



An emerging role of heterotrimeric G-proteins in nodulation and nitrogen sensing

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Abstract

Main conclusion A comprehensive understanding of nitrogen signaling cascades involving heterotrimeric G-proteins and their putative receptors can assist in the production of nitrogen-efficient plants.

Abstract Plants are immobile in nature, so they must endure abiotic stresses including nutrient stress. Plant development and agricultural productivity are frequently constrained by the restricted availability of nitrogen in the soil. Non-legume plants acquire nitrogen from the soil through root membrane-bound transporters. In depleted soil nitrogen conditions, legumes are naturally conditioned to fix atmospheric nitrogen with the aid of nodulation elicited by nitrogen-fixing bacteria. Moreover, apart from the symbiotic nitrogen fixation process, nitrogen uptake from the soil can also be a significant secondary source to satisfy the nitrogen requirements of legumes. Heterotrimeric G-proteins function as molecular switches to help plant cells relay diverse stimuli emanating from external stress conditions. They are comprised of G α , G β and G γ subunits, which cooperate with several downstream effectors to regulate multiple plant signaling events. In the present review, we concentrate on signaling mechanisms that regulate plant nitrogen nutrition. Our review highlights the potential of heterotrimeric G-proteins, together with their putative receptors, to assist the legume root nodule symbiosis (RNS) cascade, particularly during calcium spiking and nodulation. Additionally, the functions of heterotrimeric G-proteins in nitrogen acquisition by plant roots as well as in improving nitrogen use efficiency (NUE) have also been discussed. Future research oriented towards heterotrimeric G-proteins through genome editing tools can be a game changer in the enhancement of the nitrogen fixation process. This will foster the precise manipulation and production of plants to ensure global food security in an era of climate change by enhancing crop productivity and minimizing reliance on external inputs.

Keywords Legumes · Root nodule symbiosis · Calcium spiking · Nitrogen fixation · G-proteins · Nitrogen sensing · Nitrogen use efficiency

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Introduction

G-proteins also referred to as guanine nucleotide-binding proteins, are grouped structurally into two distinct classes: monomeric G-proteins and heterotrimeric G-proteins (Pandey 2020; Wang and Botella 2022; Ganotra et al. 2023). The heterotrimeric G-proteins (hereafter G-proteins) comprise three different types of subunits: $G\alpha$, $G\beta$ and $G\gamma$ (Pandey 2019; Wang and Botella 2022). The number of G-protein subunits could vary from plant to plant. For instance, the model plant *Arabidopsis thaliana* with a simpler genome harbor one canonical $G\alpha$ (*GPA1*, *At2g26300*), three non-canonical extra-large $G\alpha$ (*XLG1*, *At2g23460*; *XLG2*, *At4g34390* and *XLG3*, *At1g31930*), one $G\beta$ (*AGB1*, *At4g34460*), and three $G\gamma$ genes, including two canonical $G\gamma$ (*AGG1*, *At3g63420* and *AGG2*, *At3g22942*) and one atypical $G\gamma$ (*AGG3*, *At5g20635*) (Stateczny et al. 2016; Maruta et al. 2021a; Cantos et al. 2023). *Brassica napus* contains 2 $G\alpha$, 6 $G\beta$ and 13 $G\gamma$ genes, which contribute to a total of 21 G-protein family members (Xie et al. 2022). *M. truncatula* is a premier model legume for studies pertaining to symbiotic interactions and nitrogen fixation. A study identified two $G\alpha$, three *XLG*, one $G\beta$ and five $G\gamma$ genes in the *M. truncatula* genome (Mt4.0) (Tang et al. 2014). Another study revealed the presence of two $G\alpha$, three *XLG*, one $G\beta$ and six $G\gamma$ genes in the *P. sativum* genome (Pecrix et al. 2018; Bovin et al. 2022). Plants harboring complex genomes have enlarged networks of G-proteins, such as allotetraploid *G. max*, which has four $G\alpha$, four $G\beta$ and ten $G\gamma$ proteins (Choudhury et al. 2011; Bisht et al. 2011).

G-proteins are molecular on–off switches, and in mammalian and yeast systems, the switch characteristic is encoded by nucleotides: a guanosine triphosphate (GTP)-bound on-state and a guanosine diphosphate (GDP)-bound off-state (Ghusinga et al. 2022). The signal perception by serpentine transmembrane receptors, namely G-protein-coupled receptors (GPCR), results in an alternation in its conformation, which subsequently functions as a guanine nucleotide exchange factor (GEF) to catalyze the GDP to GTP exchange on the $G\alpha$ protein (McIntire 2009; Pandey and Vijayakumar 2018; Pandey 2019). The $G\alpha$ component separates from the $G\beta\gamma$ subunits when it binds to GTP, allowing each of these to engage with diverse effectors to initiate downstream signaling (McIntire 2009). A signal is terminated when $G\alpha$ -GTP is inactivated by GTP hydrolysis, resulting in GDP-bound $G\alpha$ being released from its effector and reassociated with the $G\beta\gamma$ complex (McIntire 2009). A protein known as a regulator of G-protein signaling (RGS) with GTPase activating protein (GAP) activity speeds up the intrinsic GTP hydrolysis on the $G\alpha$ subunit (Siderovski and Willard 2005). The G-protein signaling

pathways in plants differ from the animal paradigm due to the existence of unique receptors and effectors, altered wiring of G-protein scenarios and disparate intrinsic characteristics of specific G-protein components (Pandey and Vijayakumar 2018; Maruta et al. 2019; Ghusinga et al. 2022). Notably, it has not been established that GPCRs can activate $G\alpha$ by promoting GDP to GTP exchange in plants (Pandey 2020). The genetic and biochemical data imply that the exchange of nucleotides is less crucial for G-proteins to function in plants (Maruta et al. 2019). Furthermore, numerous receptor-like kinases (RLKs) have been implicated in the phosphorylation and dephosphorylation of G-proteins to mediate their mechanistic regulation (Jia et al. 2019; Pandey 2020). *XLG* subunit, independent of GTP-binding, has been discovered to interact with the RGS, $G\beta\gamma$ dimer and defense-related RLKs with an affinity similar to that of canonical $G\alpha$ subunits (Liang et al. 2016; Lou et al. 2020; Maruta et al. 2021a, b). The functional investigation of RGS in *A. thaliana* and *G. max* signifies its crucial activities in the control of important physiological processes (Chen and Jones 2004; Choudhury and Pandey 2015). According to Hackenberg et al. (2017), several plants do not possess an RGS protein homolog. Therefore, it is ambiguous whether RGS-mediated deactivation is the primary mechanism controlling the G-protein cycle (Hackenberg et al. 2017).

G-proteins stimulate several intracellular signaling cascades in response to various extracellular stimuli (Majumdar et al. 2023). Almost every aspect of plant growth and development is influenced by the interaction of G-proteins with specific effectors (Roy Choudhury et al. 2019). These crucial physiological processes include regulating stomatal movement, nodulation and phytohormone signaling (Chakravorty et al. 2011; Choudhury and Pandey 2013, 2015, 2022; Jose and Choudhury 2020; Bhardwaj et al. 2020; Bovin et al. 2022). Interestingly, the knock-down of a particular G-protein subunit gene and its characterization displayed different root morphologies in both monocotyledons and dicotyledons (Table 1), thereby suggesting that G-proteins have a critical function in the development of plant roots in addition to their involvement in innate immunity and stress responses in plants (Trusov et al. 2007; Ding et al. 2008; Urano et al. 2015; Subramaniam et al. 2016; Gao et al. 2019; Maruta et al. 2021a). In this review, we have discussed the signaling mechanisms that involve G-proteins to regulate plant nitrogen nutrition.

Legumes can acquire nitrogen by atmospheric nitrogen fixation through endosymbiotic associations and the uptake of mineral nitrogen from soil (Murray et al. 2017; Roy et al. 2020; Gu et al. 2022; Zhong et al. 2022). The process of symbiosis in legumes is tightly regulated depending on the nitrogen levels in the soil. Legumes presumably evolved in nutrient-poor circumstances where the capacity to fix

Table 1 List of root morphological changes conferred by mutations of G-protein subunits

G-protein subunit	Plant species	Observed root phenotype	References
GPA1 (LOC_At2g26300)	<i>Arabidopsis thaliana</i>	Decrease in the root development	Ullah et al. (2003)
XLG1 (LOC_At2g23460), XLG2 (LOC_At4g34390), XLG3 (LOC_At1g31930)	<i>Arabidopsis thaliana</i>	Increase in the lateral root development	Ding et al. (2008)
Gα (LOC_Os05g26890)	<i>Oryza sativa</i>	Decrease in the root development	Izawa et al. (2010)
Gα (LOC_Zm00001d027886)	<i>Zea mays</i>	Decrease in the root development	Urano et al. (2015)
GPA1 (LOC_Csa4G648550)	<i>Cucumis sativus</i>	Smaller cotyledons, shorter hypocotyls and fewer lateral roots	Yan et al. (2018)
AGB1 (LOC_At4g34460)	<i>Arabidopsis thaliana</i>	Increase in the lateral root development	Ullah et al. (2003)
RGB1 (LOC_Os03g46650)	<i>Oryza sativa</i>	Suppressed embryonic shoot–root axis development. Exhibits compact root architecture	Gao et al. (2019), Urano et al. (2020)
AGG1 (LOC_At3g63420) AGG2 (LOC_At3g22942)	<i>Arabidopsis thaliana</i>	Increase in the number of lateral roots	Trusov et al. (2007)
GGB1 (LOC_Solyc12g096270.1.1)	<i>Solanum lycopersicum</i>	Increase in the number of lateral roots	Subramaniam et al. (2016)

atmospheric nitrogen offers a significant growth benefit. The low soil nitrogen conditions encourage symbiotic associations of legumes with biological machines such as rhizobacteria (Murray et al. 2017; Roy et al. 2020). Gram-negative rhizobia (such as *Rhizobium*, *Sinorhizobium*, *Azorhizobium*, *Bradyrhizobium*, and *Mesorhizobium*), that associate with legume roots are diazotrophic bacteria engaged in endosymbiotic relationships to develop nodules (Geurts and Bisseling 2002; Graham and Vance 2003; Desbrosses and Stougaard 2011; Rutten and Poole 2019; Mahmud et al. 2020). Nuclear calcium oscillations are responsible for stimulating endosymbiotic programmes in response to rhizobial signals (Granqvist et al. 2012; Charpentier and Oldroyd 2013; Charpentier 2018). Moreover, calcium spiking represents one of the earliest events that can be detected and is a highly conserved component of the mutualistic signaling mechanism (Granqvist et al. 2015). This review includes information regarding the involvement of G-proteins in calcium spiking during root nodule symbiosis (RNS).

The legumes develop root cortex-based nodules in symbiosis with rhizobia (Gauthier-Coles et al. 2019). In legumes, there are two basic morphological variants of nodules, namely indeterminate and determinate. These two forms are distinguished by the existence or absence of a persistent nodule meristem, which reliably coincides with the cortical cell layers that develop nodule primordia (Kohlen et al. 2018). For instance, *Glycine max* and *Lotus japonicus* develop round determinate nodules devoid of persistent meristem (Ferguson et al. 2010; Pan and Wang 2017). In contrast, *Medicago truncatula* and *Pisum sativum* produce indeterminate nodules with a cylindrical shape in which nodule primordia generate in the inner cortex, and mature nodules retain a persistent meristem (Pan and Wang 2017; Bovin et al. 2022). Among the repertoire of proteins present in

plants, the G-proteins have significant roles to play in the emergence of leguminous root nodules in response to a symbiotic relationship with rhizobia (Choudhury and Pandey 2013, 2015; Pandey 2020; Bovin et al. 2022), which have been highlighted in this review.

A major challenge for plant survival is nutrient acquisition from soil (Bhardwaj et al. 2015; Rahman et al. 2018). In plants, the detailed mechanism of nitrogen sensing pathways linked with G-proteins remains obscure. This review summarizes the developments in the mechanistic intricacies of nitrogen signaling involving G-proteins in plants. G-proteins show the ability to influence inflorescence, root architecture, seed size, number, and germination capacity, which in turn modulates essential agronomic features such as grain yield and nitrogen use efficiency (NUE) (Zhang et al. 2015; Wu et al. 2018; Liang et al. 2018; Kaur et al. 2018; Sun et al. 2018; Cui et al. 2020; Biswal et al. 2022). In this review, insights into the participation of G-proteins in regulating NUE in plants have also been discussed. This can assist in addressing the pressing issues of increasing crop growth and yield.

Possible role of G-proteins in root nodule symbiosis

RNS is a molecular interaction between the host plant and the bacterial partner (Lazdunski et al. 2004; Mbengue et al. 2020). The outcome of a highly specific and complex signal exchange between legumes and rhizobia is the selective rhizobial colonization of legume cells within root nodules, which develop upon activation of various genes, establishing the symbiotic pathway (Desbrosses and Stougaard 2011; Das et al. 2019; Chen et al. 2021). The sensing of plant

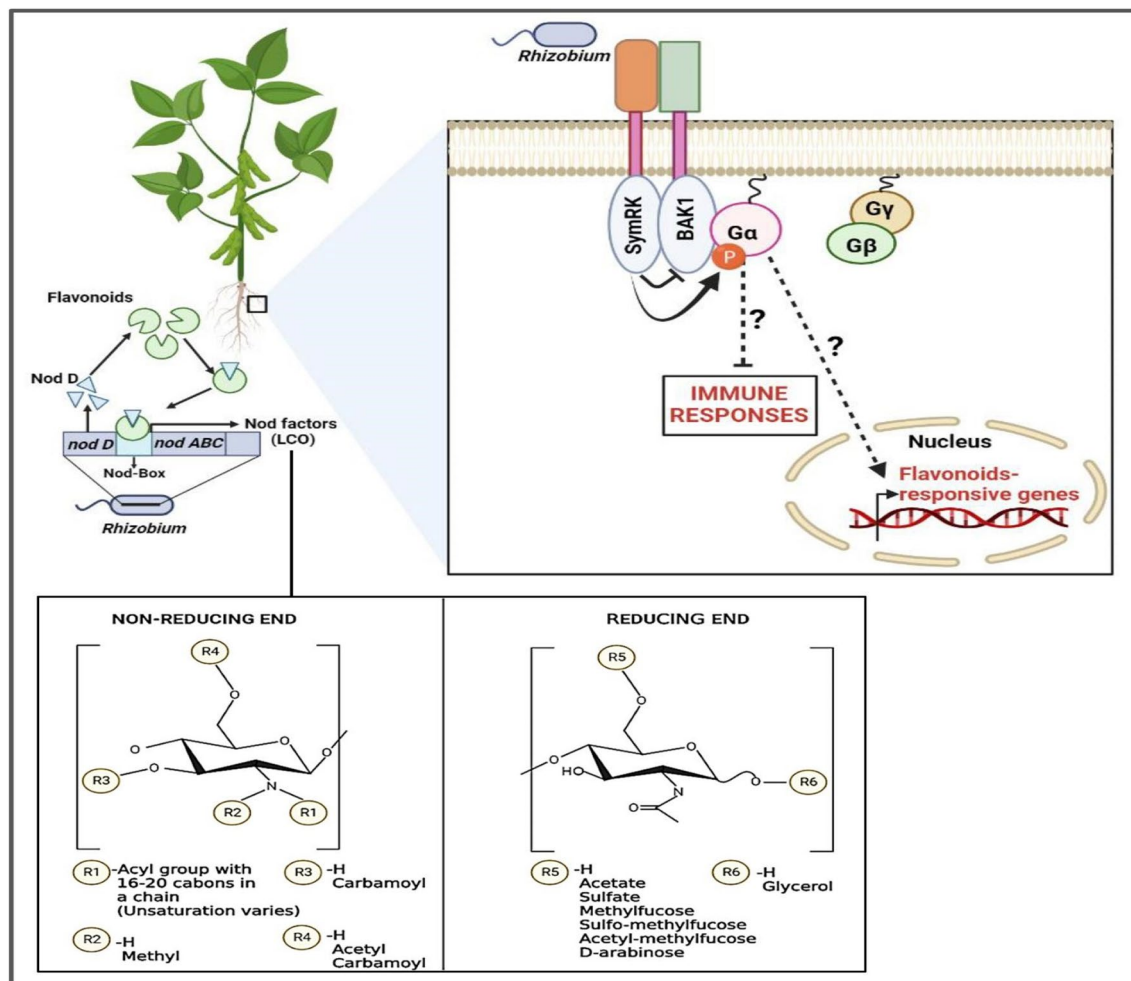


Fig. 1 Proposed model showing the role of $G\alpha$ subunit in flavonoid biosynthesis and immune responses during *G. max*-*Rhizobium* symbiosis. The signal exchange between legume and rhizobia is commenced by the secretion of flavonoids from the roots of leguminous hosts. The figure depicts that $G\alpha$ subunit is involved in the induction of flavonoids-responsive genes. The host flavonoids trigger the rhizobial Nod-cassette. The NodD protein, which is formed by rhizobia as a result of flavonoid perception, binds to the *nod* box in the promoter region of *nod* genes. This triggers the expression of the *nod* genes, which produces lipo-chitoooligosaccharides (LCO), also referred to as Nod factors (NFs). These NFs have β -1,4-linked *N*-acetyl-D-glucosamine framework with four or five reducing and non-reducing terminal glucosamine residues (Geurts and Bisseling 2002). NFs are species-

specific and undergo a range of substitutions at different positions of its non-reducing (-R1, -R2, -R3, -R4) and reducing ends (-R5, -R6) (Wang et al. 2018). *Rhizobium* releases NFs for symbiosis development. The intruding *Rhizobium* is initially perceived by the legume host as a potential pathogen, resulting in a transient defense reaction. Symbiosis receptor-like kinase (SymRK) associates directly with and suppresses the kinase activity of a positive regulator of plant immune responses, namely Brassinosteroid insensitive 1-associated receptor kinase 1 (BAK1). Further, SymRK phosphorylation of $G\alpha$ prevents it from interacting with $G\beta\gamma$ dimer. Consequently, $G\alpha$ is unable to influence host immune responses by ineffective interaction with BAK1 receptor

flavonoids by nitrogen-fixing rhizobia is one of the earliest steps in specificity between hosts and rhizobia (Fig. 1) (Hirsch and Fujishige 2012). Flavonoids are among the well-studied group of low molecular weight secondary metabolites (Hassan and Mathesius 2012; Dong and Song 2020) and are crucial for the sensitization of nitrogen-fixing bacteria (Liu and Murray 2016; Bag et al. 2022). Flavonoids are derived structurally from a 15-carbon skeleton composed of two benzene rings and are typically biosynthesized via the phenylpropanoid pathway (Liu and Murray 2016; Dong and

Song 2020). Plants produce a range of flavonoids, with up to 10,000 found across the plant kingdom (Mathesius 2018). The specific plant flavonoids that the rhizobia in the rhizosphere interact with and recognize include isoflavonoids, daidzein, genistein, coumestrol, and naringenin, among others (Liu and Murray 2016; Bosse et al. 2021). According to Sugiyama et al. (2008), the exudates from soybean roots contain genistein and daidzein, which serve as signal molecules in the chemical communication between soybean and *Bradyrhizobium japonicum*. The roots of *Phaseolus vulgaris*

when inoculated with *Rhizobium leguminosarum* shows the formation of nodules in the presence of genistein, daidzein, and coumestrol (Abd-Alla 2011). The flavonoid medicarpin, which is generated by both *Trifolium* and *Medicago* sp., has been shown to inhibit the growth of incompatible bacterial strains (Maxwell et al. 1989). Therefore, these studies imply that the ability of *Rhizobium* sp. to successfully form a symbiotic association is strongly influenced by the combination of host flavonoids present in the root exudate of legume species (Dong and Song 2020). Interestingly, in *A. thaliana*, G-protein signaling has been attributed to regulate flavonoid biosynthesis. The study revealed variations in the expression levels of several genes associated with the flavonoid biosynthesis in a knock-out mutant of the *A. thaliana* G-protein α subunit (*gpa1-5*) (Chakraborty et al. 2015). In future studies, the function of the G-protein complex signaling cascade can be determined in the legume flavonoid biosynthesis pathway using the G-protein subunit mutant studies. Consequently, establishing the involvement of the G-protein complex in the legume flavonoid synthesis cascade might pave avenues for enhancing nitrogen fixation by regulating the production of specific flavonoids using gene editing techniques.

Despite their diversity, all rhizobia contain conserved *nodABC* genes for the formation of the *N*-acylated oligosaccharide core of the lipo-chitooligosaccharides (LCO), also called Nod factors (NFs), implying that these genes are monophyletic (Debellé et al. 2001). The flavonoid perception by rhizobia results in changes in the conformation of the NodD protein, enabling it to attach to the *nod* box in the promoter region of *nod* genes. This triggers the *nod* gene expression of rhizobia, which eventually culminates in the synthesis of NFs to activate various host responses (Geurts and Bisseling 2002; Hassan and Mathesius 2012; Hirsch and Fujishige 2012; Ghantasala and Roy Choudhury 2022). The legumes possess specialized lysin-motif (LysM) and leucine-rich repeat (LRR)-containing RLKs for perceiving the rhizobial NFs (Singh and Verma 2023). These complex multicomponent receptors are localized in the plasma membrane of the root epidermal cells (Ferguson et al. 2010; Roy et al. 2020). Interestingly, the interplay between the symbiotic and defense signaling mechanisms is observed in legumes during nodulation (Cao et al. 2017; Ivanova et al. 2022). It is widely accepted that rhizobia actively inhibit the host's immune response to facilitate infection and symbiotic development (Cao et al. 2017). A study in *L. japonicus* reported that a key symbiotic component termed as Symbiosis receptor-like kinase (SymRK) aids in the rhizobial inhibition of plant innate immunity. SymRK interacts with Brassinosteroid insensitive 1-associated receptor kinase 1 (BAK1), a positive regulator of plant innate immunity, to repress BAK1 kinase activity during rhizobial infection (Feng et al. 2021). Conversely, treatment with flagellin 22 (flg22), a conserved peptide motif in the flagellar protein

of several bacteria, triggers defense responses that impede rhizobial infection and result in the delay of nodule organogenesis. This has been revealed by the adverse effect of flg22 on the spontaneous nodule production in the *L. japonicus* mutant, spontaneous-nodule-formation 1 (*snf1*). Moreover, after the symbiotic partner colonizes the host legume, the symbiotic pathway takes precedence over the defensive response (Lopez-Gomez et al. 2012). Previous studies examined the control of G-protein signaling by SymRK through protein–protein interactions and receptor-mediated in vitro phosphorylation during *G. max* nodulation (Choudhury and Pandey 2013, 2022). The studies reveal that SymRK phosphorylates $G\alpha$ to prevent the negative regulation of nodulation (Choudhury and Pandey 2015; Pandey 2020). Therefore, it suggests that $G\alpha$ might modulate host immunological responses by interacting with the SymRK-BAK1 complex, thereby affecting RNS. During RNS, the activity of G-protein subunits in the defense responses of legumes has not yet been investigated. Therefore, dissecting the specifics of all the molecular actors engaged in the RNS signaling cascade represents an exciting frontier of research. However, numerous studies illustrate the significance of G-proteins in other RNS stages, including calcium spiking and the control of nodule development in legumes (Choudhury and Pandey 2015; Mbengue et al. 2020; Pandey 2020; Bovin et al. 2022).

Involvement of G-proteins in calcium spiking

Various responses of NF signaling in the host plant include depolarization of the root hair plasma membrane, ion flux across the membrane, calcium spiking, changes in the cytoskeleton architecture, root hair curling, IT development and the production of nodule primordia (Heidstra and Bisseling 1996; Cárdenas et al. 2000; Tsyganova et al. 2018; Roy et al. 2020; Yang et al. 2022). In *L. japonicus* and *M. truncatula*, the membrane-bound Nod factor receptors (NFRs) such as LjNFR1/MtLYK3 and LjNFR5/MtNFP perceive diffusible NF released by rhizobia (Fig. 2) (Smit et al. 2007; Singh and Verma 2023). Also, LjSymRK/*M. truncatula* does not make infections 2 (MtDMI2) acts as a co-receptor with NFRs (Madsen et al. 2003; Antolín-Llovera et al. 2014; Singh and Verma 2023), which induces calcium spiking (or calcium oscillations) in and around the nucleus of root hair cells of host plants (Granqvist et al. 2015; Genre and Russo 2016).

Recently, a study in *P. sativum* reveals that calcium influx activation, which is followed by root hair deformation, involves the G-protein complex (Table 2) (Bovin et al. 2022). The interaction between LysM-RLK K1 and the PsG α 2 (*Psat5g034360*) was demonstrated in *P. sativum* with the aid of a Co-immunoprecipitation (Co-IP) assay (Bovin et al. 2022). According to a study, SYM10 has an apparently

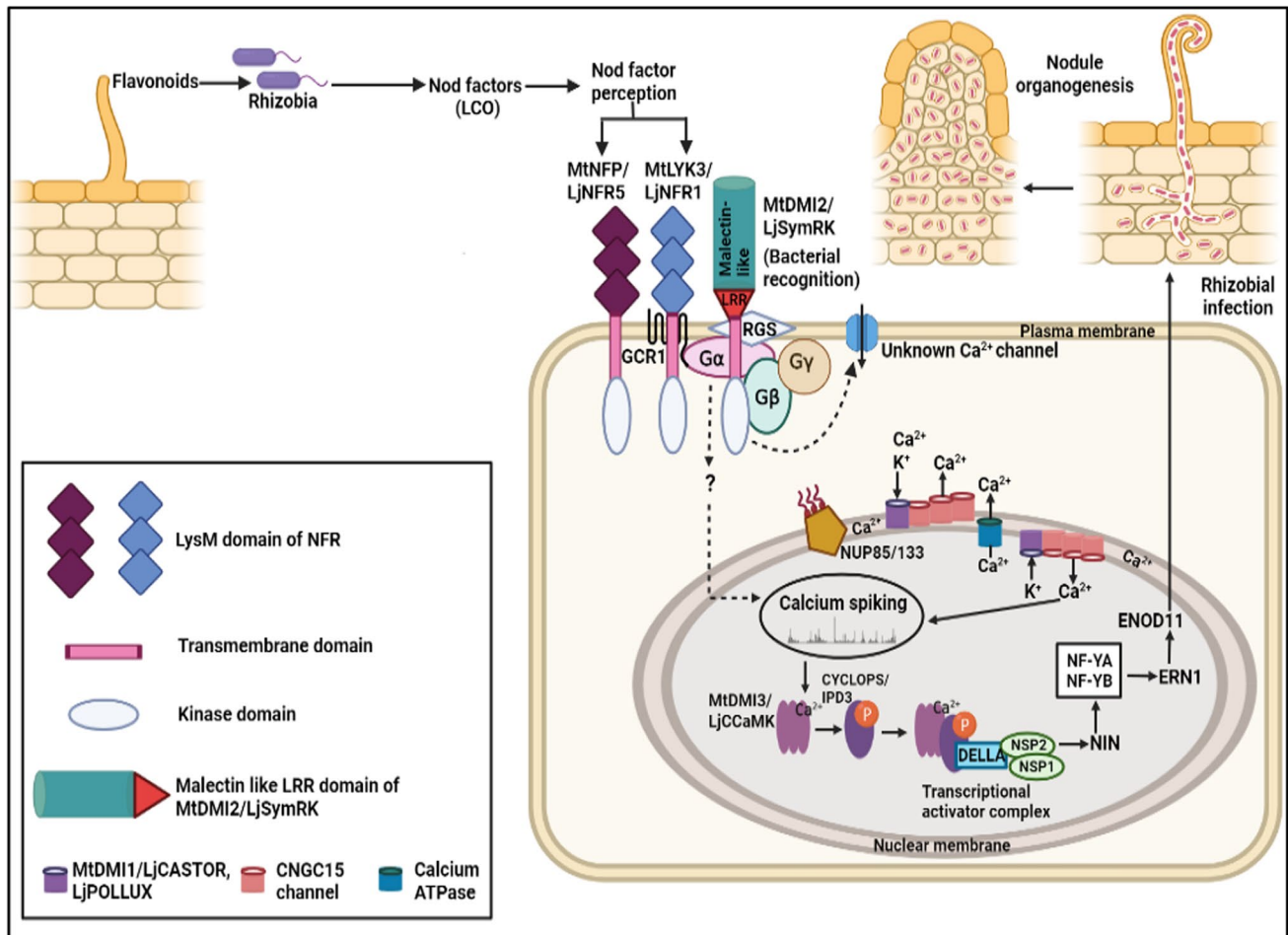


Fig. 2 Nod factor signaling cascade of events involving G-proteins occurring during legume-*Rhizobium* symbiosis. MtNFP/LjNFR5 and MtLYK3/LjNFR1 are receptor-like kinases (RLKs) involved in Nod factor (NF) sensing. *M. truncatula* Does not make infections 2 (MtDMI2)/LjSymRK functions as a co-receptor, causing intracellular signaling pathways to be activated in the legume root hair cells. The figure depicts the role of G α in mediating early symbiotic stages, including calcium (Ca²⁺) influx by regulating an unknown Ca²⁺ channel present in the plasma membrane. Consequently, cytosolic Ca²⁺ spiking occurs which further generates nuclear Ca²⁺ spikes, where nuclear pore complexes like nucleoporin 133 (NUP133) and NUP85 play a significant role (Kanamori et al. 2006). G-protein subunits interact with G-protein-coupled receptor 1 (GCR1) and Receptor of G-protein signaling (RGS) to effect nodulation. It is hypothesized

inactive kinase activity and associates with LysM-RLK K1 containing a YAQ motif in its kinase domain, making it functional in regulating symbiosis initiation (Nakagawa et al. 2011; Kirienko et al. 2018). This indicates the participation of G-proteins in calcium response followed by NF sensing in legumes during the initial developmental stages of symbiosis (Bovin et al. 2022). To positively control nodulation and expression of early nodulation genes in *G. max*, NFR1 phosphorylates RGS proteins. The phosphorylated RGS helps to initiate the signaling which results in calcium

that G-protein complexes that bind with and are phosphorylated by LjNFR1 activate the release of an unidentified secondary messenger, which would promote Ca²⁺ spiking. The MtDMI1/LjPOLLUX and LjCASTOR cation channels get activated by this unknown secondary messenger. MtDMI1/LjPOLLUX and CNGC15s influence nodule organogenesis by regulating Ca²⁺ or potassium (K⁺) ions (Venkateshwaran et al. 2012). MtDMI1/LjPOLLUX and CNGC15s influence nodule organogenesis by regulating Ca²⁺ or potassium (K⁺) ions (Venkateshwaran et al. 2012). MtDMI3/LjCCaMK phosphorylates CYCLOPS/IPD3, which further engages with transcription factors such as DELLA, nodulation signaling pathway 1 (NSP1) and NSP2 to enhance nodule inception (*NIN*) expression (Cerri et al. 2017; Diédhiou and Diouf 2018; Mbengue et al. 2020). This triggers nodule organogenesis or rhizobial infection involving *NIN*, NF-Ys, Ets2 repressor factors required for nodulation 1 (ERN1) and early nodulin 11 (ENOD11) (Laporte et al. 2014; Laloum et al. 2014)

spikes while retaining the negative regulator of nodulation (G α) in its inactive state (Choudhury and Pandey 2013, 2015; Mbengue et al. 2020). Also, in the absence of rhizobia, mastoparan 7, a G-protein agonist, mimics NF-induced early nodulin 11 (*ENOD11*) and *ENOD12* gene expression in root hair cells of *M. truncatula* during symbiosis and root hair deformation in *Vigna unguiculata*, which is prevented by the G-protein antagonist pertussis toxin (Pingret et al. 1998; Kelly and Irving 2003; Sun et al. 2007). Mastoparan 7 has also been proven to result in calcium spiking in the roots

Table 2 Various roles of G-protein subunits in nitrogen-related signaling in plants

G-protein subunits	Interacting proteins	Plant species	Functions	References
Gα2 (LOC_Psat5g034360)	Lysin-motif receptor-like kinase K1 (LysM-RLK K1)	<i>Pisum sativum</i>	In response to Nod factors, it shows involvement in calcium influx activation, which is followed by root hair deformation	Bovin et al. (2022)
Gα1 (LOC_Glyma04g05960.1)	Nod factor receptor 1 (NFR1), NFR5, Symbiosis receptor-like kinase (SymRK), Regulator of G-protein signaling (RGS)	<i>Glycine max</i>	NFR1 phosphorylation of RGS keeps Gα in an inactive state. SymRK phosphorylation of Gα prevents it from interacting with Gβγ dimer. Consequently, the Gα becomes inactive and the Gβγ dimer mediates effective nodulation	Choudhury and Pandey (2013), Choudhury and Pandey (2015), Pandey (2020)
MiGβ1 (LOC_Medtr3g116500) PsGβ1 (LOC_Psat5g006200)	Not reported	<i>Medicago truncatula</i> , <i>Pisum sativum</i>	Involvement in symbiosis pathway by regulating nodule numbers	Bovin et al. (2022)
GPA1 (LOC_At2g26300)	NRT1.1, G-protein-coupled receptor 1 (GCR1)	<i>Arabidopsis thaliana</i>	Regulates the expression of nitrate-responsive genes through ANR1	Chakraborty et al. (2015), Chakraborty et al. (2019)
DEP1 (LOC_Os09g0441900)	Not reported	<i>Oryza sativa</i>	Controls nitrate response	Sun et al. (2014), Li et al. (2023)
RGAI (LOC_Os05g26890) RGB1 (LOC_Os03g46650)	Not reported	<i>Oryza sativa</i>	Plays a role in nitrogen use efficiency Higher photosynthetic capability in <i>depl</i> variety under minimal nitrogen condition Nitrogen-mediated growth responses show inhibition upon reduced RGAI or increased RGB1 activity	Sun et al. (2014)
AGB1 (LOC_At4g34460)	Not reported	<i>Arabidopsis thaliana</i>	The high nitrogen-induced inhibition of root growth is abolished	Liang et al. (2018)
AGG3 (LOC_At5g20635)	Not reported	<i>Arabidopsis thaliana</i>	The model monocot <i>Setaria viridis</i> over-expressing <i>AtAGG3</i> showed increased root growth during the initial seedling stage of development, enhancing plant survival under conditions of low nitrogen availability	Kaur et al. (2018)

of *M. truncatula*, which are not reliant on NFP and DMI2 (Sun et al. 2007). Furthermore, an inhibitor of a downstream signaling component of G-protein namely, phospholipase D (PLD), ceases the calcium oscillations (Charron et al. 2004). According to these findings, mastoparan 7 either directly or indirectly activates PLD signaling by acting downstream of NFP and the DMI2. Accordingly, a hypothesis suggested that G-protein signaling downstream of the RLK could activate the production of an unidentified secondary messenger, which in turn causes nuclear calcium oscillations (Mbengue et al. 2020). The MtDMI1/LjPOLLUX and LjCASTOR cation channels, which show the interaction with three Cyclic Nucleotide-Gated Channels, namely CNGC15a, CNGC15b and CNGC15c, get activated by an unknown secondary messenger (Charpentier et al. 2016; Mbengue et al. 2020). Overall, G-protein subunits act as downstream elements of the NF perception pathway and trigger calcium spiking, which further activates a signal cascade involving various transcription factors including CYCLOPS/IPD3, DELLA, nodulation signaling pathway 1 (NSP1), NSP2 to enhance nodule inception (*NIN*) expression for the development of symbiotic nodules (Lévy et al. 2004; Tirichine et al. 2006; Singh et al. 2014; Laporte et al. 2014; Laloum et al. 2014; Mbengue et al. 2020; Yuan et al. 2022; Bovin et al. 2022).

Molecular basis of root nodulation involving G-proteins, their putative receptors, and associated proteins

Indications for the participation of G-proteins in the regulation of nodule development in various leguminous species have been found in several pharmacological and biochemical tests. The development of nodules is regulated by certain G-proteins subunits (Choudhury and Pandey 2013). Over-expression of G-protein components and RNAi suppression leads to a higher and lower number of nodules, respectively, confirming their functions as positive regulators of nodule development (Choudhury and Pandey 2013). One finding is direct evidence for the significance of MtG β 1 and PsG β 1 in symbiosis development regulation in *M. truncatula* and *P. sativum*, respectively, wherein the number of indeterminate nodules generated were considerably reduced by RNAi-based inhibition of *MtG β 1* (*Medtr3g116500*) and *PsG β 1* (*Psat5g006200*) (Bovin et al. 2022).

A variety of membrane-bound receptors, including GPCR, RGS and RLKs, can be coupled with G-proteins in plants (Pandey 2019; Chakraborty and Raghuram 2022). In accordance with a study, the downregulation of *GCR1* in *L. japonicus* results in a significant impact on *LjNIN*, a downstream component of the G-protein signaling network, which encodes for transcriptional factors crucial for nodulation (Rogato et al. 2016). According to Choudhury and Pandey

(2015), a RLK protein complex i.e., NFR1–NFR5–SymRK interacts and phosphorylates G-protein subunits. Enhanced GAP activity toward the G α subunit is demonstrated by phosphorylated RGS, implying that RLK-mediated phosphorylation of RGS enables the G-protein cycle to cease more rapidly (Choudhury and Pandey 2016). Therefore, NFR1 phosphorylation of RGS keeps G α in an inactive state, whereas SymRK phosphorylation of G α prevents it from interacting with G $\beta\gamma$. In this scenario, the negative regulator G α would be inactivated, and the positive regulators, i.e., G $\beta\gamma$, would lead to effective nodulation (Choudhury and Pandey 2015; Pandey 2020). A recent study demonstrated that SymRK phosphorylates G α in vitro at numerous sites, including two in the active site to prevent GTP binding (Choudhury and Pandey 2022). The two amino acids that are phosphorylated in the active region of conventional G α are conserved in the GTP-binding pocket of XLG proteins, suggesting that XLG may also be phosphorylated and influenced by SymRK (Pandey 2020). Similar to the G α subunit, the XLG subunit is also involved in interactions with G $\beta\gamma$ and RGS protein (Pandey 2020; Lou et al. 2020; Maruta et al. 2021a, b). Although XLG proteins are important regulators of defense signaling that operate in parallel with the classical G α proteins in *A. thaliana*, their role in the regulation of nodulation has not been fully investigated (Maruta et al. 2015; Liang et al. 2016; Pandey 2020). This emphasizes the need for elucidating the potential role of XLG proteins in nodule formation that can be gleaned from insights into the molecular mechanism of XLG signaling transduction cascades.

Various well-known G-protein signaling downstream components, including PLC, PLD, diacylglycerol pyrophosphate, phosphatidic acid, and G-protein-related phosphoinositide 3-kinase, have been associated with the regulation of nodulation (Misra et al. 2007; Peleg-Grossman et al. 2007; Santos-Briones et al. 2009). The Co-IP experiments have confirmed the association of G-protein subunits with PLC, implying crosstalk between G-protein and PLC-mediated symbiotic signaling pathways in both *M. truncatula* and *P. sativum* (Bovin et al. 2022). In plants, Phospholipase D α 1 (PLD α 1) is a crucial regulatory element of the G-protein signaling complex (Li et al. 2009; Lu et al. 2013) and it also interacts with G α as well as G β proteins (Zhao and Wang 2004; Gookin and Assmann 2014). According to Roy Choudhury and Pandey (2016), PLD α 1 and RGS1 are found in proximity to the G-protein complex, or perhaps PLD α 1 and G-proteins form a macromolecular complex. This model positions PLD α 1 and G α downstream of RGS1, which serves as PLD α 1 inhibitor rather than GAP and attributes the role of GAP to PLD α 1 (Roy Choudhury and Pandey 2016). Also, pharmacological and molecular methods have been employed to uncover the role of PLD α 1 in signaling during nodulation in soybean (Zhang et al. 2021). Taken

together, these observations led to the suggestion of a simplified mechanism for the roles of the RGS, PLD α 1, and G-proteins in RNS (Pacheco and Quinto 2022). According to this model, when NFs are perceived, the cytosolic kinase domain of NFR1 phosphorylates RGS and as a result, PLD α 1 is released from its inactivated state. The G-protein complex is then rendered inactive by the active PLD α 1 that act as a GAP. Consequently, the G α subunit cannot inhibit the growth of nodules, and free G $\beta\gamma$ dimers serve as positive regulators of nodule development (Pacheco and Quinto 2022). Moreover, future research can also determine the role of any member of the PLD family other than PLD α 1 in controlling the G-protein cycle (Pacheco and Quinto 2022). This indicates the necessity of further research to shed light on the molecular mechanisms underlying potential G-protein and PLD signaling during the root nodule development in legumes.

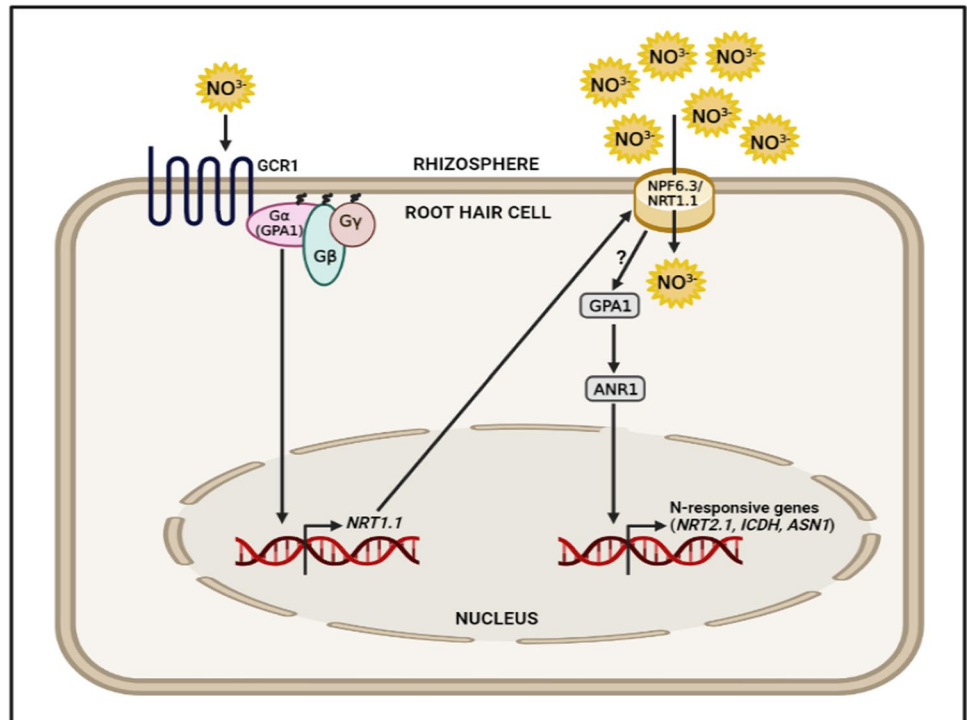
Soil nitrogen sensing in conjunction with G-proteins

In soils, numerous microbial communities engage in nitrogen transformations into plant-usable forms to regulate nitrogen mobilisation and fixation capacity (Robertson and Groffman 2007). In aerobic soils, most plants have nitrate as their principal source of nitrogen (Liu et al. 2015). The nitrate transporters, or channels, are grouped into five families in higher plants, namely, the nitrate transporter 1/peptide

transporter family (NPF or NRT1/PTR), nitrate transporter 2 (NRT2), aluminium-activated malate transporter (ALMT), slow anion channel-associated 1 homolog 3 (SLAC1/SLAH3) and chloride channel (CLC) (Krapp et al. 2014; L eran et al. 2015; Pellizzaro et al. 2017). The first identified nitrate transporter implicated in the primary nitrate response (PNR) in *A. thaliana* was the moonlighting protein AtNPF6.3 (AtNRT1.1 or CHL1) (Fichtner et al. 2021; Gu et al. 2022). Members of the NPF and NRT2 families have been shown to associate with nitrate acquisition in roots (Gu et al. 2022). Moreover, plants have developed two nitrate uptake systems: a high-affinity transport system (HATS) and a low-affinity transport system (LATS) (Wang et al. 2012). In comparison with low-affinity transporters, high-affinity transporters function better at lower concentrations of nitrate while becoming saturated at greater nitrate concentrations (Muratore et al. 2021). More importantly, except for NPF6.3 (NRT1.1 or CHL1), all members of the NRT2 family are HATS, while the majority of the NPF/NRT1 family members are LATS (Ho et al. 2009; Wang et al. 2012). NPF6.3 is considered as a dual-affinity nitrate sensor and transporter in *A. thaliana* (Ho et al. 2009; Gu et al. 2022).

Several findings reveal the importance of G-proteins in regulating nitrate uptake and metabolism encoding genes in plants (Fig. 3) (Chakraborty et al. 2015, 2019; Liu et al. 2018; Pathak et al. 2021). Researchers are increasingly aware that GPCR is found in a multitude of cells, tissues, and organs in animals, plants, and microbes and that it shows involvement in the detection of a variety of nutrients (Moran

Fig. 3 A simplified representation of the involvement of G-protein subunits in nitrate signaling in *A. thaliana*. NPF6.3 (NRT1.1 or CHL1) functions as a transporter as well as a sensor of nitrate. Depending on the nitrate levels, regulation of the expression of *NRT1.1* shows probable involvement of GCR1-GPA1 coupling followed by an interaction with the *Arabidopsis* nitrate regulated 1 (ANR1) transcription factor. This signaling subsequently regulates the nitrogen (N) responsive genes, which include nitrogen starvation/assimilation genes such as the nitrate transporter *NRT2.1*, isocitrate dehydrogenase (*ICDH*) and asparagine synthase 1 (*ASN1*)



et al. 2021; El-Defrawy and Hesham 2020; Chakraborty and Raghuram 2022). Previous findings in *A. thaliana* have shown genetic evidence of the GCR1-GPA1 linkage controlling the nitrate response (Chakraborty et al. 2015, 2019). In a report, *gpa1-5*, *gcr1-5*, and *gpa1-5gcr1-5* mutants were studied to investigate the role of three mutants in regulating nitrate uptake and metabolism encoding genes. *NRT1* was shown to be highly expressed in all three mutants as compared to the WT at low nitrate levels, but *NRT1* expression was low at high nitrate levels (Chakraborty et al. 2019). By considering *NRT1* as a low-affinity nitrate transporter, the low nitrate condition may have been insufficient due to mutations in *GCR1* and/or *GPA1*, resulting in increased expression of *NRT1*, which was not observed at a high nitrate level (Chakraborty et al. 2019). Henceforth, according to the aforementioned study, the *gcr1-5* mutant exhibits an altered dose-dependent differential nitrogen response for *NRT1* gene expression. Additionally, a study has revealed the in vitro interactions of GPCR with all three subunits of G-proteins in *P. sativum* (Misra et al. 2007). Therefore, it develops an interest in exploring whether coupling of G-proteins with GPCR in *P. sativum* also effectuates significant expression level alterations of nitrate transporters. The canonical GPCRs are often implicated in agronomically significant processes in plants (Chakraborty and Raghuram 2022), but the level of association of GPCR with the G α subunit has remained mostly unidentified (Chakraborty et al. 2019). The findings of future investigations can provide compelling scientific evidence to reassess the involvement of GPCR in plant G-protein-mediated signaling pathways.

Arabidopsis nitrate regulated 1 (ANR1), a MADS intervening keratin-like and C-terminal (MIKC)-type MADS-box transcription factor, was the first to be discovered in nitrate signaling pathways (Zhang and Forde 1998). In nitrate-rich localized areas, lateral root elongation is hindered in *ANR1* loss-of-function mutants, which affects the root system's plasticity (Zhang and Forde 1998). Later research revealed that *NRT1.1* regulates *ANR1* at the transcriptional level (Remans et al. 2006). According to Chakraborty et al. (2015), *NRT1.1* interacts with *AtGPA1*, which controls the transcription of nitrate-responsive genes through ANR1. This study employed *gpa1-5*, a novel *GPA1* knock-out mutant, to reveal that numerous nitrogen starvation/assimilation genes, such as the nitrate transporter *NRT2.1*, isocitrate dehydrogenase (*ICDH*), and asparagine synthase (*ASN1*), were up-regulated in *gpa1-5* (Chakraborty et al. 2015). It has been demonstrated that a *Triticum aestivum* G β gene, *TaNBPI* (AK332651), regulates transcription of the nitrate transporter gene (*NRT2.2*) in transgenic *Nicotiana benthamiana*, thereby indicating a role in nitrogen uptake (Liu et al. 2018). Additionally, the role of *RGA1* in nitrogen-responsive transcriptional regulation has been established by a study in *Oryza sativa* (Pathak et al. 2021). The genes encoding the

ammonium and nitrate transporters were shown to be down-regulated in the *rga1* mutant, demonstrating the relevance of *RGA1* function in nitrogen uptake. Moreover, the transcription factor network analysis of the *rga1* mutant revealed the importance of *RGA1* in regulating the nitrogen signaling cascade with several differentially expressed genes (DEGs), including *Nin-like* and *OsCIPK23*, among others (Pathak et al. 2021). Notably, the G-protein subunit mutant studies can be beneficial for translating key research findings from commercially significant crop species to legumes for deciphering the possible participation of G-proteins in nitrogen uptake.

According to Fan et al. (2017), there are reports on nitrate transporter being used for improving crop productivity. For instance, the increased expression of *OsNRT1.1B*, a low-affinity nitrate transporter, in *japonica* rice may aid in the improved sensing of varied nitrate concentrations and increasing the capacity for nitrogen accumulation inside the grain (Hu et al. 2015). In addition, rice with overexpressed *OsNRT2.3b* has a better ability to absorb other nutrients, which reduces photorespiration and promotes growth and grain yield (Fan et al. 2016). Furthermore, numerous studies demonstrate that G α subunits regulate the expression level of plant nitrate transporters (Chakraborty et al. 2015, 2019; Pathak et al. 2021). This indicates that optimizing nitrate uptake and utilization via G-protein subunits might contribute to increasing crop yield.

G-proteins and regulation of nitrogen use efficiency

NUE is a multigenic quantitative trait, including numerous nitrogen-responsive genes and mechanisms that require thorough characterization (Mandal et al. 2022). G-proteins are implicated in critical agronomic traits such as NUE, thereby directly impacting yield (Xu et al. 2016b; Stępczyński et al. 2016). There are findings on the significance of the G γ subunit in governing nitrogen assimilation and NUE. For instance, in rice, the *DEP1* (*Os09g0441900*) gene is a plant-specific G γ subunit that directs branching, density, and erectness of panicles and was also discovered to be a significant quantitative trait locus (QTL) for NUE (Huang et al. 2009; Sun et al. 2014, 2018; Xu et al. 2016a; Li et al. 2023). Both *RGA1* and *RGB1* subunits have been confirmed to interact with the *DEP1* protein in vivo (Sun et al. 2014). The G protein γ -like (GGL) domain of *DEP1* interacts with the rice *RGB1* subunit on the plasma membrane and within the nucleus. Additionally, *DEP1* binds with *RGA1*, and the von Willebrand factor type C (vWFC) domain at the C-terminus of *DEP1* may be involved in this interaction (Sun et al. 2014). According to the study, nitrogen-mediated growth responses are likewise inhibited by reduced *RGA1* or

increased RGB1 activity (Sun et al. 2014). Also, plants containing the *dep1-32* loss-of-function allele are unaffected by nitrogen availability, whereas plants with the *dep1-1* gain-of-function allele demonstrate higher nitrogen uptake even when nitrogen is scarce, suggesting that manipulating G-protein activity could be a novel strategy for regulating NUE (Sun et al. 2014). *DEP1* affects genes related to ammonium absorption and assimilation (such as *OsAMT1;1*, *OsGS1;2* and *OsNADH-GOGATI*), thereby regulating nitrogen uptake and metabolism (Huang et al. 2009). These genes showed an up-regulation in *dep1* allelic plants when nitrogen levels were low. Despite the low nitrogen availability, the *dep1* allelic plants exhibit higher glutamine synthase activity and accumulate more internal nitrogen than the *DEP1* allelic plants (Sun et al. 2014). According to a recent study in rice, the increased stomatal conductance conferred by *dep1* results in a higher photosynthetic capability under minimal nitrogen circumstances (Li et al. 2023). This research aids to comprehend the photosynthetic efficiency of the *dep1* variety in low nitrogen environments by analyzing photosynthesis, stomatal function, and nitrogen uptake and assimilation (Li et al. 2023).

Furthermore, nitrogen heterogeneity in the soil is a key factor in determining root development (Araya et al. 2016). While nitrogen deficiency stops root growth, a moderate amount of nitrogen in the soil encourages the extension of lateral roots that would otherwise be stunted in high nitrogen environments (Linkohr et al. 2002; Liu et al. 2017). Altering the root system architecture is an adaptive strategy to improve NUE (Awasthi and Laxmi 2021). According to a study, the rice G-protein mutants vary from the WT in lateral root number and high nitrogen inhibition growth (Liang et al. 2018). The high nitrogen-induced suppression of root growth was abolished in the *A. thaliana agb1-2* mutant. This indicates that G-protein modulates the root architecture in response to nitrogen availability (Liang et al. 2018). In *T. aestivum*, the nitrogen starvation response is reportedly regulated by *TaNBP1* (Liu et al. 2018). In line with its function in promoting nitrogen accumulation, *TaNBP1* overexpression in *N. benthamiana* results in enhanced phenotypic, expanded root system architecture and increased biomass for transgenic plants under nitrogen deficiency in comparison to the WT (Liu et al. 2018). Furthermore, a class C G γ subunit of *A. thaliana* termed as AGG3 may also be involved in the NUE. During the early seedling stage of development, the model monocot *Setaria viridis* overexpressing *AtAGG3* (*At5g20635*) showed improved root growth, enabling greater plant survival under limited nitrogen circumstances (Kaur et al. 2018). A study in *B. napus* revealed that under nitrogen deprivation, *G α* (*BnGAI*) and five C-type G γ genes (*BnGG9*, *BnGG10*, *BnGG11*, *BnGG12* and *BnGG13*) were initially upregulated in roots, while *G α* was initially downregulated and five C-type G γ genes were substantially expressed at

various times in leaves (Xie et al. 2022). These findings shed light on the biological processes that G-protein genes perform in response to an inadequate supply of nitrogen (Xie et al. 2022). Through genetically abrogating each of the three rice *XLGs* separately and synergistically using CRISPR/Cas9 genome editing in rice, a study uncovered a role for *XLGs* in agronomic traits. The research results reflect that non-canonical *XLGs* are crucial regulators of rice plant growth, grain filling and panicle phenotype (Biswal et al. 2022). Henceforth, deciphering the *XLG* signaling in agronomic performance can assist in establishing its association with NUE for crop improvement strategies.

Conclusions

The increasing use of nitrogen fertilizers contributes to an enormous growth in agricultural production. However, at the same time, soil quality has significantly deteriorated. Decreased soil fertility and unsustainable long-term crop yields are potential consequences of synthetic chemicals which very often suppress the symbiotic nitrogen fixation process (Aker et al. 2018; Reinprecht et al. 2020; Mórning et al. 2021). With steady population growth and climate change, it has become challenging to increase crop productivity without exacerbating environmental degradation. The development of plants that could flourish independently of expensive nitrogen fertilizers would be a significant achievement in research related to nitrogen fixation. G-proteins and their potential receptors are emerging as key players in understanding root development and nodulation process. Hence, a deeper knowledge of G-protein signaling can provide insights into the control of symbiosis and root development, thereby laying the foundation for a multitude of studies in the future. Moreover, persistent efforts to incorporate the nitrogen-fixing trait into non-legume crops, particularly cereals, which constitute a significant section of the food basket, can lessen our reliance on inorganic nitrogen fertilizers. Also, uncovering the function of G-proteins in the nitrate sensing pathway and their translation into growth-enhancing reactions is crucial for enhancing NUE, crop productivity and reducing pollution caused by chemical fertilizers. The emerging research on regulating nutrient stress is supplemented by the multiple functions of G-proteins in plant root development and nitrogen signaling. Therefore, besides alleviating plant abiotic and biotic stress, G-proteins may also aid in plant adaptation to nutrient stress. This will assist in engineering more efficient crops with improved NUE by genome modifications to maximize crop yield and limit excess nitrogen being added to the environment, thereby promoting a sustainable future for modern agriculture.

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