Journal of Food Legumes
37(3): 243-254, 2024
DOI: 10.59797/jfl.v37.i3.203

Review Paper

Heritability, signal perception and autoregulation of root nodulation in chickpea

Amrita V[1], Shanmugavadivel PS[2], Madhubala[1], Sonika[1], Rimzhim[1], Priyanka[1], Swarnalakshmi K[3] and Senthilkumar M[1*]

[1]*Division of Basic Sciences, ICAR-Indian Institute of Pulses Research, Kanpur-208 024, Uttar Pradesh, India*
[2]*Division of Plant Biotechnology, ICAR-Indian Institute of Pulses Research, Kanpur-208 024, Uttar Pradesh, India*
[3]*Division of Microbiology, ICAR-Indian Agricultural Research Institute, Pusa-New Delhi-110012, India*

[*]***Corresponding author e-mail:*** *m.kumar@icar.gov.in*

Received: *05 September 2024*
Accepted: *24 September 2024*

Handling Editor:
Dr. Narendra Kumar, ICAR-Indian Institute of Pulses Research, Kanpur, India

ABSTRACT

Chickpea (*Cicer arietinum* L.) establishes symbiotic interactions with *Mesorhizobium* to develop root nodules where nitrogen fixation occurs. This symbiotic relationship can fix atmospheric nitrogen (N_2) up to 140 kg N/ha that contribute nearly 80% nitrogen requirement of the crop. Global researchers had revealed the existence of natural variations in chickpea germplasm for nodulation traits with high heritability. Surprisingly, the contribution of environmental variation is too low for Biological Nitrogen Fixation (BNF) traits and high broad-sense heritability (>60) was observed for early nodulation, late nodule senescence and high nodule number traits. Correlation studies indicated a strong positive correlation between nodule number at flowering stage with total nodule weight and plant biomass and seed protein content. Nod Factor receptors in chickpea (CaNFR1 and CaNFR5) are characterized recently that forms phylogenetically distinct group along with *M. truncatula*, *P. sativum*, and *L. japonicus*. Critical role of cytokinin signalling through members of two component system (TCS) in nodulation was investigated in chickpea. The chickpea ortholog CaHK19 was the master spigot of cytokinin perception in chickpea. The co-expression pattern of CaHKs and CaNIN clearly indicated a link between cytokinin perception and downstream expression of CaNIN in chickpea as earlier established in *Medicago*. Genes involved in AON pathway are partially revealed in chickpea. CaRND1, CaRDN2, and CaRDN3 (*C. arietinum* Root-Determined Nodulation) function as receptors for signals produced from the roots. Revealing the molecular basis of root nodule organogenesis and their regulatory mechanisms along with identification of potential genetic stock will help on breeding or engineering chickpea genotypes with high symbiotic efficiency, extended nitrogen fixation and high symbiotic efficiency make grain legumes as nitrogen fixing factories to fertilize the soil in a sustainable way.

Key words: Chickpea, Nod Factors, Receptors, Nodule Inception, Transcription Factors, Autoregulation of Nodulation

INTRODUCTION

Plant requires nitrogen (N_2) for its growth in a larger quantity compared to other elements and N_2 makes 78% of earth's atmosphere. However, plants are unable to directly use the atmospheric nitrogen due to the stability and strong triple bond between nitrogen atoms. Plant available fertilizer nitrogen is produced by reducing N_2 through Haber–Bosch process that emits approximately 465 teragrams of carbon dioxide annually, making it a significant source of greenhouse gas emissions (IFA 2018, Brentrup *et al*. 2016, Zhang *et al*. 2013). Production of fertilizer nitrogen contributed up to 1.2% of total greenhouse emissions resulting from human activities (Wood and Cowie 2004). Additionally, the nitrification and denitrification processes in the soil release substantial amounts of nitrous oxide (N_2O), accounting for approximately 1.5% of total greenhouse emissions in agricultural systems (Eggleston *et al*. 2006). The production of agrochemicals including fertilizers heavily relies on fossil fuels and approximately 80% of the world's fossil energy being utilized. Over the past four decades, global nitrogen fertilizer usage has significantly increased, contributing to over half of the energy consumed in agriculture (Ghavam *et al*. 2021).

One of the biological processes that enhance nitrogen availability to plants is biological nitrogen fixation (BNF). Particularly, Root/stem nodulation in legumes by *Rhizobium* and VAM colonization are the most important and successful symbiosis in plants to improve nutrient availability. Legumes form symbiotic relationship with nitrogen-fixing bacteria called rhizobia that colonize legume roots, forming nodules where they convert atmospheric nitrogen into plant-usable form through nitrogenase activity. In return, legumes provide energy to the rhizobia through photosynthesis. Nitrogen fixation is significant for sustainable agriculture as it enhances soil fertility by converting inaccessible atmospheric nitrogen into a usable form. Understanding the process of biological nitrogen fixation, where certain bacteria convert atmospheric nitrogen into ammonia, is crucial for sustainable agriculture. This knowledge helps reduce reliance on synthetic fertilizers and maintain soil fertility. The symbiotic relationship between *Rhizobium* and leguminous plants plays a vital role in sustainable agriculture by facilitating access to atmospheric nitrogen, improving soil fertility, and reducing the need for chemical fertilizers. This reduces reliance on synthetic fertilizers, which have adverse environmental impacts (Coba de la Pena *et al*. 2018). Symbiotic nitrogen fixation (SNF) has the potential to address the global protein shortage by increasing nitrogen supply in agriculture. To promote sustainable agriculture, alternative approaches such as biofertilizers that utilize biological nitrogen fixation have been introduced to minimize ecological impact.

Pulses, a subset of grain legumes are being cultivated in 170 countries and covering 96 million ha area with production of 96 million tonnes (FAOSTST 2023). India evidenced the remarkable increase in pulses production with the highest production of 27.3 million tonnes during 2021-22. Among the pulses, chickpea is grown globally in 14.8 million ha with the production of 18.1 million tonnes (FAOSTAT 2023). In India, chickpea is the major rabi pulses that contribute about 47% to the total pulse basket of the country. Chickpea production plays a crucial role on "Pulses Revolution" in India making the country self sufficient in pulses (Project coordinator Report 2023-24, 2024). Chickpea establish symbiotic relationship with *Mesorhizobium* and fix up to 140 kg nitrogen/ha from atmospheric air that contribute nearly 80% of its nitrogen (N) requirement. Apart from this, substantial amount of residual nitrogen is incorporated in agricultural field for subsequent crops and improve the health of agricultural soil (Gaur *et al*. 2010). Effective establishment of SNF increased the N-status in grain which is directly associated with high protein content of chickpea grains (Tagore *et al.* 2014, Kumar *et al.* 2014). Chickpea inoculated with *Mesorhizobium* was observed with 61.1% and 11.4% greater grain N and P content, respectively (Kaur *et al.* 2015). Genotypic variability on nodulation traits were well documented in several pulses including chickpea. Hence, breeding of chickpea for efficient symbiotic nitrogen fixation can be considered as one of the alternatives to maximize/realize the benefits of the biological nitrogen fixation process and also to improve yield and protein content.

Genetic variability in root-nodulation traits

Most of the research efforts on symbiotic nitrogen fixation revealed genotypic, phenotypic and functional variations in the microsymbiont called Rhizobia. Limited germplasm sets of pulses have been screened for SNF traits that resulted in identification of host genotypes fixing higher atmospheric N_2 compared to others under similar conditions. Certain genotypes of common bean germplasm lines were very effectively fixed atmospheric nitrogen (127.7 mg N_2/plant) compared to other plants (47.8 mg N_2/plant) under phosphorous deficient conditions i.e. 70 m mol P/plant (Vadez *et al.* 1999). Similarly, screening green gram genotypes for variation in nodulation revealed that number of nodules and nodule weight varied from 16.5-28.5 and 60.23-154.35 g per plant, respectively (Mishra *et al.* 2014).

Several attempts were made by global researchers to assess the nodulation potential of chickpea that resulted in identification of high nodulating, low nodulating, and non nodulating mutants within chickpea cultivars (Rupela 1994, Tellawi *et al*. 2007, Mensah and Olukoya 2007, Gallani *et al*. 2005). In general, high nodulating selection grew better than the non nodulating and low nodulating selections of a given cultivar. Chickpea cultivar G 130, a high nodulating selection produced 31% more grains at low soil N level than its low nodulating selection (Venkateswarlu 1994). N_{15} based screening revealed fourfold differences in the total N_2 fixed (0.02 to 0.84 g/plant) within a subset of genetically diverse USDA chickpea core collection accessions and ILC 235 from Iraq was identified as the greatest nitrogen fixer (Biabani *et al*. 2011). A minimum of 21 nodule/plant was observed in accession number (ACNO) 268376

originated from Afghanistan while ACNO 451161 from Iran produced an average of 101 nodules per plant. The maximum proportion of N-fixed (PNF) was 78.1% from ACNO 451112 (Iran) while the minimum was 47.5% from ACNO 360439 (Iran). PNF is positively correlated with plant biomass (Pearson r=0.20, P<0.01). Chickpea genotypes with greater biomass provide stronger sink for nitrogen and also provide resources significantly to support the energy-intensive N fixation.

Recently, screening a set of 300 chickpea germplasm lines revealed high nodulation potential of ICC1013 and ICC16569 with high mean yield, heritability and genetic advance across the locations and considered as potential stable donors in the chickpea breeding programs for increasing biological nitrogen fixation (Chandana *et al.* 2023). A large variations for nodule number per plant (NNPP) was observed in chickpea minicore collection and variation for nodulation at early crop growth stage (S1) i.e. 15 days after sowing (DAS) is in the range of 0-18 NNPP (Renu *et al*. 2023). A few early nodulating chickpea genotypes viz. ICC12968, ICC7867, ICC13816, ICC867, ICC15264, ICC15510, and ICC283 produced >10 nodule number per plant (NNPP) at 15 as well as 30 DAS. Iran originated ICC6874 produced maximum number of root nodules i.e. 36 NNPP at 60DAS which is 253% higher than check cultivar. Nodule mass in the chickpea minicore ranged upto 850 mg/plant at 60 DAS and 2290 mg/plant at 90 DAS.

Nodulation and related traits such as number of nodule, nitrogen fixed in biomass and grain, nitrogen yield in grain, shoot and biomass, biomass and grain yield, 100 seed weight, shoot dry weight ratio, nodule dry weight, and grain protein content showed high narrow sense heritability values (Girma *et al*. 2019). Similarly, high genotypic coefficient of variations was observed for nodule fresh weight, root dry weight, number of seeds per plant and number of nodules indicating less amenability of these traits to environmental fluctuations (Chandana *et al*. 2023, Priyadarshini *et al*. 2017). Hence, greater emphasis should be given to these characters, while selecting genetic stock for future crop improvement programs.

Correlation coefficient analysis helps to measures the strength and direction of the relationship between two or more variables. In the context of quantitative traits, it helps to identify the components that influence the plant health and yield. By analyzing the correlation coefficient, researchers can determine the component traits on which selection can be based for genetic improvement in yield. Nodule fresh weight and nodule dry weight in chickpea were positively and significantly correlated with yield per plant at genotypic and phenotypic levels (Chandana *et al*. 2022, Hazra *et al*. 2021, Elias *et al.* 2009). Renu *et al.* (2023) observed a positive correlation between nodule number per plant at different stages viz S2-30DAS vs S3-60 DAS (0.27), S2 vs S4-90 DAS (0.11), and S3 vs S4 (0.45). Nodule specific weight is strongly correlated with nodule fresh weight stage-3 (0.69) as well as stage-4 (0.76), but negatively correlated with nodule number. Biabani *et al.* (2011) has reported that nodule number at flowering stage was significantly correlated only with total nodule weight and plant biomass. Other studies were also indicated that no significant correlation of nodule number/weight with N fixation and prefer only limited numbers of nodules for maximum BNF. This implies that excessive nodule formation may not necessarily lead to increased fixation in these genotypes. As a result, plants may prioritize allocating energy resources towards other essential biological processes (Hefny *et al.* 2001, Delic *et al*. 2010). Grima *et al.* (2019) had reported a significant negative association between nodule dry weight and nitrogen harvest index, number of nodule and grain nitrogen yield. Gemechu *et al.* (2013) found non-significant correlation between grain nitrogen yield and number of nodules, nodule dry weight, shoot nitrogen content, and shoot nitrogen fixation. In contrary to this correlation values, positive and highly significant associations were observed among grain nitrogen yield and biomass nitrogen yield. Surprisingly, the contribution of environmental variation is too low for the observed phenotypic variations in BNF traits and high broad-sense heritability with the value of >60 was observed for early nodulation, late nodule senescence and high nodule number. Hence, identification of potential genetic stocks for high nodulation, extended nitrogen fixation and high symbiotic efficiency help to overcome the bottlenecks and make grain legumes as nitrogen fixing factories to fertilize the soil in a sustainable way.

Renu *et al.* (2023) had consistently predicted two major clusters in chickpea minicore data set using Silhouette and Gap statistic methods. Cluster-1 comprises 126 genotypes dominated by desi type while cluster-2 consists of 74 pea type genotypes. Mahalanobis's D2 statistics is a powerful tool in quantifying the degree of variability at the genotype

level. Based on the D_2 values, Chandana *et al.* (2023) had grouped 100 chickpea genotypes into seven clusters revealing significant variations for all the characters and germplasm. Most of the genotypes (n=80) belong to Cluster I indicated the narrow genetic divergence among the genotypes tested. The near uniformity observed in the cluster I might be either due to similarity in the base population from which they were evolved or unidirectional selection for one particular trait or a group of linked traits may produce similar phenotypes that resulted to form a single cluster irrespective of their geographic origin (Parashi *et al*. 2013). High inter-cluster distance in genetic clustering suggests that the associated genotypes are highly divergent and may exhibit potential for heterosis and hence chickpea genotypes belonging to the divergent clusters need to be used in crossing programmes (Lal *et al*. 2001, Dwivedi and Lal 2001).

Signal perception during early stages of nodule organogenesis

Nod Factors (NFs) secreted by *Rhizobium* in response to flavonoids in host legume root exudate is perceived by a plasma-membrane-localized receptor complex. This receptor complex consists pseudokinase Nod Factor Perception (MtNFP) and the receptor-like kinase (RLK) LysM domain receptor-like kinase 3 (MtLYK3) in *Medicago truncatula* and Nod factor Receptors (LjNFR1& LjNFR5) in *Lotus japonicus* (Arrighi *et al*. 2006, Smit *et al*. 2007). At the plasma membrane, MtNFP and MtLYK3 form homomeric and heteromeric complexes which are important for symbiotic signal transduction (Moling *et al*. 2014). Comparative structural and functional studies revealed that regions II and IV in LysM1 of the extracellular domain in MtLYK3/LjNFR1 create a structurally defined binding pocket for the specific recognition of nod factor (NF) ligands (Bozsoki *et al.* 2020). The kinase activity of the intracellular kinase domain in MtLYK3/LjNFR1 is indispensable for symbiotic responses while LjNFR5 is phosphorylated *in vitro* by LjNFR1 (Madsen *et al*. 2011).

Palaka *et al*. (2021) had characterized CaNFR1 and CaNFR5 that showed significant homology with NFRs of other legumes. Open reading frame (ORF) of the *CaNFR1* gene consists of 1851 bp and the translated protein sequence with 616 amino acids, whereas the *CaNFR5* gene consists of 1788 bp coding 595 amino acids. CaNFR1 and CaNFR5 proteins contain a transmembrane helix extending between amino acid residues 226–248 and 247–269, respectively. NFR1 and NFR5 of *M. truncatula*, *Pisum sativum*, *C. arietinum*, *L. japonicus* and *L. pedunculatus* form a separate group (group I), which is significantly diverse group-II consisting *Abrus precatorius, Vigna radiata, Glycine max* and *Glycine soja*. The protein-protein interaction studies between the ectodomains of CaNFR1 and CaNFR5 revealed the crucial residues involved in the interactions and their possible mode binding.

Infection-thread formation and root hair curling

The plant receptor Nodule Factor Perception (NFP) protein interacts with Rho of plants-GTPases (ROP-GTPases) after being phosphorylated by LYK3 and binding to Nod Factors (NFs). This interaction triggers the activation of ROP-GTPases, leading to the production of reactive oxygen species (ROS) bursts via Respiratory Burst Oxidase Homologs (RBOHs) to induce infection thread formation. Root hair curling is a critical process in nodule organogenesis, enabling plants to respond and interact with *Rhizobium*. The process is mediated by ROP-GTPases, which interact with phosphorylated RopGEFs (guanine nucleotide exchange factors) to facilitate polar distribution of receptor complexes in root hair tips and interaction with LYK3 and NFP. In general, Receptor-like cytoplasmic kinases (RLCKs) act as downstream kinases to transduce RLK ligand signals by forming RLK–RLCK complexes. However, direct targets of these RLCKs and their role on transducing NF signals from LysM-RLK receptor kinases need to be further explored.

Critical role of cytokinin signalling through TCS members (CaHKs and TypeB RRs) in nodulation was investigated in chickpea. CaHKs were characterized for their receptor activity using complementation assays in yeast and *Medicago cre1* mutants (Tiwari *et al*. 2021). The chickpea ortholog *CaHK19* was the master spigot of cytokinin perception in chickpea. Expression analysis showed the up-regulation of *CaHKs* specifically *CaHK19*, an ortholog of *MtCRE1* and *LHK1*, after 6h of exogenous cytokinin treatment and *M. ciceri* infection. Expression analysis revealed the highest expression of *CaHK19* as compared to the other *CaHKs*, thereby suggesting its prominent role as a major sensor of cytokinin signalling. Recent study showed that the *L. japonicus*, *LHK1* (ortholog of *CaHK19*) exhibited 13 to 33 times higher expression as compared to other LHKs (Held *et al*. 2014). Additionally, *CaNIN* was also found to be up-regulated in a similar manner through cytokinin receptor 1 (CRE1) (Plet *et al.* 2011). The co-expression pattern of *CaHKs* and *CaNIN* clearly

indicated a link between cytokinin perception and the downstream expression of *CaNIN* in chickpea as earlier established in *Medicago*.

Common symbiosis signaling pathway (CSSP)

Signaling pathway in legume-*Rhizobium* symbiosis, also shared with arbuscular mycorrhizae (AM) symbiosis is activated after the perception of NF signals by NFP protein complex at the plasma membrane. This pathway conveys the signal to the nucleus to induce nucleus-associated calcium oscillations, the core pattern of CSSP, thereby initiating downstream transcriptional responses (Martin *et al*. 2017). The symbiotic plasma membrane Leucine-rich repeat Receptor-like kinase (LRR-RLK) LjSymRK serve as a co-receptor to activate the intracellular signaling pathway, which is required for symbiotic Ca^{2+} oscillations during both BNF and AM symbioses (Antolín-Llovera *et al*. 2014). Domain swapping analyses revealed that different domains of LjSymRK play different roles in root-nodule organogenesis. The intracellular cytoplasmic kinase domain is required for symbiotic signal transduction, while the extracellular Malectin-like domain and Leucine-rich repeat fine-tune nodule organogenesis (Li *et al*. 2018). DMI3 (Doesn't Make Infections 3) decodes the calcium oscillations, involves in phosphorylation of IPD3 (Interacting Protein of DMI3), forms a transcriptional activation complex with the transcription factors like DELLA, NSP2, and NSP1 that regulate downstream expression of NIN, NF-Ys, ERNs, LBD16, and the SHORT ROOT (SHR) – SCARECROW (SCR) module.

GRAS Transcription Factors-early regulators of nodule organogenesis

GRAS gene family is a plant–specific widely distributed transcription factor family with three functional members, gibberellic acid insensitive (GAI), repressor of GAI (RGA) and scarecrow (SCR). NSP1 and NSP2 were the first identified GRAS-type TFs involved in root nodulation symbiosis. The *nsp1* and *nsp2* mutants fail to form infection pockets and nodule primordia (Smit *et al*. 2005). NSP2 cannot bind directly to DNA sequences. Instead, it functions in conjunction with NSP1 to form NSP1-NSP2 complex that promote early nodulin gene expression to initiate nodule organogenesis (Hirsch *et al*. 2009). NSP2 protein also interacts with myeloblastosis transcription factors (MYB TF LjIPN2) and the loss of function of LjIPN2 reduced the frequency of rhizobial infection and the number of nodules in *L. japonicus* (Kang *et al.* 2014; Xiao *et al*. 2020). Another group of GRAS proteins, the DELLAs [aspartic acid (D)–glutamic acid (E)–Leucine (L)–Leucine (L)–alanine (A)] are key negative regulators of gibberellin signaling (Feng *et al.* 2008), promote CCaMK/DMI3-CYCLOPS/IPD3 complex formation, and interact with NSP2/NF-Ys (Fonouni-Farde *et al*. 2016, Jin *et al*. 2016, Pimprikar *et al*. 2016). MtDELLAs bridge a protein complex containing IPD3 and NSP2 to activate other NF-induced TFs such as ERN1 (Jin *et al*. 2016). Thus, DELLAs act as a hub integrating GA signaling with root nodule organogenesis. Thus, the protein complex of DMI3, IPD3, MtDELLAs, NSPs, and NF-Ys are responsible for activating downstream symbiotic gene expression.

Nodule Inception (NIN)-core regulator of nodule organogenesis

The NIN protein is a crucial regulator in nodulation that integrates signaling pathways from nodulation and plant hormones, including cytokinin and auxin, to orchestrate nodule organogenesis. NIN belongs to the NIN-like protein (NLP) subfamily of the plant-specific RWP-RK family transcription factors, which possess a characteristic DNA-binding RWP-RK motif (Lin *et al*. 2018). The distinct feature of NLP subfamily members is the presence of Phox and Bem1 (PB1) domains at their C-terminus. This is in contrast to proteins belonging to the RWP-RK domain (RKD) subfamily, which lack these domains. DNF1-complex-dependent proteolytic processing produces a C-terminal NIN fragment containing DNA binding motifs, which activate genes required for symbiosome development and nitrogen fixation (Feng *et al.* 2021a). Expression of *NIN* is mediated by several ways via., a) transcriptionally by phosphorylated CYCLOPS (Singh *et al*. 2014), b) direct binding of constitutively expressed NSP1 to the promoter of *NIN*, and c) binding of IPN2 to *IPN2-RE* motif in the *NIN* promoter through IPN2-NSP1-NSP2 protein complex (Xiao *et al*. 2020). Another transcription factor, LjSIP1, interacts with LjSymRK and can also bind to the *NIN* promoter to activate its expression (Zhu *et al*. 2008).

Cytokinin response 1 (CRE1)-mediated cytokinin signaling in inner-root cortical cells plays a crucial role in regulating the expression of *NIN* (Vernié *et al*. 2015). The *CRE1* regulatory region is located within 5 kb upstream of the *NIN* start codon (Liu *et al*. 2019c) and the region is considered as transcriptional hub that integrates multiple internal and external signals during root-nodule organogenesis. In *M. truncatula*, *NIN* activates the

expression of the Nuclear Factor-Y (NF-Y) subunit genes *NF-YA1* (formerly named *MtHAP2-1*) and *NF-YA2* (Soyano *et al.* 2013), which redundantly regulate the early stage of rhizobial infection and nodule meristem activity via the transcriptional activation of *MtERN1* (Laloum *et al*. 2014). *MTERN1* (Ethylene Response Factor Required for Nodulation 1) encodes an *AP2/ERF* transcription factor, playing a crucial role in nodulation in plants. The *MtERN!* gene is directly activated by the protein complex NSP1/2 and CYCLOPS (Cerri *et al*. 2012, 2017). MtNFYA1/MtNF-YB16/MtNF-YC2 and PvNF-YA1/PvNF-YB7/PvNFYC1 protein complexes can form heterotrimers to promote *MtERN1* or *PvERN1* expression, respectively, by recognizing the CCAAT boxes in their promoter sequences, leading to infection and nodulation (Baudin *et al*. 2015). NLP2 is recruited by legume plants to enable the expression of leghemoglobin genes, which help maintain the hypoxic environment for nitrogenase (Jiang *et al*. 2021).

Autoregulation of nodulation (AON) pathway in legumes

Autoregulation of nodulation (AON) pathway is activated at early stages of root-nodule organogenesis in legume-*Rhizobium* interaction, far before active nitrogen fixation occurs, and discrediting the idea that it functions as a feedback inhibition caused by accumulation of downstream N metabolites through nitrogen fixation. *AON* mutants of legumes are hyper-nodulating mutants under sufficient supply of nitrate nitrogen from soil, that is normally a suppressive condition for nodulation in parent genotypes. Four important components of currently known *AON* pathway are a) Signal recognition and transmission from soil NO_3^-/*Rhizobium*, b) CLE-SUNN as negative regulation pathway, c) CEP-CRA2 as positive regulation pathway and d) *miR2111/TML* module (Li *et al.* 2022).

a) **Signal recognition and transmission from soil NO_3^-/Rhizobium**

The AON pathway controls the nodulation process according to abundance of rhizobia and nitrate-N in soil and maintains the energy balance in the plant. The detection of rhizobial and nitrate ($NO3^-$) signals in the AON pathway is aided by the presence of CK (Cytokinin), Gibberlic acid (GA), and *NIN*. These signals trigger the creation of CLE and CEP signals (Li *et al.* 2022). The rhizobia signal promotes the cytokinin synthesis in root leading to

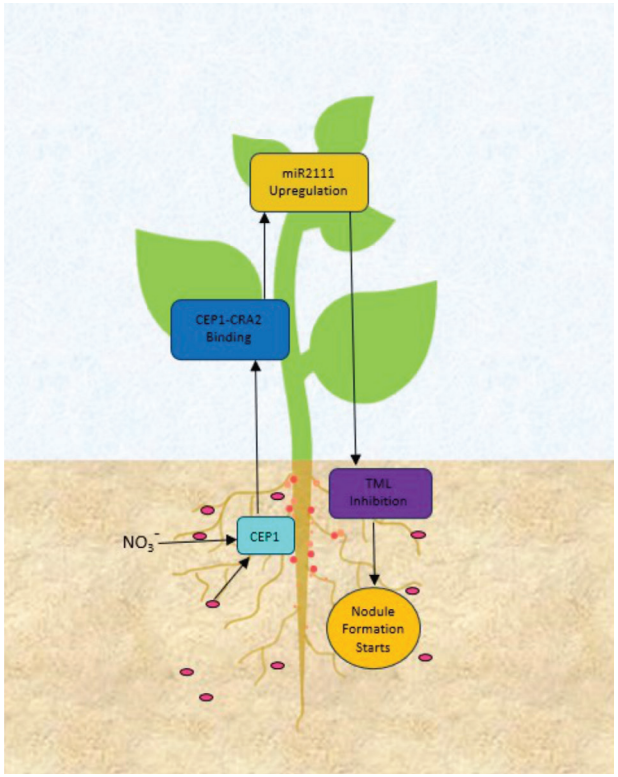

Fig. 1. Positive regulation of root-nodulation in legumes

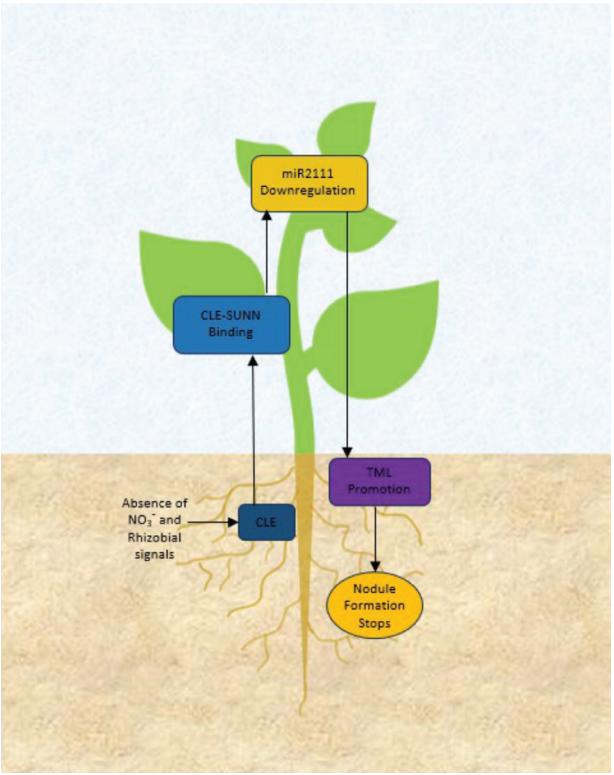

Fig. 2. Negative regulation of root-nodulation in legumes

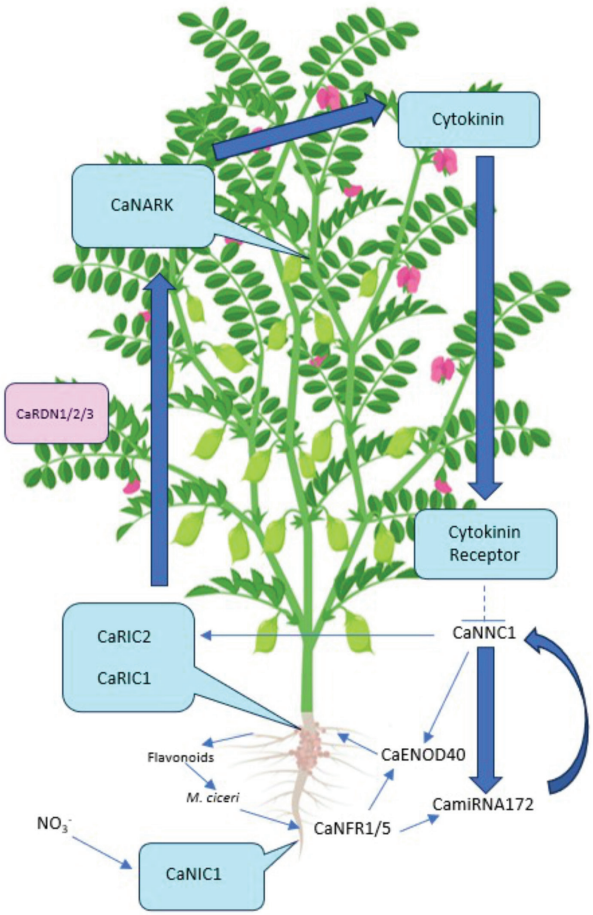

Fig. 3. Autoregulation of nodulation (AON) pathway in chickpea

nodule organogenesis. CRE-1, a cytokinin receptor recognizes the cytokinin signal and promotes the *NIN* gene expression. The transcription factor, Nodule Inception (NIN) binds to the promoters of *MtCLE13* and *LjCLE-RS1/2 (LjCLE-Root Signal1/2)* and triggers their expression (Kawaguchi *et al*. 1996, Gonzalez *et al*. 2006, Ding *et al*. 2008, Mortier *et al*. 2010, Heckmann *et al*. 2011, Soyano *et al*. 2014, Laffont *et al*. 2020). *Rizobial* signal also promote *MtCEP7* expression via the CK/CRE1 and NIN pathway and rely on *MtCRA2* to promote nodulation (Castaings *et al*. 2009). Therefore, the upregulation and downregulation of nodulation depends on the changes in the environment and the plant's requirement for NO_3^- The GA signal plays a key regulatory role in the AON pathway and inhibits nodule formation. In the process of nodule formation, GA biosynthesis in the vascular bundle of the nodule is activated and its baccumulation in root nodule induces expression of *NIN* to activate AON pathway and negatively regulate nodule formation (Kawaguchi *et al*. 1996; Akamatsu *et al*. 2021).

b) **CLE-SUNN as negative regulation pathway**

The CLavata3/Embryo-surrounding region related–Hypernodulation and Aberrant Root-1/ SUper Numeric Nodules (CLE-HAR1/SUNN) based negative regulation of nodulation in *Lotus japonicus* and *Medicago truncatula* inhibit the nodulation under sufficient soil nitrogen supply (Mohd-Radzman *et al*. 2016). Rhizobia induced the expression of CLE peptides in the roots, that also depended on the concentration of soil nitrogen (NO_3^-). These CLE peptides are encoded by *MtCLE12, MtCLE13,* and *MtCLE35* genes in *M. truncatula* which are induced because of the rhizobial signals (Mortier *et al*. 2010, Mortier *et al*. 2012, Mens *et al*. 2021, Moreau *et al*. 2021). In *L. japonicus* roots, CLE is encoded by *LjCLE-RS1, LjCLE-RS2,* and *LjCLE-RS3* Similarly, in soybean, CLE is encoded by the *GmRIC1* and *GmRIC2*. The CLE peptides like LjCLERS2, LjCLE-RS3, GmCLE1 are also be induced by the$NO3^-$ signal (Nishida *et al*. 2016). Further CLE peptides are transported to the above-ground parts through xylem and recognized by receptor kinases like GmNARK/LjHAR1/MtSUNN, which resulted on systemic inhibition of nodule formation (Mortier *et al*. 2012; Okamoto *et al*. 2013). This inhibition is associated with a lower translocation of *miR2111* from shoot to root.

c) **CEP-CRA2 as a positive regulation pathway**

The CEP-CRA2 signal works in the upregulation of the nodulation. During the nitrogen deficient condition and the absence of rhizobia, the CEP1 (C-terminally Encoded Peptide 1) is produced by the root system and transported through the xylem to the shoot buds. It is recognized by the receptor CRA2 (COMPACT ROOT ARCHITECTURE 2), which is a LRR-RLK receptor kinase which promotes nodulation (Imin *et al*. 2013, Laffont *et al*. 2019, Mens *et al*. 2021). In *Arabidopsis thaliana*, *AtCEP1* is expressed and transported through long distance root-stem signal transmission and recognized by CEPR1 (CEP Receptor 1). AtCEP1 affects the transcription of the nitrate transporters *NRT1.1, NRT2.1,* and *NRT3.1,* thereby regulating the nitrogen uptake and nitrogen status (Okamoto *et al*. 2015, Ohkubo *et al*. 2017, Chapman *et al*. 2019, Laffont *et al*. 2019).

D) miR2111/TML regulatory module

The CLE and CEP signals, which are crucial

for controlling root nodulation and development in the AON pathway, are mediated by the miR2111/*TML* regulatory module. To reduce TML expression in the roots, signals from miR2111 in the shoot bud are transmitted through the phloem. When there is insufficient nitrogen presence, the CRA2 detects the CEP1 signal, which triggers *miR2111* and encourages the creation of nodules. The CLE-SUNN signal negatively controls the expression of *miR2111* and prevents nodulation when there is enough presence of both nitrogen and plentiful rhizobia (Castaings *et al*. 2009). Two copies of the TML gene MtTML1 and MtTML2 were identified in *M. truncatula*, and the presence of miR2111/TML regulatory module in *A. thaliana* indicated that miR2111/TML regulatory module is evolutionarily conserved.

Autoregulation of nodulation pathway in chickpea

CaNFR1 and *CaNFR5* (*C. arietinum* Nod Factor Receptor) may be involved in the perception of NOD factor signals secreted by rhizobia through the root hair to initiate nodulation just like in *Medicago* (Geurts *et al*. 2005). *CaNFR1/5* controls CamiRNA172, which uses CaNNC1 to activate CaRIC1 and CaRIC2. These CaRIC1 and CaRIC2 signals are detected by CaRDN1/2/3. CaNARK is triggered by CaRDN1/2/3 to commence Shoot Derived Inhibitors (SDI). CaNNC1 is activated by cytokinin which is detected by CK Receptor. CaNNC1 directly initiates CaENOD40 (*C. arietinum* Early Nodulation) and transcriptionally represses *CamiRNA172*. *CaENOD40* may induce the division of root cortical cells, the nodule primordium and the pericycle of the root vascular bundle at early stages of nodule development just like in soybeans (Minami *et al*. 1996). *CaNARK* (*C. arietinum* Nodule Autoregulation Receptor Kinase) which is a group of CLE (CLAVATA3/Embryo) peptides is reported to be involved with cell signaling that includes *CaRIC1* (*C. arietinum* Rhizobia-Induced CLE) and *CaRIC2*. They are known to be involved in the AON signaling transduction that initiate expression solely in the roots with NOD factor-producing rhizobia and are considered as root-derived signals in AON pathway (Hastwell *et al*. 2018).

CaRDN1, CaRDN2, and CaRDN3 (*C. arietinum* Root-Determined Nodulation) may function as receptors for signals produced from the roots, initiate the induction of shoot-derived inhibitor (SDI), and release it back to the roots through phloem (Suzaki *et al*. 2015). The *CamiRNA172* (*C. arietinum microRNA172*) is regulated by *CaNFR1* and *CaNFR5* and it targets *CaNNC1*, a negative regulator of *CaRIC1* and *CaRIC2* which leads to the activation of *CaENOD40* (Jahan *et al*. 2020).

CaNARK, CaRIC1, CaRIC2, CaNIC1, CaRDN1, CaRDN3, CaENOD40, CaNNC1, CaNFR1, and *CaNFR5* was expressed in chickpea tissues 2 days after the infection of *M. ciceri*. *CamiRNA172* was expressed in the roots after six days of inoculation, whereas *CaRDN2* was expressed in the roots from the second to the sixth day following inoculation. While the remaining nodulation genes were solely expressed in the roots, the CaNARK genes were expressed in both the roots and the leaves. This suggests that *CaNARK* may have roles in controlling nodulation in root via the AON pathway. It functions in the vascular tissue of the leaf to recognize CaRIC1 and CaRIC2. This recognition of peptide lead to the production of a shoot derived inhibitor (SDI), that was translocated to roots and inhibit nodulation. According to reports, AON is turned on in the

Table 1. Genes involved in autoregulation of nodulation (AON) pathway of chickpea

Gene in *Cicer arietinum* (Chickpea)	Location in Chickpea (Chromosome number)	Resemblance with other legume gene identified
CaNFR1	Chr 2	*MtNFR*1 in *M. truncatula*
CaNFR5	Chr 8	*MtNFR5* in *M. truncatula*
CaENOD40	-	*GmENOD40* in *G. max*
CaNARK	Chr 6	*CaNARK* showed close resemblance with *M. truncatula, P. sativum,* and *L. japonicus.*
CaRIC1 and *CaRIC2* *CLE peptides*	Chr 6 and Chr 4 respectively	*PvRIC1* and *PvRIC2* CLE peptides of *P. vulgaris,* *MtCLE12* and *MtCLE13* of *M. truncatula* and, *LjCLE-RS1* and *LjCLE-RS2* genes of *L. japonicus*
CaNIC1	Chr 2	*GmNIC1* in soybean and *PvNIC1* gene in *P. vulgaris*
CaRDN1	Chr 2	*GmRDN1A , GmRDN1B* of soybean, *LjRDN of L. japonicus and MtRDN1 of M. truncatula*
CaRDN2	Chr 7	*MtRDN2 of M. truncatula. LjRDN2 of L. Japonicus GmRDN2A* and *GmRDN2B of soybean*
CaRDN3	Chr 4	*MtRDN*3 in *M. truncatula*
CaNNC1	Chr 6	*MtNNC1* in *M. truncatula*

cortical cells of roots during rhizobial infection and stays turned on during the development of nodule primordium and nodule maturation (Muhammad *et al*. 2020). *CaNIC1* (*C. arietinum* Nitrate-Induced CLE) that are reported to act locally in the roots to suppress nodulation by nitrate inhibition (Muhammad *et al*. 2020, Lim *et al*. 2014).

CONCLUSION

Limited research on exploring chickpea germplasm revealed the existence of natural variability and heritability of nodulation traits. Execution of a systematic and fully designed phenotyping approach is required to screen and identify new donors for improving symbiotic nitrogen fixation. The genes involved at different stages in root nodule organogenesis have been characterized in model legumes like *L. japonicus* and *M. truncatula* and to some extent in *G. max*. Though chickpea is the major rabi pulses that contribute about 47% to the total pulse basket of India and plays a crucial role on "Pulses Revolution" in the country, knowledge on molecular basis of root-nodule organogenesis in chickpea is limited. Most of the studies were on *Mesorhizobium* instead of molecular and genomic regions controlling nodulation in chickpea genotypes. However, recent studies on protein-protein interaction using CaNFRs revealed their mode of binding and crucial amino acid residues involved. In future, it will help to design of a novel strategy to improve efficacy on binding with signal, as well as in understanding the selectivity of ligand binding of NFRs. *In vitro* gene silencing, site-specific mutagenesis and *in vitro* binding assay studies will help in confirming the exact binding profile of NFs to their respective receptors. Understanding on the molecular basis of autoregulation of root nodulation in chickpea can help to engineer the host genotype and make chickpea crop as nitrogen fixing factories in agricultural fields.

REFERENCES

Akamatsu A, Nagae M, Nishimura Y, Montero DR, Ninomiya S, Kojima M, Takebayashi Y, Sakakibara H, Kawaguchi M and Takeda N. 2021. Endogenous gibberellins affect root nodule symbiosis via transcriptional regulation of NODULE INCEPTION in *Lotus japonicus*. Plant Journal **105:** 1507–1520.

Antolín-Llovera M, Ried MK and Parniske M. 2014. Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod factor receptor 5. Current Biology **24:** 422–427.

Ariel F, Brault-Hernandez M, Laffont C, Huault E, Brault M, Plet J et al. 2012. Two direct targets of cytokinin signalling regulate symbiotic nodulation in *Medicago truncatula*. Plant Cell **24:** 3838–3852.

Arrighi JF, Barre A, Ben Amor B, Bersoult A, Soriano LC, Mirabella R, de Carvalho-Niebel F, Journet EP, Ghérardi M, Huguet T, Geurts R, Dénarié J, Rougé P and Gough C. (2006). The *Medicago truncatula* lysin motif-receptor-like kinase gene family includes NFP and new nodule-expressed genes. Plant Physiology **142:** 265–279.

Biabani A, Carpenter Boggs L, Katozi M and Sabouri H. 2011. Effects of seed deterioration and inoculation with *Mesorhizobium ciceri* on yield and plant performance of chickpea. Australian Journal of Crop Sciences **5:** 66-70.

Boivin S, Kazmierczak T, Brault M, Wen J, Gamas P, Mysore KS and Frugier F 2016. Different cytokinin histidine kinase receptors regulate nodule initiation as well as later nodule developmental stages in *Medicago truncatula.* Plant Cell Environment. **39:** 2198–2209.

Bozsoki Z, Gysel K, Hansen SB, Lironi D, Krönauer C, Feng F, de Jong N, Vinther M, Kamble M, Thygesen MB, Engholm E, Kofoed C, Fort S, Sullivan JT, Ronson CW, Jensen KJ, Blaise M, Oldroyd G, Stougaard J, Andersen KR and Radutoiu S. 2020. Ligand-recognizing motifs in plant LysM receptors are major determinants of specificity. Science **369:** 663–670.

Brentrup F, Hoxha A and Christensen B. 2016. Carbon footprint analysis of mineral fertilizer production in Europe and other world regions. In: Proceedings of the 10th International Conference on Life Cycle Assessment of Food (LCA Food 2016), Dublin, Ireland, 19–21.

Castaings L, Camargo A, Pocholle D, Gaudon V, Texier Y, Boutet-Mercey S, Taconnat L, Renon JP, Daniel-Vedele F, Fernandez E, Meyer C and Krapp A. 2009. The nodule inception-like protein 7 modulates nitrate sensing and metabolism in *Arabidopsis*. Plant Journal **57:** 426–435.

Cerri MR, Wang Q, Stolz P, Folgmann J, Frances L, Katzer K, Li X, Heckmann AB, Wang TL, Downie JA, Klingl A, de Carvalho-Niebel F, Xie F and Parniske M. 2017. The ERN1transcription factor gene is a target of the CCaMK/CYCLOPS complex and controls rhizobial infection in *Lotus japonicus*. New Phytology **215:** 323–337.

Chandana BS, Mahto RK, Singh RK, Singh KK, Kushwah S, Lavanya GR, Tripathi S, Hegde VS and Kumar R. 2023. Unclenching the potentials of global core germplasm for root nodulation traits for increased biological nitrogen fixation and productivity in chickpea (*Cicer arietinum* L.). Indian Journal of Genetics and Plant Breeding **83(4):** 526-534.

Chapman K, Taleski M, Ogilvie HA, Imin N and Djordjevic MA. 2019. CEP-CEPR1 signalling inhibits the sucrose-dependent enhancement of lateral root growth. Journal of Experimental Botany **70:** 3955–3967.

Coba de la Pena T, Fedorova E, Pueyo JJ and Lucas MM. 2018. The symbiosome: Legume and rhizobia co-evolution toward a nitrogen-fixing organelle? Front Plant Sci **8:** 2229. doi: 10.3389/fpls.2017.02229. PMID: 29403508; PMCID: PMC5786577.

Ding YL, Kalo P, Yendrek C, Sun JH, Liang Y, Marsh JF, Harris JM and Oldroyd GED. 2008. Abscisic acid coordinates Nod factor and cytokinin signaling during the regulation of nodulation in *Medicago truncatula*. Plant Cell **20:** 2681–2695.

Eggleston HS, Buendia L, Miwa K, Ngara T and Tanabe K. 2006. IPCC Guidelines for National Greenhouse Gas Inventories. Available online: https://www.osti.gov/etdeweb/biblio/20880391.

Elias N. 2009. Optimizing chickpea nodulation for nitrogen fixation and yield in north eastern, Australia. PhD thesis, University of Western Sydney. 10.1038/nature00842.

FAOSTAT. 2023. F.A.O. Crops. Food and Agriculture Organization of the United Nations.

Feng J, Lee T, Schiessl K and Oldroyd GE. 2021. Processing of NODULE INCEPTION controls the transition to nitrogen fixation in root nodules. Science **374:** 629–632.

Feng S, Martinez C, Gusmaroli G, Wang Y, Zhou J, Wang F, Chen L, Yu L, Iglesias-Pedraz JM, Kircher S, Schäfer E, Fu X, Fan LM and Deng XW. 2008. Coordinated regulation of *Arabidopsis thaliana* development by light and gibberellins. Nature **451:** 475–479.

Fonouni-Farde C, Tan S, Baudin M, Brault M, Wen J, Mysore KS, Niebel A, Frugier F and Diet A. 2016. DELLA-mediated gibberellin signalling regulates Nod factor signalling and rhizobial infection. Nature Communications **7:** 12636.

Gallani R, Dighe JM, Sharma RA and Sharma PK. 2005. Relative performance of different chickpea (*Cicer arietinum* L.) genotypes grown on vertisols. Agricultural Science Digest - A Research Journal **6:** 211-213.

Ghavam S, Vahdati M, Wilson IAG and Styring P. 2021. Sustainable Ammonia Production Processes. Frontiers in Energy Research 9:580808, https://doi.org/10.3389/fenrg.2021.580808

Girma N, Mekbib F, Asnake K, Gemechu G and Ojiewo C. 2019. Estimate of heritability and correlation analysis for nitrogen fixation, yield and associated traits in chickpea (*Cicer arietinum* L.). International Journal of Science and Research **10(5):** 2229-5518.

Gonzalez-Rizzo S, Crespi M and 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.

Hastwell A 2018. Functional characterization of novel peptide hormones in legume nodulation and plant development. PhD thesis. The University of Queensland. 2018

Hazra KK, Nath CP, Singh SS et al. 2021. Categorization of chickpea nodules and their relation with plant growth. National Academy Science Letters **44:** 91–95.

Heckmann AB, Sandal N, Bek AS, Madsen LH, Jurkiewicz A, Nielsen MW, Tirichine L, Stougaard J. 2011. Cytokinin induction of root nodule primordia in *Lotus japonicus* is regulated by a mechanism operating in the root cortex. Molecular Plant-Microbe Interactions **24:** 1385–1395.

Held M, Hou H, Miri M, Huynh C, Ross L, Hossain MS et al. 2014. *Lotus japonicus* cytokinin receptors work partially redundantly to mediate nodule formation. Plant Cell **26:** 678–694.

Hirsch S, Kim J, Muñoz A, Heckmann AB, Downie JA and Oldroyd GE. 2009. GRAS proteins form a DNA binding complex to induce gene expression during nodulation signaling in *Medicago truncatula*. Plant Cell **21:** 545–557.

IFA. 2009. Energy Efficiency and CO_2 Emissions in Ammonia Production. Available online: https://www.fertilizer.org/images/Library_Downloads/2009_IFA_energy_efficiency.pdf.

Imin N, Mohd-Radzman NA, Ogilvie HA and Djordjevic MA. 2013. The peptide-encoding CEP1 gene modulates lateral root and nodule numbers in *Medicago truncatula*. Journal of Experimental Botany **64:** 5395–5409.

Jiang S, Jardinaud MF, Gao J, Pecrix Y, Wen J, Mysore K, Xu P, Sanchez-Canizares C, Ruan Y, Li Q, Zhu M, Li F, Wang E, Poole PS, Gamas P and Murray JD. 2021. NIN-like protein transcription factors regulate leghemoglobin genes in legume nodules. Science **374:** 625–628.

Jin Y, Liu H, Luo D, Yu N, Dong W, Wang C, Zhang X, Dai H, Yang J and Wang E. 2016. DELLA proteins are common components of symbiotic rhizobial and mycorrhizal signalling pathways. Nature Communications. **7:** 12433.

Kang H, Chu X, Wang C, Xiao A, Zhu H, Yuan S, Yang Z, Ke D, Xiao S, Hong Z and Zhang Z 2014. A MYB coiled-coil transcription factor interacts with NSP2 and is involved in nodulation in Lotus japonicus. New Phytology **201:** 837–849.

Kaur N, Sharma P and Sharma S 2015. Co-inoculation of *Mesorhizobium* sp. and plant growth promoting rhizobacteria *Pseudomonas* sp. as bio-enhancer and bio-fertilizer in chickpea (*Cicer arietinum* L.). Legume Research **38**(3): 367-374.

Kawaguchi M, Imaizumi Anraku H, Fukai S and Syono K 1996. Unusual branching in the seedlings of Lotus japonicus—Gibberellins reveal the nitrogen-sensitive cell divisions within the pericycle on roots. Plant Cell Physiology **37**: 461–470.

Kumar D, Arvadiya LK, Kumawat AK, Desai KL and Patel TU. 2014. Yield, protein content, nutrient content and

uptake of chickpea (*Cicer arietinum* L) as influenced by graded levels of fertilizers and bio-fertilizers. Research Journal of Chemical and Environmental Sciences **2**(6): 60-64.

Laffont C, Huault E, Gautrat P, Endre G, Kalo P, Bourion V, Duc G and Frugier F. 2019. Independent Regulation of Symbiotic Nodulation by the SUNN Negative and CRA2 Positive Systemic Pathways. Plant Physiology **180**: 559–570.

Laffont C, Ivanovici A, Gautrat P, Brault M, Djordjevic MA and Frugier F. 2020. The NIN transcription factor coordinates CEP and CLE signaling peptides that regulate nodulation antagonistically. Nature Communications **11**: 3167.

Lal D, Ram Krishna and Gurpreet S. 2001. Genetic divergence in chickpea. Indian Journal of Pulses Research **14**(1): 63-64.

Laloum T, Baudin M, Frances L, Lepage A, Billault - Penneteau B, Cerri M R, Ariel F, Jardinaud M F, Gamas P, de Carvalho Niebel F and Niebel A. 2014. Two CCAAT-box-binding transcription factors redundantly regulate early steps of the legume rhizobiaendo symbiosis. Plant Journal. 79: 757–768.

Li H, Chen M, Duan L, Zhang T, Cao Y and Zhang Z. 2018. Domain swap approach reveals the critical roles of different domains of SYMRK in root nodule symbiosis in Lotus japonicus. Frontiers Plant Science **9**: 697.

Li Y, Pei Y, Shen Y, Zhang R, Kang M, Ma Y, Li D and Chen Y. 2022. Progress in the self-regulation system in legume nodule development-AON (autoregulation of nodulation). International Journal of Molecular Sciences **23:** 6676.

Lim CW, Lee YW, Lee SC and Hwang CH. 2014. Nitrate inhibits soybean nodulation by regulating expression of CLE genes. Plant Science **229**: 1-9.

Lin JS, Li X, Luo Z, Mysore KS, Wen J and Xie F. 2018. NIN interacts with NLPs to mediate nitrate inhibition of nodulation in Medicago truncatula. Nature Plants **4**: 942–952.

Liu J, Rutten L, Limpens E, van der Molen T, van Velzen R, Chen R, Chen Y, Geurts R, Kohlen W, Kulikova O and Bisseling T 2019. A remote cis-regulatory region is required for NIN Expression in the Pericycle to Initiate Nodule Primordium Formation in *Medicago truncatula*. Plant Cell **31**(1): 68-83.

Madsen EB, Antolín-Llovera M, Grossmann C, Ye J, Vieweg S, Broghammer A, Krusell L, Radutoiu S, Jensen ON, Stougaard J and Parniske M. 2011. Autophosphorylation is essential for the in vivo function of the Lotus japonicus Nod factor receptor 1 and receptor-mediated signalling in cooperation with Nod factor receptor 5. Plant Journal **65**: 404–417.

Martin FM, Uroz S and Barker DG. 2017. Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria. Science 356: eaad4501.

Mens C, Hastwell AH, Su HN, Gresshoff PM, Mathesius U and Ferguson BJ. 2021. Characterisation of *Medicago truncatula* CLE34 and CLE35 in nitrate and rhizobia regulation of nodulation. New Phytologist **229**: 2525–2534.

Mensah JK and Olukoya RT. 2007. Performance of mungbean (*Vigna mungo* L. hepper) grown in mid-west Nigeria. American-Eurasian Journal of Agricultural and Environmental Sciences **2(**6): 696-701.

Minami E, Kouchi H, Cohn JR, Ogawa T and Stacey G. 1996. Expression of the early nodulin, ENOD40, in soybean roots in response to various lipo-chitin signal molecules. The Plant Journal **10**(1): 23-32.

Mishra A, Mohanty SK, Mishra S, Samal KC and Das S. 2014. Character association and genetic diversity in rainfed green gram [(*Vigna radiata* L.) Wilczek]. Indian Journal of Dryland Agricultural Research and Development **29**(1): 45-51.

Mohd-Radzman NA, Laffont C, Ivanovici A, Patel N, Reid D, Stougaard J, Frugier F, Imin N and Djordjevic MA. 2016. Different Pathways act downstream of the CEP peptide receptor CRA2 to regulate lateral root and nodule development. Plant Physiology **171**: 2536–2548.

Mortier V, DeWever E, Vuylsteke M, Holsters M and Goormachtig S. 2012. Nodule numbers are governed by interaction between CLE peptides and cytokinin signaling. Plant Journal **70**: 367–376.

Moling S, Pietraszewska-Bogiel A, Postma M, Fedorova E, Hink MA, Limpens E, Gadella T and Bisseling T. 2014. Nod factor receptors form heteromeric complexes and are essential for intracellular infection in Medicago nodules. Plant Cell **26**: 4188–4199.

Moreau C, Gautrat P and Frugier F. 2021. Nitrate-induced CLE35 signaling peptides inhibit nodulation through the SUNN receptor and miR2111 repression. Plant Physiology **185**: 1216–1228.

Mortier V, Den Herder G, Whitford R, Van de Velde W, Rombauts S, D'haeseleer K, Holsters M and Goormachtig S. 2010. CLE peptides control *Medicago truncatula* nodulation locally and systemically. Plant Physiology **153**: 222–237.

Nishida H, Handa Y, Tanaka S, Suzaki T and Kawaguchi M. 2016. Expression of the CLE-RS3 gene suppresses root nodulation in Lotus japonicus. Journal of Plant Research **129**: 909–919.

Ohkubo Y, Tanaka M, Tabata R, Ogawa-Ohnishi M and Matsubayashi Y. 2017. Shoot-to-root mobile polypeptides involved in systemic regulation of nitrogen acquisition. Nature Plants **3**: 17029.

Okamoto S, Shinohara H, Mori T, Matsubayashi Y and Kawaguchi M. 2013. Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase. Nature Communications **4**: 2191.

Okamoto S, Suzuki T, Kawaguchi M, Higashiyama T and

Matsubayashi Y. 2015. A comprehensive strategy for identifying long distance mobile peptides in xylem sap. Plant Journal **84**: 611–620.

Palaka BK, Vijayakumar S and Roy Choudhury S. 2021. Exploring nod factor receptors activation process in chickpea by bridging modelling, docking and molecular dynamics simulations. International Journal of Biological Macromolecules **189**: 965-979.

Parashi VS, Lad TB, Maser LB, Kute NS and Sonawane CJ. 2013. Genetic diversities studies in Chickpea (*Cicer arietinum* L.). Bioinfolet **10**(1B): 337-341.

Pimprikar P, Carbonnel S, Paries M, Katzer K, Klingl V, Bohmer MJ, Karl L, Floss DS, Harrison MJ, Parniske M and Gutjahr C. 2016. A CCaMK-CYCLOPS-DELLA complex activates transcription of RAM1 to regulate arbuscule branching. Current Biology **26**: 1126.

Plet J, Wasson A, Ariel F, Le Signor C, Baker D, Mathesius U, Crespi M et al. 2011. MtCRE1-dependent cytokinin signaling integrates bacterial and plant cues to coordinate symbiotic nodule organogenesis in *Medicago truncatula*. Plant Journal **65**: 622–633.

Rupela OP. 1994. Screening for intracultivaral variability for nodulation of chickpea and pigeonpea. In: Linking biological nitrogen fixation research in Asia: report of a meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes. 6-8 Dec 1993, ICRISAT Asia Center, India. Pp. 75.

Shah Jahan M, Ibrahim Tahir M, Inam Ul Haq M, Shahzad U and Nemenzo Calica P. 2020. Characterisation of genes involved in the autoregulation of nodulation in chickpea (*Cicer arietinum* L.). Journal of Engineering Environment and Agriculture Research **2**: 51-58.

Singh S, Katzer K, Lambert J, Cerri M and Parniske M. 2014. CYCLOPS, a DNA-binding transcriptional activator, orchestrates symbiotic root nodule development. Cell Host Microbe **15**: 139–152.

Smit P, Limpens E, Geurts R, Fedorova E, Dolgikh E, Gough C and Bisseling T. 2007. Medicago LYK3, an entry receptor in rhizobial nodulation factor signaling. Plant Physiology **145**: 183–191.

Smit P, Raedts J, Portyanko V, Debellé F, Gough C, Bisseling T and Geurts R. 2005. NSP1 of the GRAS protein family is essential for rhizobial Nod factor-induced transcription. Science **308**: 1789–1791.

Snyder C, Bruulsema T, Jensen T and Fixen P. 2009. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. Agriculture Ecosystem Environment **133:** 247–266.

Soyano T, Hirakawa H, Sato S, Hayashi M and Kawaguchi M. 2014. Nodule Inception creates a long-distance negative feedback loop involved in homeostatic regulation of nodule organ production. Proceedings of National Academy of Science USA **111**: 14607–14612.

Soyano T, Kouchi H, Hirota A and Hayashi M. 2013. Nodule inceptiondirectly targets NF-Y subunit genes to regulate essential processes of root nodule development in Lotus japonicus. Public Library of Science Genetics **9**: e1003352.

Suzaki T, Yoro E and Kawaguchi M. 2015. Leguminous plants: inventors of root nodules to accommodate symbiotic bacteria. International Review of Cell and Molecular Biology **316**: 111-158.

Tagore GS, Sharma SK and Shah SK. 2014. Effect of microbial inoculants on nutrient uptake, yield and quality of chickpea genotypes. International Journal of Agricultural Sciences and Veterinary Medicine **2**(2): 18-23.

Taheripour F, Zhao X and Tyner WE. 2017. The impact of considering land intensification and updated data on sbiofuels land use change and emissions estimates. Biotechnology for Biofuels and Bioproducts **10**: 191.

Tiwari M, Yadav M, Singh B, Pandey V, Nawaz K and Bhatia S 2021. Evolutionary and functional analysis of two-component system in chickpea reveals CaRR13, a TypeB RR, as positive regulator of symbiosis. Plant Biotechnology Journal **19**(12): 2415-2427.

Vadez V, Lasso JH, Beck DP and Drevon JJ. 1999. Variability of N_2-fixation in common bean (*Phaseolus vulgaris* L.) under P deficiency is related to P use efficiency. Euphytica **106**(3): 231-242.

Venkateswarlu B and Katyal JC. 1994. Improvement of nitrogen fixation in groundnut by host plant selection. In: Linking Biological Nitrogen Fixation Research in Asia; report of a meeting of the Asia Working Group on-Biological Nitrogen Fixation in Legumes. Pp. 6-8.

Vernié T, Kim J, Frances L, Ding Y, Sun J, Guan D, Niebel A, Gifford ML, de Carvalho-Niebel F and Oldroyd GE. 2015. The NIN transcription factor coordinates diverse nodulation programs in different tissues of the Medicago truncatula root. Plant Cell **27**: 3410–3424.

Wood S and Cowie A. 2004. A review of greenhouse gas emission factors for fertilizer production. IEA Bioenergy Task **38**: 1–20.

Xiao A, Yu H, Fan Y, Kang H, Ren Y, Huang X, Gao X, Wang C, Zhang Z, Zhu H and Cao Y. 2020. Transcriptional regulation of NIN expression by IPN2 is required for root nodule symbiosis inLotus japonicus. New Phytology **227**: 513–528.

Zhang WF, Dou ZX, He P, Ju XT, Powlson D, Chadwick D, Norse D, Lu YL, Zhang Y, Wu L, et al. 2013. New technologies reduce greenhouse gas emissions from nitrogenous fertilizer in China. Proceedings of National Academy of Science USA **110**: 8375–8380.

Zhu H, Chen T, Zhu M, Fang Q, Kang H, Hong Z and Zhang Z. 2008. A novel ARID DNA-binding protein interacts with SymRK and is expressed during early nodule development in Lotus japonicus. Plant Physiology **148**: 337–347.