Plant Science 274 (2018) 320–331

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review article

Roles of nitrogen and cytokinin signals in root and shoot communications in maximizing of plant productivity and their agronomic applications

Junfei Gu[a], Zhikang Li[a], Yiqi Mao[a], Paul C. Struik[b], Hao Zhang[a], Lijun Liu[a], Zhiqin Wang[a], Jianchang Yang[a,*]

[a] *Jiangsu Key Laboratory of Crop Genetics and Physiology / Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China*

[b] *Centre for Crop Systems Analysis, Department of Plant Science, Wageningen University, PO Box 430, Wageningen, 6700 AK, The Netherlands*

ARTICLE INFO

Keywords:
Cytokinin transport
Long-distance signaling
Nitrogen status
Root - shoot relationship
Source-sink relationship

ABSTRACT

Nitrogen is an essential, often limiting, factor in plant growth and development. To regulate growth under limited nitrogen supply, plants sense the internal and external nitrogen status, and coordinate various metabolic processes and developmental programs accordingly. This coordination requires the transmission of various signaling molecules that move across the entire plant. Cytokinins, phytohormones derived from adenine and synthesized in various parts of the plant, are considered major local and long-distance messengers. Cytokinin metabolism and signaling are closely associated with nitrogen availability. They are systemically transported via the vasculature from plant roots to shoots, and vice versa, thereby coordinating shoot and root development. Tight linkage exists between the nitrogen signaling network and cytokinins during diverse developmental and physiological processes. However, the cytokinin-nitrogen interactions and the communication systems involved in sensing rhizospheric nitrogen status and in regulating canopy development remain obscure. We review current knowledge on cytokinin biosynthesis, transport and signaling, nitrogen acquisition, metabolism and signaling, and their interactive roles in regulating root-shoot morphological and physiological characteristics. We also discuss the role of spatio-temporal regulation of cytokinins in enhancing beneficial crop traits of yield and nitrogen use efficiency.

1. Introduction

Plant productivity relies heavily on nitrogen fertilization. Nitrogen is usually taken up by plants in forms of nitrate and ammonia in aerobic soil and water-logged soil, respectively. Amino acids are abundant in some soils, and plants have been found to possess the ability to take up amino acid [1–4]. Uptake of amino acids and peptides is of interest in specific conditions, but is insignificant in agricultural systems. Under field environments, the quantity and accessibility of nitrogen in the rhizosphere varies, triggering systemic signals that shape plant growth and development [5]. Thus, plants adapt their morphology and physiology to the soil nutrient status. These morphological and physiological adaptations depend on coordination of metabolism at the whole plant level, especially in terms of root-shoot-root long-distance signaling [6–8]. Recent studies suggest that nitrogen signals are partly dependent on cytokinins, which act both as a local and as long-distance messengers [6,9,10]. Cytokinins regulate the proliferation and differentiation of plant cells. They have pleiotropic effects on various growth and development processes, for example by affecting cell division and differentiation, apical dominance, senescence, grain number, root nodulation, transmission of nutritional signals and nutrient homeostasis [11–13]. However, the communication and interaction systems between cytokinin and nitrogen involved in sensing rhizospheric nitrogen status and regulating root and canopy development are not clear [14]. In this review, we summarize cytokinin biosynthesis, transport, and signaling, nitrogen acquisition and metabolism, and their interactive roles in regulating root - shoot morphology and physiology. We also discuss possible agronomic strategies aimed at improving the efficiency of nitrogen acquisition and utilization in the context of interactions with the cytokinin regulatory circuitry.

2. Nitrogen acquisition, metabolism and signaling

Nitrogen is an important limiting factor of plant performance. Under changing nitrogen supply, plants elaborate physiological and morphological responses aiming at adjusting growth and development.

---

[*] Corresponding author at: Jiangsu Key Laboratory of Crop Genetics and Physiology / Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China.

E-mail address: jcyang@yzu.edu.cn (J. Yang).

https://doi.org/10.1016/j.plantsci.2018.06.010
Received 31 January 2018; Received in revised form 13 June 2018; Accepted 13 June 2018

Plant roots possess uptake systems for both nitrate and ammonium, with different transporter affinities suited to the dynamic and heterogeneity variations with different nitrate and ammonium levels [5,15]. Nitrogen transporters are classified into two groups, NITRATE TRANSPORTERS (NRTs) and AMMONIUM TRANSPORTERS / METHYLAMMONIUM PERMEASES (AMTs / MEPs) [1]. The nitrate transporters are further divided into four classes: the nitrate transporter 1 family (NRT1 / NPF), nitrate transporter 2 family (NRT2 / NAR2), CHLORIDE CHANNEL FAMILY (CLC), and SLOW ACTION ANION CHANNEL-ASSOCIATED HOMOLOGUES (SLAC / SLAH) [16–18]. Most NRT1s have low affinity for nitrate, except for NRT1.1 (CHL1 / NPF6.3), a dual-affinity transporter [16,19,20], which functions as a transceptor (transporter and receptor) of nitrate and even amino acids [17,20], regulating lateral root emergence under nitrate shortage [16,18,21,22]. In contrast, the affinity of all AtNRT2s is high, but they only transport nitrate. In most higher plants, NRT2s function in transportation of nitrates in the presence of a partner, NAR2.1 (NRT3.1) [17,23,24]. For example, in rice (*Oryza sativa*), OsNRT2 family genes (*OsNRT2.1*, *OsNRT2.2*, *OsNRT2.3a*, and *OsNRT2.5*) partner with *OsNAR2.1*, regulating nitrate uptake at varying concentrations [5,25–27]. Meanwhile, NITRATE TRANSPORTER1/PEPTIDE TRANSPORTER 3.1 (NPF3.1 / Nitr), localized in the plasma membrane, transports nitrite and nitrate in *Arabidopsis* [17,28,29]. Interestingly, these transporters (e.g. NRT2.1, NRT2.2, NPF2.4, and NRT1.1b) are mainly expressed in the root epidermis and emerged lateral roots, tissues involved in nitrogen sensing, suggesting roles in sensing external nitrate signals. Meanwhile, CLCs consist of two related protein families, CLCa and CLCb, and are mostly expressed in vacuoles and the tonoplast. While CLCa can transport nitrate, stimulating nitrate accumulation in the vacuoles [30], nitrate loading and transporting activity in the tonoplast remains unclear [17,31]. The anion channels of the SLAC / SLAH family (including SLAC1, SLAH3, and SLAH2) also exhibit a preference for nitrate [32–35].

Plants also possess a meticulous system for ammonium uptake and transportation, with AMTs / MEPs playing an important role. The AtAMT family consists of six members, while the OsAMT family includes at least 10 members grouped into the OsAMT1 and OsAMT2 subfamily [36,37]. AtAMT1;1, AtAMT1;2, AtAMT1;3, and AtAMT2;1 are strongly expressed in the roots and can induce ammonium-dependent lateral roots [38,39]; however, while AtAMT1;1 is a known transceptor of ammonium, AtAMT1;1-dependent downstream ammonium signaling remains unclear [40]. Meanwhile, the mechanisms of MEP2, another member of the AMT family, have been extensively studied; MEP2 is thought to be transmitted via activation of a protein kinase A (KPA)-related signaling system [41,42]. In rice, OsAMT1;1 is strongly expressed in the root epidermis and stele, playing an important role in ammonium uptake. The activity of OsAMT1;1 not only contributes to approximately 25% of total ammonium uptake from the soil, but is also important in acropetal ammonium transport [43]. For amino acid transporters, AAP1 [44], AAP5 [45], LHT1 [46] and ProT2 [47] have been identified as crucial components of amino acid root uptake in *Arabidopsis*.

Initial nitrate uptake involves NRTs in the roots, followed by transport to the shoot. In shoot, it is first reduced to nitrite by nitrate reductase (NR) in the cytoplasm, then to ammonium by nitrite reductase (NIR) in the plastids and glutamine synthetase (GS) in the plastids and cytoplasm. In the roots, a small amount of the absorbed nitrate is reduced to ammonium; most is directly transported to the shoot by NRTs [5,18,48]. While for nitrogen-fixing plants, the ammonium is excreted by *Rhizobium* bacteroids into the host cell cytoplasm, where it is assimilated and used in the synthesis of organic N and then transported to the shoot through the xylem sap [49]. Initial production of most ammonium assimilation involves amino acids via the GS / glutamine-2-oxoglutarate amino transferase (GOGAT) cycle [18]. Thus, as expected, the majority of nitrate-specific response genes are involved in nitrate metabolism and synthesis of amino acids (e.g. *Gln*, *Glu*, *Asp*, *Asn*) [6].

Nitrate also serves as a signaling molecule that regulates numerous processes of plant development. Elements involved in the nitrate signaling pathway have been identified, including the nitrate transceptor, calcium signaling, kinases, transcription factors, and various peptides and proteins [50,51]. Nitrate is perceived by the NRT1.1 (CHL1/ NPF6.3) [19]. As a dual-affinity transporter, NRT1.1 can switch between high and low affinities in response to external nitrate concentrations through phosphorylation and dephosphorylation at the threonine 101 site [52]. Two CALCINEURIN B-LIKE (CBL)- INTERACTION PROTEIN KINASES (CIPKs) interact with NRT1.1 and differentially regulate nitrate signaling. CIPK8 regulates NRT1.1 under high-nitrate conditions, whereas CIPK23 phosphorylates the transceptor under low-nitrate conditions [19,53].

After nitrate is sensed by NRT1.1, the signal needs to be transmitted to the nucleus and magnified through cytosolic regulators through a calcium-dependent or calcium-independent pathway. In the calcium-dependent pathway, PHOSPHOLIPASE C (PLC) was activated by NRT1.1 to increase cytoplasmic calcium concentration, accompanied with INOSITOL 1,4,5-TRISPHOSPHATE (IP_3) accumulation. The calcium signal is transmitted downstream by three Ca^{2+}-sensor protein kinases (CPKs): CPK10, CPK30, and CPK32. CPKs can phosphorylate NIN-LIKE PROTEIN 7 (NLP7), which is the master regulator to regulate other transcription factors in nitrate signaling [54,55]. For the calcium-independent pathway, AUXIN SIGNALING F-BOX 3 (AFB3) is transcriptionally regulated in response to nitrate [56].

However, the signal transduction pathway is still not well understood and is highly complex. Several transcription factors have been identified as important regulatory factors in the nitrate response [50,51]. Among them, NLP7, BASIC LEUCINE ZIPPER 1 (bZIP1), TGACG MOTIF-BINDING FACTOR 1 (TGA1), and TEOSINTE BRANCHED 1/ CYCLOIDEA /PROLIFERATING CELL FACTOR 20 (TCP20) have been confirmed for direct interactions with NO_3^--responsive genes.

In addition to local N signaling, systemic signaling allows the plant to adapt root morphologic and metabolic responses to overall N status [57,58]. Nitrate is distributed heterogeneously in the soil. In a split-root system, local and systemic signals in roots and shoots are orchestrated, thereby stimulating nitrate acquisition in the nitrate-rich patch [59]. The secreted small signaling peptide C-TERMINALLY ENCODED PEPTIDE 1 (CEP1) are produced by roots under N deficiency, and transported to the shoot via the xylem to interact with the leucine-rich repeat receptor kinases CEP RECEPTOR 1 and 2 (CEPR1 and CEPR2). This leads to induction of the non-secreted small signaling peptides CEP DOWNSTREAM 1 (CEPD1) and CEPD2. CEPD1 and CEPD2 are then transported to the roots. When CEPD1 and CEPD2 are integrated with the local N-rich signal, NRT2.1 is induced, leading to enhanced nitrate acquisition at the nitrogen-rich site of a split-root system [59]. Opposite to CEP, *Rhizobium*-induced CLAVATA 3 /EMBRYO-SURROUNDING REGION-related (CLE3) peptides interact with their receptor kinase CLAVATA1 (CLV1) to suppress nodulation [60]. Besides, *Arabidopsis* ELONGATED HYPOCOTYL5 (HY5), a bZIP transcription factor that mediates light-regulated coupling of shoot growth, root growth and nitrate uptake [61]. HY5 is a shoot-to-root mobile signal. Shoot-derived HY5 auto-activates root *HY5*. In the shoot, HY5 promotes carbon assimilation and translocation, whereas in the root, HY5 activation of NRT2.1 expression and nitrate uptake is potentiated by increased carbon photo-assimilate (sucrose) levels.

Recent studies further suggest that nitrate also regulates cytokinin synthesis and metabolism, with cytokinins acting as secondary messengers, communicating between organs and regulating development and metabolism. One example of this is communication between photosynthesizing leaves and nutrient-absorbing roots, which is crucial in ensuring coordinated growth under fluctuating environmental conditions [6]. Understanding cytokinin biosynthesis, transport and signaling as well as its communication with nitrogen is therefore essential in modulating plant development.