REVIEW PAPER

How does nitrogen shape plant architecture?

Le Luo1,2, Yali Zhang1,2 and Guohua Xu1,2,*

1 State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China
2 China MOA Key Laboratory of Plant Nutrition and Fertilization in Lower-Middle Reaches of the Yangtze River, Nanjing 210095, China.

* Correspondence: ghxu@njau.edu.cn

Received 15 December 2019; Editorial decision 7 April 2020; Accepted 9 April 2020

Editor: Hideki Takahashi, Michigan State University, USA

Abstract

Plant nitrogen (N), acquired mainly in the form of nitrate and ammonium from soil, dominates growth and development, and high-yield crop production relies heavily on N fertilization. The mechanisms of root adaptation to altered supply of N forms and concentrations have been well characterized and reviewed, while reports concerning the effects of N on the architecture of vegetative and reproductive organs are limited and are widely dispersed in the literature. In this review, we summarize the nitrate and amino acid regulation of shoot branching, flowering, and panicle development, as well as the N regulation of cell division and expansion in shaping plant architecture, mainly in cereal crops. The basic regulatory steps involving the control of plant architecture by the N supply are auxin-, cytokinin-, and strigolactone-controlled cell division in shoot apical meristem and gibberellin-controlled inverse regulation of shoot height and tillering. In addition, transport of amino acids has been shown to be involved in the control of shoot branching. The N supply may alter the timing and duration of the transition from the vegetative to the reproductive growth phase, which in turn may affect cereal crop architecture, particularly the structure of panicles for grain yield. Thus, proper manipulation of N-regulated architecture can increase crop yield and N use efficiency.

Keywords: Amino acids, ammonium, architecture, flowering time, nitrate, panicle structure, phytohormones, shoot branching, tillering, transcription factor, transporter.

Introduction

Nitrogen (N) is quantitatively the most important mineral nutrient in plants. N is acquired as nitrate (NO$_3^-$) and/or ammonium (NH$_4^+$) from soil (Xu et al., 2012). High-yield cultivation relies heavily on the use of N fertilizers. Excessive application of N fertilizers not only causes energy waste and increases production costs but also aggravates soil acidification and water eutrophication, as well as contributing to greenhouse gas emissions (Guo et al., 2010; Sutton et al., 2011). Therefore, there is an urgent need to breed crop varieties that use N efficiently in order to minimize N input for the sake of sustainable plant production.

For efficient acquisition of N from soil, plants have developed sophisticated regulatory mechanisms of root development and N transport. Several recent reviews (Forde et al., 2014; Giehl et al., 2014; Kiba et al., 2016; O’Brien et al., 2016; Xuan et al., 2017; Taleski et al., 2018; Yang et al., 2019) and two reviews in this Special Issue (Liu et al., 2020; Jia and von Wirén, 2020) have described root adaptation to altered N supply and the prospects for genetically engineering ideal root phenotypes. Meanwhile, the growth and development of aboveground plant parts are systematically regulated by N status (Chen et al., 2016; O’Brien et al., 2016; Xuan et al., 2017). Architectural features that are affected by N, such as plant height, branches, and panicles not only affect yield but also determine N distribution in various organs as well as the efficiency of N use (Hu et al., 2015; Chen et al., 2017). Although the basic regulatory mechanisms of
plant architecture have been characterized (Wang and Li, 2008; Xing and Zhang, 2010; Wang et al., 2018a), reports concerning the N regulation of plant architecture, particularly by different forms of N at the cellular and molecular levels, are limited and dispersed in the literature. In this review, we summarize how the N supply shapes plant architecture and discuss the possible relationships between plant architecture, growth duration, and N use efficiency (NUE), mainly in cereal crops.

Nitrogen regulation of growth and development in different phases

The hormonal and genetic control of plant architecture, including shoot apical meristem (SAM) activity, axillary meristem formation and elongation, inflorescence structure, and plant height, has been characterized in Arabidopsis, rice, pea, maize, and tomato (Wang and Li, 2008; Xing and Zhang, 2010; Wang et al., 2018a). During developing phases, the plant architecture changes in several aspects, such as stem elongation, branch development, stem and leaf angle, and inflorescence development (Wang et al., 2018a). For cereal crops, the branch number and panicle structure (inflorescence) are two of the most important traits that directly determine the grain yield (Kyozuka et al., 2014). The number of branches (or tillers in rice and wheat) is determined by the initiation of axillary meristem and thereafter via the elongation of axillary buds (Bennett and Leyser, 2006). The mechanism controlling axillary bud outgrowth in apical dominance has been extensively studied; auxin is the main player involved in axillary bud regulation (Teale et al., 2006). The panicle structure is also determined by meristem activity. Floral meristem is the final phase wherein the meristem activity ceases. In grass species, the basic panicle structure is determined by spikelets, the small branches for producing flowers (Itoh et al., 2005; Kellogg et al., 2013). It has been shown that the inflorescence in rice is mainly determined by the floral meristem, which controls the timing of phase transition from the vegetative to the reproductive stage, thereby influencing panicle size (Itoh et al., 2005). Early transition decreases the number and length of branches and panicles, while delayed transition results in more and longer branches as well as larger panicles (Kyozuka et al., 2014).

The plant architecture is greatly influenced by aspects of the growth environment, particularly the duration and intensity of light, and supplies of nutrients and water (Kudoyarova et al., 2015; de Wit et al., 2016; Feng et al., 2016; Wang et al., 2018a). N is one of the major determinants of plant growth and development that affect the major components of plant architecture such as tiller number and panicle structure (Ladha et al., 1998; Zhang et al., 2009, 2017; Tian et al., 2017; Wang et al., 2018a; Yi et al., 2019; Yang et al., 2019). The plant N uptake rate varies during different growth and development stages. In rice, total N accumulation rapidly increases during the vegetative and early reproductive stages, then reaches a plateau before declining slightly during grain filling and ripening (Hashim et al., 2015). The root uptake rate and concentration of N during early growth stages are critical for forming effective tillers. During the ripening stage, the N that is used for grain formation and seed filling is transferred mainly from culms and leaves (Hashim et al., 2015). Therefore, varying the rate of N application at different stages (sowing, tillering, panicle initiation, and heading) can alter the yield components of rice. The N demand for forming effective tillers and for grain filling (weight) may vary among different varieties, probably due to their differences in growth and development (Thu et al., 2014).

Changes in plant architecture in response to N supply may vary among plant species and even accessions of the same species. We have observed natural variation in the response of the different components of plant architecture to N fertilization
among rice accessions in a core collection grown in a paddy field (data not shown). Nevertheless, N limitation suppresses rice growth, decreases height, and limits tiller number (Fig. 1A, B). Notably, the N demand for maintaining height and tiller number is not the same as that for the branch number of spikelets, and the growth of secondary branches rather than primary branches is sensitive to the supply of N (Fig. 1C, D).

In general, the effect of the level of N supply on plant height can be predicted, whereas its effect on other architecture components, such as tiller number, filled grains per panicle, 1000-grain weight, and grain yield, is complicated. Sufficient N supply stimulates shoot elongation and ensures that the plant will reach the expected height in both rice and wheat (Wu et al., 2020), while excess N prevents secondary cell wall formation, resulting in poor lodging resistance (Wu et al., 2017; Zhang et al., 2017). The tiller number is affected by the N supply level and growth stage. The effective tiller number can be increased by increasing the N supply to an appropriate level and can be decreased by the excessive application of N (Haque et al., 2016). In rice, N deficiency suppresses bud elongation rather than initiation (Luo et al., 2017). The most critical time for N fertilization for rice grain yield is at the panicle initiation stage (Yoshida et al. 2006). The N supply affects inflorescence development, panicle length, and the number of flowers per panicle (Yoshida et al. 2006; Makino, 2011). In wheat, N accumulation at anthesis was found to be positively correlated with the onset of flag-leaf senescence, and thus total N accumulated at anthesis is an important trait for enhancing grain yield and NUE under low to moderate N supply (Nehe et al., 2018).

For efficient use of light, water, and nutrient resources, it is imperative that plants have the phenotypic plasticity to be able to adapt to varied environmental conditions. Interestingly, super-high-yield rice cultivars show high morphological acclimation in leaf dispersion and orientation to different agronomic practices, including N application (Wang et al., 2019a). The efficient phenotypic adaptation of rice is coordinated with improved N uptake and assimilation; the shoot photosynthetic productivity of a given rice phenotype is closely and positively related to leaf N concentration and total N accumulation (Wang et al., 2019a).

**Nitrate in the regulation of shoot branching and flowering**

The mechanism of N regulation of plant architecture has been partially elucidated during the past decades. In wheat, He et al. (2015) isolated a NO$_3^-$-inducible and cereal-specific NAC (NAM, ATAF, and CUC) transcription factor, TaNAC2-5A. Limited NO$_3^-$ supply enhances the expression of TaNAC2-5A in shoots and roots. TaNAC2-5A can directly bind to the promoter regions of the genes encoding NO$_3^-$ transporters and glutamine synthetase, consequently enhancing N acquisition and assimilation. Overexpression of TaNAC2-5A can increase tiller numbers, spikelet number, and 1000-grain weight, resulting in higher grain yield (He et al., 2015). In rice, OsMADS57, a MADS-box transcription factor whose expression is enhanced by NO$_3^-$ supply, interacts with TEOSINTE BRANCHED1 (TB1) and targets Dwarf14 (D14) to control the outgrowth of axillary buds (Guo et al., 2013; Huang et al., 2019a). OsMADS57 can also bind to the CArG motif (CATTTTATAG) within the promoter of OsNRT2.3a that functions in NO$_3^-$ translocation; knockout of OsMADS57 suppresses the distribution of NO$_3^-$ from root to shoot (Tang et al., 2012; Huang et al., 2019a). These results suggest that OsMADS57 may participate in NO$_3^-$-regulated tiller bud outgrowth of rice plants.

Some NO$_3^-$ transporters have been reported to participate in the modulation of plant architecture (tiller number and panicle architecture), mainly through changing N uptake and translocation to reproductive organs. Overexpression of OsNRT2.3b, but not of OsNRT2.3a, increases panicle size parameters including panicle length, number of primary and secondary rachises, number of seeds per panicle, and seed-setting rates under different N treatments (Fan et al., 2016). Chen et al. (2016, 2017) reported that overexpression of a high-affinity NO$_3^-$ transporter gene, OsNRT2.1, driven either by the promoter of OsNAR2.1 (encoding a nitrate transport accessory protein) or by its own promoter can increase post-anthesis N uptake and translocation from vegetative organs to grains, resulting in greater panicle length and seed set, more grains per panicle, and higher grain yield.

Several members of the NO$_3^-$ and peptide transporter family (NPF) in rice have been characterized with regard to their functions in regulating shoot branching and panicle structure. OsNPF7.7 has two splicing variants, OsNPF7.7-1 and OsNPF7.7-2, that show similar expression responses to N in axillary buds (Huang et al., 2018). Enhanced expression of OsNPF7.7-1 and OsNPF7.7-2 increases NO$_3^-$ and NH$_4^+$ influx, respectively, while both OsNPF7.7-1 and OsNPF7.7-2 promote the outgrowth of axillary buds and increase the numbers of tillers, effective panicles, and filled grains per plant, resulting in higher grain yield (Huang et al., 2018). In addition, both OsNPF7.1 and OsNPF7.4 function in NO$_3^-$ uptake, but they show opposite expression patterns in axillary buds (Huang et al., 2019b). Overexpression of OsNPF7.1 or knockout of OsNPF7.4 can increase axillary bud outgrowth, especially for the second bud, and subsequently tiller number in rice. Moreover, OsNPF7.2, a low-affinity nitrate transporter, can positively alter cell division in tiller buds to increase tiller number and grain yield (Wang et al., 2018b).

Very recently, the indica allele of the nitrate reductase gene OsNR2, which encodes a NADH/NADPH-dependent nitrate reductase, has been shown to promote NO$_3^-$ uptake via feed-forward interaction with a NO$_3^-$ transporter, OsNRT1.1B, thereby enhancing rice yield potential and NUE (Gao et al., 2019). Notably, effective tiller number is increased in Nipponbare plants expressing indica OsNR2 (cv. 9311) and decreased by reduced OsNR2 expression, probably via alteration of the expression of rice OsTB1, a gene controlling tiller bud formation and elongation (Gao et al., 2019). The feed-forward interaction of OsNR2 and OsNRT1.1B may explain the effect of OsNRT1.1B in altering tiller number in rice (Hu et al., 2015).

Another aspect of nitrate-dependent regulation of plant architecture may be associated with flowering time. N
fertilization influences the length of different growth phases and results in varied architecture, even within the same genotype (Leng et al., 2020; Hall et al., 2014). The transition from the vegetative to the reproductive phase is the end of leaf generation on the main stem; this influences the axillary meristem number. The rice branch number is commonly increased by late flowering and decreased by early flowering (Leng et al., 2020). Excess N application commonly causes a delay in the flowering time, resulting in later ripening. In Arabidopsis, the influence of N supply on flowering time has been well characterized (Castro Marín et al., 2011; Vidal et al., 2014). Both extreme deficiency and excess of N result in postponement of flowering time (Lin and Tsay, 2017). SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) serves as a central integrator for multiple flowering pathways in the plant architecture. We have shown that mutation of asparagine synthetase 1 (ASN1) in rice decreased the concentration of asparagine, while total N was unchanged (Luo et al., 2019). Knockout of OsASN1 suppressed tiller bud outgrowth and tiller number, suggesting that OsASN1 is involved in the regulation of rice development (Luo et al., 2019). Rice cytosolic glutamine synthetase OsGS1;2, which is involved in the primary assimilation of NH$_4^+$ in roots, is also involved in the regulation of plant development (Funayama et al., 2013; Ohashi et al., 2015, 2017). It has been demonstrated that OsGS1;2 contributes to tiller bud outgrowth by regulating N-dependent CK biosynthesis (Ohashi et al., 2017).

In addition to their functions as basic compounds for growth and development, amino acids may function as signaling molecules (Dinkeloo et al., 2018). For example, serine acts as a signal in brain tissue and in mammalian cancer cells. The biosynthesis of serine is highly active and restricted to proliferating cells of the primary meristem (Häuser et al., 2014). Serine in the meristems has been suggested to regulate targets of rapamycin signaling in plants (Benstein et al., 2013; Cascales-Minana et al., 2013; Menand et al., 2002). Moreover, it is very likely that there are amino acid transceptors that are involved in the regulation of plant development (Dinkeloo et al., 2018).

### Amino acids in the regulation of plant architecture

Plant roots can directly acquire a large portion of NH$_4^+$ and amino acids in addition to NO$_3^-$ in the soil, particularly under highly N-fertilized or irrigated paddy conditions. After absorption, most of the NH$_4^+$ and part of the NO$_3^-$ are assimilated to amino acids in the roots; therefore, the transportation and distribution of N inside plants occur mainly in the form of amino acids (Xu et al., 2012). Although NH$_4^+$ could regulate root architecture (Liu and von Wirén, 2017), it is not clear whether NH$_4^+$ itself can directly regulate shoot architecture; however, several studies have shown that some transporters and synthetases of amino acids are directly involved in the regulation of plant architecture.

Amino acids are essential components of plant metabolism, not only as constituents of proteins but also as precursors of important secondary metabolites and as carriers of organic N between the organs of the plant (Dinkeloo et al., 2018). Amino acids in the roots are transported from the cortex or endodermis cells to the vasculature to circumvent the Casparian strip and then translocated to the aboveground tissues (Dinkeloo et al., 2018; Tegeder and Masclaus-Daubrevesse, 2018). Amino acid transporters play roles in amino acid uptake by roots, xylem and phloem loading, xylem–phloem transfer, and intracellular transport (Dinkeloo et al., 2018). It has been recently shown that amino acid transporters function in the regulation of tiller growth and the entire plant architecture in addition to altering N distribution and NUE. In rice, blocking amino acid permease 3 (AAP3) can stimulate bud outgrowth and effective tiller number, leading to higher grain yield; in contrast, overexpressing OsAAP3 results in an enriched amount of amino acids and inhibition of bud outgrowth (Lu et al., 2018). Amino acid permease 5 (OsAAP5) can also affect tiller number and grain yield through the regulation of cytokinin (CK) biosynthesis (Wang et al., 2019b).

The synthesis of amino acids may be involved in regulating the plant architecture. We have shown that mutation of asparagine synthetase 1 (ASN1) in rice decreased the concentration of asparagine, while total N was unchanged (Luo et al., 2019). Knockout of OsASN1 suppressed tiller bud outgrowth and tiller number, suggesting that OsASN1 is involved in the regulation of rice development (Luo et al., 2019). Rice cytosolic glutamine synthetase OsGS1;2, which is involved in the primary assimilation of NH$_4^+$ in roots, is also involved in the regulation of plant development (Funayama et al., 2013; Ohashi et al., 2015, 2017). It has been demonstrated that OsGS1;2 contributes to tiller bud outgrowth by regulating N-dependent CK biosynthesis (Ohashi et al., 2017).

In addition to their functions as basic compounds for growth and development, amino acids may function as signaling molecules (Dinkeloo et al., 2018). For example, serine acts as a signal in brain tissue and in mammalian cancer cells. The biosynthesis of serine is highly active and restricted to proliferating cells of the primary meristem (Häuser et al., 2014). Serine in the meristems has been suggested to regulate targets of rapamycin signaling in plants (Benstein et al., 2013; Cascales-Minana et al., 2013; Menand et al., 2002). Moreover, it is very likely that there are amino acid transceptors that are involved in the regulation of plant development (Dinkeloo et al., 2018).

### Nitrogen-regulated small signaling peptides in plant development

Small signaling peptides have been identified in plant cell-to-cell communication and plant growth regulation (Czyzewicz et al., 2013; Tavormina et al., 2015; Oh et al., 2018). Two small peptide families, CLAVATA3/EMBYO SURROUNDING REGION (CLE) and C-TERMINALLY ENCODED PEPTIDE (CEP), function in both local N-status-dependent signaling and systemic N signaling (Okamoto et al., 2016; de Bang et al., 2017; Taleski et al., 2018). CEPs, which are widely distributed among seed plants, are expressed in N-starved root parts and transported to the shoots, where they bind to the CEP receptor (CEPR) (Tabata et al., 2014). This signal triggers the expression of class III glutaredoxins as mobile signals transported from shoots to roots via phloem to induce the expression of the NO$_3^-$ transporter NRT2.1 in the N-replete portion of the roots (Ohkubo et al., 2017).

Small signaling peptides have been identified to play important roles in the regulation of root morphology, while their functions in regulating shoot architecture have received less attention. In Arabidopsis, perturbed CEP expression leads to...
changes in plant height and leaf shape (Roberts et al., 2013). The CEP genes show different functions in regulating the shoot and root response to the growth conditions tested (Delay et al., 2013). For example, the overexpression (ox) line of CEP2 (CEP2ox) shows fewer rosette leaves, delay of flowering, and alteration of leaf morphology in comparison to the wild type (WT); CEP3ox and CEP4ox display a similar phenotype characterized by epinasty, leaf yellowing, and reduced rosette size; CEP6ox and CEP9ox show milder changes. These results indicate that CEPs may interact with different receptors and may play distinct roles in shoot development (Delay et al., 2013).

In rice, there are 17 OsCEP genes, and OsCEP6.1ox also has negative effects on rice shoot development (Sui et al. 2016). Compared with the WT line, OsCEP6.1ox transgenic lines exhibit reduced height, lower tiller number, shorter panicle length and smaller seed size (Sui et al., 2016). Further functional analysis demonstrated that the regulatory activity of CEPs on panicle development may be related to the alteration of cell size but not cell number (Sui et al., 2016). However, the downstream signaling components of OsCEPs and their response to N status remain largely undetermined in rice.

### Nitrogen regulation of cell division and expansion for shaping architecture

The regulation of the cell cycle by N was reported decades ago. Limited N supply suppresses DNA synthesis, cell division, cell growth, and bud growth at similar rates (Rivin and Fangman, 1980). Long-term N starvation results in the cessation of cell division and associated growth of branches in rice (Luo et al., 2017). Increasing N supply levels accelerate cell division and expansion, resulting in greater biomass accumulation. Notably, the effect of the form of N supply on cell division and expansion is not significant in the short term. In Arabidopsis, the provision of either NO$_3^−$ or NH$_4^+$ causes the same effects on shoot branching (de Jong et al., 2014). In rice, shoot branching is influenced significantly by the concentration but not the form of N during the vegetative stage (Luo et al., 2017).

The NO$_3^−$ supply level influences the synthesis and distribution of CKs and their downstream transcription factors, which further regulate cell division in plants (Landrein et al., 2018). Macadam et al. (1989) found that high N fertilization increased the rate of division of mesophyll cells and increased epidermal cell elongation of tall fescue leaf blades. In pea, the expression levels of cell-cycle-related genes (PCNA, cyclinB, cdk2, and histone H4) are enhanced in axillary tiller buds when the buds grow (Devitt and Stafstrom, 1995; Shimizu and Mori, 1998). N deficiency resulted in the dormancy of tiller buds, probably via altering the expression of cell-cycle-related genes (Luo et al., 2017). However, it is not known whether the suppression of cell division in response to N deficiency is a direct effect of N or rather results from a signal that is transmitted to the tiller bud.

Amino acids may also influence cell division. In human tumor cells, asparagine was found to be an important regulator of amino acid homeostasis, anabolic metabolism, and proliferation (Krall et al., 2016). The loss of function of asparagine synthetase (ASNS) resulted in the suppression of cell proliferation and inhibition of tumor growth in human gastric cancer cells, melanoma cells, and epidermoid carcinoma cells (Li et al., 2016; Yu et al., 2016). Silencing of ASNS arrested cell cycle progression at the G0/G1 phase, probably through regulation of the expression of cell cycle molecules such as CDK2 and cyclin E1 (Miao et al., 2013). These results all shed light on the possible relationship between amino acids and cell division in plants. Conducting such studies may uncover new mechanisms involved in the control of shoot architecture by N.

### Nitrogen regulation of phytohormone synthesis and distribution for shaping architecture

Plants integrate internal systemic signals, such as hormones, that provide information on the N status of organs to finely adjust the growth and development of shoots and roots (Wang et al., 2018a). Among these phytohormones, auxin, CKs, strigolactones (SLs), and GAs are of vital importance for regulating plant architecture. The dynamic balance between cell division and cell differentiation controls organ shape and size.

#### Auxin

Fluctuation of the N supply has a significant effect on auxin distribution. A decrease in N supply commonly increases indole-3-acetic acid (IAA) accumulation in the root of plants including Arabidopsis, soybean, durum wheat, and maize (Caba et al., 2000; Walch-Liu et al., 2006; Tian et al., 2008). The establishment of auxin distribution within plant tissues constitutes its function in plant morphogenesis, and this mainly depends on the function of auxin efflux facilitators of the PIN-FORMED (PIN) family (Friml et al., 2003; Woodward and Bartel, 2005).

Plant N status may also be related to auxin synthesis and/or distribution in aboveground parts. For example, decreased NO$_3^−$ supply to rice can down-regulate the expression of multiple OsPIN genes and decrease $^3$H-IAA transport from shoots to roots, resulting in increased IAA content in the youngest leaves and decreased IAA content in the shoot base and roots (Sun et al., 2014). In addition, the provision of a mixture of NO$_3^−$ and NH$_4^+$, in comparison to a single form of N, increases the IAA concentration in leaves and roots and increases the expression of both OsAUX1 and OsPIN genes (Song et al., 2011).

The molecular mechanisms of N-induced auxin distribution in shaping shoot architecture are still obscure. We have shown that N deficiency inhibits the expression of seven OsPINs (OsPIN1b/1c/2/5a/5b/9/10a) in the roots of rice (Sun et al., 2014). Since rice tiller numbers are increased by overexpression of OsPIN2 and OsPIN3 (Chen et al., 2012; Zhang et al., 2012) and are decreased by knockout of OsPIN10a (Zhang et al., 2012), these PIN members may be involved in N regulation of tiller bud outgrowth. However, transgenic plants overexpressing OsPIN1 and OsPIN5b show inverse aboveground phenotypes (Xu et al., 2005; Lu et al., 2013), probably due to the disturbance of auxin-mediated bud inhibition or other secondary messengers such as CKs (Barbier et al., 2001).
IPT3, the regulator system, NLP/NIGT1, controls CYP735A (2011). Recent findings indicate that a transcriptional regulator of gene expression in Arabidopsis in response to NO$_3$ is isopentenyltransferase, may play a critical role in mediating IPT3, encoding adenosine phosphate- the synthesis and distribution of CKs.

Supply (Kamada-Nobusada et al., 2004; Song et al., 2013) has been shown to reduce NO$_3$-specific increase of ZR level in barley (Samuelson and Larsson 1993), while a decrease of total N concentration in tiller buds reduces the active CK content (Liu et al., 2011; Ohashi et al., 2017). These results suggest that entire N status or N assimilation, rather than NO$_3^-$ or NH$_4^+$ alone, determine the synthesis and distribution of CKs.

Plants possess multiple regulatory pathways of N-dependent CK biosynthesis to modulate growth. The CK synthesis gene IPT3, encoding adenosine phosphate-isopentenyltransferase, may play a critical role in mediating NO$_3^-$-induced CK synthesis in Arabidopsis and rice plants (Takei et al., 2004; Song et al., 2013) and possibly in N-controlled plant architecture. IPT3 is regulated by inorganic N sources in a NO$_3^-$-specific manner. Miyawaki et al. (2006) have shown that the phenotype of dramatically reduced shoot apical meristems and short, thin aerial shoots of *attipt3/5/6/7* mutants can be complemented by expressing IPT3. Remarkably, IPT3 was mainly regulated by NO$_3^-$, and ipt3 mutants failed to sense NO$_3^-$ signals to produce CKs (Takei et al., 2004). NO$_3^-$-induced expression of IPT3 is partly dependent on NRT1.1/CHL1 (Liu et al., 1999; Ho et al., 2009; Wang et al., 2009; Kiba et al., 2011). Recent findings indicate that a transcriptional regulatory system, NLP/NIGT1, controls IPT3 and CYP735A gene expression in Arabidopsis in response to NO$_3^-$ (Maeda et al., 2018). Nevertheless, the function of IPT3 in regulating N-controlled plant architecture at different developmental stages still needs to be investigated. In addition to IPT3, other IPT members may also be involved in N-regulated CK biosynthesis. In rice, glutamine or a related metabolite rather than NO$_3^-$ or NH$_4^+$ can enhance the expression of OsIPT4, OsIPT5, OsIPT7, and OsIPT8, with accompanying accumulation of CKs. Repressing the expression of OsIPT4, the dominant IPT in rice roots, significantly reduces the N-dependent increase of CKs in the xylem sap and retards shoot growth despite a sufficient N supply (Kamada-Nobusada et al., 2013).

### Cytokinins

Increasing evidence indicates that elevated CK content restricts root growth and promotes shoot growth, influencing plant height, shoot branching, flowering, and seed production (Liu et al., 2017). It has been shown that both the biosynthesis and distribution of CKs are closely linked to N availability during shoot and root development. Increasing NO$_3^-$ supply to barley roots can rapidly stimulate the biosynthesis and acropetal transportation of zeatin riboside (ZR), a naturally occurring CK, while NH$_4^+$ has less effect than NO$_3^-$ on the increase of ZR (Samuelson and Larsson 1993). In rice, NO$_3^-$ supply increases the concentrations of six CK forms in xylem sap, as well as leading to their high accumulation in both roots and leaves (Song et al., 2013). Notably, pretreatment with either nitrate reductase or glutamine synthetase inhibitor can prevent the NO$_3^-$-stimulated increase of ZR level in barley (Samuelson and Larsson 1993), while a decrease of total N concentration in tiller buds reduces the active CK content (Liu et al., 2011; Ohashi et al., 2017). These results suggest that entire N status or N assimilation, rather than NO$_3^-$ or NH$_4^+$ alone, determine the synthesis and distribution of CKs.

### Gibberellins

GA is involved in the regulation of the inverse relationship between plant height and tiller number. Exogenous application of GA reduces tiller number in cereal plants (Zhuang et al., 2019). GA promotes plant height by stimulating the degradation of the DELLA protein SLR1 (SLENDER RICE 1) (Murase et al., 2008; Sasaki et al., 2003; Liao et al., 2019). Since the tiller number regulator MONOCULM 1 (MOC1) relies on binding to SLR1 to avoid degradation, GAs trigger both the degradation of SLR1, leading to stem elongation, and the degradation of MOC1, leading to a lower tiller number (Liao et al., 2019).

In current commonly cultivated reduced height (Rht) wheat, DELLAs are resistant to GA-stimulated destruction (Peng et al., 1999), whereas the semi-dwarfish rice sd1

### Strigolactones

SLs have been identified more recently as a group of plant hormones that modulate plant architecture (Umehara et al., 2008; Sun et al., 2014; Barbier et al., 2019). The function of SLs in altering shoot architecture, including involvement in plant stature, axillary tiller bud outgrowth, and tiller angle, has been partially characterized (Seto and Yamaguchi, 2014; Sang et al., 2014). Small sections of stem tissue are able to supply sufficient SLs to inhibit branching in mutant shoots that are unable to synthesize SLs (Dun et al., 2009), suggesting that SLs may act at very low concentrations.

Enhancement of the biosynthesis and exudation of SLs by N deficiency has been observed in several plant species (Yoneyama et al., 2007, 2012; Xie et al., 2010; Sun et al., 2014). In sorghum plants, limited N or phosphorus largely increases the amount of 5-deoxystyrigol in the root exudates (Yoneyama et al., 2007). In rice, N deficiency results in high endogenous SLs and degradation of D53 protein, a key repressor in the SL signaling pathway—the same effect as that caused by exogenous supply of the SL analogue GR24 (Sun et al., 2016). These results clearly demonstrate that SLs are involved in N-regulated rice development. However, the effect of N deficiency on the synthesis of SLs depends on the plant type and experimental conditions (Yoneyama et al., 2007, 2012; Sun et al., 2014). For example, SL contents in the roots of red clover and alfalfa are not significantly affected by altering the N supply (Yoneyama et al., 2012).

The effect of N supply on branching in Arabidopsis is comparable between WT and mutants of SL biosynthesis (*max1* and *max3*) and signaling (*max4*) (de Jong et al., 2014). Even though N limitation reduces branching in both SL mutants and WT, the mutants still produce more secondary shoots than WT under the same N-limiting condition. These results suggest that the ability to maintain N-regulated branching in Arabidopsis is at least partially dependent on SLs. In rice, the SL signaling gene *D53* can repress ideal plant architecture 1 (IPA1), a key regulator of architecture, thereby functioning as a downstream transcription factor (Song et al., 2017). Thus, it is an intriguing question whether there are other targets that bind to D53 in rice plants and, if so, whether the target genes, including *IPA1*, are involved in SL participation in N-regulated plant development.
Nitrogen shapes plant architecture | 4421

allele reduces the abundance of bioactive GA (Itoh et al., 2002; Asano et al., 2011). Notably, growth-regulating factor 4 (GRF4) can bind to GRF-interacting factor 1 (GIF1) and activate the genes related to N uptake and assimilation, while DELLA protein inhibits the binding of GRF4 to GIF1; DELLA protein accumulation thus inhibits growth and N uptake and assimilation in rice and wheat (Li et al., 2018b). Moreover, N stimulation of tillering in rice is regulated by N-mediated tiller growth response 5 (NGR5) (Wu et al., 2020), an APETALA2 (AP2)-domain transcription factor previously known as SMOS1 (SMALL ORGAN SIZE1) and RLA1 (REDUCED LEAF ANGLE1) (Aya et al., 2014; Hirano et al., 2017; Qiao et al., 2017). NGR5 is a target of the GA receptor GID1; thus, NGR5 abundance is negatively associated with GA level. Mutation of NGR5 results in the insensitivity of tillering number to N supply. NGR5 regulates N-promoted H3K27me3 modification by recruiting PRC2 (POLYCOMB REpressive COMPLEX 2) to methylate the sites of D14 (encoding Dwarf14, an SL receptor protein) and OsSPL14 (encoding SQUAMOSA PROMOTER BINDING PROTEIN LIKE-14) and other tillering inhibition genes. Thus, in response to N supply, NGR5 inhibits the expression of the shoot-branching-inhibitory genes D14 and OsSPL14 and promotes tillering in rice (Wu et al., 2020).

Perspectives

N fertilization in the field has primary effects on plant growth and development. Based on the most recent findings, we have drawn an outline of N regulatory pathways in altering flowering time, shoot branching, and panicle size under varied NO₃⁻ and/or NH₄⁺ supply (Fig. 2). The genes directly or indirectly involved in the N regulation of plant architecture are summarized in Table 1. It should be noted that the N regulation of different components of plant architecture and yield is affected by environmental conditions and agricultural practices. The interaction effects of planting density and N fertilization on architecture and yield are worth further investigation from both physiological and molecular genetic perspectives. In addition, the relationship between N-regulated growth duration,
| Gene name      | Gene locus    | Host plant | Protein type                                                                 | Spatial expression                                           | Transcriptional regulation by N                               | Effect on plant development                                    | Reference            |
|----------------|---------------|------------|-------------------------------------------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|----------------------------------------------------------------|----------------------|
| TaNAC2-5A      | AY625683      | Wheat      | Transcription factor that can directly bind to the promoter regions of TaNRT2.1-B1, TaNPF7.1-D1, and TaGS2-2A | Mainly expressed in old leaves and flag leaves               | Induced by nitrate                                          | Promotes root and shoot growth and grain yield                  | He et al., 2015      |
| OsMADS57      | LOC_Os02g49840 | Rice       | MADS-box transcription factor, interacts with OsTB1 (TEO-SINTE BRANCHED1) and targets D14 (Dwarf14) | Root stellar, sheath, leaves, shoot apical meristem          | Induced by nitrate regardless of nitrate concentrations, but not by ammonium | Controls the outgrowth of axillary buds                         | Huang et al., 2019a; Guo et al., 2013 |
| OsNRT2.3b      | LOC_Os1g50820 | Rice       | pH-sensitive high-affinity nitrate transporter                                | Mainly expressed in the phloem                              | Induced by nitrate                                          | High expression of OsNRT2.3b improves rice growth and NUE       | Fan et al., 2016      |
| OsNRT1.1B      | LOC_Os10g40600 | Rice       | Nitrate transporter                                                          | Root hair, epidermis, and vascular tissues                  | Regulated by the concentration of external N sources         | Increases tiller number, grain yield, biomass, and NUE          | Hu et al., 2015       |
| OsNPF7.1       | LOC_Os07g41250 | Rice       | Member of the NPF family                                                     | Root and axillary buds                                      | Regulated by the concentration of external N sources         | Promotes axillary bud growth and increases tiller number        | Huang et al., 2019b  |
| OsNPF7.2       | LOC_Os02g47090 | Rice       | Low-affinity nitrate transporter                                              | Mainly expressed in elongation and maturation zones of roots | Induced by high nitrate                                      | Improves seedling growth, root development, and grain yield     | Wang et al., 2018b    |
| OsNPF7.3       | LOC_Os04g50950 | Rice       | Peptide transporter                                                          | Root tip, lateral root, outgrowth bud, leaf blade, stem, and panicle | Induced by organic N                                       | Increases the number of panicles per plant, filled grain numbers per panicle, and grain N content, and enhances grain yield | Fang et al., 2017    |
| OsNPF7.4       | LOC_Os04g50940 | Rice       | Member of the NPF family                                                     | Root and axillary buds                                      | Regulated by the concentration of external N sources         | Inhibits seedling biomass, tillering and yield                  | Huang et al., 2019b  |
| OsNPF7.7       | LOC_Os10g42870 | Rice       | Putative nitrate transporter                                                  | Highly expressed in panicles, also in root, leaf, bud, basal part, and culm | Suppressed by high external N concentration                   | Promotes the outgrowth of axillary bud; increases nitrate influx and concentration | Huang et al., 2018    |
| OsNRT2.1       | LOC_Os02g02170 | Rice       | High-affinity nitrate transporter                                             | Root, leaf sheath, leaf blade, internode, seed, palea, and lemma | Induced by nitrate                                          | Increases biomass, grain yield, seed setting rate, grain number per panicle, and NUE | Chen et al., 2016    |
| SMZ            | At3g54990     | Arabidopsis| AP2-type transcription factor                                                | Unknown                                                      | Induced by nitrate                                          | Represses flowering                                             | Gras et al., 2018     |
| SNZ            | At2g39250     | Arabidopsis| AP2-type transcription factor                                                | Unknown                                                      | Induced by nitrate                                          | Represses flowering                                             | Gras et al., 2018     |
| FNR1           | At5g66190     | Arabidopsis| Oxidizes the final reduced product of the photosynthetic electron transport chain, ferredoxin, to reduce NADP⁺, resulting in ATP production | Expressed in leaf                                           | Induced by low nitrogen conditions                           | Regulates expression of the circadian clock genes               | Yuan et al., 2016    |
| Gene name | Gene locus | Host plant | Protein type | Spatial expression | Transcriptional regulation by N | Effect on plant development | Reference |
|-----------|------------|------------|--------------|-------------------|-------------------------------|-----------------------------|-----------|
| NLP7      | At1g24020  | Arabidopsis| Transcription factor, NIN-LIKE PRO-TEIN | Unknown           | Responds to nitrate signaling | A main regulator of nitrate signaling | Olas et al., 2019 |
| NLP6      | At1g64530  | Arabidopsis| Transcription factor, NIN-LIKE PRO-TEIN | Unknown           | Responds to nitrate signaling | A main regulator of nitrate signaling | Olas et al., 2019 |
| OsAAP3    | LOC_Os06g36180 | Rice       | Amino acid permease | Root, leaf, leaf sheath, culm, and panicle | Unknown | Suppresses tiller outgrowth and decreases yield | Lu et al., 2018 |
| OsAAP5    | LOC_Os01g65660 | Rice       | Amino acid permease | Root, tiller basal part, leaf sheath, leaf blade, and young panicle | Unknown | Suppresses tiller outgrowth and decreases yield | Wang et al., 2019b |
| OsGS1:2   | LOC_Os03g12290 | Rice       | Glutamine synthetase | Root, basal part of shoot, leaf sheath, and leaf blade | Induced by ammonium | Reduces axillary bud outgrowth, tiller number, height, panicle number; disorder of metabolic balance and decreases grain filling | Funayama et al., 2013; Ohashi et al., 2015; Ohashi et al., 2017 |
| OsASN1    | LOC_Os03g18130 | Rice       | Asparagine synthetase | Root, leaf, leaf sheath, and basal part of shoot | Induced by ammonium | Promotes tiller bud elongation and tiller number | Luo et al., 2019 |
| OsCEP6.1  | LOC_Os08g37070 | Rice       | Mature post-translationally modified peptide of 15 amino acids | Root, shoot, lemma, palea, stamen, pistil, leaf, and panicles | Induced by low nitrogen condition | Reduces plant height, tiller number, grain number, and grain size | Sui et al., 2016 |
| OsPIN2    | LOC_Os06g44970 | Rice       | Member of the auxin efflux carrier protein family | Root and the base of shoot | Induced by nitrate | Increases tiller number | Chen et al., 2012; Sun et al., 2014 |
| OsPIN 3t (OsPIN10a) | LOC_Os01g45550 | Rice       | Member of the auxin efflux carrier protein | Vascular tissue | Induced by nitrate | Promotes root length and adventitious root growth; decreases effective tillers, seed setting rates, and thousand-kernel weight yield per plant | Zhang et al., 2012; Sun et al., 2014 |
| OsPIN1    | LOC_Os02g50960 | Rice       | Member of the auxin efflux carrier protein | Expressed in the vascular tissues and root primordia | Induced by nitrate | Plays an important role in auxin-dependent adventitious root emergence and tillering | Xue et al., 2005; Sun et al., 2014 |
| OsPIN5b   | LOC_Os08g41720 | Rice       | Endoplasmic reticulum-localized protein that participates in auxin homeostasis, transport, and distribution in vivo | Mainly expressed in panicle, culm, and leaf | Induced by nitrate | Changes auxin homeostasis, transport and distribution | Lu et al., 2015; Sun et al., 2014 |
| IPT3      | At3g63110   | Arabidopsis| Adenosine phosphates-isopentenyl transferase | All organs in the seedlings | Responds to nitrate availability under N-limited conditions | Enhances leaf size with an increased number of cells; impairs root development | Takei et al., 2004; Song et al., 2013; Wu et al., 2020 |
| OsNGR5    | LOC_Os05g32270 | Rice       | Transcriptional regulator; recruits PRC2 to alter H3K27me3 methylation of targeted nitrogen-related genes | Nucleus | Increased transcription and abundance by N | Increases tiller number and grain yield | Li et al., 2018 |
| OsGRF4    | LOC_Os02g47280 | Rice       | Transcriptional regulator, regulates expression of multiple nitrogen-metabolism genes | Nucleus | Promoted by low N supplementation | Increases culm diameter, wall thickness, spike length, grain numbers per spike, and biomass accumulation | Li et al., 2018 |
plant architecture, and NUE should be further investigated in crop production studies.

The most important traits that influence the yield are the number of branches, panicle number, and seed size, while other aspects of plant architecture such as height, tiller angle, and leaf angle are also important for plant growth. Therefore, the trade-off among different architecture components regulated by different forms and concentrations of N should be considered for both high yield and NUE. Since the concept of “ideotype” was first put forward, the influence of environmental factors, including N fertilization, on the ideal plant architecture has received much less attention than expected, and has not been characterized in detail. To sustain the highest yield potential of the cultivars with ideotype, the architecture is expected not to be largely altered by varied N supplies in the field. Therefore, revealing the N-dependent mechanisms modulating plant architecture is helpful for molecular breeding of the ideotype with high NUE.

The influence of N fertilization on plant architecture can be monitored in real time at different scales in the field by the use of recently developed unmanned aerial vehicle (UAV)-based active canopy sensors. The modern UAV technique for providing phenotypic data shows great applicability and flexibility in the estimation of crop N status and in the analysis of plant architecture (Zaman-Allah et al., 2015; Watanabe et al., 2017; Elsayed et al., 2018; Li et al., 2018; Bucailleot et al., 2019; Cen et al., 2019; Lu et al., 2019). As improvement of these real-time monitoring and data-modeling techniques continues, the remote-sensing technology may be extensively applied in the future to predict the responses of the plant, including plant architecture, to N application.

To better understand the direct N regulatory pathways affecting plant architecture, identification of key quantitative trait loci and the genes controlling N-sensitive or -insensitive responses of certain components of plant architecture is expected in the future. Principal component analysis in genome-wide association studies is an effective means of extracting key information from phenotypically complex traits, and has been performed for analyzing rice architecture (Yano et al., 2019). This method has been broadly used for the analysis of N-related phenotyping in some other crops (Zhang et al., 2015; Monostori et al., 2017; Nigro et al., 2019; Steketeet al., 2019) and it can be applied to isolate the key genes involved in the N regulation of plant architecture.

Acknowledgements

This study was supported by the National Key Research and Development Program of China (2016YFD0100700), the Natural Science Foundation of China (31930101, 31972501, and 31672225), the Innovative Research Team Development Plan of the Ministry of Education of China (IRT_17R56; KYT201802), and the 111 project (B12009). We appreciate the constructive suggestions of Dr Zhongmin Fang, Guizhou University of China, for this review.

References

Asano K, Yamasaki M, Takuno S, et al. 2011. Artificial selection for a green revolution gene during japonica rice domestication. Proceedings of the National Academy of Sciences, USA 108, 11034–11039.

Aya K, Hobo T, Sato-Izawa K, Ueguchi-Tanaka M, Kitano H, Matsuoka M. 2014. A novel AP2-type transcription factor, SMALL ORGAN SIZE1, controls organ size downstream of an auxin signaling pathway. Plant & Cell Physiology 55, 897–912.

Barbier FF, Dun EA, Kerr SC, Chabikwa TG, Beveridge CA. 2019. An update on the signals controlling shoot branching. Trends in Plant Science 24, 220–236.

Bennett T, Leyser O. 2006. Something on the side: axillary meristems and plant development. Plant Molecular Biology 60, 843–854.

Benstein RM, Ludewig K, Wulfert S, Wittek S, Gigolashvili T, Frerighm H, Gierth M, Flügge UI, Krueger S. 2013. Arabidopsis phosphoglycerate dehydrogenase1 of the phosphoserine pathway is essential for development and required for ammonium assimilation and tryptophan biosynthesis. The Plant Cell 25, 5011–5029.

Buchailot ML, Gracia-Romero A, Vergara-Diaz O, Zaman-Allah MA, Tarekegne A, Cairns JE, Prasanna BM, Araus JL, Kefauver SC. 2019. Evaluating maize genotype performance under low nitrogen conditions using RGB UAV phenotyping techniques. Sensors 19, 1815.

Caba JM, Centeno ML, Fernández B, Gresshoff PM, Ligeró F. 2000. Inoculation and nitrate alter phytohormone levels in soybean roots: differences between a supernodulating mutant and the wild type. Planta 211, 98–104.

Cascales-Miñana B, Muñoz-Bertomeu J, Flores-Torner M, Anoman AD, Pertusa J, Alaiz M, Osorio S, Fernie AR, Segura J, Ros R. 2013. The phosphorylated pathway of serine biosynthesis is essential both for male gametophyte and embryo development and for root growth in Arabidopsis. The Plant Cell 25, 2084–2101.

Castro Marín I, Loej I, Bartetzko L, Searle I, Coupland G, Stitt M, Osuna D. 2011. Nitrate regulates floral induction in Arabidopsis, acting independently of light, gibberellin and autonomous pathways. Planta 233, 539–552.

Cen H, Wan L, Zhu J, et al. 2019. Dynamic monitoring of biomass of rice under different nitrogen treatments with a lightweight UAV with dual frame snapshot cameras. Plant Methods 15, 32.

Chen J, Fan X, Qian K, Zhang Y, Song M, Liu Y, Xu G, Fan X. 2017. pOsNAR2.1:OsNAR2.1 expression enhances nitrogen uptake efficiency and grain yield in transgenic rice plants. Plant Biotechnology Journal 15, 2820–2833.

Chen X, Yao Q, Gao X, Jiang C, Harberd NP, Fu X. 2016. Shoot-to-root mobile transcription factor HYS coordinates plant carbon and nitrogen acquisition. Current Biology 26, 640–646.

Chen Y, Fan X, Song W, Zhang Y, Xu G. 2012. Over-expression of OsPIN2D leads to increased tiller numbers, angle and shorter plant height through suppression of OsLAZY1. Plant Biotechnology Journal 10, 139–149.

Czyzewicz N, Yue K, Beeckman T, De Smet I. 2013. Message in a bottle: small signalling peptide outputs during growth and development. Journal of Experimental Botany 64, 5281–5296.

de Bang TC, Lay KS, Scheible WR, Takahashi H. 2017. Small peptide signaling pathways modulating macronutrient utilization in plants. Current Opinion in Plant Biology 39, 31–39.

de Jong M, George G, Ongaro W, Williamson L, Willetts B, Ljung K, McCulloch H, Leyser O. 2014. Auxin and strigolactone signaling are required for modulation of Arabidopsis shoot branching by nitrogen supply. Plant Physiology 166, 384–395.

de Wit M, Galvão VC, Fankhauser C. 2016. Light-mediated hormonal regulation of plant growth and development. Annual Review of Plant Biology 67, 513–537.

Delay C, Imin N, Djordjevic MA. 2013. CEP genes regulate root and shoot development in response to environmental cues and are specific to seed plants. Journal of Experimental Botany 64, 5383–5394.

Devitt ML, Stafstrom JP. 1995. Cell cycle regulation during growth-dormancy cycles in pea axillary buds. Plant Molecular Biology 29, 255–265.

Dinkeloo K, Boyd S, Pilot G. 2018. Update on amino acid transporter functions and on possible amino acid sensing mechanisms in plants. Seminars in Cell & Developmental Biology 74, 105–113.

Dun EA, Brewer PB, Beveridge CA. 2009. Strigolactones: discovery of the elusive shoot branching hormone. Trends in Plant Science 14, 364–372.

Elsayed S, Barmeier G, Schmidhalter U. 2018. Passive reflectance sensing and digital image analysis allows for assessing the biomass and
nitrogen status of wheat in early and late tillering stages. Frontiers in Plant Science 9, 1478.

Fan X, Tang Z, Tan Y, Zhang Y, Luo B, Yang M, Lian X, Shen Q, Miller AJ, Xu G. 2016. Overexpression of a pH-sensitive nitrate transporter in rice increases crop yields. Proceedings of the National Academy of Sciences, USA 113, 7118–7123.

Fang ZM, Bai GX, Huang WT, Wang ZX, Wang XL, Zhang MY. 2017. The rice peptide transporter OsNPF7;3 is induced by organic nitrogen and contributes to nitrogen allocation and grain yield. Frontiers in Plant Science 8, 1338.

Feng W, Lindner H, Robbins NE 2nd, Dinneny JR. 2016. Growing out of stress: the role of cell- and organ-scale growth control in plant water-stress responses. The Plant Cell 28, 1769–1782.

Forde BG. 2014. Nitrogen signalling pathways shaping root system architecture: an update. Current Opinion in Plant Biology 21, 30–36.

Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgens G. 2003. Efflux-dependent auxin gradients establish the apical–basal axis of Arabidopsis. Nature 426, 147–153.

Funayama K, Kojima S, Tabuchi-Kobayashi M, Sawa Y, Nakayama Y, Hayakawa T, Yamaya T. 2013. Cytosolic glutamine synthetase1:2 is responsible for the primary assimilation of ammonium in rice roots. Plant & Cell Physiology 54, 934–943.

Gao Z, Wang Y, Chen G, et al. 2019. The indica nitrate reductase gene OsNPR2 allele enhances rice yield potential and nitrogen use efficiency. Nature Communications 10, 5207.

Giehl RF, von Wirén N. 2014. Root nutrient foraging. Plant Physiology 166, 509–517.

Gras DE, Vidal EA, Undurraga SF, Riveras E, Moreno S, Dominguez-Figueroa J, Alabadi D, Blázquez MA, Medina J, Gutiérrez RA. 2018. SMZ/SNZ and gibberellin signaling are required for nitrate-elicited delay of flowering in Arabidopsis thaliana. Journal of Experimental Botany 69, 619–631.

Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX, Zhang WF, Christie P, Goulding KW, Vitousek PM, Zhang FS. 2010. Significant acidification in major Chinese croplands. Science 327, 1008–1010.

Guo SY, Xu YY, Liu HH, Mao ZW, Zhang C, Ma Y, Zhang QR, Meng Z, Chong K. 2013. The interaction between OsMADS57 and OsTB1 modulates rice tillering via DWARF4. Nature Communications 4, 1566.

Hall AJ, Savin R, Slafer GA. 2016. Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. Plant & Cell Physiology 57, 707–714.

Häusler RE, Ludewig F, Krueger S. 2001. CHL1 functions as a nitrate sensor responsible for the primary assimilation of ammonium in rice roots. Plant & Cell Physiology 42, 882–895.

Hashim MM, Yusop MK, Othman R, Wahid SA. 2015. Characterization of the apical–basal axis of Arabidopsis, Nature 426, 147–153.

Huang WT, Bai GX, Wang J, Zhu W, Zeng QS, Lu K, Sun SY, Fang ZM. 2018. Two splice variants of OsNPF7;7 regulate shoot branching and nitrogen utilization efficiency in rice. Frontiers in Plant Science 9, 300.

Itoh J, Nonomura K, Ikeda K, Yamaki S, Inukai Y, Yamagishi H, Kitano H, Nagato Y. 2005. Rice plant development: from zygote to spikelet. Plant & Cell Physiology 46, 23–47.

Itoh H, Ueguchi-Tanaka M, Sato Y, Ashikari M, Matsuoka M. 2002. The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. The Plant Cell 14, 57–70.

Jia Z, von Wirén N. 2020. Signaling pathways underlying nitrogen-dependent changes in root system architecture: from model to crop species. Journal of Experimental Botany 71, 4393–4404.

Kamada-Nobusada T, Makita N, Kojima M, Sakakibara H. 2013. Nitrogen-dependent regulation of de novo cytokinin biosynthesis in rice: the role of glutamine metabolism as an additional signal. Plant & Cell Physiology 54, 1881–93.

Kellogg EA, Camara PEAS, Rudall PJ, Ladd P, Malcomber ST, Whipple CJ, Doust AN. 2013. Early inflorescence development in the grasses (Poaceae). Frontiers in Plant Science 4, 250.

Kim T, Krapp A. 2016. Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. Plant & Cell Physiology 57, 707–714.

Kudo K, Tudo K, Toida M, Sakakibara H. 2011. Hormonal control of nitrogen acquisition: roles of auxin, abscisic acid, and cytokinin. Journal of Experimental Botany 62, 1399–1409.

Kral AS, Xu S, Graeber TG, Braas D, Christofk HR. 2016. Asparagine promotes cancer cell proliferation through use as an amino acid exchange factor. Nature Communications 7, 11457.

Ladha JK, Kirch GJ, Bennett J, Peng S, Reddy CK, Reddy PM, Singh U. 1998. Opportunities for increased nitrogen-use efficiency from improved lowland rice germplasm. Field Crops Research 56, 41–71.

Landrein B, Formosa-Jordan P, Malivert C, Melnyk CW, Yang W, Turnbull C, Meyerowitz EM, Locke JCW, Jönsson H. 2018. Nitrate modulates stem cell dynamics in Arabidopsis shoot meristems through cytokinins. Proceedings of the National Academy of Sciences, USA 115, 1382–1387.

Leng Y, Gao Y, Chen L, et al. 2020. Using Heading date 1 preponderant alleles from indica cultivars to breed high-yield, high-quality japonica rice varieties for cultivation in south China. Plant Biotechnology Journal 18, 119–128.

Li H, Zhou F, Du W, et al. 2016. Knockdown of asparagine synthetase by RNAi suppresses cell growth in human melanoma cells and epidermoid carcinoma cells. Biotechnology and Applied Biochemistry 63, 329–333.

Li S, Ding X, Kuang Q, Ata-Ul-Karim ST, Cheng T, Liu X, Tian Y, Zhu Y, Cao W, Cao Q. 2018a. Potential of UAV-based active sensing for monitoring rice leaf nitrogen status. Frontiers in Plant Science 9, 1834.

Li S, Tian Y, Wu K, et al. 2018b. Modulating plant growth–metabolism co-ordination for sustainable agriculture. Nature 560, 595–600.

Liao Z, Yu H, Duan J, et al. 2018b. Modulating plant growth–metabolism co-ordination for sustainable agriculture. Nature 560, 1881–93.

Lin YL, Tsay YF. 2017. Influence of differing nitrate and nitrogen availability on flowering control in Arabidopsis. Journal of Experimental Botany 68, 2603–2609.

Liu J, Moore S, Chen C, Lindsey K. 2017. Crosstalk complexities between auxin, cytokinin, and ethylene in Arabidopsis root development: from experiments to systems modeling, and back again. Molecular Plant 10, 1490–1496.

Liu KH, Diener A, Lin Z, Liu C, Sheen J. 2020. Primary nitrate responses via calcium signaling and diverse protein phosphorylation. Journal of Experimental Botany 71, 4428–4441.
Luo KH, Huang CY, Tsay YF. 1999. CHL1 is a dual-affinity nitrate transporter of Arabidopsis involved in multiple phases of nitrate uptake. The Plant Cell 11, 855–867.

Liu Y, Tarkowski YF, Wang QS, Meng DX, Wang SH. 2011. Effects of nitrogen and 6-benzylaminopurine on rice tiller bud growth and changes in endogenous hormones and nitrogen. Crop Science 51, 786–792.

Liu Y, von Wirén N. 2017. Ammonium as a signal for physiological and morphological responses in plants. Journal of Experimental Botany 68, 2581–2592.

Lu G, Coneva V, Casaretta JA, Ying S, Mahmood K, Liu F, Nambara E, Bi YM, Rothstein SJ. 2015. OsPIN5b modulates rice root (Oryza sativa) plant architecture and yield by changing auxin homeostasis, transport and distribution. The Plant Journal 83, 913–925.

Lu K, Wu B, Wang J, Zhu W, Nie H, Qian J, Huang W, Fang Z. 2018. Blocking amino acid transporter OsAAP3 improves grain yield by promoting outgrowth buds and increasing tiller number in rice. Plant Biotechnology Journal 16, 1710–1722.

Lu N, Zhou J, Han Z, Li D, Cao Q, Yao X, Tian Y, Zhu Y, Cao W, Cheng T. 2019. Improved estimation of aboveground biomass in wheat from RGB imagery and point cloud data acquired with a low-cost unmanned aerial vehicle system. Plant Methods 15, 17.

Luo L, Pan S, Liu XH, Wang XU, Xu GH. 2017. Nitrogen deficiency inhibits cell division-determined elongation, but not initiation, of rice tiller buds. Israel Journal of Plant Sciences 64, 32–40.

Luo L, Qin RY, Liu T, Yu M, Yang TW, Xu GH. 2019. OsASN1 plays a critical role in asparagine-dependent rice development. International Journal of Molecular Sciences 20, 130.

Macadam JW, Voleneck JJ, Nelson CJ. 1989. Effects of nitrogen on mesophyll cell division and epidermal cell elongation in tall fescue leaf blades. Plant Physiology 89, 549–556.

Maeda Y, Konishi M, Kiba T, Sakuraba Y, Sawaki N, Kurai T, Ueda Y, Sakakibara H, Yasunaga S. 2018. A NIGT1-centred transcriptional cascade regulates nitrate signalling and incorporates phosphorus starvation signals in Arabidopsis. Nature Communications 9, 1376.

Makino A. 2011. Photosynthesis, grain yield, and nitrogen utilization in rice and wheat. Plant Physiology 155, 125–129.

Menand B, Desnos T, Nussaume L, Berger F, Bouchez D, Meyer C, Robaglia C. 2002. Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene. Proceedings of the National Academy of Sciences, USA 99, 6422–6427.

Miao J, Guo D, Zhang J, Huang G, Qin G, Zhang X, Wan J, Gu H, Qu LJ. 2013. Targeted mutagenesis in rice using CRISPR-Cas system. Cell Research 23, 1233–1236.

Miyawaki K, Tarkowski P, Matsumoto-Kitano M, Kato T, Sato S, Tarkowska D, Tabata S, Sandberg G, Kakimoto T. 2006. Roles of Arabidopsis ATP/ADP isotransferases and RNA isotransferases in cytokinin biosynthesis. Proceedings of the National Academy of Sciences, USA 103, 16598–16603.

Monostori I, Szira F, Tondelli A, Árendás T, Gierczik K, Cattivelli L, Galiba G, Vágújfali V. 2017. Genome-wide association study and genetic diversity analysis on nitrogen use efficiency in a Central European winter wheat (Triticum aestivum L.) collection. PLoS One 12, e0189265.

Murase K, Hirano Y, Sun TP, Hakoshima T. 2008. Gibberellin-Induced DELLA recognition by the gibberellin receptor GID1. Nature 456, 459–463.

Nehe AS, Misra S, Murchie EH, Chinnathambi K, Foulkes MJ. 2016. Nitrate supply affects root growth differently in two rice cultivars differing in nitrogen use efficiency. Plant and Soil 343, 357–368.

Song WJ, Makeen K, Wang DS, Zhang CM, Xu YH, Zhao HJ, Tu E, Zhang YL, Shen QR, Xu GH. 2011. Nitrate supply affects root growth differentially in two rice cultivars differing in nitrogen use efficiency. Plant Biology 13, 1–6.

Shimada A, Ueguchi-Tanaka M, Nakatsu T, Nakajima M, Naoe Y, Ohmiya H, Kato H, Matsuoka M. 2008. Structural basis for gibberellin recognition by its receptor GID1. Nature 456, 520–523.

Singh J, Smith S, De Rybel B, Van Den Broeke J, Smet W, De Coker S, Mispleaere M, De Smet I, Beeckman T. 2013. The CEP family in plant lands: evolutionary analyses, expression studies, and role in Arabidopsis shoot development. Journal of Experimental Botany 64, 5371–5381.

Sasakura H, Yanagisawa S. 2018. A NIGT1-centred transcriptional network controls nitrogen starvation affect root development. Nature Communications 9, 2550–2561.

Roberts I, Smith S, De Rybel B, Van Den Broeke J, Smet W, De Coker S, Mispleaere M, De Smet I, Beeckman T. 2013. The CEP family in plant lands: evolutionary analyses, expression studies, and role in Arabidopsis shoot development. Journal of Experimental Botany 64, 5371–5381.

Rivin CJ, Fangman WL. 1980. Cell cycle phase expansion in nitrogen-limited cultures of Saccharomyces cerevisiae. Journal of Cell Biology 85, 96–107.

Shimizu S, Mori H. 1998. Analysis of cycles of dormancy and growth in pea axillary buds based on mRNA accumulation patterns of cell cycle-related genes. Plant Cell & Physiology 39, 255–262.

Song WJ, Li J, Sun HW, Huang SJ, Gong XP, Ma QY, Zhang YL, Xu GH. 2013. Increased photosynthetic capacity in response to nitrate is correlated with enhanced cytokinin levels in rice cultivars with high responsiveness to nitrogen nutrients. Plant and Soil 373, 981–993.

Song WJ, Makeen K, Wang DS, Zhang CM, Xu YH, Zhao HJ, Tu E, Zhang YL, Shen QR, Xu GH. 2011. Nitrate supply affects root growth differentially in two rice cultivars differing in nitrogen use efficiency. Plant and Soil 343, 357–368.

Song X, Lu Z, Yu H, et al. 2017. IPA1 functions as a downstream transcription factor repressed by D53 in strigolactone signaling in rice. Cell Research 27, 1128–1141.

Srikanth A, Schmid M. 2011. Regulation of flowering time: all roads lead to Rome. Cellular and Molecular Life Sciences 68, 2013–2037.

Steketee CJ, Sinclair TR, Riar MK, Schapaugh WT, Li Z. 2019. Unraveling the genetic architecture for carbon and nitrogen related traits and leaf hydraulic conductance in soybean using genome-wide association analyses. BMC Genomics 20, 811.

Sui ZP, Wang TY, Li HQ, Zhang M, Li YY, Xu RB, Xing GF, Ni ZF, Xin MM. 2016. Overexpression of peptide-encoding OsCZEP1 results in pleiotropic effects on growth in rice (O. sativa). Frontiers in Plant Science 7, 288.

Sun H, Bi Y, Tao J, et al. 2016. Strigolactones are required for nitric oxide to induce root elongation in response to nitrogen and phosphate deficiencies in rice. Plant, Cell & Environment 39, 1473–1484.

Sun H, Tao J, Liu S, Huang S, Chen S, Xie X, Yoneyama K, Zhang Y, Xu G. 2014. Strigolactones are involved in phosphate- and
nitrate-deficiency-induced root development and auxin transport in rice. Journal of Experimental Botany 65, 6735–6746.

Sutton MA, Oenema O, Erisman JW, Leip A, van Grinsven H, Winiwarter W. 2011. Too much of a good thing. Nature 472, 159–161.

Tabata R, Sumida K, Yoshii T, Ohyama K, Shinohara H, Matsuhashi Y. 2014. Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. Science 346, 343–346.

Takei K, Ueda N, Aoki K, Kuromori T, Hirayama T, Shinozaki K, Yamaya T, Sakakibara H. 2004. AtIPT3 is a key determinant of nitrate-dependent cytokinin biosynthesis in Arabidopsis. Plant & Cell Physiology 45, 1035–1062.

Taleski M, Imin N, Djordjevic MA. 2018. CEP peptide hormones: key players in orchestrating nitrogen-demand signaling, root nodulation, and lateral root development. Journal of Experimental Botany 69, 1829–1836.

Tang Z, Fan X, Li Q, Feng H, Miller AJ, Shen Q, Xu G. 2012. Knockdown of a rice stelar nitrate transporter alters long-distance translocation but not root influx. Plant Physiology 160, 2052–2063.

Tavormina P, De Coninck B, Nikonorova N, De Smet I, Cammue BP. 2015. The Plant peptidome: an expanding repertoire of structural features and biological functions. The Plant Cell 27, 2005–2118.

Teale WD, Paponov IA, Palme K. 2006. Auxin in action: signalling, transport and the control of plant growth and development. Nature reviews. Molecular cell biology 7, 847–859.

Tegeder M, Masclaux-Daubresse C. 2018. Source and sink mechanisms of nitrogen transport and use. New phytologist 217, 35–53.

Thu TTP, Yamakawa T, Moe K. 2014. Effect of nitrogen application timing on growth, grain yield and eating quality of the KD18 and TH3-3 rice varieties. Journal of the Faculty of Agriculture Kyushu University 59, 55–64.

Tian G, Gao L, Kong Y, Hu X, Xie K, Zhang R, Ling N, Shen Q, Guo S. 2017. Improving rice population productivity by reducing nitrogen rate and increasing plant density. PLoS One 12, e0182310.

Tian Q, Chen F, Liu J, Zhang F, Mi G. 2008. Inhibition of maize root growth by high nitrate supply is correlated with reduced IAA levels in roots. Journal of Plant Physiology 165, 942–951.

Umehara M, Hanada A, Yoshida S, et al. 2008. Inhibition of shoot branching by new terpenoid plant hormones. Nature 455, 195–200.

Vidal EA, Moyano TC, Canales J, Gutierrez RA. 2014. Nitrogen control of developmental phase transitions in Arabidopsis thaliana. Journal of Experimental Botany 65, 5611–5618.

Walch-Liu P, Ivanov II, Filleur S, Gan Y, Remans T, Forde BG. 2006. Nitrogen regulation of root branching. Annals of Botany 97, 875–881.

Wang B, Smith SM, Li J. 2018a. Genetic regulation of shoot architecture. Annual Review of Plant Biology 69, 437–468.

Wang D, Fahad S, Saud S, Kamran M, Khan A, Khan MN, Hammad HM, Nasim W. 2019a. Morphological acclimation to agronomic manipulation in Arabidopsis. Journal of Plant Research 132, 499–510.

Wang J, Lu K, Nie H, Zeng Q, Wu B, Qian J, Fang Z. 2018. Rice nitrate transporter OsNPF7.2 positively regulates tiller number and grain yield. Rice 11, 12.

Wang J, Wu B, Lu K, Wei Q, Qian J, Chen Y, Fang Z. 2019b. The amino acid permease S (OsAAP5) regulates tiller number and grain yield in rice. Plant Physiology 180, 1031–1045.

Wang R, Xing X, Wang Y, Tran A, Crawford NM. 2009. A genetic screen for nitrate regulatory mutants captures the nitrate transporter gene NRT1.1. Plant Physiology 151, 472–478.

Wang Y, Li J. 2008. Molecular basis of plant architecture. Annual Review of Plant Biology 59, 253–279.

Watanabe K, Guo W, Arai K, et al. 2017. High-throughput phenotyping of sorghum plant height using an unmanned aerial vehicle and its application to genomic prediction modeling. Frontiers in Plant Science 8, 421.

Woodward AW, Bartel B. 2005. Auxin: regulation, action, and interaction. Annals of Botany 95, 707–735.

Xue M, Zhu L, Shou H, Wu P. 2005. A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. Plant & Cell Physiology 46, 1674–1681.

Xuan W, Beeckman T, Xu G. 2017. Plant nitrogen nutrition: sensing and signaling. Current Opinion in Plant Biology 39, 57–65.

Yang JT, Schneider HM, Brown KM, Lynch JP. 2019. Genotypic variation and nitrogen stress effects on root anatomy in maize are node specific. Journal of Experimental Botany 70, 5311–5325.

Yano K, Morinaka Y, Wang F, et al. 2019. GWAS with principal component analysis identifies a gene comprehensively controlling rice architecture. Proceedings of the National Academy of Sciences, USA 116, 21262–21267.

Yi J, Gao J, Zhang W, Zhao C, Wang Y, Zhen X. 2019. Differential uptake and utilization of two forms of nitrogen in japonica rice cultivars from north-eastern China. Frontiers in Plant Science 10, 1061.

Yoneyama K, Xie X, Kim HI, Kisugi T, Nomura T, Sekimoto H, Yokota T, Yoneyama K. 2012. How do nitrogen and phosphorus deficiencies affect strioloaglone production and exudation? Planta 235, 1197–1207.

Yoneyama K, Xie X, Kusumoto D, Sekimoto H, Sugimoto Y, Takeuchi Y, Yoneyama K. 2007. Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystigrol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. Planta 227, 125–132.

Yoshida H, Horie T, Shiraiwa T. 2006. A model explaining genotypic and environmental variation of rice spikelet number per unit area measured by cross-sectional experiments in Asia. Field Crops Research 97, 337–343.

Yu Q, Wang X, Wang L, Zheng J, Wang J, Wang B. 2016. Knockdown of asparagine synthetase (ASNS) suppresses cell proliferation and inhibits tumor growth in gastric cancer cells. Scandinavian Journal of Gastroenterology 51, 1220–1226.

Yuan S, Zhang ZW, Zheng C, et al. 2016. Arabidopsis cryptochrome 1 functions in nitrogen regulation of flowering. Proceedings of the National Academy of Sciences, USA 113, 7661–7666.

Zaman-Allah M, Vergara O, Araus JL, et al. 2015. Unmanned aerial platform-based multi-spectral imaging for field phenotyping of maize. Plant Methods 11, 35.

Zhang N, Gibon Y, Wallace JG, et al. 2015. Genome-wide association of carbon and nitrogen metabolism in the maize nested association mapping population. Plant Physiology 168, 575–583.

Zhang Q, Li J, Zhang W, Yan S, Wang R, Zhao J, Li Y, Qi Z, Sun Z, Zhu Z. 2012. The putative auxin efflux carrier OsPIN3 is involved in the drought stress response and drought tolerance. The Plant Journal 72, 806–816.

Zhang W, Wu L, Ding Y, et al. 2017. Nitrogen fertilizer application affects lodging resistance by altering secondary cell wall synthesis in japonica rice (Oryza sativa L.). Journal of Plant Research 130, 859–871.

Zhang YL, Fan JB, Wang DS, Shen QR. 2009. Genotypic differences in grain yield and physiological nitrogen use efficiency among four rice cultivars. Plosphere 19, 681–691.

Zhuang L, Ge Y, Wang J, Yu J, Yang Z, Huang B. 2009. Genotypic variation of asparagine synthetase (ASNS) suppresses cell proliferation and inhibits tumor growth in gastric cancer cells. Scandinavian Journal of Gastroenterology 51, 1220–1226.

Wu K, Wang S, Song W, et al. 2020. Enhanced sustainable green revolution yield via nitrogen-responsive chromatin modulation in rice. Science 367, eaaz2048.

Wu L, Zhang W, Ding Y, et al. 2017. Shading contributes to the reduction of stem mechanical strength by decreasing cell wall synthesis in japonica rice (Oryza sativa L.). Frontiers in Plant Science 8, 881.

Xie Y, Yoneyama K, Yoneyama K. 2010. The strigolactone story. Annual Review of Phytopathology 48, 93–117.

Xing Y, Zhang Q. 2010. Genetic and molecular bases of rice yield. Annual Review of Plant Biology 61, 421–442.

Xu G, Fan X, Miller AJ. 2012. Plant nitrogen assimilation and use efficiency. Annual Review of Plant Biology 63, 153–182.

Wu M, Zhu L, Shou H, Wu P. 2005. A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. Plant & Cell Physiology 46, 1674–1681.