Trends in Plant Science

CellPress

Review

Nitrogen Systemic Signaling: From Symbiotic Nodulation to Root Acquisition

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Plant nutrient acquisition is tightly regulated by resource availability and metabolic needs, implying the existence of communication between roots and shoots to ensure their integration at the whole-plant level. Here, we focus on systemic signaling pathways controlling nitrogen (N) nutrition, achieved both by the root import of mineral N and, in legume plants, through atmospheric N fixation by symbiotic bacteria inside dedicated root nodules. We explore features conserved between systemic pathways repressing or enhancing symbiotic N fixation and the regulation of mineral N acquisition by roots, as well as their integration with other environmental factors, such as phosphate, light, and CO_2 availability.

Root–Shoot–Root Communication Is an Essential Circuit to Regulate Plant N Acquisition

Plant metabolism combines light-energy capture and carbon (C) fixation by photosynthetic shoots with water and nutrient acquisition by roots. These two organs are challenged by very different local environments, but need to cooperate to optimize nutrient supply and ensure plant growth. To do so, in addition to the local perception of different nutrition-related cues in the environment, dedicated systemic signaling networks integrating nutrient availability/needs with growth/developmental status operate at the whole-plant level [1]. Plant vascular tissues play an essential role in the long-distance communication between roots and shoots, with the directionality of nutrient and mobile signal exchanges achieved by xylem and phloem vessels [2]. Exhaustive analyses of xylem and phloem sap revealed, besides nutrients and metabolic products of their assimilation, a diversity of molecules ranging from hormones to small and long RNAs, signaling peptides, and proteins [3–7]. Understanding the role in systemic root and shoot regulation of signals moving within this complex sap composition, as well as their origins and targets, is a challenging but crucial objective to improve the efficiency of nutrient use and homeostasis at the whole-plant level.

Plant N acquisition by roots is achieved by the import of various mineral N sources from the soil as well as, in some specific plants such as legumes, by the fixation of atmospheric N_2 through a symbiotic interaction with soil bacteria, collectively referred to as rhizobia, in a dedicated root lateral organ, the nodule. Both root and nodule N acquisition are under the control of long-distance homeostatic signaling, whose original characterization at a physiological level is described in Box 1 for nodules and in Box 2 for roots. These regulatory pathways share similarities as they both aim to regulate the plant N nutrition from different sources (atmospheric N_2, soil mineral NO_3^-, or NH_4^+) depending on: (i) environmental N availability; and (ii) plant N needs and assimilation capacities that are notably driven by C metabolism. Symbiotic N_2 fixation in root nodules is a unique model to study this cost–benefit control of N acquisition by long-distance signals. Indeed, a new organ dedicated to N acquisition is formed, the nodule, that is nonessential when mineral N is available, in contrast to roots that have many other functions in addition to N nutrition. This specificity allowed efficient genetic screens to be performed to unravel the genetic basis of symbiotic root nodulation [8]. In this review, the systemic pathways regulating N fixing root

Highlights

Nitrogen (N) nutrition relies on root acquisition of mineral resources, as well as on symbiotic N_2 fixation by soil bacteria in legume plants.

Both processes are regulated by systemic signaling pathways aiming to adjust N acquisition depending on plant N needs and assimilation capacities.

Similar effectors acting in systemic signaling pathways are shared between these two N nutrition modes, including hormones such as cytokinins and peptide hormones, as well as related NIN-like protein (NLP) transcription factors.

Recent advances highlighted that systemic signaling pathways linked to N fixation and acquisition are tightly related to phosphate systemic signaling.

Light and/or CO_2 shoot environmental factors impact N systemic signaling, suggesting mechanisms allowing the integration of N and C signaling and metabolism.

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Box 1. Systemic Control of the N_2-Fixing Nodule Symbiosis in Legume Plants

Nodule organogenesis and maintenance are energetically costly; thus, legumes must tightly control their number and activity to balance nutrient gain and loss, thanks to a dedicated network of signaling pathways [119]. The first evidence of systemic control of nodulation, in the early 1950s, came from nodule excision, variety or mutant grafting, and split-root experiments in soybean, which highlighted that the host plant limits nodule numbers by integrating shoot and root cues and regulations [120–122]. This systemic negative regulation of nodulation was named AON [123], as it originally corresponded to a restriction of nodule number following a first wave of rhizobial infection events. Later, the observation that the nodulation of super/hypernodulating mutants was also NO_3^- resistant expanded the delineation of AON to nodulation-repressive conditions mediated by high NO_3^-, which for the plant is a cheaper source of N than N-fixing rhizobia [21]. More recent results showed that systemic AON restricts rhizobial infections, which is likely to be by impairing the perception of rhizobial signals [66,70,124]. N repression involves not only AON-dependent mechanisms, but also independent local and/or systemic pathways controlling nodule number and rhizobial infections as well as later nodulation stages, including N fixation and assimilation in nodules [38,125–127].

N limitation is a mandatory factor for the promotion of root nodule symbiosis. Split-root experiments showed that new nodule formation and nodule expansion, rather than an increase of N-fixation activity in existing nodules, which seems to be already at its maximum capacity, is systemically compensated in distal non-N-limited areas [126,128]. The stimulation of mature nodule metabolism and expansion would act through an independent pathway and rely on rapid reallocation of sucrose to sustain nodule metabolism along with the repression of nodule senescence and plant defense markers [129].

nodulation in legumes will be used as a core to intertwine them with the knowledge gained on N acquisition in roots of the non-nodulating reference plant arabidopsis (*Arabidopsis thaliana*). We successively explore: (i) mechanisms ensuring the homeostatic repression of N acquisition; (ii) mechanisms promoting N acquisition under low-N conditions where plant N needs are high; and (iii) how shoot environmental conditions and C metabolism capacities may impact N systemic signaling.

Repressing Root Nitrogen Fixation and Acquisition by Long-Distance Signaling

The autoregulation of nodulation (AON) homeostatic systemic signaling pathway restricts the number of N-fixing nodules that form on legume roots dependent on previous rhizobial infections and nodules (Box 1). In nodulated roots, AON signaling initiates with the production of specific small secreted peptides named CLAVATA3/EMBRYO-SURROUNDING REGION RELATED (CLE) peptides (Figure 1A). These 12-amino-acid-long peptides are conserved among legumes and their expression is induced by rhizobia and high mineral N availability. AON-related CLE

Box 2. Systemic Control of Nitrogen Acquisition in Roots

Plants can acquire N from a range of mineral sources, NO_3^- and NH_4^+, as well as organic compounds (e.g., amino acids) [130–132]. The acquisition of mineral N relies on root transporters belonging to three main gene families: NRT2s and NPFs/NRT1s for NO_3^- and AMTs for NH_4^+ [133,134]. Their characterization advanced our understanding of N acquisition regulation by environmental cues, including the fluctuating availability of the ions themselves [135,136]. Moreover, an intricate relationship was established with a role for some transporters in the control of root development independent of their transport activity [137–139]. N acquisition (i.e., root transporters and development) is tightly regulated by a network of local but also systemic signaling, integrating N external availability with global plant N needs, also in coordination with other nutrient resource pools [140,141].

Early work using split-root experiments in rice, corn, and barley revealed that a systemic compensation response to N deprivation exists in the distant root system comprising increased NO_3^- uptake and root development [142,143]. Going along with these physiological responses, NO_3^--supplied roots display a rapid increase in the expression of genes involved in NO_3^- transport and assimilation, observed in split-root experiments performed on either arabidopsis or medicago [60,127,144]. These physiological and molecular responses are controlled by systemic N-demand signaling that is likely to include several pathways related to the heterogeneous NO_3^- availability at the root-system level and/or to the NO_3^-/N-limited status of shoots [60,92,97,144–147]. Local NH_4^+ availability is not compensated by an increase of NH_4^+ uptake in arabidopsis [127,144] but rather by AMT1.3-dependent enhanced proliferation of lateral roots [138], indicating some specificity of the N systemic signaling with respect to the N source. However, all of these N acquisition components are similarly repressed by a systemic N supply [60,127,148], suggesting a lower specificity for the repressive mechanisms. For instance, amino acid supply represses N acquisition, and a role as a systemic inhibitory signal for N acquisition was thus proposed based on their ability to circulate inside plants [149,150].

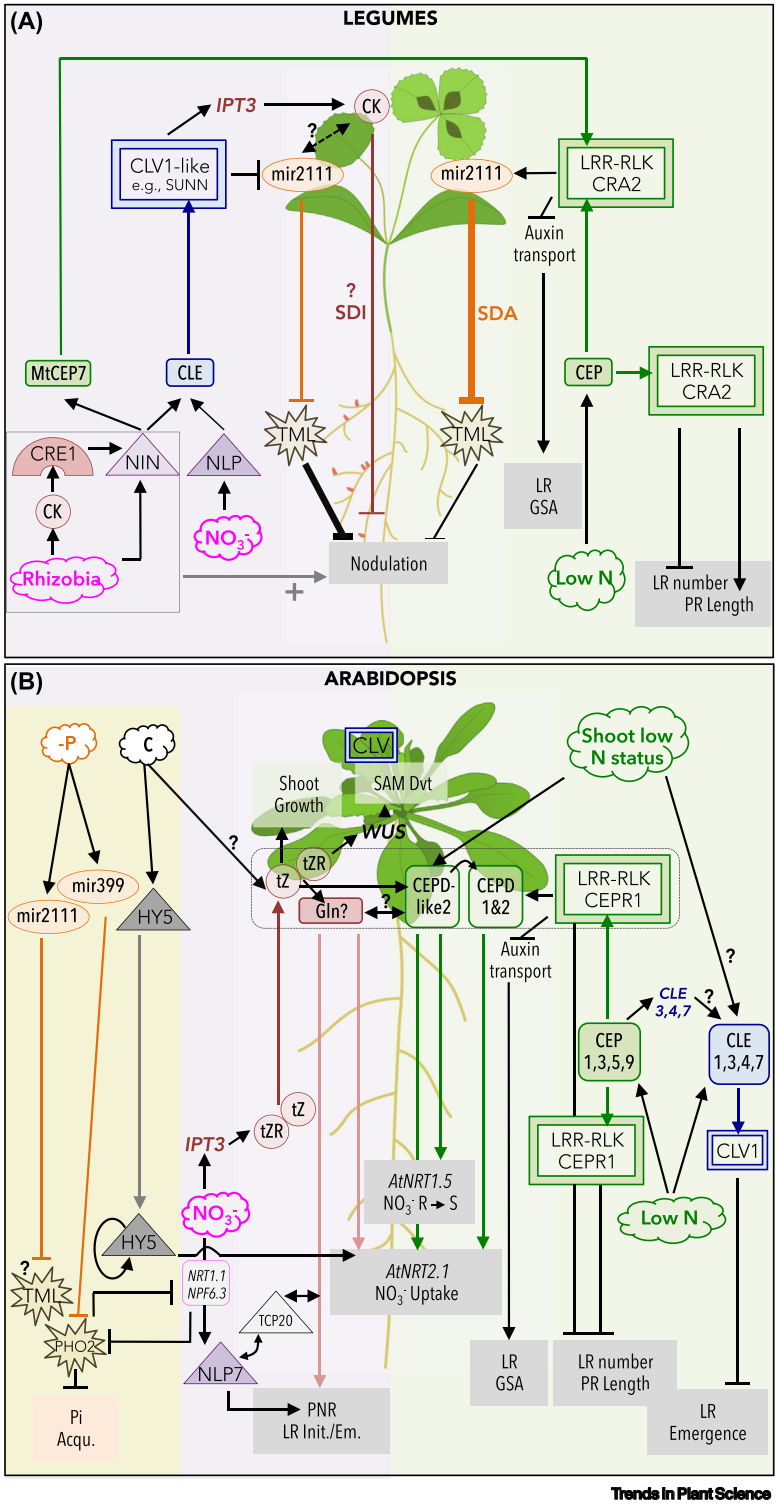

Figure 1. Regulation of Nitrogen Fixation and Acquisition by Systemic Signaling Pathways in Legumes and in Arabidopsis (*Arabidopsis thaliana*). (A) In legumes, CLAVATA3/EMBRYO-SURROUNDING REGION RELATED (CLE)-

(Figure legend continued at the bottom of the next page.)

peptides are encoded by the rhizobium-induced *MtCLE12*, *MtCLE13*, and *MtCLE35* genes in *Medicago truncatula* [9–11,160], the rhizobium-induced *LjCLE-Root Signal1* (*RS1*), *LjCLE-RS2*, and *LjCLE-RS3* in *Lotus japonicus* (the two latter genes being also induced by NO_3^-) [12,13], and the rhizobium-induced *Rhizobia-Induced CLE1* (*GmRIC1*) and *GmRIC2* genes and the NO_3^--induced *Nitrate-Induced CLE1* (*GmNIC1*) gene in soybean [14]. Peptide hormones' activity and/or stability frequently rely on post-translational modifications, and accordingly, some of these CLE peptides were reported to be hydroxyprolinated and tri-arabinosylated in roots, by the Root Determined Nodulation1 (MtRDN1) enzyme for MtCLE12 [15–17] and by LjPLENTY for LjCLE-RS2 and LjCLE-RS3 [18–20]. CLE peptides can be detected in the xylem sap and were thus proposed to be root-to-shoot mobile signals, perceived in shoots by the CLAVATA1-like (CLV1-like) leucine-rich repeat receptor-like kinases (LRR-RLKs) MtSUNN (SUper Numeric Nodules) [9,21], LjHAR1 (Hypernodulation and Aberrant Root 1) [19,22–25], and GmNARK (Nodule Autoregulation Receptor Kinase) [14,26] (Figure 1A). Direct binding of LjCLE-RS2 to LjHAR1 was demonstrated [19] and several co-receptors (LjKLAVIER, MtCLAVATA2, MtCORYNE) were shown to interact with MtSUNN/LjHAR1 [27–29].

dependent long-distance signaling represses N-fixing nodulation (blue arrow). Symbiotic rhizobial infection triggers this systemic signaling partially through the cytokinin (CK) signaling pathway involving the activity of the CK receptor CRE1 and the Nodule Inception (NIN) transcription factor that are also required for nodulation (+). Nitrate (NO_3^-) also triggers this CLE systemic signaling through the activity of NIN-like protein (NLP) transcription factors. CLE peptide signals are perceived in shoots by a CLAVATA1 (CLV1)-like receptor [leucine-rich repeat receptor-like kinases (LRR-RLKs); SUNN in *Medicago truncatula*]. This induces the downstream expression of *ISOPENTENYLTRANSFERASE 3* (*IPT3*) and thus CK biosynthesis, a putative nodulation 'shoot-derived inhibitor' (SDI) signal. In the *M. truncatula* legume, C-terminally encoded peptide (CEP)-dependent long-distance signaling promotes N-fixing nodulation under low-N conditions (green arrow). CEP peptide signals are perceived in shoots by a LRR-RLK receptor named Compact Root Architecture2 (CRA2). The MtCEP/MtCRA2 pathway also negatively regulates lateral root (LR) development locally in roots and promotes primary root (PR) growth. This pathway also systemically regulates the LR gravitropic set angle (GSA) by inhibiting shoot–root auxin transport. These CLE and CEP systemic signaling pathways respectively repress and induce the expression of the mobile miRNA miR2111, a nodulation 'shoot-derived activator' (SDA) signal that targets the accumulation of transcripts encoding an E3 ubiquitin ligase, Too Much Love (TML), inhibiting nodulation. The thickness of arrows and lines represents the strength of the signaling pathway. In addition, rhizobia promote through CK/CRE1 and NIN the expression of MtCEP7, promoting nodulation depending on MtCRA2, thus potentially allowing the switch from negative to positive regulation of nodulation depending on changes in N availability and plant N status. (B) N provision (left) and N deficiency (right) systemic signaling in arabidopsis. On the left (with the light-purple background), NO_3^- signaling requires the NRT1.1/NPF6.3 transceptor and NLP7 in roots. The Teosinte branched1/Cycloidea/Proliferating cell factor1-20 (TCP20) transcription factor interacts with NLP7 and intersects with N systemic signaling to control the primary nitrate response (PNR) and LR development. In roots, NO_3^- induces *IPT3* expression and thus the biosynthesis of CK precursor [*trans*-Zeatin riboside (*t*ZR)] and active [*trans*-Zeatin (tZ)] forms, followed by their translocation into shoots. Their perception in shoots promotes shoot growth (*t*Z) and shoot apical meristem development (SAM dvt) (*t*ZR), through induction of the expression of the transcription factor *WUSCHEL* (*WUS*), known to coordinate, with the CLAVATA (CLV) pathway, stem cell proliferation with differentiation. *t*Z and *t*ZR shoot integration controls shoot-to-root signaling enhancing PNR, LR development, and NO_3^- transport and modulating in shoots the expression of glutamine (Gln) metabolism, which may have a role in N systemic signaling. On the right (with the light-green background), low N triggers a CEP/LRR-RLK pathway similar to that in *M. truncatula*. The CEPR1 receptor homologous to MtCRA2 represses LR and PR development dependent on its activity in roots and in shoots. Repression of LR emergence is also controlled by a root CLE/CLV1 signaling pathway induced by low (shoot) N status and potentially also by CEP peptides. Low N availability also triggers in shoots the expression of class III glutaredoxins, named CEP-Downstream (CEPD) 1/2, acting downstream of the CEP/CEPR1 pathway, and of CEPD-like2 depending on shoot low-N status. These CEPD shoot-to-root mobile signals enhance the expression of the *AtNRT2.1* NO_3^- transport gene and thus NO_3^- uptake. CEPDL also enhances *AtNRT1.5* expression and thus NO_3^- root-to-shoot transport (R➔S). *t*Z/*t*ZR being required for *CEPD-like2* expression, the interaction in shoots between 'NO_3^-/CK' and 'CEP/CEPD/CEPD-like' systemic signaling may fine-tune N acquisition depending on root NO_3^- availability and N demand. At the extreme left (with the yellow background), the integration between phosphate (P/Pi) and carbon (C) signaling with the N systemic signaling network is depicted, via: (i) potentially the conserved miR2111/TML pathway, paralleling the miR399/PHO2 pathway known to control Pi acquisition (Pi acqu.) in relation to the regulation of NRT1.1/NPF6.3; (ii) the shoot-to-root Elongated Hypocotyl5 (HY5) transcription factor controlling its own expression, *AtNRT2.1*, and NO_3^- uptake; and (iii) possible direct or indirect regulation of the expression of these various shoot systemic effectors (arrow pointing to the gray-broken-line frame).

In arabidopsis, local developmental functions of CLE peptides in meristems are extensively described, but only a few links with nutrient and systemic regulations have been reported [30,31]. A role for the CLE–CLV1 signaling pathway in the regulation of lateral root development by N provision was, however, suggested [32]. Under N-limited conditions repressive for lateral root growth, the expression level of *CLE1*, *3*, *4*, and *7* is induced compared with N-supply conditions and the overexpression of these peptides represses the emergence of lateral root primordia in a CLV1-dependent manner [32]. These CLE peptides and their receptor being expressed in root pericycle cells and phloem companion cells, respectively, it was proposed that this signaling module may negatively regulate N acquisition locally in roots, and would be the target of an unknown systemic low-N signal (Figure 1B). Interestingly, in *L. japonicus*, the *har1* mutant, affected in the LRR-RLK most closely related to AtCLV1, also has a root architecture phenotype, comprising shorter roots and an increased number of lateral roots [22]. The existence of a root-to-shoot CLE-related signaling pathway regulating root architecture and N acquisition remains to be further explored in arabidopsis, as well as the function in shoots of the AtCLV1 receptor in relation to N-related CLE peptides.

In legumes, the induction of *CLE* gene expression by NO_3^- and rhizobia relies on transcription factors of the NODULE INCEPTION (NIN) family. In both *M. truncatula* and *L. japonicus*, the induction of *MtCLE13* and *LjCLE-RS1/2* expression by rhizobia depends on NIN, which binds to their promoters [10,33,34]. Interestingly, cytokinin (CK) hormones, which are essential for the establishment of nodules, activate NIN and MtCLE13 or LjCLE-RS1/2 expression [9,33,35–37] (Figure 1A). A subset of NIN-LIKE PROTEIN (NLP) similarly mediates the CLE-dependent NO_3^- inhibition of nodulation in both *L. japonicus* and *M. truncatula* [38,39,160] (Figure 1A). *Nitrate unResponsive Symbiosis 1* (*NRSYM1*)/*LjNLP4* binds the *LjCLE-RS2* promoter and activates its expression in response to NO_3^-, as well as the NITRITE REDUCTASE 1 (NIR1)-encoding gene involved in NO_3^- assimilation but not nodulation, highlighting shared symbiotic and nonsymbiotic root functions of this N/NLP/CLE module [38]. In *M. truncatula*, *MtNLP1* and *MtNLP4* redundantly accumulate in the nucleus in response to NO_3^- and interact with NIN to potentially inhibit its function, including hampering the activation of the CK receptor *MtCRE1* expression that is required for nodule initiation [39].

In arabidopsis, regulators of CLE peptide expression in response to N provision remain unknown. Given that AtNLP7 is an important hub for the NO_3^- root responses [40–43], its involvement may be speculated, although no CLE peptide whose expression is induced by NO_3^- provision has as yet been identified in arabidopsis. Among the hundreds of genes identified as NLP7 targets based on the combination of a ChIP approach with microarray transcriptomic analysis of *nlp7* mutants and on an inducible NLP7 variant used to identify stable and transient targets in isolated root cells by RNA-seq analysis, none corresponded to a CLE peptide [42,44]. It remains to be explored whether other NLPs could nevertheless regulate the expression of *CLE* genes linked to N signaling. In addition, several NLPs interact together via their Phox and Bem1 (PB1) domain, as shown for MtNIN and MtNLP1, or with other transcription factors, which may allow coordination of the root response to different N environments [45,46]. AtNLP7 notably interacts with Teosinte branched1/Cycloidea/Proliferating cell factor1-20 (AtTCP20) to regulate the response of NO_3^--responsive genes and root meristem growth depending on N availability [46]. Interestingly, AtTCP20 is a target in roots of N-demand systemic signaling [47], indicating that the AtNLP7/AtTCP20 transcriptional complex may integrate local and systemic N signaling pathways (Figure 1B).

Downstream of the AON-related CLE receptor activation in legume shoots, shoot-to-root systemic effectors inhibiting root nodulation have been identified. In *L. japonicus*, the expression

of the *ISOPENTENYLTRANSFERASE3* (*IPT3*) gene and accumulation of the intermediate forms of CKs [N^6-(Δ^2-isopentenyl) adenine riboside 5′-phosphates (iPRPs)] increase in shoots in response to rhizobium inoculation depending on the LjCLE-RS/LjHAR1 signaling pathway. Moreover, relatively high CK concentrations [6-benzylaminopurine (BAP) at 10^{-6} M] applied to shoots are transported through the phloem towards the roots to inhibit nodulation, independent of LjHAR1. Taking these findings together, it was hypothesized that CKs could be a shoot-to-root signal inhibiting root nodulation in addition to their local roles in roots, including as an activator of *CLE* gene expression [34,48,49] (Figure 1A). In soybean, a *LjIPT3* ortholog, *GmIPT5*, is also shoot induced by symbiotic conditions but independent of GmNARK, and CK application on either shoots or roots similarly promotes or inhibit nodulation, depending on the low [10^{-7} M BAP, iP, or *trans*-Zeatin (tZ)] or high (10^{-4} M BAP, iP, or tZ) concentration used, respectively [50]. Thus, the negative role of shoot CKs on root nodulation downstream of the AON pathway proposed in *L. japonicus*, implying a potential systemic action of CKs, may not be a general feature of AON in all legumes.

In arabidopsis, *AtIPT3* expression is triggered by NO_3^- [51]. In roots, this ultimately leads to the accumulation of the CK precursor *t*Z riboside (*t*ZR) and of the biologically active *t*Z, which are then translocated to shoots by the ABCG14 transporter [52–54]. Both *t*ZR and *t*Z act as systemic signals to promote shoot growth in response to NO_3^- [55–58]. The shoot apical meristem fate is likely to be modulated through the action of *t*ZR on *WUSCHEL* expression [56,57] (Figure 1B), *WUSCHEL* being directly activated by a subset of type B authentic response regulators (see [59] for an overview). Moreover, in split-root experiments mimicking NO_3^- heterogeneity, the integration in shoots of *t*Z/*t*ZR transported from roots is also required to enhance lateral root development and NO_3^- transport in NO_3^--supplied roots, thus compensating for the partial N deficiency [60–62] (Figure 1B). As shown in legumes, CKs are proposed to act at several levels of N signaling since they also regulate locally the NO_3^--dependent primary root growth [63], lateral root development, and NO_3^- transport [64,65].

In legumes, another shoot-to-root signal conserved between *L. japonicus* and *M. truncatula* was more recently proposed, corresponding to the miRNA miR2111, whose expression is shoot specific and repressed by rhizobium inoculation, dependent on LjHAR1/MtSUNN [66,67] (Figure 1A). This miRNA regulates post-transcriptionally the accumulation of *Too Much Love* (*TML*) genes (*LjTML* in *L. japonicus* [66,68,69] and *MtTML1*/*MtTML2* in *M. truncatula* [67,70]), which encode nuclear E3 ubiquitin ligases acting in roots to inhibit nodulation. Although TML targets remain unknown, one possibility is that they regulate the stability of symbiotic regulators, and notably of NIN, which is a homeostatic target of the NIN/CLE/AON pathway [33]. Overall, whereas the initial AON model suggested a nodulation 'shoot-derived inhibitor' (SDI) signal, as could be CKs in *L. japonicus* (see earlier), the miR2111 shoot-to-root systemic effector behaves as a nodulation 'shoot-derived activator' (SDA) signal that is repressed by the AON pathway (Figure 1A).

In arabidopsis, the miR2111/TML regulatory module is evolutionarily conserved, suggesting possible recruitment in the regulation of root responses to N availability beyond nodulation. One of the two TML homologs (At3g27150) encoding an uncharacterized E3 ubiquitin ligase is targeted by miR2111, which was detected in arabidopsis phloem sap in agreement with a function as a systemic shoot-to-root effector [71]. However, miR2111 accumulation is induced under phosphate (Pi)-limited conditions and not by N limitation [71], in contrast to legumes (Figure 1B). Interestingly, other F-box proteins that are also regulated by systemic miRNAs control nutrient homeostasis depending on the combined N and P availability. The E3 ubiquitin ligase NITROGEN LIMITATION ADAPTATION (NLA) and the E2 ubiquitin conjugase PHOSPHATE2 (PHO2)

are targeted by the shoot-to-root mobile miR827 and miR399 miRNAs, respectively, under Pi-limiting conditions, to inhibit their activity and to favor Pi acquisition [72–74]. Furthermore, the phosphate starvation response (PSR) is conditional to NO_3^- provision [75–77], depending on the reciprocal regulation of the transceptor NPF6.3/NRT1.1 and of PHO2 [75] (Figure 1B). This is achieved by local but also systemic N-related signals suggesting, that they are both mandatory to forage for P acquisition [75].

Promoting Root Nitrogen Acquisition and Fixation by Long-Distance Signaling

As a mirror of the negative AON systemic pathway, a positive systemic pathway promoting nodulation was more recently evidenced in *M. truncatula*, involving another class of peptide hormone, from the C-terminally encoded peptide (CEP) family, whose expression is generally enhanced in N-deficient roots [78] (Figure 1A). CEPs increase the number of nodules formed [78] through a systemic pathway involving the activity in shoots of the Compact Root Architecture2 (MtCRA2) LRR-RLK receptor [79,80]. Under N-deficiency conditions, this systemic MtCEP/MtCRA2 pathway upregulates in shoots the expression of the miR2111 shoot-to-root systemic effector, promoting in roots the cleavage of *MtTML* transcripts that encode an inhibitor of nodulation (see above) [67]. The MtCEP/MtCRA2 pathway thus actively maintains roots competent for nodulation under N-deficiency conditions (Figure 1A). Interestingly, the previously described rhizobium- and N-induced MtSUNN AON pathway and the MtCRA2 pathway act independently [81], but modulate antagonistically the same miR2111/TML regulatory module, which is thus a central hub to regulate dynamically nodule number depending on environmental N availability and on the plant N metabolic status [67]. The promotion of nodulation exerted by the MtCEP/MtCRA2 pathway also involves the inhibition of the ethylene signaling pathway mediated by SICKLE (SKL)/Ethylene Insensitive 2 (EIN2), which inhibits rhizobial infections potentially though an interaction between MtCRA2 and MtSKL/EIN2 [80,82,83].

Interestingly, the MtCEP/MtCRA2 pathway also regulates locally root system architecture, inhibiting lateral root development and being required for primary root growth depending on a YUCCA-dependent local auxin biosynthesis pathway [78,79,81,83,84] (Figure 1A). *cra2* mutant roots perceive changes in the N environment but have altered responses to these conditions [83]. Instead of repressing root growth and increasing lateral root density, NO_3^- promotes *cra2* mutant root growth and represses its lateral root density. In addition, this pathway regulates systemically from the shoots the gravitropic set-point angle of lateral roots by limiting shoot auxin transport to roots, ultimately allowing a wider foraging area for the root system [85] (Figure 1A).

In arabidopsis, the first CEP peptide, AtCEP1, was identified *in silico* and then by liquid chromatography–mass spectrometry (LC-MS) as a 15-amino-acid-long hydroxyproline peptide [86]. A subset of AtCEP peptides, including notably AtCEP3, 5, and 9, accumulates in N-deficient roots and inhibits primary root growth (AtCEP3 and 5) through a reduction of meristematic cell number and of the size of the meristem, as well as the emergence of lateral roots (AtCEP3 and 5) and/or the gravitropic set-point angle of lateral roots (AtCEP3) [85,87–91] (Figure 1B). In addition, AtCEP1 upregulates systemically the expression of the *AtNRT2.1* high-affinity NO_3^- transporter depending on the LRR-RLKs CEP receptor 1 and 2 (AtCEPR1/AtCEPR2) [92], closely related to MtCRA2 in *M. truncatula*, although such a functional link was not established in the latter plant (Figure 1B). It is noteworthy that the binding of the AtCEP1 peptide to these two CEPR receptors was demonstrated [92]. A shoot-to-root systemic signal upregulating *AtNRT2.1* expression in roots downstream of the AtCEP/CEPR1 pathway was identified as belonging to the class III glutaredoxin family, and so-called CEP Downstream1 (AtCEPD1) and AtCEPD2. Split-root experiments revealed that these shoot-produced AtCEPDs are translocated

through the phloem in the root system to upregulate *AtNRT2.1* expression only in roots locally supplied with NO_3^-, through a still unknown mechanism implying an interaction with local N conditions [93] (Figure 1B). Interestingly, it was previously shown that the class III glutaredoxins AtGRX3/4/5/8, homologous to CEPDs, are regulated by NO_3^- and CK, and negatively control primary root growth [94], suggesting a complex involvement of GRXs in the NO_3^--dependent root plasticity. Overall, the CEP/CEPR/CEPD pathway is a root-shoot-root circuit allowing plants to adapt to heterogeneous NO_3^- supply in soils.

In medicago, CEPD homologs were recently identified to be upregulated by N deficiency conditions depending on the MtCRA2 receptor, both in shoots and in roots, in agreement with the role of MtCRA2 in shoots and roots to regulate nodule or lateral root development, respectively [67,79,81], but it remains to be established whether *MtNRT2.1* homologs as well as nodulation are targeted by a MtCRA2/MtCEPD pathway. Conversely, a relationship between the arabidopsis CEP/CEPR pathway and the conserved miR2111/TML-like module remains to be explored.

Finally, in arabidopsis, another member of the class III GRX family expressed predominantly in shoots under N deficiency, and denominated AtCEDPlike2 (AtCEPDL2), was recently shown to regulate root NO_3^- uptake and root-to-shoot transport [95] (Figure 1B). AtCEPDL2 acts independent of AtCEPR1/2 but complements *cepd1/2* mutations for NO_3^- uptake [95]. Interestingly, the maximal induction of *AtCEPDL2* expression in shoots requires *t*Z accumulation [95] (Figure 1B). As described in the previous section, *t*Z/*t*ZR is a N-supply root-borne signal whose translocation in shoots has a positive effect on NO_3^- uptake, including on *AtNRT2.1* upregulation, and on lateral root growth [61]. Together, these results show that multiple systemic signals coexist to positively regulate NO_3^- uptake in plants. This would allow the integration of local N-deprivation pathways (i.e., CEP/CEPR/CEPD) with global N-deprivation pathways (i.e., CEPDL) and local NO_3^- supply (i.e., *t*Z/*t*ZR) to finely modulate N acquisition in NO_3^--heterogeneous environments (Figure 1B).

Influence of Shoot Environment on N-Related Long-Distance Signaling

The systemic regulation of root N acquisition and fixation relies on the perception of root-borne signals modulated by local environmental soil conditions and by their integration in shoots. This suggests that N acquisition may be modulated depending on the nutrient status of shoots; that is, the N status but also C resources that can be provided to sustain root and nodule

Box 3. Differential Plant N Acquisition Efficiencies in Response to Elevated CO_2

The Anthropocene era is synonymous with increased greenhouse gas emissions. In the 1750–2011 period, CO_2 concentrations increased from 280 to 380 ppm, with a dramatic acceleration of emissions in the past 40 years (+2 ppm/year). The consequences of associated climate changes on plant responses have been evaluated during these years, notably using the Free-Air CO_2 Enrichment (FACE) system that allows the effects of a CO_2-enriched atmosphere to be simulated at a whole-ecosystem scale and for several years [151]. Increasing CO_2 concentrations in the atmosphere are expected to promote plant biomass and yield due to a 'CO_2 fertilization effect' that increases the photosynthesis rate, but several studies have also revealed a limited beneficial effect in the long term in some plants, such as C3 grains (wheat, rice) and legumes (field pea, soybean), leading to lower yields, protein content, and even nutritional food quality, because of CO_2/photosynthesis acclimation [151–153]. Whether elevated CO_2 consequences for plant growth and protein content also differ depending on the N inorganic form remains under debate [154,155]. Some studies point to inhibition of photorespiration and consequently of NO_3^- assimilation [156,157], whereas other studies report no differences in N assimilation and growth in C3 plants supplied with NO_3^- or NH_4^+ [158].

CO_2/photosynthesis acclimation also strongly relies on the strength of the sink organ's ability to consume photosynthesis C products, since reduced sink strength negatively feeds back on photosynthesis [151]. The N_2-fixing symbiosis is particularly interesting in the context of a CO_2-enriched atmosphere since nodules are strong C sinks and since the nodule N_2 fixation capacity depends on C allocation. Recent studies suggest that nodule N_2 fixation activity may avoid the negative feedback on photosynthesis provoked by elevated-CO_2 conditions [159]. In this way, symbiotic N-fixing legumes are anticipated to achieve higher yields and biomass productivity under elevated CO_2, a condition that will be the future agricultural context to consider.

development as well as N assimilation, and thus by plant C fixation capacities and C allocation to roots. Aboveground atmospheric environmental conditions, including CO_2 levels as well as light quality and quantity, are then anticipated to impact systemic signaling pathways targeting N acquisition. The increase of atmospheric CO_2 concentrations associated with global climate change has various consequences depending on plant species and their N acquisition mode, mineral N versus symbiotic acquisition (Box 3).

Data indicating that C provision is likely to impacts N systemic signaling effectors and pathways regulating N acquisition have recently emerged. In *M. truncatula*, in addition to their transcriptional upregulation in response to low NO_3^-, the expression of most *CEP* genes is cumulatively induced by low N and high CO_2 (800 ppm) conditions [78]. In arabidopsis, the root architecture phenotype of the *cep3* mutant is observed not only under low-N but also under low-light conditions [87]. In addition, a subset of *AtCEP* genes (*AtCEP5–AtCEP9*) is upregulated by treatment with metabolizable sugars, such as sucrose, and AtCEP5 restricts the promotion of lateral root growth in response to sucrose or high light depending on AtCEPR1 activity in both roots and shoots [96]. This suggests that the arabidopsis CEP/CEPR1 pathway, and thus likely, by analogy, the *M. truncatula* CEP/CRA2 pathway, integrates N and C signals to modulate root system architecture and growth (Figure 1B). In addition, the role of the AtCEPR1/MtCRA2 pathway in the regulation by C provision of root NO_3^- transport and assimilation should be investigated. Photosynthesis products stimulate NO_3^- uptake and NO_3^- transporter gene expression [97,98]. To date, the regulation of *AtNRT2.1* and *AtNPF6.3/NRT1.1* is linked to the oxidative pentose phosphate pathway (OPPP) [99,100]. In addition, glucose itself promotes NRT2.1 protein accumulation [100]. Interestingly, redox metabolism, including the OPPP output, may regulate NO_3^- transport depending on N and C signaling (see [101] for a review). On this line, the CEPD/CEPDL shoot-born mobile signals, being class III GRXs (ROXY6–9), may thus participate in such redox integration of C and N regulation, although the role of these small proteins as sensors or modulators of the redox state still needs to be investigated [102].

In arabidopsis, a systemic shoot-to-root function of the transcription factor Elongated Hypocotyl5 (AtHY5) was also proposed to control N acquisition and shoot/root development depending on the C status of shoots [103]. AtHY5 was initially described as a positive regulator of photomorphogenesis, modulating hypocotyl growth depending on light availability, but was also linked to various aspects of root development and to redox regulation [104–108]. Recently, AtHY5 was shown to upregulate the expression of sucrose biosynthesis and efflux genes in light-grown shoots (*AtTPS1* for trehalose-6-phosphate synthase; *SWEET11* and *SWEET12*) [103]. In addition, AtHY5 proteins were shown to be translocated systemically from shoots to roots through the phloem and to bind in roots the *AtNRT2.1* promoter to enhance its expression and root NO_3^- uptake [103] (Figure 1B). Interestingly, in *L. japonicus*, LjHY5/BZip ring finger (LjBzf) inhibits nodulation but not lateral root development [109], although it is unknown whether this occurs locally and/or systemically.

Finally, hormones are anticipated to mediate the integration of shoot local environmental C-related signals with N systemic signaling. For instance, CK is a positive regulator of chlorophyll synthesis as well as of chloroplast development [110,111] and might potentially favor C allocation to roots and thus N acquisition. Conversely in arabidopsis, high-CO_2 treatment, or treatment with sucrose or glucose, induces in roots the expression of the *IPT3*, *CYP735A2*, and *ABCG14* genes encoding CK biosynthesis enzymes and a root-to-shoot CK transporter, leading to CK accumulation in roots but also in shoots, and to the promotion of shoot growth [112]. By promoting CK biosynthesis, elevated CO_2 may thus modulate CK-dependent systemic signaling to enhance NO_3^- acquisition in roots [61,95] (Figure 1B).

In arabidopsis shoots, an overrepresented number of genes encoding enzymes involved in glutamine metabolism show differential expression depending on NO_3^- availability, and this regulation relies on *t*Z/*t*ZR translocated from roots [61]. The role of amino acids as a nutrient status reporter at the whole-plant level, and in particular of glutamine, which is the first amino acid issued from C and N assimilation, was a longstanding hypothesis that could however never be validated experimentally [113]. By contrast, glutamine, whose accumulation level may also rely on photosynthetic activity, is also known to influence CK biosynthesis [112,114]. Together, this highlights that a reciprocal CK–glutamine interaction in shoots may be a potential hub in systemic signaling to control root responses and integrate C and N responses.

Concluding Remarks and Future Perspectives

In legumes, a mechanistic framework for the N-related systemic control of nodulation has recently emerged involving antagonistic CEP/CRA2 and CLE/SUNN–HAR1 pathways differentially activated depending on N availability and/or on the whole-plant N status to regulate nodulation, and likely more globally, root system architecture and N acquisition. This new knowledge allowed a reconsideration of the historical AON model, which is activated not only by previously established nodules but also by NO_3^-, indicating that rhizobium is considered as an N source by the plant. In addition, the initially proposed shoot-to-root systemic signal inhibiting nodulation, which may involve CKs in some legume plants, also comprises the repression of a positive regulator of nodulation produced only in shoots, the miR2111 miRNA. Strikingly, the production of this miRNA is actively maintained under low-N and/or N-deficit conditions by the CEP/CRA2 pathway (Figure 1A). In addition to these two antagonistic systemic pathways regulating rhizobial infections at the onset of nodule initiation, AON-independent systemic signaling pathways also exist to regulate later nodulation stages including N fixation and assimilation (Box 1).

Whether through N_2 fixation in symbiotic nodules of legume plants or NO_3^- import from the soil at the root surface of all plants, N acquisition is constantly modulated by a combination of local and long-distance systemic signals. The parallel between the legume and arabidopsis knowledge that is at the basis of this review allows us to highlight similarities and overlaps between N-related systemic pathways regulating N-fixing nodules and root N acquisition. The respective roles of the miR2111, CEPD/CEPDL, CK, and HY5 shoot-to-root signals remain, however, to be better analyzed in parallel between arabidopsis and legume plants, as well as the potential role of NLP transcription factors to regulate CLE and/or CEP signaling peptides related to N systemic pathways (see Outstanding Questions). In addition, functional differences that exist between arabidopsis and legume orthologous genes linked to N systemic pathways deserve to be explored. For example, the CEP peptide regulation of root development occurs both systemically from shoots and locally in roots in arabidopsis, whereas it acts only locally in *M. truncatula* roots [80,96]. Another example is the LjHAR1/MtSUNN and AtCLV1 receptors that, despite being the most closely related proteins, potentially diverged regarding their role in shoot and floral meristems versus N-related systemic signaling [115].

Mechanisms regulating inorganic N acquisition exist in all plants and are thus expected to be conserved in legumes, including the regulation of NO_3^- transport (Box 2). The acquisition of nodulation, implying the co-option of local and systemic N- (or P-) related regulatory circuits, thus required the integration of N-fixing nodules and root N acquisition regulatory pathways. This also ensures the robustness of repressive mechanisms against the detrimental effects of exaggerated root and nodule development resulting from C over-allocation, as exemplified by the phenotypes of peptide receptor mutants; either 'supernodulating', as *sunn/har1*, or 'superrooting', as *cra2* (see Outstanding Questions).

Outstanding Questions

What are the respective roles and relevance of evolutionarily conserved regulatory modules, including the miR2111, CEPDs/CEPDLs, CK, and HY5 mobile signals, in different plants? Combined evolutionary and functional approaches are needed to understand the origin of these systemic signaling pathways and how they have evolved in different plants to ensure robust regulation of the different N acquisition modes.

What are the roles and target overlaps of N-related NLP transcription factors, notably regarding the regulation of CLE and/or CEP signaling peptides?

The coexistence of various systemic signals, such as peptides and hormones, questions how these molecular effectors interact and impact N systemic signaling.

How are the various roles of different types of CKs, locally in shoots versus roots, and systemically, decoded depending on the N availability/whole-plant N status?

How are low-N/N-deficiency signals and high-N/N-satiety signals, as well as local and systemic regulation, integrated?

How are the multiplicity of N-availability signals that plants continuously perceive in different parts of their bodies together with the whole-plant metabolic status integrated, as well as in relation to other nutrients than N (e.g., C, P)? How are shoot systemic effectors (miR2111, CEPDs/CEPDLs, CKs, etc.) regulated by these other nutrient inputs? Such questions will require the use of integrated analyses.

Some systemic signals, such as CK, are known to regulate vascular system development. This raises the possibility that systemic signals may by themselves modulate vascular channeling and thus long-distance communication.

This review highlights in addition that we know little about the interactions between these different systemic signaling pathways and how they integrate the multiplicity of signals that plants continuously perceive in their various organs (e.g., rhizobium, N limitation, NO_3^- provision in roots) (see Outstanding Questions). For example, in arabidopsis the study of the potential interactions between the CK (*tZ*/*tZR*) and CEPD/CEPDL pathways in shoots remains in its infancy (Figure 1B). Interestingly, results obtained on the antagonistic regulation of the miR2111 by CRA2 and SUNN illustrate the integration of the balance between permissive and repressive nodulation states according to N provision (i.e., N limitation and rhizobium) [67] (Figure 1A). On the same line, a specific *M. truncatula CEP* gene, *MtCEP7*, systemically promoting nodulation through the MtCRA2 receptor was recently shown to be induced by rhizobium and CKs, depending on the MtCRE1 CK receptor and on the MtNIN transcription factor [34] (Figure 1A). Interestingly, MtNIN binds both the *MtCLE13* and *MtCEP7* promoters and induces their expression through NIN-binding sites required for their activation in response to rhizobium [33,34]. MtNIN thus coordinates the regulation of specific CLE and CEP peptides from two different families antagonistically regulating nodulation, potentially to allow switching from negative to positive regulation of nodulation depending on changes in N availability and of the plant N status [34]. In addition to the coregulation of specific *CLE* and *CEP* genes by a single transcription factor, these peptide hormones may also regulate each other's expression. In arabidopsis, AtCEP3 upregulates the expression of *AtCLE3*, *4*, and *7*, suggesting that the AtCEP3/AtCEPR1-dependent low-N response may promote the low-N-dependent AtCLV1/CLE signaling pathway [88] (Figure 1B). Finally, different types of CKs have various roles in shoots versus roots as well as locally versus systemically, but how these specificities are decoded depending on the N availability/whole-plant N status remains to be explored.

The integration of local and systemic signaling associated with the heterogeneous distribution of nutrients other than N is also likely to be critical (see Outstanding Questions). Herein, we covered interactions between C availability and N systemic signaling, but other nutrients also have an influence. A role of N in Pi-limitation signaling has already been reported [75–77] and one can anticipate a large integration between these two major nutrient signaling pathways. Interestingly, the low-Pi regulation of nodulation and of the arbuscular endomycorrhizal symbiosis also requires the CLE/SUNN systemic pathway, thus mirroring the AON pathway and so-called autoregulation of mycorrhization (AOM) [116–118]. The integration of these different systemic pathways ensuring integrated plant nutrition through the root acquisition of various mineral nutrients and through mutualistic symbiosis establishment is key to understanding how plants adapt to fluctuating and heterogeneous environments (see Outstanding Questions).

Acknowledgments

We thank Ambre Miassod (IPS2) for plant illustrations used in Figure 1 and Pascal Ratet (IPS2) and Benjamin Péret (B&PMP) for critical reading of the manuscript. P.G., C.L., and F.F.'s work is supported by the Agence Nationale de la Recherche (ANR) PSYCHE project and 'Saclay Plant Sciences' (SPS) Graduate School of Research. P.G. received a PhD fellowship from the University of Paris-Saclay. S.R.'s work is supported by the National Science Foundation (IOS 1339362-NutriNet).

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