Improving Nitrogen Acquisition and Utilization Through Root Architecture Remodelling: Insight from Legumes

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Abstract

In crop species, nutrient deficiency severely damages plant growth and developmental processes, leading to end-yield penalties. Root architecture remodelling is considered a key factor underpinning nutrient-poor soil environments. Adequate nitrogen (N) supply can play a significant role in sustaining crop productivity on nutrient-deficient soils. However, excessive application of nitrogenous fertilizer may pollute the soil and increase the production cost for the growers. To tackle this problem, crop breeders have made tremendous efforts to improve the N-use efficiency of agricultural crops. This article summarizes the recent progress in identifying QTLs/genes, regulatory pathways, and hormonal crosstalk involved in the growth and development of legumes roots system. Moreover, we have described the progress in microbe–root symbiosis via QTLs/genes regulations, which results in improved N acquisition. Understanding the molecular mechanisms that regulate the root architecture in response to N availability may help to strengthen the root system of legumes and promote environmental friendly and sustainable agriculture.

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Introduction

Legumes play an essential role in contributing to food security and environmental maintenance (Graham et al. 2003). They are usually intercrop with cereals to improve land productivity through soil amelioration. In crop rotation, legumes as N_2-fixing plant contribute to diversify the cropping system. Although legumes fix nitrogen; about 50% of soil-nitrogen still needs to utilize efficiently by plants to improve and maintain crops yield at optimal levels. Nitrogen (N) is an essential mineral required in massive quantity by plants to produce sufficient energy for sustaining vegetative growth and achieving economic grain yield (Zörb et al. 2018; Wang et al. 2018).

The root is the primary organ modified in plants to acquire nutrients and water from the soil (Ramakrishna et al. 2019). The soil medium in which plant roots penetrate is highly heterogeneous in the distribution of minerals and water reservoirs. Plants have adopted various tactics to alter their root architecture in response to these heterogeneous distributions (Xu et al. 2022; Poitout et al. 2018). The root system architecture is affected by various abiotic and biotic

factors (Najeeb et al. 2015), including water starvation, N, potassium, and at phosphorus levels. Plants have evolved to survive under low N levels through long-term evolution, which might have involved the modifications in the root configuration, such as the primary root (PR) length, lateral root (LR) system, and root hairs structure (Rao et al. 2016; Qin et al. 2021).

Untangling the molecular mechanism, including various regulatory networks, proteins, and genes responding to low N stress, significantly improve the low N stress tolerance crop. A large number of genes are involved in the modification of root systems to enhance minerals acquisition in different plant species, such as maize (Li et al. 2011, 2019), *arabidopsis* (Yu et al. 2016), soybean (Kong et al. 2019), and rice (Sun et al. 2019; Kitomi et al. 2020). Furthermore, regulatory proteins (Araya et al. 2014), transcription factors (Devaiah et al. 2007; Architecture et al. 2012; Miura et al. 2020), and miRNAs also contribute to regulatory networks associated with the root system to improve nutrient-use efficiency (Meng et al. 2010; Vidal et al. 2010). In addition, the identification of numerous root architecture QTLs for N starvation in different crops including bean/common bean (Cichy et al. 2009), rice (Shimizu et al. 2008; Li et al.2009), maize (Li et al. 2015), and soybean (Liang et al. 2010) highlights the potential to improve nutrient use efficiency via marker-assisted breeding. This review provides a comprehensive overview of root architectural alterations under N-deficit conditions. It will help to modulate N deficiency molecular mechanisms for developing N-use efficient varieties (NUE) with superior root architecture.

Importance of Legumes in Conservation Agriculture

Grain and forage legumes are grown on approximately 180 million ha or 12% to 15% of the earth's farmland (Graham et al. 2003). Legumes account for 27% of the total global primary crop production, contributing 33% to the human needs for dietary proteins (Graham et al. 2003). Further, legumes cultivation improve soil fertility and organic matter contents and enhance soil water retention and nutrients circulation (Rasheed et al. 2010; Graham et al. 2003). Therefore, sustainable increase in legumes production is critical to feed the world's growing population. Likewise, by adopting sustainable agricultural production methods, we can minimize greenhouse gas emissions, food losses, and wastes, improve crop productivity and the global supply chain, and provide nutritional food to communities suffering from hunger and malnutrition. Therefore, introducing legumes in sustainable agriculture may offer a solid foundation for food safety, and security along with environmental quality (Vanlauwe et al. 2019).

The key advantages of legumes are; they fix atmospheric N, high quality of organic matter released into the soil, and improve soil C/N ratio (carbon-to-nitrogen ratio). Additionally, grain legumes have a robust and deep root system that encourages mycorrhizal mineral solubilization, recycling/ uptake, and water transport in deeper soil layers (Graham et al. 2003). Recently, some legumes such as faba bean, pea, chickpea, soybean, and lentil have widely been adopted in sustainable cropping systems and conservation agriculture in Turkey, Australia, Brazil, and North America (Stagnari et al. 2017).

Nitrogen (N) and Sustainable Crop Production

Sustainable agriculture refers to crop production which does not harm biodiversity, quality of crops, and the environment. Sustainable crop production relies on minimizing the pesticide usage through integrated pest management. Thus, it protects biodiversity, improves soil health, and ensures food quality and safety. N is an essential constituent of a plant cell as a mineral element and directly promotes protein formation in grain seeds (Worku et al. 2007; Perchlik and Tegeder 2020). It is also linked with crop yield and its accumulated amount in the plant is an essential factor for crop productivity (Khan et al. 2020). Considering this, NUE is a crucial parameter for crops yield, unfortunately, its usage efficiency is relatively low, e.g. accounts for 30% in China, 50–60% in developed countries, and approximately 59% globally (Williamson 2011). This significant loss has been causing a worse impact on our natural environment in the form of acid rain, soil acidification, polluting freshwater streams, and even air pollution, which ultimately raises human health issues (Bouwman et al. 2013; Udvardi et al. 2015).

Recently, various new high-yielded cultivars have been developed and they are primarily dependent on high input of nitrogenous fertilizers. These cultivars have been introduced into the cropping systems according to the preferences of breeders and farmers. However, increased application of nitrogenous fertilizers led to several problems, such as decreased N-use efficiency, high input cost, environmental pollution, and N fertilizer loss (Zhang et al. 2016). Consequently, it is now a consensus that it is necessary to balance the benefit of N usage to improve yield and reduce its adverse impacts by reducing the N fertilizer input and soil pollution. Hence, developing cultivars with tolerance to low N stress or improved NUE are critical aims for future crop breeding (Zhang et al. 2016). Achieving these goals will necessitate a comprehensive knowledge and understanding of N metabolism under N deficiency. Root-related traits have considerable influence on N capturing from the soil. However, there are still discrepancies over the impact of the

N deficit on root growth, length, and LR. Therefore, new cultivars with improved root architecture that can be grown under low N are prerequisites for sustainable agriculture (Xu et al. 2012).

Root Architecture Remodelling for Better N Acquisition

Plants alter their root system to extract N from the soil (Lay-Pruitt and Takahashi 2020). They can adjust to low N supply by directing root development towards N-rich patches and increasing the root absorptive surface area. Considering the low nitrate availability and high mobility within a given soil area, the definition of a more efficient root system may not be specific. It may differ and depends on soil type, plant species, and other environmental factors (Postma et al. 2014). Modelling techniques proposed that an effective capturing of nitrate results from a trade-off between the total volume of soil explored and the speed of N acquisition (Dunbabin et al. 2003).

A superior root architecture helps plants to explore soil and promote nutrients absorption. Therefore, the improvement of root architecture has widely been documented and considered an essential strategy to improve N uptake under N-deficient environments, which may accelerate the performance of roots in low N soils. N is an integral component of organic compounds in plants comprising protein, chlorophyll, and nucleic acid (Amtmann and Armengaud 2009). Thus, N deficiency is the main factor inhibiting plant growth and development, resulting in a significant drop in crops yield and productivity. Plants use different N forms in the rhizosphere, including nitrate, organic compounds (soluble N-containing), and ammonium (Tegeder and Rentsch 2010). In arabidopsis, for instance, the roots are adapted to different N supply levels and types, including stimulation of root branching, inhibition of LR initiation under a high C/N ratio, systemic/local regulation of LR growth, and inhibition of PR growth induced by exogenous L-glutamate (Zhang et al. 2007). In response to low N supply, nutrient acquisition is mainly enhanced through root growth and the deeper root system to absorb nitrate, which is one of the most movable nutrient ions in the soil (Ju et al. 2015; Rasmussen et al. 2015; Yu et al. 2015). Literature suggests that genotypes with deeper roots are more efficient in absorbing N from N-deficient soils (Saengwilai et al. 2014). Therefore, an ideotype of root architecture with strong LR, deeper roots, and robust nitrate response was proposed for effective N acquisition in intensive cropping systems (Zhang et al. 2007). A field trial-based study supported this by revealing that a broad and deep root system is a prerequisite for high N consumption in *Zea mays* (Mi et al. 2010). In legumes, modifications in root system architecture facilitate N and phosphorus acquisition and nodule formation (Egamberdieva et al. 2017). In a study, Egamberdieva et al. (2017) observed a significant positive relationship between the N content in plant tissue, the number of nodules, and the root system architecture of soybean.

Molecular Mechanisms of Root Architecture Remodelling in Response to N

Understanding the molecular mechanism of modifications in the root architecture of plants in response to specific nutrients would facilitate the genetic improvement of nutritional efficiency. In past years, many experiments have been designed to identify multiple QTLs/genes in plants related to changes in root architecture to cope with nutrient supply (Table 1).

Plants absorb N from the soil in two forms: organic (amino acids) or inorganic (ammonium or nitrate) (Hao et al. 2020). In ammonium foam, root growth is controlled by ammonium transporters (ATMs), they are crucial to optimize ammonium concentration in plants and regulate root responses to avoid ammonium toxicity (Hao et al. 2020). Legumes AMTs have functionally been characterized, and their transport properties are similar to those identified in arabidopsis (D'Apuzzo et al. 2004). In arabidopsis, two ATM families (ATM1 and ATM2) were identified (Yuan et al. 2007) and ATM2 family is involved in regulatory mechanisms. This is essential in recovering ammonium lost from nodule cells by efflux, whilst ATM1 regulates ammonium acquisition and transport. Two ATM2s and three ATM1s have been identified in *Lotus japonicus* (Salvemini et al. 2001). Under N-deficit conditions, *LjAMT1;*1 and *LjAMT1;2* are up-regulated, *LjAMT1;3* is down-regulated by higher ammonium concentrations and it regulates the root response to ammonium toxicity (Apuzzo et al. 2004; Rogato et al. 2010). *LjAMT2;1* controls ammonium loss from cellular efflux in nodules, whereas *LjATM2;2* is required for N transport and acquisition during AMF associations (Simon-rosin et al. 2003). Ammonium negatively influences the nodule formation by repressing the expression of *NIN (NODULE INCEPTION)*, an essential gene for nodulation (Barbulova et al. 2007). When ammonium is the sole N source, primary and LR elongation suppression is a commonly observed indicator of ammonium toxicity (Araya et al. 2016).

On the other hand, nitrate is the primary N source and absorbs through roots by various nitrate transporters. Several aspects of nitrate-dependent root architecture regulation have been discussed and reported in recent reviews (Imin et al. 2013; Forde 2014; Giehl et al. 2014). The modulation of root architecture in an N-deficit environment depends on the environmental conditions (day length and light intensity) and the strength of N limitation. The root development is

Table 1 QTLs/genes associated with root architecture in legume crops

Legume crops	QTLs/genes	Role	Reference
Soybean	*GmNRT1.2a, GmNTR1.2b*	Improve nodule numbers	(Guo-ji et al. 2020)
	GmEXPB2	Improve lateral root numbers	(Li et al. 2020)
	GmEXPB1	Improve lateral root	(Kong et al. 2019)
	GmNAC109	Improve lateral root formation	(Yang et al. 2019)
	RR-Gm01, RR-Gm03, RR-Gm04, RR-Gm08, RR-Gm020	Increase fibrous root and surface area	(Abdel-Haleem et al. 2011)
	SA_Gm06, TRL_Gm06, RDL3_Gm07	Improve root growth and length	(Prince et al. 2015)
	TRL_Gm08, LRN_Gm08	Improve lateral root numbers	(Manavalan et al. 2015)
Chickpea	*Ca-AFP*	Improve root growth and biomass	(Kumar et al. 2019)
	RLD4, RLD6, RDWR4	Improve root length and surface area	(Jaganathan et al. 2015)
Common bean	*SIN1*	Improve lateral root growth	(Battaglia et al. 2020)
	Brg1.1, Brg5.1, Brg5.2	Basal root angle	(Liao et al. 2004)
Cowpea	*Brg10, RD1, MW6, WA10*	Improve root diameter and basal root angle	(Burridge et al. 2016a, b)
Lotus japonicus	*LjHAR1*	Improve root structure	(Buzas and Ã 2007)
	LjNPF2.9	Improve root growth and biomass	(Sol et al. 2019)
	MAMI	Improve root growth and development	(Volpe et al. 2013)
Alfalfa	*Micro-RNA166*	Improve root development	(Boualem et al. 2008)
	SUNN	Improve root elongation and growth	(Schnabel et al. 2005)
	LATD	Improve root growth and development	(Bright et al. 2005)
	Micro-RNA160	Involve in root growth	(Bustos-sanmamed et al. 2012)
	NIP/LATD	Improve root architecture	(Harris and Dickstein 2010), (Yendrek et al. 2010)
	CEP1	Improve lateral root growth	(Imin et al. 2013)
	CDC16	Improve lateral root growth	(Kuppusamy et al. 2009)
	NOOT/COCH	Improve root growth and development	(Couzigou et al. 2012)
	CRA2	Involve in root growth	(Huault et al. 2014)

restricted under severe N stress, leading to a reduced number of LR and shorter PR, whereas LR and PR length increased under mild N stress (Araya et al. 2014; Gruber et al. 2020). In a past study, arabidopsis transcriptome data comprising about 100 root growth-related genes revealed that N stress triggers *MDR4/PGP4* and *WAK4* expression levels (Lally et al. 2001; Terasaka et al. 2005; Giehl et al. 2014). Both genes modulate PR growth and LR development and might be involved in response to mild N stress. Whilst, down-regulation of *ACR4* and *AXR5* reduced LR formation, it might depict that these two genes are constraints to LR formation under severe N stress (Fig. 1B; Yang et al. 2004; Smet et al. 2008). Two families of transporters genes (*NRT1* and *NRT2*) participate in nitrate transport in root and shoot cells (Criscuolo et al. 2012). Nitrate transporters with sensing functions are involved in signalling pathways. For instance, *NRT1.1* acts as a transceptor of NO_3^- have a dual-sensor/transporter role in signalling pathways and could regulate LR formation under the low/absence of NO_3^-. While in N starvation, *AtNRT2.1* acts as NO_3^- signalling component or sensor inhibiting LR initiation. Although the definite molecular mechanism is yet uncertain, negative role of *NRT2.1* and

NRT1.1 in LR initiation and formation may represent a distinct systematic pathway under N starvation. Nitrate sensor/transporter *NRT1.1* is also involved in the auxin-mediated signalling pathway related to root structure changes. In contrast, nitrate sensor/transporter *NRT2.1* has dual functions relevant to the coordination of nitrate availability in nitrate absorption and LR growth (Remans et al. 2006b). The *NRT1/NRT2* families are also reported in the model legumes, including *L. japonicus* (Criscuolo et al. 2012). *L. japonicus* was found to have 92 *NRT1* genes and four *NRT2* genes. The expression pattern of three *NRT2*-type transporters in another model legume species, namely *M. truncatula* have also been reported (Pellizzaro et al. 2014). Some family members require a protein partner (*NAR2 or NRT3*) to function in legumes (Tong et al. 2005). Moreover, in soybean, overexpression of *GmNRT1.2a* and *GmNRT1.2b* in leaves induced by nitrate reported to increase the nodule numbers (Guo-ji et al. 2020).

Recent literature also suggests that *beta-expansion protein gene* plays an important role in regulating nodulation and root architecture. For example, in soybean overexpression of *GmEXPB2* resulted in a longer root, larger root hair
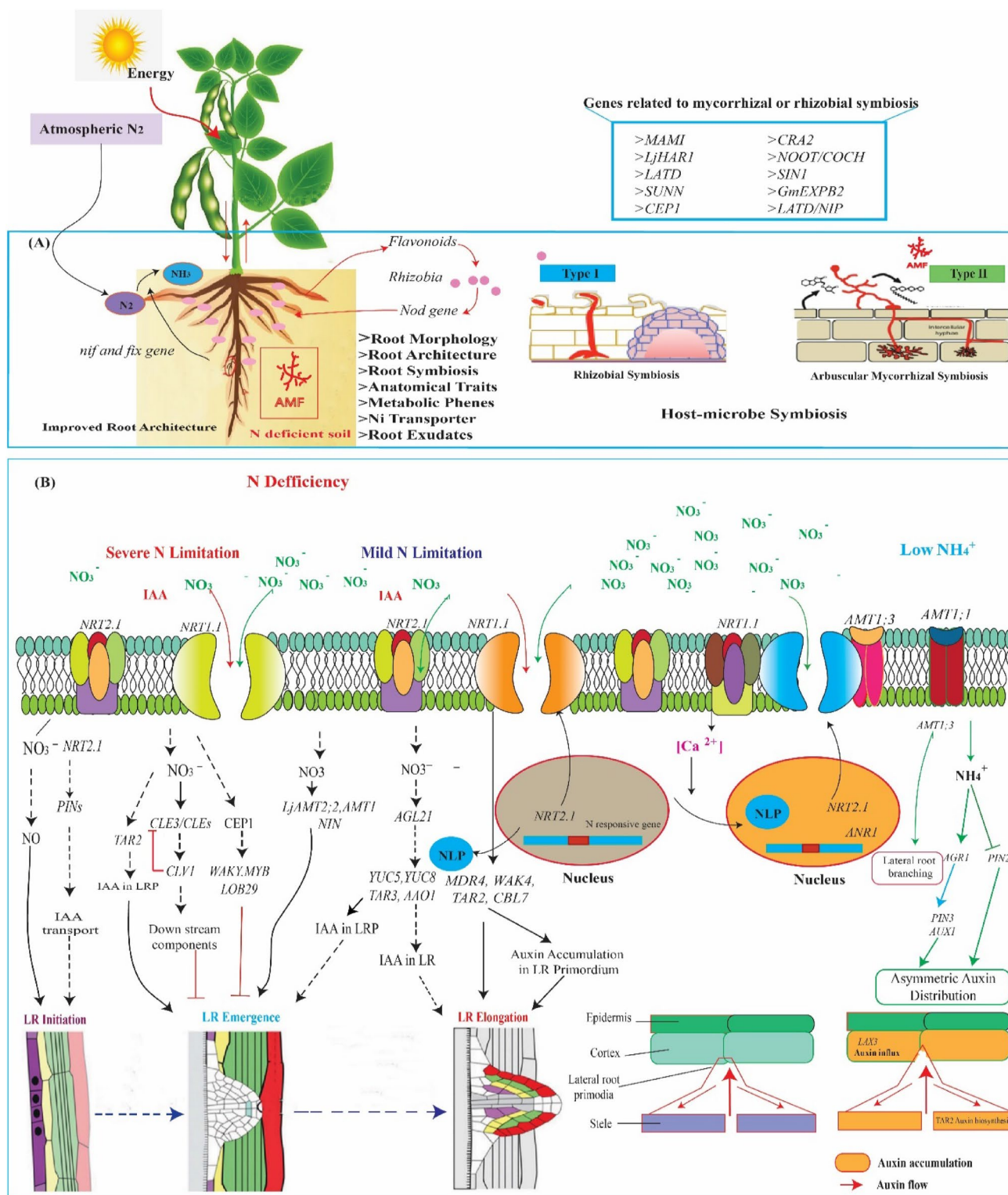
Fig. 1 Improved root architecture for better N acquisition. **A** Diagrammatic representation of modified root architecture for enhanced N uptake and symbiotic host–microbe interactions in response to N availability. **B** Signalling pathways control LR initiation, emergence, and elongation in response to low NO-3 or N-deficit response. Blunted lines and arrows represent interactions that are either negative or positive

area, and dense root hair and significantly improved the frequency of root hairs attachment of rhizobia (Li et al. 2020).

The auxin biosynthesis *TAR2* gene regulates low N-mediated reprogramming of root architecture in arabidopsis (Ma et al. 2014), supporting the vital role of auxin in regulating root architecture in response to N accessibility. Despite the evidence from the literature that several signalling pathways link soil N accessibility and plant N status to root structure (Fig. 1B; Walch-liu et al. 2005; Zhang et al. 2007), only a few genes were observed to play a role in this process. For example, in arabidopsis, plasma membrane proton pump along with CLE peptide, *AHA2* and *CLV1* regulate the response of root growth to N availability (Araya et al. 2014). Besides these mechanisms, *NRT1.1* also signals to activate gene expression of a MADS-box transcription factor *ANR1* to promote LR elongation in response to local NO_3^- supply (Remans et al. 2006a). The induction of *NRT2.1* and potentially *ANR1* gene expression occurs downstream of NO_3^- and *NRT1.1*-induced Ca^{2+} signalling, promoting the nuclear localization of the transcription factor *NLP7* (Fig. 1B; Krapp et al. 2014; Zhang et al. 2020). In *Lotus japonicus*, *MAMI* gene is evolutionarily relevant to *GARP* transcription factor aided in root development (Volpe et al. 2013). Large number of genes have been identified which are involved in regulating legume nodulation and root architecture remodelling. For example, *LjHAR1* in *Lotus japonicas* and *SIN1* in common bean play an essential role in root and nodule development (Battaglia et al. 2020). Furthermore, many genes, including *LATD* (Bright et al. 2005), *SUNN* (Schnabel et al. 2005), *LATD/NIP* (Harris and Dickstein 2010; Yendrek et al. 2010), *NOOT/COCH* (Couzigou et al. 2012), microRNAs (*miRNA160/166*) (Boualem et al. 2008; Bustos-sanmamed et al. 2012), and genes encoding cell cycle proteins (*cell division cycle 16/CEP1/CRA2*) were identified, which affect the growth and development of roots and nodules in *Medicago truncatula* (Kuppusamy et al. 2009; Huault et al. 2014). Imin et al. (2013) reported that *MtCEP1* expression was up-regulated under N-deficit conditions and *MtCEP1* overexpression causes the inhibition of LR development in *Medicago truncatula*, showing that *MtCEP1* could negatively regulate LR development under N starvation. Moreover, RNA sequencing results revealed that *MYB, bZIP, and WRKY* transcription factors and *SUPERROOT2 and LOB29* homologs may act downstream of *MtCEP1*.

In addition, microRNAs also have role in response to nutrient deficiency stress. In arabidopsis, two microRNAs, *miR393*, and *miR167*, were reported to regulate AFB3 (*miR393*) and ARF8 (*miR167*) adjust the changes in root architecture to cope with the changes in N supply (Vidal et al. 2010). In soybean, *miR172* regulates root nodulation (Yan et al. 2013). Furthermore, an exogenous application of synthetic *miPEP172c* has been proven to stimulate actual *miR172c* expression, increases the number of formed nodules (Couzigou et al. 2016). Though role of miRNAs in the regulation of nodulation has been examined in previous studies; however, a link between nodulation and N status in legumes has yet to be defined. This is a novel area of research, and the detailed function of miRNAs in regulating root structure remains to be explored.

Improvement of Root Architecture Remodelling Associated with Symbiosis

Globally, more than 80% of plants including agricultural crops colonized through arbuscular mycorrhizal fungi (AMF) (Gianinazzi-Pearson et al. 1995). They expand the ability of roots to excavate necessary micro- and macronutrients from the soil and establish a symbiotic relationship that can significantly promote plant growth. In symbiosis, hyphae pass through the root's cortical cells to form well-differentiated arbuscules and exchanges minerals with the host root and AMF (Fig. 1A; Parniske 2008). Another crucial step in the symbiotic relationship is to promote the acquisition of N and formation of legume nodules, where rhizobia fix N and provides plants with N-containing organic compounds. The first symbiosis event is just an interaction of chemical signals between bacteria and roots. Afterwards, bacteria attach to the roots, potentially cause hairs to curl, and thus produce colonies and form N-fixing nodules (Fig. 1A) (Oldroyd and Downie 2008). Studies show that AMF colonization of deep root soybean genotypes were more significant under low phosphorus, and the nodulation effect was better than that of shallow root genotypes under high phosphorus conditions (Wang et al. 2011). These results may suggest that the root architecture is linked in forming a symbiotic relationship between root rhizobia and AMF.

Under low nutrient supply, there are two types of root architecture modifications associated with rhizobia or AMF (Fig. 1A). In type I, AMF promotes root growth, improves density and length of LR, and increases the dry weight of roots (Berta et al. 1995; Yu et al. 2015). In type II, root-rhizobium symbiotic association, like in soybean infected by AMF, inhibits root growth with reduced total root length, surface area, and root volume (Wang et al. 2011). This growth inhibition may be due to the carbon cost of nodule formation, maintaining N_2 fixation and the effect of AMF on the plant- or fungal species-dependent root architecture remodelling.

Further, the effect of AMF on plants probably fluctuates by the changes in root architecture. For example, in rice, larger rather than thinner LR were mainly colonized through AMF (Gutjahr et al. 2009), and the mycorrhizal colonization rate of taproots were higher than fibrous roots (Yang et al. 2015). These findings indicate that the taproots may be more suitable for mycorrhizal colonization than the fibrous

roots. Also, rhizobia-inoculated legumes could change their root architecture, producing smaller roots than un-inoculated plants (Yang et al. 2015). One possible explanation is that nodules compete with host roots for carbohydrates because nodules and N fixation consume more carbohydrates and nutrients (Aleman et al. 2009; Reich et al. 2013). Therefore, in the nodulation process, better root growth and sufficient supply of mineral nutrients may contribute to develop healthy nodules and, thus, improve final crops yield.

Hormonal Remodelling of Root Architecture in Legumes in Response to N

Plant endogenous hormones perform a decisive role in regulating root growth and LR formation (Fig. 2; Vissenberg et al. 2020; Ubeda-Toma's et al. 2020). Auxin and cytokinin were found to crosstalk antagonistically or synergistically during plant growth and development. For instance, auxin and cytokinin have a positive role in root/stem growth (Schaller et al. 2015; Liu et al. 2017). Contrastingly, both hormones have a hostile function during LR development.

Auxin is an important hormone that plays a noteable role in nitrogen-mediated root growth and regulates developmental processes in plants. A high N level is assumed to decrease the local auxin accumulation, suggesting that auxin is a central element in the shoot-root signal of N availability (Okushima et al. 2011). Higher shoot N levels are believed to inhibit shoot-root auxin transport, resulting in lower LR numbers in arabidopsis (Reed et al. 1998; Forde 2002). However, studies on *Medicago truncatula* show that higher N levels in the shoot have improved the auxin shoot-root transport (Jin et al. 2020). This response was further emphasized by the sunn-1 Medicago mutant, which has insensitive auxin shoot-to-root transport regardless of N concentration. In *sunn-1*, no correlation was observed between LR regulation and N-mediated auxin transport. However, *SUNN*-mediated auxin shoot-root movement only applies to nitrate-dependent LR remodelling but not in nodulation, indicating that auxin-mediated N regulation of nodule acts locally in the root (Jin et al. 2020). Auxin-mediated root growth also includes interaction with other hormones, such as ethylene.

Ethylene, a gaseous phytohormone, is a positive regulator of roots and engages in local nitrate-mediated root development. In many plant species, a higher nitrate level promotes root-ethylene progression (Caba et al. 1998; Tian et al. 2009). On the other hand, high ethylene limits LR development and nodule formation, whilst low ethylene level improves the LR growth and nodulation (Fig. 2; Nukui et al. 2000; Oldroyd et al. 2001). Many rhizobia species limit localized ethylene by producing ethylene precursor or creating aminocyclopropane deaminase, which breaks

down aminocyclopropane to promote nodulation response (Ma et al. 2002). Additionally, ethylene imparts positional control of nodulation because nodule formation is favoured by higher ethylene levels in the opposing phloem poles (Heidstra et al. 1997). The regulation of nodules and LR by ethylene, most likely to occur through the regulation of cell cycle pathways (Dan et al. 2003; Spadafora et al. 2012). Ethylene influences the cell cycle by interacting with cytokinin, which is also involved in nitrate-mediated root development (Spadafora et al. 2012). **Cytokinin**, another crucial plant hormone that regulates the cell cycle and mediates N status between roots and shoots via the phosphorelay pathway (Sakakibara et al. 2000). The cytokinin synthesis in N-starved roots is improved by nitrate supply, which goes into the shoots and signals the N status in roots. Cytokinin is moved back to roots to signalling the low N level in shoots as shoots N supply decrease (Sakakibara et al. 2000; Ruffel et al. 2011). Cytokinin acts directly on LR founder cells to inhibit LR initiation (Fig. 2; Li et al. 2006; Laplaze et al. 2007). However, higher cytokinin levels stimulate LR elongation once LR differentiation occurs (Li et al. 2006). Elongation of LR is also triggered via higher nitrate; it could be fascinating to study further nitrate-cytokinin crosstalk during LR formation. In legumes, exogenous cytokinin application triggers several genes related to nodulation, as cytokinin acts as an upstream component of the nodule formation pathway (Fang and Hirsch 1998; Gonzalez-rizzo et al. 2006). According to studies, *Medicago* and *Lotus* cytokinin mutants had diminished nodule formation, whereas mutants with repaired function showed increased nodule formation (Gonzalez-Rizzo et al. 2006; Murray et al. 2007; Tirichine et al. 2007).

Approaches for Improvement of low N Tolerance

Recent advancements in plant biotechnology could provide a way to identify genomic regions possessing natural genetic variations (NGV) and transform these targeting alleles into new elite breeding lines to generate NUE lines. QTLs associated with root architecture systems (RSA) have also been identified in many major legume crops, including soybean, chickpea, lentil, and alfalfa (Abdel-Haleem et al. 2011). Some QTLs/genes could have lost during domestication processes, maybe due to linkage with negative loci or a pleiotropic effect on yield. It is therefore necessary to carry out a fine mapping that may investigate the precise cause of the adverse impact on the yield, caused by pleiotropy influence or linkage with any other adverse loci. For that, a sizeable segregant population can be used to break the tight linkage amongst them. Furthermore, functional characterization of these genes using NGV could be an appropriate technique to thoroughly understand the physiological and molecular
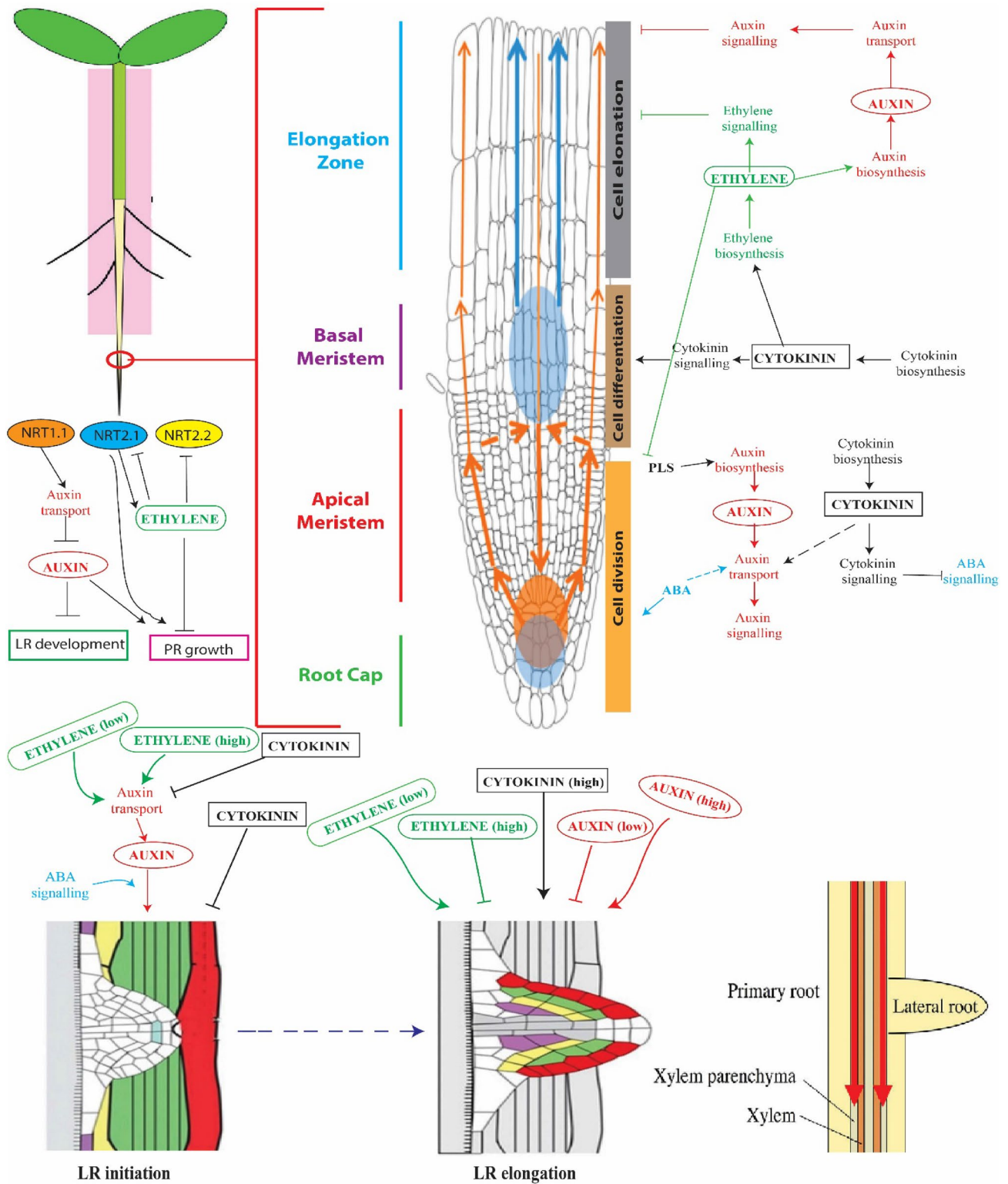
Fig. 2 Hormonal regulation of root development and current understanding of hormones interactions. The active involvement of hormonal-mediated signalling in controlling all crucial aspects of root development, elongation, differentiation, and cell division can be seen below. Hormonal networks are colour coded (Red; auxin; Green; ethylene; Black; cytokinin) (Color figure online)

pathways of stress adaption mechanisms to improve N-use efficiency in legumes.

Novel high-throughput genotyping and phenotyping approaches have the potential to monitor RSA and are indispensable for enhancing a better understanding of N-use efficiency. These field-based, cost-effective approaches have recently been utilized in some major field crops such as maize, wheat, and rice for phenotyping of RSA (Trachsel et al. 2011; York and Lynch 2015; Richard et al. 2015; Wissuwa et al. 2016) and also in some legume species, namely cowpea, common bean, and soybean (Manavalan et al. 2015; Prince et al. 2015). Furthermore, the efficiency and accuracy of RSA phenotyping can be accelerated by adopting high-throughput image analysis through digital imaging of root traits (DIRT) (Das et al. 2015).

In the past decade, legumes were considered amongst minor crops with limited genomic resources. However, as sequencing technology has improved, the scenario for legume crops has entirely transformed, and they now belong to a group of crops that are rich in genomic resources. This has resulted in a new initiative to enhance the world food production by breeding legumes, which is mainly based on genomic-assisted breeding (GAB) projects. Marker-assisted selection (MAS) and genomic-based selections are the most prevalent methods in legumes breeding (Pandey et al. 2016). The MAS technique can effectively improve traits controlled by only one or two genes. But, MAS is not considered suitable for complex traits as a genomic technique. In comparison, genomic-based selection has recently been applied to improve complex breeding traits due to its efficiency.

Breakthroughs in high-precision genotyping and phenotyping have allowed a more accurate selection of targeted genotypes and reduced the breeding cycle time by eliminating unnecessary phenotyping observations. Genomic selection technique integrates the minor and massive impact on genetic factors, and it could be advantageous for speeding effective selection gain. Additionally, cost-effective genotyping has created a diversified gene pool in legume crops, which is crucial for breeding new genetic traits (Jain et al. 2013). With emerging gene-editing tools like the CRISPR/Cas9 system, plant scientists have a better chance of reaching their goals, especially for the specific traits they want to change (Jacobs et al. 2015; Sun et al. 2015). CRISPR/Cas9-mediated genome editing has been reported in *Lotus japonicus* (Wang et al. 2016), *Vigna unguiculata* (Ji et al. 2019), *Medicago truncatula* (Meng et al. 2016), and *Glycine max* with some desirable results (Cai et al. 2015; Sun et al. 2015). So, this new revolution in genome editing could modify RSA-related alleles to enhance their functionality in the required breeding environment. With these advanced genomic sequences, a massive database for various legume crops has been generated to identify critical regulatory genes, transcripts, and proteins, as well as gene families that contribute in stress tolerance. The availability of wider genetic diversity for major legume crops may smooth the way for digging out traits that can facilitate haplotype-assisted breeding. Therefore, incorporating broader variability of targeted traits from newly found genomic resources and technologies into advance breeding programmes would improve crop yield under nutrient-deficit conditions.

Advances in Breeding for low N Tolerance

Plants with a robust root system can extract nutrients from the soil efficiently and enable them to maintain normal growth in N-deficit conditions. For instance, root architecture of soybean is the key characteristic for sustaining yield in N-deficient environments. A better subsoil exploration owing to stronger and deeper roots has been proven to enhance the yield of chickpeas under stress conditions (Kashiwagi et al. 2015). Several root traits, including root diameter, length, root volume, and surface area were studied to determine the importance of root architecture in sustaining legume yield in a nutrient-limited environment (Fried et al. 2019). Root traits directly impact legume productivity under N-deficit conditions, suggesting that root architecture is a desired trait for further breeding initiatives. Although many recent studies have been conducted to identify QTLs/genes and genetic diversity that influence root architecture, but fewer attempts to exploit these resources in breeding programmes. Efforts are currently being made to introduce the genomic region to various other elite legume lines (Pandey et al. 2016). Legumes are revealed to have abundant genetic resources for root architecture. Unfortunately, these genetic resources have not yet been exploited in crop stress breeding programs to develop better lines. Thus, genotypes with a better and robust root system should be exploited to develop breeding lines with improved root architecture under nutrient-poor environments.

Conclusion and Future Prospects

Extensive and rapid root growth is crucial for plant survival in unfavourable environments, allowing plants to access nutrients and water more efficiently. Furthermore, root growth angle, which is connected to the extraction of water and nutrients; is a fundamentally significant characteristic impacting the root system architecture of legumes. Therefore, a greater understanding of key genes associated with root structure and their control would allow researchers to use marker-assisted selection to develop new genotypes with robust root system. Significant progress has recently been made in understanding the genetic bases of the root

system in legumes, primarily through the identification of QTLs.

Many genes that regulate root architecture have been identified, but the molecular mechanisms of root hair formation, LR development, and root elongation are still poorly understood. With the rapid advancements in molecular approaches, including transcriptomics, genomics, phenomics, and proteomics, researchers will be able to understand the underlying molecular mechanisms which regulate the roots system. A proposed approach to integrate the above-mentioned multidisciplinary strategies for enhancing legumes NUE is summarized in Fig. 3. Despite the fact that identifying the targeted QTLs with moderate effects is difficult, but cloning these genes using QTL analysis is an effective strategy to select candidate genes for future breeding programs. In addition, genome-wide association studies,
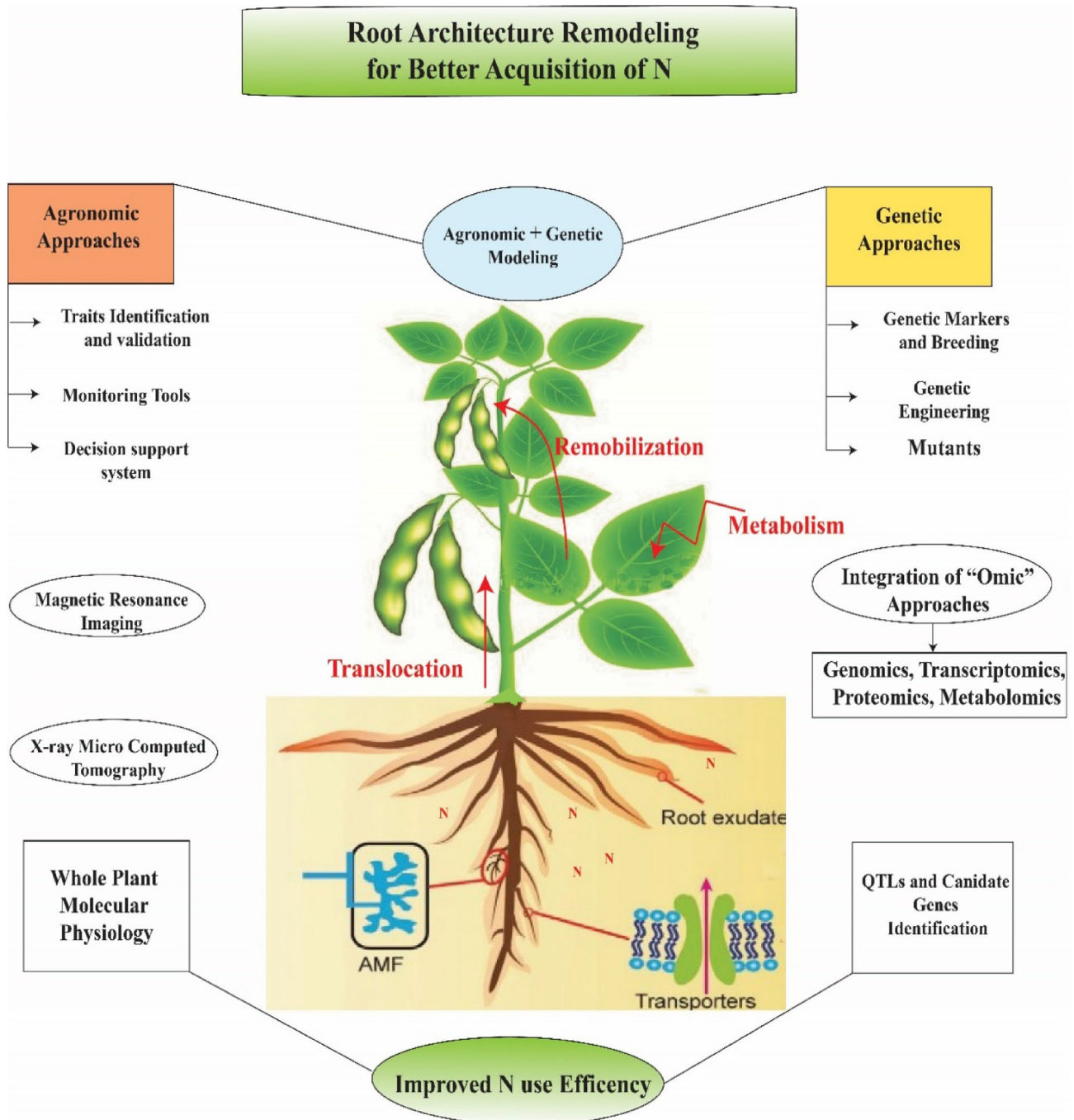
Fig. 3 A proposed strategy for improving N acquisition and utilization by integrating multidisciplinary approaches

often known as GWAS, have been proven a realistic and feasible way to find alleles of existing genotypes, which can be exploited in molecular breeding. Using these new strategies, numerous QTLs have been identified comprising candidate genes for root development. Unfortunately, GWAS and QTL mapping have limitations in cloning genes which regulate the root development. The root phenotypes grown in agar/gel or hydroponic conditions do not precisely reflect their growth when sown in the soil; this constraint makes it tougher to perform root phenotyping in natural soil environment. Exploring the symbiotic relationship between microorganisms (rhizobia and AMF) and host plants would be another impotent approach to speed up NUE. Roots are the primary site of rhizobial and AMF infection; therefore, the root system may play a vital role in colonization. Although the molecular mechanisms of the rhizobial and AMF symbiosis and signalling transduction have been identified, complex regulatory mechanisms and various other components are still unknown. To develop NUE cultivars, it is necessary to combine omics approaches with traditional breeding and effective agronomic strategies (Fig. 3). For a more precise assessment of root system, it is also necessary to investigate the root architecture in complex and natural soils using cutting-edge techniques, like magnetic resonance imaging and X-ray microcomputed tomography.

Declarations

Conflict of interest The authors declare no competing financial interest.

References

Abdel-Haleem H, Lee GJ, Boerma RH (2011) Identification of QTL for increased fibrous roots in soybean. Theor Appl Genet 122:935–946. https://doi.org/10.1007/s00122-010-1500-9

Aleman L, Ortega JL, Martinez-Grimes M, Seger M, Holguin FO, Uribe DJ, Garcia-Ibilcieta D, Sengupta-Gopalan C (2009) Nodule-enhanced expression of a sucrose phosphate synthase gene member (MsSPSA) has a role in carbon and nitrogen metabolism in the nodules. Planta 231:233–244. https://doi.org/10.1007/s00425-009-1043-y

Amtmann A, Armengaud P (2009) Effects of N, P, K and S on metabolism: new knowledge gained from multi-level analysis. Curr Opin Plant Biol 12:275–283. https://doi.org/10.1016/j.pbi.2009.04.014

Araya T, Miyamoto M, Wibowo J, Suzuki A, Kojima S, Tsuchiya YN, Sawa S, Fukuda H, Von Wirén N, Takahashi H (2014) CLE peptides regulate lateral root development in response to nitrogen nutritional status of plants. Plant Signal Behav ISSN 2324:1559–2324. https://doi.org/10.4161/psb.29302

Araya T, Kubo T, von Wirén N, Takahashi H (2016) Statistical modeling of nitrogen-dependent modulation of root system architecture in Arabidopsis thaliana. J Integr Plant Biol 58:254–265. https://doi.org/10.1111/jipb.12433

Barbulova A, Rogato A, D'Apuzzo E, Omrane S, Chiurazzi M (2007) Differential effects of combined N sources on early steps of the Nod factor dependent transduction pathway in Lotus japonicus. Am Phytopathol Soc 20:994–1003

Battaglia M, Rípodas C, Clúa J, Baudin M, Aguilar OM, Niebel A, Zanetti ME, Blanco FA (2020) A nuclear factor Y interacting protein of the GRAS family is required for nodule organogenesis, infection thread progression, and lateral root growth. Plant Physiol 164:1430–1442. https://doi.org/10.1104/pp.113.230896

Boualem A, Laporte P, Jovanovic M, Laffont C, Plet J, Combier JP, Niebel A, Crespi M, Frugier F (2008) MicroRNA166 controls root and nodule development in Medicago truncatula. Plant J 54:876–887. https://doi.org/10.1111/j.1365-313X.2008.03448.x

Bouwman L, Goldewijk KK, Van Der Hoek KW, Beusen AH, Van Vuuren DP, Willems J, Rufino MC, Stehfest E (2013) Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. Proc Natl Acad Sci 110:20882–20887. https://doi.org/10.1073/pnas.1206191109

Bright LJ, Liang Y, Mitchell DM, Harris JM (2005) The LATD gene of Medicago truncatula is required for both nodule and root development. Am Phytopathol Soc 18:521–532

Burridge J, Jochua CN, Bucksch A, Lynch JP (2016a) Legume shovelomics: High-throughput phenotyping of common bean (Phaseolus vulgaris L.) and cowpea (Vigna unguiculata) root architecture in the field. Field Crops Res 192:21–32. https://doi.org/10.1016/j.fcr.2016.04.008

Burridge JD, Schneider HM, Huynh BL, Roberts PA, Bucksch A, Lynch JP (2016b) Genome-wide association mapping and agronomic impact of cowpea root architecture. Theor Appl Genet. https://doi.org/10.1007/s00122-016-2823-y

Bustos-Sanmamed P, Mao G, Deng Y, Elouet M, Khan GA, Bazin J, Turner M, Subramanian S, Yu O, Crespi M, Lelandais-Brière C (2012) Overexpression of miR160 affects root growth and nitrogen-fixing nodule number in Medicago truncatula. Funct Plant Biol 40:1208–1220

Buzas DM, Gresshoff PM (2007) Short and long-distance control of root development by LjHAR1 during the juvenile stage of *Lotus japonicus*. J Plant Physiol 164:452–459. https://doi.org/10.1016/j.jplph.2006.03.006

Caba JM, Recalde L, Ligero F (1998) Nitrate-induced ethylene biosynthesis and the control of nodulation in alfalfa. Plant Cell Environ 21:87–93

Cai Y, Chen L, Liu X, Sun S, Wu C, Jiang B, Han T, Hou W (2015) CRISPR/Cas9-mediated genome editing in soybean hairy roots. PLoS ONE. https://doi.org/10.1371/journal.pone.0136064

Cichy KA, Blair MW, Galeano Mendoza CH, Snapp SS, Kelly JD (2009) QTL analysis of root architecture traits and low phosphorus tolerance in an andean bean population. Crop Sci 49:59–68. https://doi.org/10.2135/cropsci2008.03.0142

Couzigou JM, Zhukov V, Mondy S, Abu el Heba G, Cosson V, Ellis TN, Ambrose M, Wen J, Tadege M, Tikhonovich I, Mysore KS (2012) NODULE ROOT and COCHLEATA maintain nodule development and are lLegume oOrthologs of Arabidopsis BLADE-ON-PETIOLE genes. Plant Cell 24:4498–4510. https://doi.org/10.1105/tpc.112.103747

Couzigou JM, André O, Guillotin B, Alexandre M, Combier JP (2016) Use of microRNA-encoded peptide miPEP172c to stimulate nodulation in soybean. New Phytol 211(2):379–381

Criscuolo G, Valkov VT, Parlati A, Alves LM, Chiurazzi M (2012) Molecular characterization of the Lotus japonicus NRT1 (PTR) and NRT2 families. Plant Cell Environ 35(9):1567–1581

D'Apuzzo E, Rogato A, Simon-Rosin U, El Alaoui H, Barbulova A, Betti M, Dimou M, Katinakis P, Marquez A, Marini AM, Udvardi MK (2004) Characterization of three functional high-affinity ammonium transporters in Lotus japonicus with differential transcriptional regulation and spatial expression. Plant Physiol 134:1763–1774

Dan H, Imaseki H, Wasteneys GO, Kazama H (2003) Ethylene stimulates endoreduplication but inhibits cytokinesis in cucumber hypocotyl epidermis. Plant Physiol 133:1726–1731. https://doi.org/10.1104/pp.103.025783

Das A, Schneider H, Burridge J, Ascanio AK, Wojciechowski T, Topp CN, Lynch JP, Weitz JS, Bucksch A (2015) Digital imaging of root traits (DIRT): a high-throughput computing and collaboration platform for field-based root phenomics. Plant Methods 11(1):1–12

Devaiah BN, Karthikeyan AS, Raghothama KG (2007) WRKY75 transcription factor is a modulator of phosphate acquisition and root development in Arabidopsis. Plant Physiol 143:1789–1801. https://doi.org/10.1104/pp.106.093971

Dunbabin V, Diggle A, Rengel Z (2003) Is there an optimal root architecture for nitrate capture in leaching environments? Plant Cell Environ 26(6):835–44

Egamberdieva D, Wirth S, Jabborova D, Räsänen LA, Liao H (2017) Coordination between Bradyrhizobium and Pseudomonas alleviates salt stress in soybean through altering root system architecture. J Plant Interact ISSN 12:100–107. https://doi.org/10.1080/17429145.2017.1294212

Fang Y, Hirsch AM (1998) Studying early nodulin gene ENOD40 expression and induction by nodulation factor and cytokinin in transgenic Alfalfa. Plant Physiol 116:53–68

Forde BG (2002) The role of long-distance signalling in plant responses to nitrate and other nutrients. J Exp Bot 53:39–43. https://doi.org/10.1093/jexbot/53.366.39

Forde BG (2014) Nitrogen signalling pathways shaping root system architecture: an update. Curr Opin Plant Biol 21:30–36. https://doi.org/10.1016/j.pbi.2014.06.004

Fried HG, Narayanan S, Fallen B (2019) Evaluation of soybean (Glycine max L.) genotypes for yield, water use efficiency, and root traits. PLoS ONE 14:1–18. https://doi.org/10.1371/journal.pone.0212700

Gianinazzi-Pearson V, Gollotte A, Tisserant B, Franken P, Dumas-Gaudot E, Lemoine MC, Tuinen DV, Gianinazzi S, Lherminier J (1995) Cellular and molecular approaches in the characterization of symbiotic events in functional arbuscular mycorrhizal associations. Canad J Bot 73(S1):526–532

Giehl RF, Gruber BD, von Wirén N (2014) It's time to make changes: modulation of root system architecture by nutrient signals. J Exp Bot 65:769–778. https://doi.org/10.1093/jxb/ert421

Gonzalez-Rizzo S, Crespi M, Frugier F (2006) The Medicago truncatula CRE1 cytokinin receptor regulates lateral root development and early symbiotic interaction with Sinorhizobium meliloti. Plant Cell 18:2680–2693. https://doi.org/10.1105/tpc.106.043778

Graham PH, Vance CP (2003) Legumes: importance and constraints to greater use. Plant Physiol 131(3):872–877

Gruber BD, Giehl RF, Friedel S (2020) Plasticity of the arabidopsis root system under nutrient deficiencies. Plant Physiol 163:161–179. https://doi.org/10.1104/pp.113.218453

Guo-Ji LI, Lin ZH, Jin-Shan CA, You-Ning WA (2020) Cloning and functional analysis of GmNRT1.2a and GmNRT1.2b in soybean. Acta Agron Sin 46:1025–1032. https://doi.org/10.3724/SP.J.1006.2020.94152

Gutjahr C, Casieri L, Paszkowski U (2009) Glomus intraradices induces changes in root system architecture of rice independently of common symbiosis signalling. New Phytol 182:829–837

Hao DL, Zhou JY, Yang SY, Qi W, Yang KJ, Su YH (2020) Function and regulation of ammonium transporters in plants. Int J Mol Sci 21(10):3557

Harris JM, Dickstein R (2010) Control of root architecture and nodulation by the LATD/NIP transporter. Plant Signal Behav. https://doi.org/10.4161/psb.5.11.13165

Heidstra R, Yang WC, Yalcin Y, Peck S, Emons A, Van Kammen A, Bisseling T (1997) Ethylene provides positional information on cortical cell division but is not involved in Nod factor-induced root hair tip growth in Rhizobium-legume interaction. Development 124:1781–1787

Huault E, Laffont C, Wen J, Mysore KS, Ratet P, Duc G, Frugier F (2014) Local and systemic regulation of plant root system architecture and symbiotic nodulation by a receptor- like kinase. PLoS Genet. https://doi.org/10.1371/journal.pgen.1004891

Imin N, Mohd-Radzman NA, Ogilvie HA, Djordjevic MA (2013) The peptide-encoding CEP1 gene modulates lateral root and nodule numbers in medicago truncatula. J Exp Bot 64:5395–5409. https://doi.org/10.1093/jxb/ert369

Jacobs TB, LaFayette PR, Schmitz RJ, Parrott WA (2015) Targeted genome modifications in soybean with CRISPR/Cas9. BMC Biotechnol 15:1–10. https://doi.org/10.1186/s12896-015-0131-2

Jaganathan D, Thudi M, Kale S, Azam S, Roorkiwal M, Gaur PM, Kishor PB, Nguyen H, Sutton T, Varshney RK (2015) Genotyping-by- sequencing based intra-specific genetic map refines a "QTL- hotspot" region for drought tolerance in chickpea. Mol Genet Genomics 290:559–571. https://doi.org/10.1007/s00438-014-0932-3

Jain M, Misra G, Patel RK, Priya P, Jhanwar S, Khan AW, Shah N, Singh VK (2013) A draft genome sequence of the pulse crop chickpea (Cicer arietinum L.). Plant J 74(5):715–729

Ji J, Zhang C, Sun Z, Wang L, Duanmu D, Fan Q (2019) Genome Editing in Cowpea Vigna unguiculata Using CRISPR-Cas9. Int J Mol Sci 20:2471

Jin J, Watt M, Mathesius U (2020) The autoregulation gene SUNN mediates changes in root organ formation in response to nitrogen through alteration of shoot-to-root auxin transport. Plant Physiol 159:489–500. https://doi.org/10.1104/pp.112.194993

Ju C, Buresh RJ, Wang Z, Zhang H, Liu L, Yang J, Zhang J (2015) Root and shoot traits for rice varieties with higher grain yield and higher nitrogen use efficiency at lower nitrogen rates application. Field Crops Res 175:47–55. https://doi.org/10.1016/j.fcr.2015.02.007

Kashiwagi J, Krishnamurthy L, Purushothaman R, Upadhyaya HD, Gaur PM, Gowda CL, Ito O, Varshney RK (2015) Scope for improvement of yield under drought through the root traits in chickpea (*Cicer arietinum* L.). Field Crops Res 1(170):47–54

Khan A, Najeeb U, Xiangjun K, Munsif F, Jie Z, Zhou R (2020) Leaf nitrogen metabolism during reproductive phase is crucial for sustaining lint yield of densely populated cotton genotypes. J Agron 112(5):4031–4044

Kitomi Y, Hanzawa E, Kuya N, Inoue H, Hara N, Kawai S, Kanno N, Endo M, Sugimoto K, Yamazaki T, Sakamoto S (2020) Root angle modifications by the DRO1 homolog improve rice yields in saline paddy fields. Proc Natl Acad Sci 107:21242–21250. https://doi.org/10.1073/pnas.2005911117

Kong Y, Wang B, Du H, Li W, Li X, Zhang C (2019) GmEXLB1, a soybean Expansin-Like B gene, alters root architecture to improve phosphorus acquisition in Arabidopsis. Front Plant Sci 10:808. https://doi.org/10.3389/fpls.2019.00808

Krapp A, David LC, Chardin C, Girin T, Marmagne A, Leprince AS, Chaillou S, Ferrario-Méry S, Meyer C, Daniel-Vedele F (2014) Nitrate transport and signalling in Arabidopsis. J Exp Bot 65:789–798. https://doi.org/10.1093/jxb/eru001

Kumar M, Yusuf MA, Yadav P, Narayan S, Kumar M (2019) Overexpression of chickpea defensin gene confers tolerance to water deficit stress in Arabidopsis thaliana. Front Plant Sci 10:1–18. https://doi.org/10.3389/fpls.2019.00290

Kuppusamy KT, Ivashuta S, Bucciarelli B, Vance CP, Gantt JS, VandenBosch KA (2009) Knockdown of CELL DIVISION CYCLE16 reveals an inverse relationship between lateral root and nodule numbers and a link to auxin in Medicago truncatula. Plant Physiol 151:1155–1166. https://doi.org/10.1104/pp.109.143024

Lally D, Ingmire P, Tong HY, He ZH (2001) Antisense expression of a cell wall-associated protein kinase, WAK4, inhibits cell elongation and alters morphology. Plant Cell 13:1317–1331

Laplaze L, Benkova E, Casimiro I, Maes L, Vanneste S, Swarup R, Weijers D, Calvo V, Parizot B, Herrera-Rodriguez MB, Offringa R (2007) Cytokinins act directly on lateral root founder cells to inhibit root initiation. Plant Cell 19:3889–3900. https://doi.org/10.1105/tpc.107.055863

Lay-Pruitt KS, Takahashi H (2020) Integrating N signals and root growth: the role of nitrate transceptor NRT1.1 in auxin-mediated lateral root development. J Exp Bot 71:4365–4368. https://doi.org/10.1093/jxb/eraa243

Li X, Mo X, Shou H, Wu P (2006) Cytokinin-mediated cell cycling arrest of pericycle founder cells in lateral root initiation of Arabidopsis. Plant Cell Physiol 47:1112–1123. https://doi.org/10.1093/pcp/pcj082

Li J, Xie Y, Dai A, Liu L, Li Z (2009) Root and shoot traits responses to phosphorus deficiency and QTL analysis at seedling stage using introgression lines of rice. J Genet Genomics 36:173–183. https://doi.org/10.1016/S1673-8527(08)60104-6

Li Z, Gao Q, Liu Y, He C, Zhang X, Zhang J (2011) Overexpression of transcription factor ZmPTF1 improves low phosphate tolerance of maize by regulating carbon metabolism and root growth. Planta 233:1129–1143. https://doi.org/10.1007/s00425-011-1368-1

Li P, Chen F, Cai H, Liu J, Pan Q, Liu Z, Gu R, Mi G, Zhang F, Yuan L (2015) A genetic relationship between nitrogen use efficiency and seedling root traits in maize as revealed by QTL analysis. J Exp Bot 66:3175–3188. https://doi.org/10.1093/jxb/erv127

Li J, Chen F, Li Y, Li P, Wang Y, Mi G, Yuan L (2019) ZmRAP2.7, an AP2 transcription factor, is involved in maize brace roots development. Front Plant Sci 10:1–11. https://doi.org/10.3389/fpls.2019.00820

Li X, Zhao J, Tan Z, Zeng R, Liao H (2020) GmEXPB2, a cell wall B-Expansin, affects soybean nodulation through modifying root architecture and promoting nodule formation and development. Plant Physiol 169:2640–2653. https://doi.org/10.1104/pp.15.01029

Liang Q, Cheng X, Mei M, Yan X, Liao H (2010) QTL analysis of root traits as related to phosphorus efficiency in soybean. Ann Bot 106:223–234. https://doi.org/10.1093/aob/mcq097

Liao H, Yan X, Rubio G, Beebe SE, Blair MW, Lynch JP (2004) Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. Funct Plant Biol 31:959–970

Liu J, Moore S, Chen C, Lindsey K (2017) Crosstalk complexities between auxin, cytokinin, and ethylene in Arabidopsis root development: From experiments to systems modelling, and back again. Mol Plant 10:1480–1496. https://doi.org/10.1016/j.molp.2017.11.002

Ma W, Penrose DM, Glick BR (2002) Strategies used by rhizobia to lower plant ethylene levels and increase nodulation. Can J Microbiol 48:947–954. https://doi.org/10.1139/W02-100

Ma W, Li J, Qu B, He X, Zhao X, Li B, Fu X, Tong Y (2014) Auxin biosynthetic gene TAR2 is involved in low nitrogen- mediated reprogramming of root architecture in Arabidopsis. Plant J 78:70–79. https://doi.org/10.1111/tpj.12448

Manavalan LP, Prince SJ, Musket TA, Chaky J, Deshmukh R, Vuong TD, Song L, Cregan PB, Nelson JC, Shannon JG, Specht JE (2015) Identification of novel QTL governing root architectural traits in an interspecific soybean population. PLoS Genet 10:1–18. https://doi.org/10.1371/journal.pone.0120490

Meng Y, Ma X, Chen D, Wu P, Chen M (2010) Biochemical and Biophysical Research Communications MicroRNA-mediated signalling involved in plant root development. Biochem Biophys Res Commun 393:345–349. https://doi.org/10.1016/j.bbrc.2010.01.129

Meng Y, Hou Y, Wang H, Ji R, Liu B, Wen J, Niu L, Lin H (2016) Targeted mutagenesis by CRISPR / Cas9 system in the model legume Medicago truncatula. Plant Cell Rep. https://doi.org/10.1007/s00299-016-2069-9

Mi G, Chen F, Wu Q, Lai N, Yuan L, Zhang F (2010) Ideotype root architecture for efficient nitrogen acquisition by maize in intensive cropping systems. Sci China Life Sci 53:1369–1373

Miura K, Lee J, Gong Q, Ma S, Jin JB, Yoo CY, Miura T, Sato A, Bohnert HJ, Hasegawa PM (2020) SIZ1 regulation of phosphate starvation-induced root architecture remodelling involves the control of auxin accumulation. Plant Physiol 155:1000–1012. https://doi.org/10.1104/pp.110.165191

Murray JD, Karas BJ, Sato S, Tabata S, Amyot L, Szczyglowski K (2007) A cytokinin perception mutant colonized by rhizobium in the absence of nodule organogenesis. Science 315(5808):101–104. https://doi.org/10.1126/science.1132514

Nukui N, Ezura H, Yuhashi KI, Yasuta T, Minamisawa K (2000) Effects of ethylene precursor and inhibitors for ethylene biosynthesis and perception on nodulation in Lotus japonicus and Macroptilium atropurpureum. Plant Cell Physiol 41:893–897

Okushima Y, Inamoto H, Umeda M (2011) A high concentration of nitrate causes temporal inhibition of lateral root growth by suppressing cell proliferation. Plant Biotechnol J 28:413–416. https://doi.org/10.5511/plantbiotechnology.11.0722a

Oldroyd GE, Downie JA (2008) Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu Rev Plant Biol 59:519–546. https://doi.org/10.1146/annurev.arplant.59.032607.092839

Oldroyd GE, Engstrom EM, Long SR (2001) Ethylene inhibits the Nod factor signal transduction pathway of Medicago truncatula. Plant Cell 13:1835–1849

Pandey MK, Roorkiwal M, Singh VK, Ramalingam A, Kudapa H, Thudi M, Chitikineni A, Rathore A, Varshney RK (2016) Emerging genomic tools for legume breeding: Current status and future prospects. Front Plant Sci 7:1–18. https://doi.org/10.3389/fpls.2016.00455

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775. https://doi.org/10.1038/nrmicro1987

Pellizzaro A, Clochard T, Cukier C, Bourdin C, Juchaux M, Montrichard F, Thany S, Raymond V, Planchet E, Limami AM, Morère-Le Paven MC (2014) The nitrate transporter MtNPF6.8 (MtNRT1.3) transports abscisic acid and mediates nitrate regulation of primary root growth in Medicago truncatula. Plant Physiol 166:2152–2165

Perchlik M, Tegeder M (2020) Improving plant nitrogen use efficiency through alteration of amino acid transport processes. Plant Physiol 175:235–247

Poitout A, Crabos A, Petřík I, Novák O, Krouk G, Lacombe B, Ruffel S (2018) Responses to systemic nitrogen signaling in arabidopsis roots involve trans-zeatin in shoots. Plant Cell 30:1243–1257. https://doi.org/10.1105/tpc.18.00011

Postma JA, Dathe A, Lynch JP (2014) The optimal lateral root branching density for maize depends on nitrogen and phosphorus. Plant Physiol 166:590–602. https://doi.org/10.1104/pp.113.233916

Prince SJ, Song L, Qiu D, Maldonado dos Santos JV, Chai C, Joshi T, Patil G, Valliyodan B, Vuong TD, Murphy M, Krampis K (2015) Genetic variants in root architecture-related genes in a Glycine soja accession, a potential resource to improve cultivated soybean. BMC Genomics 16:1–20. https://doi.org/10.1186/s12864-015-1334-6

Qin Y, Wang D, Fu J, Zhang Z, Qin Y, Hu G, Zhao J (2021) *Agrobacterium rhizogenes*-mediated hairy root transformation as an efficient system for gene function analysis in *Litchi chinensis*. Plant Methods 17:103. https://doi.org/10.1186/s13007-021-00802-w

Ramakrishna P, Ruiz Duarte P, Rance GA, Schubert M, Vordermaier V, Vu LD, Murphy E, Vilches Barro A, Swarup K, Moirangthem K, Jørgensen B (2019) EXPANSIN A1-mediated radial swelling of pericycle cells positions anticlinal cell divisions during lateral root initiation. PNAS 116:8597–8602. https://doi.org/10.1073/pnas.1820882116

Rao IM, Miles JW, Beebe SE, Horst WJ (2016) Root adaptations to soils with low fertility and aluminium toxicity. Ann Bot 118:593–605. https://doi.org/10.1093/aob/mcw073

Rasheed M, Jilani G, Shah IA, Najeeb U, Iqbal T (2010) Improved lentil production by utilizing genetic variability in response to phosphorus fertilization. Acta Psychiatr Scand 60(6):485–493

Rasmussen IS, Dresbøll DB, Thorup-Kristensen K (2015) Winter wheat cultivars and nitrogen (N) fertilization effects on root growth, N uptake efficiency and N use efficiency. Eur J Agron 68:38–49. https://doi.org/10.1016/j.eja.2015.04.003

Reed RC, Brady SR, Muday GK (1998) Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. Plant Physiol 118:1369–1378

Reich PB, Hungate BA, Luo Y (2013) Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. Annu Rev Ecol Evol Syst 37:611–636. https://doi.org/10.1146/annurev.ecolsys.37.091305.110039

Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E, Tillard P, Forde BG, Gojon A (2006a) The Arabidopsis NRT1.1 transporter participates in the signalling pathway triggering root colonization of nitrate-rich patches. Proc Natl Acad Sci 103:19206–19211

Remans T, Nacry P, Pervent M, Girin T, Tillard P, Lepetit M, Gojon A (2006b) A central role for the nitrate transporter NRT2.1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. Plant Physiol 140:909–921. https://doi.org/10.1104/pp.105.075721

Richard CA, Hickey LT, Fletcher S, Jennings R, Chenu K, Christopher JT (2015) High-throughput phenotyping of seminal root traits in wheat. Plant Methods 11(1):1–11

Rogato A, D'Apuzzo E, Chiurazzi M (2010) The multiple plant response to high ammonium conditions. Plant Signal Behav 5:1594–1596. https://doi.org/10.4161/psb.5.12.13856

Ruffel S, Krouk G, Ristova D, Shasha D, Birnbaum KD, Coruzzi GM (2011) Nitrogen economics of root foraging : Transitive closure of the nitrate – cytokinin relay and distinct systemic signalling for N supply vs. demand. Proc Natl Acad Sci 108:18524–18529

Saengwilai P, Tian X, Lynch JP (2014) Low crown root number enhances nitrogen acquisition from low-nitrogen soils in maize. Plant Physiol 166:581–589. https://doi.org/10.1104/pp.113.232603

Sakakibara H, Taniguchi M, Sugiyama T (2000) His-Asp phosphorelay signaling : a communication avenue between plants and their environment. Plant Mol Biol 42:273–278

Salvemini F, Marini AM, Riccio A, Patriarca EJ, Chiurazzi M (2001) Functional characterization of an ammonium transporter gene from lotus japonicus. Gene 270:237–243

Schaller GE, Bishopp A, Kieber JJ (2015) The Yin-yang of hormones: cytokinin and auxin interactions in plant development. Plant Cell 27:44–63. https://doi.org/10.1105/tpc.114.133595

Schnabel E, Journet EP, de Carvalho-Niebel F, Duc G, Frugoli J (2005) The Medicago truncatula SUNN gene encodes a CLV1-like leucine-rich repeat receptor kinase that regulates nodule number and root length. Plant Mol Biol 58:809–822. https://doi.org/10.1007/s11103-005-8102-y

Shimizu A, Kato K, Komatsu A, Motomura K, Ikehashi H (2008) Genetic analysis of root elongation induced by phosphorus deficiency in rice (Oryza sativa L.): fine QTL mapping and multivariate analysis of related traits. Theor Appl Genet 117:987–996. https://doi.org/10.1007/s00122-008-0838-8

Simon-Rosin U, Wood C, Udvardi MK (2003) Molecular and cellular characterisation of LjAMT2;1, an ammonium transporter from the model legume Lotus japonicus. Plant Mol Biol 51:99–108

De Smet I, Vassileva V, De Rybel B, Levesque MP, Grunewald W, Van Damme D, Van Noorden G, Naudts M, Van Isterdael G, De Clercq R, Wang JY (2008) Receptor-like kinase ACR4 restricts formative cell divisions in the Arabidopsis root. Science 322 (5901): 594–597Dunbabin V, Diggle A, Rengel Z (2003) Is there an optimal root architecture for nitrate capture in leaching environments ? Plant Cell Environ 26:835–844

Sol S, Valkov VT, Rogato A, Noguero M, Gargiulo L, Mele G, Lacombe B, Chiurazzi M (2019) Disruption of the Lotus japonicus transporter LjNPF2.9 increases shoot biomass and nitrate content without affecting symbiotic performances. BMC Plant Biol 19:1–14

Spadafora ND, Parfitt D, Marchbank A, Li S, Bruno L, Vaughan R, Nieuwland J, Buchanan-Wollaston V, Herbert RJ, Bitonti MB, Doonan J (2012) Perturbation of cytokinin and ethylene-signalling pathways explain the strong rooting phenotype exhibited by Arabidopsis expressing the Schizosaccharomyces pombe mitotic inducer, cdc25. BMC Genom 12:1–15

Stagnari F, Maggio A, Galieni A, Pisante M (2017) Multiple benefits of legumes for agriculture sustainability: an overview. Chem Biol Tech Agri 4:2

Sun X, Hu Z, Chen R, Jiang Q, Song G, Zhang H, Xi Y (2015) Targeted mutagenesis in soybean using the CRISPR-Cas9 system. Sci Rep 5:1–10. https://doi.org/10.1038/srep10342

Sun H, Guo X, Xu F, Wu D, Zhang X, Lou M, Luo F, Xu G, Zhang Y (2019) Overexpression of OsPIN2 regulates root growth and

formation in response to phosphate deficiency in rice. Int J Mol Sci 20:5144

Tegeder M, Rentsch D (2010) Uptake and partitioning of amino acids and peptides. Mol Plant 3:997–1011. https://doi.org/10.1093/mp/ssq047

Terasaka K, Blakeslee JJ, Titapiwatanakun B, Peer WA, Bandyopadhyay A, Makam SN, Lee OR, Richards EL, Murphy AS, Sato F, Yazaki K (2005) PGP4, an ATP binding cassette P-glycoprotein, catalyzes auxin transport in Arabidopsis thaliana roots. Plant Cell 17:2922–2939. https://doi.org/10.1105/tpc.105.035816.2

Tian QY, Sun P, Zhang WH (2009) Ethylene is involved in nitrate-dependent root growth and branching in Arabidopsis thaliana. New Phytol 184:918–931

Tirichine L, Sandal N, Madsen LH, Radutoiu S, Albrektsen AS, Sato S, Asamizu E, Tabata S, Stougaard J (2007) A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. Science 315(5808):104–107. https://doi.org/10.1126/science.1132397

Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2011) Shovelomics: high throughput phenotyping of maize (Zea mays L.) root architecture in the field. Plant Soil 341:75–87. https://doi.org/10.1007/s11104-010-0623-8

Ubeda-Tomás S, Beemster GT, Bennett MJ (2020) Hormonal regulation of root growth: integrating local activities into global behaviour. Trends Plant Sci 17:326–331. https://doi.org/10.1016/j.tplants.2012.02.002

Udvardi M, Brodie EL, Riley W, Kaeppler S, Lynch J (2015) Impacts of agricultural nitrogen on the environment and strategies to reduce these impacts. Procedia Environ Sci 29:303. https://doi.org/10.1016/j.proenv.2015.07.275

ULLAH NAJEEB LX, ALI S, IQBAL N, ZHOU W (2015) Mat rush (juncus effusus L.) trounces manganese toxicity through ultra-morphological modifications and manganese restriction in roots. Pak J Bot 47(1):241–246

Vanlauwe B, Hungria M, Kanampiu F, Giller KE (2019) The role of legumes in the sustainable intensification of African smallholder agriculture: Lessons learnt and challenges for the future. Agric Ecosyst Environ 284:106583

Vidal EA, Araus V, Lu C, Parry G, Green PJ, Coruzzi GM, Gutiérrez RA (2010) Nitrate-responsive miR393/AFB3 regulatory module controls root system architecture in Arabidopsis thaliana. Proc Natl Acad Sci 107:4477–4482. https://doi.org/10.1073/pnas.0909571107

Vissenberg K, Claeijs N, Balcerowicz D, Schoenaers S (2020) Hormonal regulation of root hair growth and responses to the environment in Arabidopsis. J Exp Bot 71:2412–2427. https://doi.org/10.1093/jxb/eraa048

Volpe V, Dell'Aglio E, Bonfante P (2013) The Lotus japonicus MAMI gene links root development, arbuscular mycorrhizal symbiosis and phosphate availability. Plant Signal Behav 2324:e23414–e23421. https://doi.org/10.4161/psb.23414

Walch-Liu P, Filleur S, Gan Y, Forde BG (2005) Signaling mechanisms integrating root and shoot responses to changes in the nitrogen supply. Photosynth Res. https://doi.org/10.1007/s11120-004-2080-9

Wang X, Pan Q, Chen F, Yan X, Liao H (2011) Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. Mycorrhiza 21:173–181. https://doi.org/10.1007/s00572-010-0319-1

Wang L, Wang L, Tan Q, Fan Q, Zhu H, Hong Z, Zhang Z, Duanmu D (2016) Efficient inactivation of symbiotic nitrogen fixation related genes in Lotus japonicus using CRISPR-Cas9. Front Plant Sci 7:1–13. https://doi.org/10.3389/fpls.2016.01333

Wang W, Hu B, Yuan D, Liu Y, Che R, Hu Y, Ou S, Liu Y, Zhang Z, Wang H, Li H (2018) Expression of the nitrate transporter gene OsNRT1.1A/OsNPF6.3 confers high yield and early maturation in rice. Plant Cell 30:638–651. https://doi.org/10.1105/tpc.17.00809

Williamson JM (2011) The role of information and prices in the nitrogen fertilizer management decision: New evidence from the agricultural resource management survey. J Agric Econ 36:552–572

Wissuwa M, Kretzschmar T, Rose TJ (2016) From promise to application: root traits for enhanced nutrient capture in rice breeding. J Exp Bot 67:3605–3615. https://doi.org/10.1093/jxb/erw061

Worku M, Bänziger M, Erley GS, Friesen D, Diallo AO, Horst WJ (2007) Nitrogen uptake and utilization in contrasting nitrogen efficient tropical maize hybrids. Crop Sci 47(2):519–528

Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol 63:153–182. https://doi.org/10.1146/annurev-arplant-042811-105532

Xu L, Cao M, Wang Q, Xu J, Liu C, Ullah N, Li J, Hou Z, Liang Z, Zhou W, Liu A (2022) Insights into the plateau adaptation of Salvia castanea by comparative genomic and WGCNA analyses. J Adv Res. https://doi.org/10.1016/j.jare.2022.02.004

Yan Z, Hossain MS, Wang J, Valdés-López O, Liang Y, Libault M, Qiu L, Stacey G (2013) miR172 regulates soybean nodulation. Mol Plant Microbe Interact 26:1371–1377

Yang X, Kim MY, Ha J, Lee S-H, (2019) Overexpression of the soybean NAC gene GmNAC109 increases lateral root formation and abiotic stress tolerance in transgenic Arabidopsis plants. Front Plant Sci 10, 1–12. https://doi.org/10.3389/fpls.2019.01036

Yang X, Lee S, So JH, Dharmasiri S, Dharmasiri N, Ge L, Jensen C, Hangarter R, Hobbie L, Estelle M (2004) The IAA1 protein is encoded by AXR5 and is a substrate of SCF. Plant J 40:772–782. https://doi.org/10.1111/j.1365-313X.2004.02254.x

Yang H, Zhang Q, Dai Y, Liu Q, Tang J, Bian X, Chen X (2015) Effects of arbuscular mycorrhizal fungi on plant growth depend on root system: A meta-analysis. Plant Soil 389:361–374. https://doi.org/10.1007/s11104-014-2370-8

Yendrek CR, Lee YC, Morris V, Liang Y, Pislariu CI, Burkart G, Meckfessel MH, Salehin M, Kessler H, Wessler H, Lloyd M (2010) A putative transporter is essential for integrating nutrient and hormone signaling with lateral root growth and nodule development in Medicago truncatula. Plant J 62:100–112. https://doi.org/10.1111/j.1365-313X.2010.04134.x

York LM, Lynch JP (2015) Intensive field phenotyping of maize (Zea mays L.) root crowns identifies phenes and phene integration associated with plant growth and nitrogen acquisition. J Exp Bot 66:5493–5505. https://doi.org/10.1093/jxb/erv241

Yu P, Li X, White PJ, Li C (2015) A large and deep root system underlies high nitrogen-use efficiency in maize production. PLoS ONE 3:1–17. https://doi.org/10.1371/journal.pone.0126293

Yu LH, Wu J, Tang H, Yuan Y, Wang SM, Wang YP, Zhu QS, Li SG, Xiang CB (2016) Overexpression of Arabidopsis NLP7 improves plant growth under both nitrogen-limiting and -sufficient conditions by enhancing nitrogen and carbon assimilation. Nat Publ Gr. https://doi.org/10.1038/srep27795

Yuan L, Loque D, Kojima S, Rauch S, Ishiyama K, Inoue E, Takahashi H, von Wirén N (2007) The organization of high-affinity ammonium uptake in Arabidopsis roots depends on the spatial arrangement and biochemical properties of AMT1-type transporters. Plant Cell 19(8):2636–2652

Zhang H, Rong H, Pilbeam D (2007) Signalling mechanisms underlying the morphological responses of the root system to nitrogen in Arabidopsis thaliana. J Exp Bot 58:2329–2338. https://doi.org/10.1093/jxb/erm114

Zhang J, Dong S, Dai X, Wu T, Wang X, Bai H, Wang L, He M (2016) Combined effect of plant density and nitrogen input on grain yield, nitrogen uptake and utilization of winter wheat. Vegetos 29:2. https://doi.org/10.5958/2229-4473.2016.00023.9

Zhang X, Cui Y, Yu M, Su B, Gong W, Baluška FE, Komis G, Šamaj J, Shan X, Lin J, (2020) Phosphorylation-mediated dynamics of

nitrate transceptor NRT1.1 regulate auxin flux and nitrate signaling in lateral root growth. Plant Physiol 181:480–498. https://doi.org/10.1104/pp.19.00346

Zörb C, Ludewig U, Hawkesford MJ (2018) Perspective on wheat yield and quality with reduced nitrogen supply. Trends in Plant Sci 23:1029–1037. https://doi.org/10.1016/j.tplants.2018.08.012