News & Views

Phytohormonal networks facilitate plant root developmental adaptations to environmental changes

Hui Liu [a], Xiangdong Fu [a,b,*]

[a] New Cornerstone Science Laboratory, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China
[b] College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

In vascular plants, roots are important underground organs that anchor the plant in the ground and absorb water and many elemental nutrients from the soil. Root system architecture (RSA) plays critical roles in not only determining a plant's ability to acquire soil resources but also improving plant survival under various dynamic environmental conditions. Acquisition of water and nutrients is essential for crop performance, but low levels of water and nutrient availability in most agricultural soils limit crop production. Therefore, modulation of RSA enables enhanced water- and nutrient-use efficiency for future agricultural sustainability and food security. The current studies have shown that root morphological plasticity, including primary root elongation, lateral root formation, and root hair emergence and growth, is considered closely linked to plant hormone signaling pathways [1]. The advancements in understanding molecular mechanisms of hormonal networks involved in triggering plant root developmental adaptations to various environmental conditions can be exploited to improve sustainable productivity in agriculture.

The adaptive phenomenon of root tips sensing moisture gradient in soil and growing towards higher water potential is known as root hydrotropism, which is crucial for plant survival under drought conditions. Cytokinins (CKs) have been shown to play important roles in directing root growth orientation in response to hydrostimulation (a moisture gradient). The lower water potential side of the root tips exhibits a higher cytokinin response than the higher water potential side. Two cytokinin type-A Arabidopsis response regulators (ARRs), ARR16 and ARR17, are shown to be up-regulated at the lower water potential side, which causes increased cell division in the meristem zone, allowing the roots to bend towards the higher water potential side [2]. Abscisic acid (ABA) has also been implicated in the hydrotropism response of roots. Subclass III Snf1-related kinases (SnRK2s) and mizu-kussei1 (MIZ1) are required for plant ABA response and are specifically expressed in the root cortex of the elongation zone and are responsible for hydrotropism. Heterogeneous moisture distribution at the root tips triggers MIZ1-mediated calcium (Ca^{2+}) signals. This Ca^{2+} wave propagates through the phloem to the elongation zone, resulting in asymmetric distribution of Ca^{2+} based on the water gradient. MIZ1 also is involved in the asymmetric re-distribution of cytokinins driven by hydrotropism. In addition, low concentrations of ABA induce root growth and promote hydrotropism by both inhibiting the activity of protein phosphatase 2C (PP2C) phosphatases and enhancing root apoplastic H^+ efflux via H^+-ATPase2 under salt and drought conditions [3]. Auxin response factor 7 (ARF7) is known to be essential for gravitropic responses through regulation of auxin homeostasis, and the previous study has demonstrated that ARF7 is involved in regulating root hydropatterning [4]. In lateral root founder cells, ARF7 induces asymmetric expression of *lateral organ boundaries domain16* (*LBD16*). The expression pattern of *LBD16* is regulated by post-translational modification of ARF7 through small ubiquitin-like modifier (SUMO) protein. The SUMOylation of ARF7 is required for recruiting the Aux/IAA repressor protein IAA3 but blocking ARF7 SUMOylation interferes with IAA3 recruitment and root hydropatterning [4] (Fig. 1 a).

Nitrogen (N) is one of the most important plant nutrients affecting almost all aspects of plant growth and development, and plays a powerful role in the promotion of crop production. Nitrate (NO_3^-) and ammonium (NH_4^+) are two major forms of inorganic N uptake for plants. NO_3^- represents the most readily available forms of N for root absorption from the aerobic soil. Besides its role as a nutrient, NO_3^- has been shown to act as a signal molecule in regulating plant root development and metabolic adaptations to changes in N availability. RSA plasticity is a vital characteristic that enables plants to survive and sustain growth under different nutrient environments [5]. Indeed, NO_3^- deficiency and NO_3^- excess have a dual impact on plant root growth and development. Low NO_3^- concentrations (0.2 to 0.55 mmol/L) promote primary and lateral root (LR) growth, whereas severe NO_3^- deficiency (<0.1 mmol/L) inhibits root system growth, particularly in terms of LR elongation in *Arabidopsis*. The moderate supply of NO_3^- (0.55 to 1 mmol/L) can enhance the initiation of lateral roots, whereas excessive NO_3^- concentrations (>10 mmol/L) induce inhibition of root elongation. Phytohormones serve as intermediaries, triggering the perception and transduction of N signals of plants and thus dynamically adjusting RSA. Among these, auxin plays a central role in regulating LR emergence and elongation in response to NO_3^- deficiency (Fig. 1b).

When NO_3^- deficiency becomes severe, nitrate transporter 1.1 (NRT1.1, also termed as NPF6.3) functions as a NO_3^--controlled
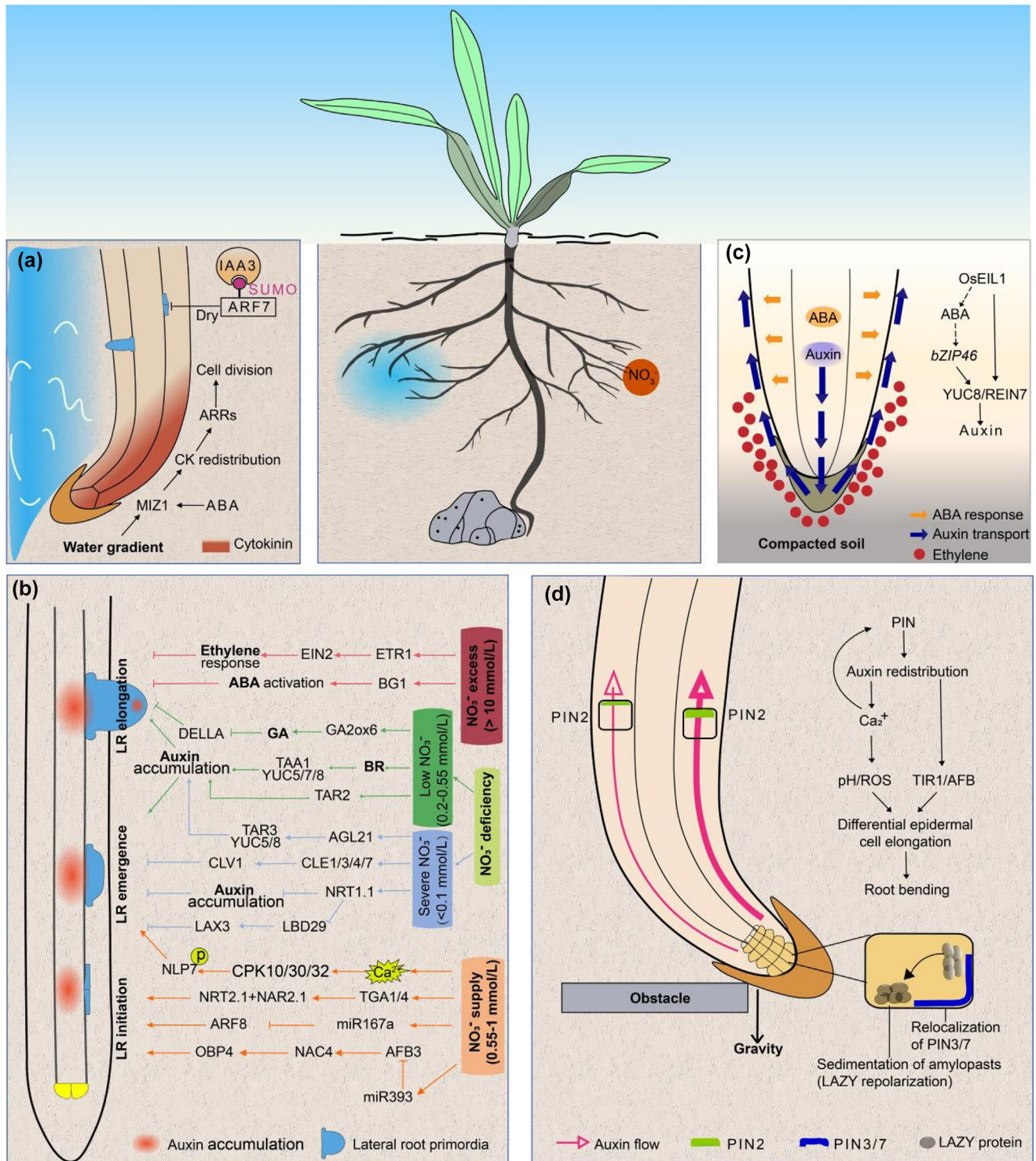
Fig. 1. Plant root system architecture in response to changes in soil environment. (a) The hydrotropism in roots. When the roots encounter an environment with varying water potential, cytokinins tend to accumulate on the side with lower water potential and that leads to the up-regulation of *ARRs*, specifically *ARR16* and *ARR17*, resulting in increased cell division on the lower water potential side, thus consequently exhibiting a bending response towards the side with higher water potential. (b) Phytohormones modulate the root system architecture in response to different concentrations of nitrogen. Under nitrate (NO_3^-) limitation, the growth of both primary and lateral root are promoted. When nitrogen supply is ample, primary and lateral root growth are suppressed. The multiple signal transduction pathways participate in regulating primary root (PR) elongation and lateral root (LR) development in response to different NO_3^- supply. Only the pathways highlighted in the present review are depicted. (c) The effects of soil compaction on root growth response. When the soil is compacted, the diffusion of ethylene is restricted, which leads to OsEIL1 accumulation in the tips of roots, thus activating *YUC8* and promoting the biosynthesis of auxin in epidermal cells. OsEIL1 also activates ABA biosynthesis in cortical cells, causing radial expansion of root cortical cells and causing root swelling. In addition, increased ABA activates OsbZIP46, which in turn promotes the expression of *YUC8*, increasing auxin biosynthesis and thus inhibiting the elongation of epidermal cells and root elongation, consequently resulting in shorter and swollen roots. (d) Gravity sensing occurs in the columella cells of the root cap. The sedimentation of starch-filled plastids (amyloplasts) triggers a pathway that leads to the relocation of PIN proteins to the lower side of the cell. The PIN proteins facilitate the efflux of auxin. The TIR1/AFB-mediated auxin signaling pathway plays a crucial role in both root gravitropism and thigmotropism by regulating the differential elongation of epidermal cells, resulting in root bending. Ca^{2+} waves have been identified as facilitators of root bending, operating through downstream pH and ROS signals during root gravitropism and obstacle avoidance.

auxin transporter, resulting in inhibiting LR emergence by reducing local auxin accumulation at LR primordia (LRP) tips. In addition, NRT1.1/NPF6.3 inhibits LR emergence through negative regulation of *LBD29* and *like-AUX3* (*LAX3*) [6]. Clavata3/endosperm surrounding region-related (CLE) peptides constitute a regulatory module in the NO_3^- signaling, exerting negative regulation on LR emergence [6]. Severe NO_3^- deficiency up-regulates *agamous-like21* (*AGL21*), a MADS-box transcription factor, to maintain auxin homeostasis during LR elongation. This process is impaired in the *agl21* mutant [6]. Auxin biosynthesis enzyme tryptophan aminotransferase related 2 (TAR2) catalyzes the conversion of L-Trp to indole-3-pyruvic acid, initiating the IPyA pathway that branches from Trp-dependent auxin biosynthetic pathway [7]. When grown under mild N deficiency, *TAR2* expression is induced in the pericycle and vasculature of mature root zones. Additionally, the *tar2* mutant exhibits a decrease in the number of lateral roots [7]. The up-regulation of auxin biosynthesis genes *tryptophan aminotransferase of arabidopsis1(TAA1)* and *yucca* (*YUC3, 5, 7, 8*) serves as a central component connecting with brassinosteroid (BR) in the modulation of LR development in response to low concentrations of NO_3^- [8]. However, the molecular mechanism underlying the crosstalk between BR and auxin in regulating RSA plasticity remains unclear. Furthermore, crosstalk exists between NO_3^- and gibberellic acid (GA) signaling pathway. Low NO_3^- reduces the abundance of the growth-repressing DELLA proteins by increasing the expression of bioactive GA metabolism genes, thus promoting root elongation [9]. Recent studies have shown that the key component of GA signaling, growth-regulating factor4 (GRF4), coordinates and integrates plant growth, carbon fixation, and N assimilation by co-regulating expressions of those genes involved in cell cycle control, photosynthesis, and N metabolism. Most importantly, both N-use efficiency and grain yield of high-yield cereal crops are increased by tipping the GRF4-DELLA balance towards increased GRF4 abundance [10].

Auxin modulates LR development in response to homogeneous high NO_3^- supply. The high NO_3^- supply induces the expression levels of the auxin receptor gene *auxin signaling F-box 3* (*AFB3*) that is inhibited by miR393, which in turn activates the downstream gene *NAC4* and its target gene *OBF binding protein 4* (*OBP4*), thereby promoting LR initiation [6]. Intriguingly, *AFB3* has been identified as a target of (nodule inception)-like protein 7 (NLP7), which acts as NO_3^- sensor or downstream regulator of NRT1.1/NPF6.3-mediated NO_3^- signaling. NO_3^- also promotes auxin signaling and LR initiation in the pericycle through the miR167-ARF8 regulatory module. Specifically, the products of NO_3^- assimilation, glutamine, and glutamate, reduce the expression level of miR167 in the pericycle, allowing *ARF8* to accumulate and consequently stimulating LR initiation and outgrowth [6]. Furthermore, NO_3^- responsive TGACG-sequence-specific binding-protein1 (TGA1) and TGA4 directly bind to and activate the NO_3^- transporter genes *NRT2.1* and *NRT2.2*, triggering LR initiation. However, the partial reduction of LR density observed in the *nrt2.1/nrt2.2* double mutant when compared to the *tga1/tga4* double mutant suggests that TGA1 and TGA4 may target other genes to support NO_3^--induced LR initiation. TGA1/4 can directly regulate the expression of the root hair-specific gene caprice (CPC), leading to an increase in root hair density in *Arabidopsis* [6]. Ca^{2+} signaling plays an important role in the stimulatory effect of NO_3^- on lateral root formation. Intracellular NO_3^-, transported by NRT1.1/NPF6.3, induces cytosolic Ca^{2+} waves, which are decoded by the subgroup III Ca^{2+}-sensor protein kinases (CPK10/30/32). These CPKs phosphorylate NLP6/7, enabling them to retain their nuclear localization, thereby promoting the progression and emergence of LRP [11]. Although an excessive supply of NO_3^- inhibits LR elongation, this inhibitory effect can be relieved in ethylene signaling mutants, for example, *Arabidopsis ethylene resistant1-3* (*etr1-3*) and *ethylene insensitive2-1* (*ein2-1*).

The expression levels of *NRT1.1/ NPF6.3* and *NRT2.1* in *etr1-3* and *ein2-1* mutants are insensitive to changes in NO_3^- availability, suggesting that ethylene is required for LR development in response to excessive NO_3^- supply [12]. When NO_3^- levels increases in the root environment, it triggers the gradual accumulation of ABA in the root tips. This process ultimately regulates NO_3^- uptake and assimilation. The increase in ABA levels in the root tips is caused by the activity of ER-localized β-glucosidase 1 (BG1), which releases bioactive ABA from the inactive ABA-glucose ester, resulting in promoting the expression of NO_3^- metabolism-associated genes and thus activating a negative feedback loop simultaneously [13]. Over the past few years, the underlying mechanisms of N-responsive RSA have been investigated and are found to be highly conserved across various plant species. for instance, modulating the wheat ortholog of *Arabidopsis TAR2* enables enhanced LR branching and grain yield [14], suggesting that the advancements in knowledge from model plants can be exploited to improve crop N-use efficiency.

Soil compaction is a significant global issue that causes insufficient root development and low crop production worldwide. In agricultural soils, compaction is primarily caused by excessive vehicular traffic, the use of farm equipment that surpasses the soil's bearing capacity, and tilling when the soil moisture content is unsuitable, particularly in wet soils. Rice, a semi-aquatic plant, thrives in water-saturated environments for most of its life cycle. Therefore, rice is more vulnerable to soil compaction [15]. The rice *EIN3*-like gene, *OsEIL1*, encodes a crucial transcription factor in the ethylene signaling. Both ethylene and soil compaction promote the accumulation of OsEIL1 protein in roots. Previous studies have shown that OsEIL1 inhibits root growth via directly binding to the promoter of *YUC8/REIN7*, activating its expression and resulting in auxin accumulation in roots. In recent research, Qin et al. [16] demonstrated that ABA mediates *OsbZIP46* to activate the expression of *YUC8/REIN7*, thereby regulating root system growth. Therefore, *YUC8/REIN7* serves as a key role in integrating ethylene and ABA to regulate root growth in response to soil compaction (Fig. 1c).

Plants perceive their inclination relative to the direction of gravity and adjust their growth direction accordingly, a process known as geotropism. The roots of plants are stimulated by mechanical resistance as they grow downwards, which is crucial for plants to respond to these stimuli and modify their growth patterns, as it can regulate agricultural traits such as drought tolerance and mineral nutrient absorption. Normally, gravitropism encompasses three key processes: gravity sensing, signal transduction, and differential growth response. According to the "starch-equilibrium stone" hypothesis, when the orientation of plants changes relative to the gravity vector, starch bodies (containing plastids) within equilibrium stone cells (root tip column cells and stem endothelial layer cells) settle to the new bottom of these cells, thus initiating the transmission of gravity signals. The subsequent transmission of gravity signals causes an asymmetric distribution of auxin in roots or stems, thereby regulating their curved growth [17]. Two new studies have elucidated the molecular mechanisms behind the "starch equilibrium stone" hypothesis. When plants deviate from the direction of gravity, starch bodies can carry LAZY proteins through their surface TOC (translocon at the outer envelope membrane of chloroplasts) proteins and settle together, guiding LAZY to form new polar distributions on the cell membrane along the direction of gravity. This process ultimately regulates plant gravitropism [18,19]. When the roots detect an obstacle, a stepped growth pattern occurs where the roots grow parallel to the obstacle while maintaining contact with it. During this period, the roots undergo two consecutive bending reactions, which are associated with the asymmetric growth of cells in the elongation zone of the root tips. The interplay between touch and gravity signals

directs root growth to circumvent obstacles in the soil while generally maintaining a downward trajectory (Fig. 1d) [20]. Indeed, auxin biosynthesis, polar transport, and signal transduction play important roles in controlling the gravitropic curvature of the roots. Most *Arabidopsis* mutants related to auxin exhibit curling of the main root during growth. The polar auxin transport carriers, auxin1/like-aux1 (AUX1/LAX) and pin-formed (PIN) families, play key roles in the response of the gravity process within the columella cells of root tips. Upon gravistimulation, PIN3 and PIN7, initially positioned symmetrically, quickly relocate to the new bottom side of the columella cells in the root apex, thus consequently facilitating the redirection of auxin flow towards the lower side of the root tips. Subsequently, PIN2 aids in further transportation of auxin towards the elongation zone. The asymmetric auxin flow is reinforced by PIN2 stabilization in cells along the lower side of the roots and its destabilization on the opposite side. Similar to root gravitropism, asymmetric auxin flow is also observed during root obstacle avoidance. As a consequence, auxin inhibits growth on the lower side of the roots through the swift action of transport inhibitor response 1/auxin-signaling F-box (TIR1/AFB) auxin signaling in gravitropism. Thigmotropism, which involves root bending, also relies on TIR1/AFB-mediated auxin signaling. However, the specific downstream cellular processes that regulate root growth in both types of tropism remain unclear [20,21]. Apart from auxin, other signals such as Ca^{2+}, pH, and reactive oxygen species (ROS) also contribute to the responses of root gravitropism and thigmotropism processes. During root bending, the concentration of Ca^{2+} rapidly increases in the epidermal cells on the protruding side of the roots, leading to the generation of apoplastic ROS and cytoplasmic acidification, both of which are involved in promoting cell elongation. The interference of Ca^{2+} signaling can disrupt PIN-mediated auxin redistribution during obstacle avoidance, suggesting the presence of a feedback loop between the Ca^{2+} wave and auxin distribution [20] (Fig. 1d).

In terrestrial plants, the root is responsible for sensing diverse environmental cues and integrating these signals into intrinsic genetic regulators, thus optimizing plant response and adaptation to its environment. The morphology of the root systems of different plant species exhibits a great level of diversity under various environmental conditions, which ranges from deep-penetrating vertical roots to shallow horizontal roots, with various combinations of theses root types with each other. Within plant communities, these morphological diversities result in different plants having their roots placed in distinct layers that prevent plant roots from growing nearby, thus reducing competition for water and nutrients. Indeed, RSA of the major crops is also a crucial agronomic trait. Dicotyledonous crops such as soybean possess a taproot system, where the main root repeatedly branches out to produce numerous lateral roots. On the other hand, cereal crops have fibrous root systems, for example, rice has a root system composed of numerous crown roots (adventitious roots) that grow from the stem and root nodes. In low-input agricultural soils characterized by low nutrient availability, less soil moisture and higher soil hardness, an ideal RSA consists of longer lateral roots, thicker root diameters and longer and denser root hairs, allowing plants to better capture water and nutrients and adapt to harsh environment. Recent progress in unlocking the genetic basis of greener Green Revolution within the broader context of plant systems biology has provided insights into the plant growth-metabolic

coordination and inspired the implementation of a new breeding strategy to improve sustainable productivity in agriculture [10]. Identifying key components that crop plants respond to and adapt to their surroundings along with the use of precision gene modification will optimize crop breeding strategies and launch a new generation of sustainable Green Revolution.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Hui Liu received her Ph.D. degree from the Department of Agricultural Engineering, Shihezi University in 2021. She then served as a postdoctoral fellow (with Prof. Xiangdong Fu) at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. Since June 2023, she has been a research scientist at the same institute. Her research interests focus on the molecular mechanisms of integrative hormonal signalling in regulating plant root development.

Xiangdong Fu joined the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences as a principal investigator in 2005, and is currently a professor at the University of Chinese Academy of Sciences. His research interests include understanding the molecular mechanisms of how plants integrate and coordinate growth, carbon and nitrogen assimilation, and identifying new genes for improving grain yield and nitrogen-use efficiency in crops.