINASE 1* and BR biosynthesis gene *DWARF1*, respectively, promotes root foraging for N acquisition[35,36]. The functional divergence between different *BRASSINOSTEROID SIGNALING KINASE 3* genotypes, a BR signaling kinase gene, is responsible for the variable root responses to low N condition[36]. Also, CALMODULIN-LIKE-38 interacts with PEP1 RECEPTOR 2 to regulate root growth under low N conditions by integrating nitrate and BR signals[37]. Under severe nitrate deficiency condition, NRT2.1 suppresses PIN7 auxin transport activity by direct interaction, hindering auxin influx to root tip, and thereby inhibiting primary root elongation[38].

4.2 N-dependent lateral root growth

Lateral root elongation is inhibited by ammonium but branching promoted. This regulation is largely mediated by ammonium transporter AMT1;3[7,74]. Ammonium uptake is coupled with proton efflux via H$^+$-ATPase, causing acidification of the apoplast. Subsequently, auxin undergoes protonation and is imported into cortical and epidermal cells overlying lateral root primordia by auxin importers AUX1 (AUXIN RESISTANT 1) and LAX3 (LIKE AUX 3). Consequently, auxin-induced expression of gene related to cell wall remodeling causes cell wall loosening and reduces the mechanical resistance for lateral root outgrowth[39].

Under conditions of free-N or low nitrate (< 0.5 mmol·L^{-1}), reduced auxin concentration within lateral root region suppresses lateral root growth, which is resulted from NRT1.1-mediated auxin efflux from primordia and young lateral root tips[40,41]. During this process, phosphorylation of Thr-101 in NRT1.1 is critical for its plasma membrane localization and further integration into functional membrane microdomains in lateral root cells, thereby enhancing the auxin efflux[75]. Beyond directly promoting auxin efflux, NRT1.1 also suppresses auxin biosynthesis and influx by reducing the expression of auxin synthetic gene *TAR2* (*Tryptophan aminotransferase related 2*) and auxin influx carrier gene *LAX3*, respectively, maintaining lower auxin levels in lateral roots[76]. Additionally, NRT1.1 collaborates with a receptor kinase QSK1 (Qian Shou Kinase 1) and H$^+$-ATPase AHA2 to form a complex in plasma membrane, leading to decreased AHA2 proton pump activity by phosphorylation of AHA2 at Ser899. Eventually, inhibition of H$^+$ efflux from cytoplasm to apoplast represses lateral root growth by releasing apoplast acidification[42]. Under limited nitrate and ammonium mixed conditions, lateral root growth is stimulated by the induced expression of *TAR2*, which causes auxin accumulation in lateral root primordia[43].

Under mild nitrate deficiency conditions, the auxin transport activity of NRT1.1 is suppressed, leading to auxin accumulation in lateral roots, thereby promoting their elongation[40]. Additionally, auxin content is also promoted by nitrate-induced auxin synthesis genes via multiple pathways, including NLP7-mediated induction of *TAR2*[77], and the upregulation of *YUC8*, and *TAA1* (*Tryptophan aminotransferase of Arabidopsis 1*), which acts downstream of N deficiency-activated BR signaling cascade[44]. Also, NRT1.1-NLP7-ANR1 signaling module stimulates nitrate-induced lateral root growth[16,40,45,46]. While inhibiting growth of primary roots, nitrate induced expression of *AFB3* in pericycle area, the initiation region of lateral roots, causes the activated expression of *NAC4*, a key component that regulating nitrate-responsive pathway, thereby enhancing lateral root emergence[33,47]. A regulatory module downstream of the AUXIN RESPONSE FACTOR (ARF) 7 and 19 comprises XTH9 (Xyloglucan endotransglucosylase/hydrolase 9), a regulator of cell wall biosynthesis, and OBP4 (OBF Binding Protein 4), a Dof transcription factor. XTH9 activates lateral root development and OBP4 suppresses it by directly inhibiting expression of *XTH9*[48]. Remarkably, it is N metabolites, such as glutamine/glutamate, rather than nitrate that mediate nitrate suppressed lateral root initiation. This intricate process is fine-tuned by microRNA167/*ARF8* module[49].

The excessive supply of nitrate suppresses both lateral root length and number, concurrently stimulating the accumulation of ethylene (ET) content. The nitrate-inhibited lateral root growth is partially rescued in defected ET signaling mutants, *etr1* and *ein2*, indicating that ET is involved in high nitrate-regulated lateral root growth[50]. Additionally, ABA pathway also contributes to the inhibition of lateral root elongation by nitrate[78].

It has also been reported that the transcription factors TGA1, TGA4, AGL21, TCP20 and high-affinity nitrate transporter NRT2.1 are involved in nitrate-modified lateral root growth under various conditions[79–82].

4.3 N-dependent root hair growth

Root hairs, which is crucial for N capture, are tightly modulated by both the form and concentration of N[83,84]. Maintaining a balanced NH_4^+ content in the cytoplasm is essential for outgrowth of root hairs. Elevated cytoplasmic NH_4^+ concentration triggers the activation of a tonoplast-localized $[Ca^{2+}]_{cyt}$-associated protein kinase, which phosphorylates unidentified targets, thereby enhancing the translocation of NH_4^+ from cytoplasm to vacuole and maintaining the optimal cytoplasmic NH_4^+ concentration and root hair growth[51]. Under low N conditions, auxin content in root tip is increased due to the upregulation of *TAA1* and *YUC8*. Then, auxin is transported to root hair differentiation region by auxin transporter, AUX1 and PIN2, activating the expression of *RHD6* (*ROOT HAIR DEFECTIVE 6*)-*LRL3* (*LOTUS JAPONICA ROOT HAIRLESS-LIKE 3*) module by AUXIN RESPONSE FACTOR 6 and 8 to promote root hair elongation[52]. In addition, GLABRA 2-ET OVERPRODUCER 1 (ETO1) module, that GLABRA 2, a transcriptional factor, directly regulates the expression of *ETO1*, an ET production regulator, fine-tunes the expression of numerous genes involved in ET-dependent root hair growth[53].

4.4 Genes/loci involved N-regulated root system architecture in crops

Similar to that in *Arabidopsis*, several phytohormone-related components have been reported to regulate N-dependent modification of RSA in crops (Table 1). The interaction of OsNAR2.1, a partner protein of high-affinity nitrate transporter, and nitrilases, OsNIT1 and OsNIT2, which facilitate biosynthesis of the main auxin form indole-3-acetic acid, is necessary for both primary and lateral root growth in rice[54,55]. REGULATOR OF N-RESPONSIVE RSA ON CHROMOSOME 10 functions as a negative regulator for rice root response to N by stabilizing DULL NITROGEN RESPONSE1, a suppressor of auxin biosynthesis. Mutants of *rnr10* exhibit enhanced root response to N and grain field with different N treatment[56]. Both in rice and maize, auxin efflux proteins modulate root development by altering auxin distribution within root[85,86]. In maize, low N induced root elongation is regulated through multiple pathways. ARF19-activated expression of *AUXIN/INDOLE-3-ACETIC ACID14* inhibits root growth. Conversely, ZmNLP3.2 directly interacts with ARF19, attenuating this expression, thereby promoting root growth under low N conditions[57]. In addition, low-N induced auxin shoot-to-root translocation causes acidification of apoplast and alters rapamycin pathway, enhancing root growth[87]. During this process, induced expression of *ZmPIN1a* enhances auxin accumulation in primary root tip but reduces the accumulation in lateral root primordia. Consequently, the primary root length is increased and the lateral root initiation is inhibited[58]. As the homolog of

Arabidopsis TAR2 in wheat, TaTAR2.1 also modifies the lateral root growth by manipulating auxin biosynthesis. Overexpression of *TaTAR2.1* significantly promotes lateral root growth and grain yield under varying N conditions[59]. A low nitrate level elevates strigolactones (SL) content, which increases seminal root length but decreases lateral root density, mediated by the SL signaling component D3[60]. When nitrate is supplied, the key component in SL signaling pathway and the suppressor of the transcriptional activity of SPL14/17 (SQUAMOSA PROMOTER BINDING PROTEIN LIKE 14/17), D53 is degraded, releasing SPL14/17 to activate the expression of *OsPIN1b*, which enhances root elongation with sole nitrate supply[61]. MicroRNA444a targets and reduces the stability of mRNA of *OsMADS23*, *OsMADS27a*, *OsMADS27b*, and *OsMADS57*, the homologs of *ANR1*, thereby altering nitrate dependent RSA[62]. With sole ammonium supply, the mRNA stability of MADS-box transcription factors is interrupted by microRNA444, leading to the induced expression of a BR biosynthetic gene, *OsBRD1* (*BR-deficient dwarf 1*). The microRNA444-OsMADSs-OsBRD1 module causes overaccumulation of BR content, further inhibiting root growth with ammonium supply[63]. However, an understanding of the involvement of ammonium-induced microRNA444-OsMADS-OsBRD1 module in nitrate-regulated root growth remains elusive.

Given the complexity of root architecture, various factors are involved in regulation of root plasticity. Notably, the transcriptional factor N-mediated heading date 1 not only regulates flowering time, but also promotes root growth and N utilization by activating *OsNRT2.4* and *OsAMT1.3*[64]. A plastid-localized argininosuccinate lyase that converts glutamine to arginine, enables the alleviation of ammonium-inhibited root elongation[65]. Additionally, both mutants of nitrate transporter TaNPF2.12, bZIP transcriptional factor OsbZIP1, and ZmTGA in wheat, rice and maize, respectively, exhibit increased root length under low N condition[66–68]. The N-regulated root hair growth in rice is modulated by Cellulose synthase-like D1[69].

Differing from the cereals, legumes have symbiotic root nodulation, a process regulated by ambient nitrate level, as a pivotal strategy to enhance nitrogen acquisition, particularly under low N conditions[88]. In soybean, expression of CLE peptide coding genes, *NIC1a*/*b*, is directly induced by NLP1/NLP4 with high-nitrate supply. As a result, root nodulation was inhibited and nitrate uptake was enhanced[70]. Except for nitrogen fixation in root nodules, altered root architecture with increased primary root length, lateral root density, and thus increased root biomass is achieved by induced expression of *GmGLP20.4*, a germin-like protein coding gene, to cope with low nitrogen stress[71].

5 Prospects

In summary, the majority of studies elucidating the molecular mechanisms that govern N-dependent RSA have been undertaken in *Arabidopsis*. However, advancements in comprehending this process in crops, the primary consumers of nitrogen fertilizer worldwide, remain constrained. Only few loci that enables increase N use efficiency in the field have been verified[56,59,65]. One obstacle is the difficulty in collecting direct or non-destructive phenotypes of crop roots *in situ*, the subterranean component of plants. Fortunately, incorporation of X-ray computed tomography in plants research has facilitated the *in situ*, non-destructive collection of such phenotypes[89]. Coupled with high throughput phenotyping systems, this approach can provide precise root phenotype for genome-wide association study, similar to those performed to identify loci modifying N-dependent root growth in *Arabidopsis*[36]. Another challenge arises from the cell-type-specific functionality of certain genes in roots. When studying these genes using whole roots as the study unit, their effects can be diluted, which is common challenge for all life science studies. Single cell analysis, such as single cell sequencing, is a power technology to overcome this limitation, especially for precise understanding of local and systemic N signaling pathways at the cellular level. Through such analysis, certain biological processes, patterns of gene expression and gene function within distinct cells will be revealed. In agriculture, plants RSA is coordinately regulated by multiple soil sources and cultural management. Managements, such as relay strip intercropping (including maize/soybean, maize/peanut and tea/soybean/rapeseed intercropping) and straw incorporation, significantly bolster N use efficiency and yield of crops in the field, which largely benefits from altered N distribution and microbial communities in soil, and thus RSA[90–94]. Nevertheless, related researches mainly performed at physiology level, the molecular responses and genetic underpinnings of crop performance under different management regimes are largely unexplored. Therefore, it will be important to decipher the genetic basis of interaction of genotype, environment and management in order to develop improved RSA ideotypes for optimized crop performance.

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Compliance with ethics guidelines

Xiujie Liu, Kai Huang, and Chengcai Chu declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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