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The Role of Different Genes Involoved in Symbiotic Nitrogen Fixation - Review

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Abstract: On the contrast to the well known, that there are other different genes involved in symbiotic nitrogen fixation in addition to *nod*, *nif* and *fix* genes. In this review we will discuss up to date the function of genes that can play a significant role in symbiotic N_2 fixation such as nodulation, nitrogen fixation, production of exopolysaccharides, Type I to Type VI secretion systems and other different genes. Among the other genes that involved in this process, are genes that could be related to host specificity, competition and infection of nodulation process. In addition to the signals from the host plant that are important for plant-*Rhizobium* communications system. This review article will highlight the discovering of new symbiotic genes and their roles in nitrogen fixation.

Key words: Symbiotic nitrogen fixation · *nod* · *nif* · *tts* · *virB* · Signals between legume-symbionts · Host specificity and infection process

INTRODUCTION

Nitrogen is one of the most important major limiting nutrients for most crops and other plant species. There is now little doubt that the world will face severe food shortages in the not to distant future, in part due to excessive population growth and negative environmental impacts associated with the increase of population. Biological Nitrogen Fixation (BNF) is an ecologically important phenomenon that can support an amount of nitrogen to compensate the difficiencies of this element. In this biologically-mediated process, a specific group of bacteria, collectivelly called rhizobia, fixed atomospheric dinitrogen (N_2) via symbioses with legumes. Other free living bacteria fix nitrogen in the soil or in non specific-association with plants. Atmospheric N_2 fixed symbiotically by the association between *Rhizobium* species and legumes represent a renewable source of N for agriculture. This biological process between *Rhizobium* strains and their legume partners can be happened under low level of available nitrogen with help of many different genes such as *nod*, *nif*, *fix*, production of polysaccharides, competition, infection process, host specificity, Type I to Type VI secretion, signals of host and many other different genes that recently have been reported by scientists. Our main target in this review is to focous on the new genes that reported to do significant role in symbiotic nitrogen fixation up to date.

Nitrogen Fixation Process and *nif* Genes: Environmental symbiotic N_2-fixation requires the coordinate interaction of two major classes of genes present in rhizobia, the *nif* genes and *fix* genes. The *nif* genes have structural and functional-relatedness to the N_2 fixation genes found in *Klebsiella pneumonia*. The structural *nif* genes from taxonomically diverse microbes are nearly identical and function in a similar manner to encode nitrogenase [1]. A majority of the *nif* genes are plasmid borne in the rhizobia, but are located on chromosome in the *Bradyrhizobium*. Nitrogen fixation in symbionts and free-living microbes is catalyzed by nitrogenase, an enzyme complex encoded *nifDK* and *nifH* genes. Nitrogenase itself consists of a molybdenum-iron protein (MoFe), subunit I and an iron-containing protein (Fe) subunit II. The MoFe Protein subunits are encoded by *nifK* and *nifD* and a FeMo cofactor (FeMo-Coo) is

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Table 1: *nif* genes products and their role in Nitrogen fixation

Nif genes	Role in Nitrogen fixation
nifH	Dinitrogenase reductase. Obligate electron donar to dinitrogenase during dinitrogenase turnover. Also is required for FeMo-Co biosynthesis and apodinitrogenase maturation.
nifD	α-subunit of dinitrogenase. Forms an a_2B_2 tetramer with B-subunit interface. FeMo-co, the site substrate reduction, is present buried within the α-subunit of dinitrogenase.
nifK	B subunits of dinitrogenase. B clusters are present at B subunit-interface.
nifT	Unknown
nifY	In *K. pneumoniae*, aids in the insertion of FeMo-co into apodinitrogenase.
nifE	Forms a_2B_2 tetramer with nifN. Required for FeMo-co synthesis.
nifN	Required for FeMo-co synthesis.
nifx	Involved in FeMo-co synthesis.
nifU	Involved in mobilization of Fe-S cluster synthesis and repair.
nifS	Involved in mobilization of S for Fe-S cluster synthesis and repair.
nifV	Homocitrate synthesis involved in FeMo-co synthesis.
nifW	Involved in stability of dinitrogenase. Proposed to protect dinitrogenase from O_2 inactivation.
nifZ	Unknown
nifM	Required for the maturation of *nifH*.
nifF	Flavodoxin, Physiologic electron donor to *nifH*.
nifL	Negative regulatory element.
nifA	Positive regulatory element.
nifB	Required FeMo-co synthesis. Metabolic product. NifB-co is the specific Fe and S donor to FeMo-co.
fdxN	Ferredoxin serves as electron donor to nitrogenase.
nifQ	Involved in FeMo-co synthesis. Proposed to function in early $MoO4^2$ processing.
nifJ	Pyruvate flavodoxin (ferredoxin) oxidoreductase involved in electron transport to nitrogenase.

Klipp *et al*., (2004)

required for activation of the MoFe protein. This is assembled from *nifB*, *V*, *N* and *nilfE* genes. The Fe subunit protein is encoded by the *nifH* gene. The organization and complexity of *nif* genes in microorganisms varies tremendously [2]. For example in the free-living *K. pneumonia*, at least 20 *nif* genes are organized in about 8 operons [3]. In most systems, however, the regulation of all *nif* genes is controlled by *NifA* (a positive activator of transcription) and *NifL* (the negative regular). Environmentally, *nif* gene expression is regulated by both oxygen and nitrogen levels [4]. For example, elevated soil ammonia (NH_3 or NH_4) concentrations allows NifL to act as a negative controller or gene expression by preventing *NifA* to act as activator. In addition, elevated O_2 concentrations inhibit *FixJ*, which in turn prevents increases in *nifA*. Since *nifA* is the transcriptional activator of the other *nif* genes elevated O_2 results in a net decreases in the synthesis of nitrogenase and a decrease in, or abolition of symbiotic N_2 fixation [5]. In addition to *nif* genes, many other microbial genes are involved in symbiotic nitrogen fixation, these collectively referred to as fix genes. Moreover, several other genes have been reviewed that they play direct or indirect role in nitrogen fixation such as exoploysaccharides [6], hydrogen uptake [7], glutamine synthase [8], dicarboxylate transport [9], nodulation efficiency [10], B-1,2 Glucans [11] and lipopolysaccharides [8]. Different kinds of *nif* genes that have been identified and their functions are listed in (Table 1) and published by Klipp *et al.* [12].

Mechanism of Nitrogenase Enzyme: The nitrogenase reaction has two essential steps: (1) electron activation by a suitable donor or adenosine di-phosphate (ADP) and (2) substrate reduction. These two steps of the reaction take place at different sites on the nitrogenase molecule but are interdependent. Purified preparations of nitrogenases are highly sensitive to oxygen, specially the Fe protein part of the enzyme. However, it is believed that an undefined respiratory system exists in *Azotobacter* near the site of nitrogen fixation which actively 'scavenges' oxygen so as to prevent the inactivation of nitrogenase. Energy requirements for nitrogenase reaction come from the cellular metabolic cycles in the form of adenosine triphosphate or ATP (roughly 12 to 20 moles of ATP per mole of molecular nitrogen reduced). Pyruvate functions both as an electron donor and an energy source. In the phosphoroclastic reaction, pyruvate forms acetyl phosphate which in the presence of adenosine diphosphate or ADP gives rise to ATP. The reductants are the strongly reducing naturally occurring electron carrier proteins, ferredoxin and flavodoxin. Dithionite ($Na_2S_2O_4$) and certain dyes such as methyl viologen and benzyl viologen can also serve as artificial extracellular

sources of electron donors. Since all nitrogen-fixing microorganisms contain hydrogenase, this enzyme system in cells catalyzes the transfer of electrons from pyruvate or hydrogen to ferredoxin or flavodoxin. Ferredoxins are naturally occurring iron-sulphur (Fe-S) electron carrier proteins capable of undergoing reversible oxidation and reduction. The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH. In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to $H_2N\text{-}NH_2$ and this in turn is reduced to $2NH_3$.

Signals from the Microsymbionts

Nod Factors: Are the most important elements for *Rhizobium* legume communication during the first step of nodule formation. Nod factors or known as lipochitoligosaccharides (LCOs) produced and excreted by more than 30 different *nod*, *nol*, *noe* genes and their corresponding proteins from the microsymbionts. The first identified Nod factors were from *Rhizobium meliloti* [13] and *Rhizobium leguminosarum* sv. *viciae* [14]. The general structure of Nod factors are N-acetylglucosamine backbone with four or five GlcNAc residues with different substituents at 9 different positions such as N-methyl, O-carbamyl, O-acetyl, O-sulfyl, α-linked fucosyl, 2-O-methylfucosyl, 4-O-acetyl-2-O-methylfucosyl, 3-O-sulfate-2- O-methylfucosyl, ethyl, glyceryl, mannosyl and N-glycosyl groups. A major other residues variable is the fatty acid group attached to the nitrogen of the non-reducing end of the Nod factor. Fatty acids with 16 to 18 carbons and a different degree of unsaturation in different positions of the double bonds are mainly present. Also C18 to C22 (ώ-1) hydroxyl fatty acids, which perhaps intermediates in the synthesis of C23 (ώ-1) hydroxyl fatty acyl groups in lipopolysaccherides, can be present in Nod factors of *Sinorhizobium meliloti* [15]. A novel lipochitin oligosaccharides has recently been found in *Rhizobium etli* KIM5s [16]. This is the first case that the major LCO contains 6 oligosaccharide residues and differs by this point from all other rhizobia analyzed. An additional specificity was that the chitin backbone was deactivated in one or two of the Glena moieties, although these were only minor compounds. The fatty acids of these Nod factors were C16:0, C16:1, C18:0, C18:1 and C17:1. In this respect the fatty acids are much more variable than those of *Rhizobium etli* strain CE3. With *Sinorhizobium meliloti* it has been shown that an enzymatic N-deacetylation of the Nod factors decreases their biological activity, but increases stability in the rhizosphere [17]. In all *Rhizobium* species the *nodABC* genes are essential for the synthesis of the core LCO: NodC synthesis the chito-oligosaccharide backbone and *nodB* removes N-acetyl groups from the sugar at its non-reducing end. All other *nod*, *nol* and *noe* genes are responsible for the modification of this general structure. NodD is appositive transcription regulator from the Lysr family and present in all rhizobia. In some rhizobial species such as *Sinorhizobium meliloti*, *nodD* genes are present in multiple forms and their proteins respond to different groups of flavonides. *NodG* has the enzymatic activity of an 3-oxoacyl-acyl carrier protein reductase and is thereby homologous to FabG involved generally in fatty acids elongation [18].

Cyclic Glucans: Cyclic glucans in rhizobia are small molecule linked either by β- (1,2) glycosidic bonds with 17 to 40 units in *Rhizobium* and *Sinorhizobium* or by β- (1,3) and β- (1,6) glycosidic bonds in *Bradyrhizobium japonicum*. Dominant substituents can be either sn-1-phosphoglycerol [11] or phosphocholine [19]. The function of the cyclic glucans in *Rhizobium*, *Sinorhizobium* and *Brayrhizobium* is to protect against hypoosmotic conditions. Rhizobia produce, also large quantities of cyclic glucans in the endosymbiotic stage. A specific function during this stage is assumed to be an increase in the solubility of falvonoids and Nod factors [20]. Another hypothesis is, that β-glucans play a decisive role in the suppression of the host plant defense response with rhizobia, compared to phytopathogenic bacteria.

Lipopolysaccharides: Lipopolysaccharides of rhizobia have been studied only in a few species such as *Rhizobium etli* and *Rhizobium trifolii*. The structure contains three parts, the lipid A, the core chain and the repeat unit of the O-antigen chain. All three parts are very variable. Typical features of rhizobial LPS are the very long chain hydroxy fatty acids [21]. The genes of the LPS core and O-antigen synthesis have been localized on a plasmid. A mutation in a glycosyltransferase produced a rough colony phenotype with a disruption of the O-antigen biosynthesis. The LPS in rhizobia may be involved in the infection process [22]. Their function is perhaps not in the first stages of symbiosis development but in the release of the bacteria from infection thread and the first steps of the symbiosome membrane development. Also for the LPS, a function in the suppression of the host plant defense response has been assumed comparable to the LPS functions in plant pathogens [23].

Exopolysaccharides: The exoploysaccharides have been studied in details by a large number of rhizobial strains [24]. *Sinorhizobium meliloti;* two types of EPS forms could be discriminated, EPS as succinoglucan and EPS II as a galactoglucan with two size classes in each form, one with thousands of saccharide units and a low molecular weight class with 8 to 40 saccharide units. All genes involved in the biosynthesis of the repeating units have been identified. Exopolysaccharides play a major role in the primary stage of the infection of the host plant. It has been suggested that EPS are involved in the suppression of a defense response by the host plant and EPS mutants are eliciting a pronounced plant defense response [25]. There are linkages between the lipopolysaccharides and extracellular polysaccharide synthesis. A Konockout of the dTDP-L-rhamnose synthase affects liopopolysaccharide and extracellular polysaccharide production, as shown for *Azorhizobium caulinodans* [26]. The mutation affecting this gene induced only ineffective nodular structures on the host *Sesbania rostrata*, with no bacteroides and leghemoglobin present in the nodules. The bacteria were trapped in thick-walled infection threads.

Signals from Legume Plants: The relation between *Rhizobium* and its symbiotic partner usually start by producing biochemical signals from both sides. The plants released falvonids that induced by *nod* factor from the micro-symbiont. The later is mentioned in details in the part of signals from the micro-symbionts so this part will focus on signals from plants.

Flavonoids occur widely in plants and are a biologically major and chemically diverse group of secondary metabolites that can be divided into subgroups including anthocyanidins, flavonols, flavones, flavanols, flavanones, chalcones, dihydrochalcones and dihydroflavonols. Isoflavonoids. All flavonoids are phenoloic compounds consist of two benzene rings linked through a heterocyclic pyran or pyrone ring. Specific substitutions on the ring produce flavonols, flavones, flavanones, as well as iso flavonoids, which are derived from a migration of the B ring from the 2 to the 3 position. More than 4,000 different flavonoids have been identified in vascular plants and a particular subset of them is involved in mediating host specificity in the legumes [27].

To start the communication dialogue between *Rhizobium* strains and their legume partners, this usually happened by producing compounds called flavonoids as inducers for Nod factors from the microsymbionts. Approximately 30 nod gene-inducing flavonoids have been isolated from nine legume genera under axenic conditions [28]. They are either glycones or aglycones from a variety of flavonoid subclasses including chalcones, flavones, flavanones, isoflavones and coumestans.

Isoflavones daidzein and genistein, which are inducers for *B. japonicum* and *Rhizobium* sp. NGR234 but anti inducers for *Rhizobium leguminosarum* sv. *trifolii* and sv. *viciae*. Jasmonates are nod inducers that can stimulate *nod* gene expression in *R. leguminosarum* and *B. japonicum*, either alone or synergistically in combination with a flavonoid inducer [29, 30].

In addition to flavonoids, plant signals to rhizobia include betaines, aldonic acids, xanthones, simple phenolics and jasmonates, all of which have been shown to act as *nod* gene inducers [31]. Compounds synthesized by rhizobia encompass not only Nod factors and surface polysaccharides but also types I, III and IV secreted proteins, N-acyl homoserine lactones (AHL), bradyoxetin, hopanoids, lumichrome, indole-3-acetic acid (IAA) and a host of proteins (both characterized and putative) whose contributions to the dialogue, if any, have yet to be defined. *Bradyrhizobium japonicum nod* gene transcription can also be induced by xanthones [32]. Finally, the simple phenolics vanillin and isovanillin from a non legume, wheat, are capable of inducing *nod* genes in *Rhizobium* sp. NGR234 [33].

Several non-flavonoids can be induced by rhizobial *nod* gene transcription. Among them betaines such as stachydrine and trigonelline from seeds of *Medicago* species [34], they are considered as co-inducers, with NodD2 in *S. meliloti*. Lupin seeds also released *nod* inducers named aldonic acids erythronic and tetronic acid [35]. It was reported that the flavanone naringenin can stimulate the colonisation of wheat roots by diazotrophic bacteria via lateral root crack invasion by *Azorhizobium caulinodans* and increased the number of lateral roots per plant [36].

Other Genes Involved in Nodulation and Nitrogen Fixation: A large number of bacterial genes that are playing role in the formation of nodules on leguminous plants have been identified. Lately, there are more than 65 nodulation genes have been identified in rhizobia, each strain can carry one or more of these genes. Several investigators [37, 38, 39] gave explanation about the possible function of the common genes involved in nodulation process. As previously mention in the section of signals from the symbionts and nitrogen fixation process, the *nod*, *nif* and *fix* genes are the main important cluster genes that control this biological process. There are different types of *nod* genes designated as *nodA*,

Table 2: Different genes involved in BNF (Sadowsky *et al*., 2012)

Gene code	Function	References
hsn	Host specificity nodulation	Horvath *et al*., 1986
gsn	Genotypic specific nodulation	Sadowsky *et al*., 1991
exo	Exopolysaccharides	Becker and Puhler 1998
hup	Hydrogen uptake	Maier 1986
gln	Glutamine synthase	Carlson *et al*., 1987
dct	Dicarboxylate transport	Finan *et al*., 1983
nfe	Nodulation formation efficiency	Sanjuan and Olivares 1989
ndv	ß, 1,2 Glucans	Breedveld and Miller 1998
lps	Lipopolysaccharide	Carlson *et al*., 1987
bacA	Bacteroid development	Glazebook *et al*., 1993
tts	Type III secretion system	Kruase *et al*., 2002
virB	Type IV secretion system	Hubber *et al*., 2004
acds, rtx	Inhibition of plant ethylene biosynthesis	Ma *et al*., 2003
pur	Purine biosynthesis	Giraud *et al*., 2007
rosR	Cell surface and competitiveness	Bittinger and Handelsamn 2000
iol	Inositol catabolism (competitivness)	Kohler *et al*., 2010
tfx	Trifolitoxin (competitivness)	Robleto *et al*., 1998
moc	Rhizopine catabolism (competitiveness)	Murphy *et al*., 1995
enod1, enod12 and enod40	Nodulin genes	Van de Sande *et al*., 1997
lectin	Interact with LCos	Berwin and Kardailsky 1997
Rj2 and *Rfg1*	Responsible for host specificity with legumes	Yang *et al*., 2012
KnOx	Cytokinin hormone plays important role in symbiotic nodule development and nodule organogenesis	Ariel *el al*., 2012
ACC	Aminocyclopropane 1-carboxylate deaminase plays viotal role in ACC deaminase activity in legume-*Rhizobum* symbiosis and \ nodule senescence	Nukui *et al*., 2006
ESN1	Contribute in nodule senescence and symbiotic nitrogen fixation	Xi *et al*., 2013
LHK1	Coding for Lotus Histidine Kinase this is important in nodule initiation and primordium	Suzaki *et al*., 2013
Cre1	Contribute in nodule formation, mutant strain of this gene can not form nodules because it is defective in cytokinin response	Gonzalez-Rizzo *et al*., 2006
	Continue of Table 2	
MtNIN	MtNIN functions downstream of the early NF signaling pathway to coordinate and regulate the correct temporal and spatial formation of root nodules	Marsh *et al*., 2007
CLE	CLE-RS glycopeptides are the long sought mobile signals responsible for the initial step of autoregulation of nodulation.	Okamoto *et al*., 2013
CelC2	Revealed that CelC2 fulfils an essential role in the primary infection process required for development of the canonical nitrogen-fixing *R. leguminosarum* sv. *trifolii*-white clover symbiosis.	Robeldo *et al*., 2008
nap and nos	Genes involved in nitrate reductase plays vital role in nodule regulation	Sanchez *et al*., 2013

nodB and *nodC*. Collectively, they are responsible for the biosynthesis of chitin backbone while *nodD* is a regulatory gene that activates the transcription of other inducible *nod* genes [40]. The essential nodulation genes in *B. japonicum* strain USDA 110 are located on the chromosome in several transcriptional units and ordered as: *nolZ*, *nolA*, *nodD2*, *nodD1*, *nodYABCSUIJmolMNO* [41]. Unlike other rhizobial *nodD* genes, the *B. japonicum nodD1* is induced by the flavonoids genistein and daidzein [42,43] and by xanthones [44]. Intriguingly, *Bradyrhizobium* strains BTAi1 and ORS278, which induce nodules on both the root and stem of the aquatic legume *Aeschynomene*, do not possess functional *nodABC* genes [45]. This indicates that *nodABC* genes and Nod factor are not required for symbiosis in these strains and implies that an alternative nodulation strategy exists in the *Bradyrhizobium-Aeschynomene* symbiotic interaction [45, 46]. For more information about different types of *nod* genes and their structure that excreated during the initation of nodule development with different species of legume plants [47, 48].

Rather than these three main important cluster of genes, there are different genes identified as essential genes involved in nodulation and nitrogen fixation [49] (Table 2) such as gene designated host specific nodulation (*hsn*) responsible for controlling nodulation of specific legume genera and identified in *E. meliloti* [50], *Rhizobium leguminosarum* sv. *trifolii* [51]. For example, Göttfert, Hitz and Hennecke [52] identified another types of *hsn* gene in *B. japonicum* USDA 110 called *nodVW* that is essential for the nodulation of siratro, mungbean and cowpea but not soybean.

Genotypic specific nodulation genes (*gsn*) identified by Sadowsky *et al.*[53] are allowing nodulation of specific plant genotypes within a given legume species. For example strain TOM nodulating roots of cultivar Afghanistan genotype of pea (*Pisum sativum*), but *R. leg*. sv. *viciae* isolated from European countries failed to nodulate this host. Gene *nodM* in *R. leguminosarum* sv. *trifolii* is a *gsn* which prevents effective nodulation of subterraneum cv. Woogenellup [54].

Rhizobia can produce four main types of surface polysaccharides which noticed to contribute in various stages of symbiotic development including root colonization, host recognition, infection thread formation and nodule invasion. They comprised extracellular polysaccharides (EPS), LPS, K polysaccharides (K-antigens, capsular polysaccharides or KPS) and cyclic glucans. EPS is essential for the development of fully functioning nodules [55]. Comprehensive reviews of the synthesis, structures and functions of all classes of rhizobial surface polysaccharide are available [56, 57].

Maier [58] found that the *hup* (hydrogenase structural gene) responsible for hydrogen uptake increases the efficiency of nitrogen fixation by recycling hydrogen produced by nitrogenase enzyme. Glutamine synthase (*gln*) playing an essential role of nodule metabolism, as it regulate the antioxidant defenses in response to nitric oxide around the roots [8]. The presence of a functional C4-dicarboxylic acid transport (*dct*) system is essential for N_2 fixation to occur in pea nodules [9]. Sanjuan and Olivars [10] they identified a new symbiotic region of about 5 kb called nodule formation efficiency (*nfe*). The mutation of this part with Tn3HoHo1 reduced the nodulation competitiveness in *Medicago stiva*. LCOs are liopo-chitin oligosaccharides that induced as a result of flavonoids released during the early stages of nodules infection. The core of LCOs is synthesized by *nodA*, *nodB* and *nodC*. The C18:1 is the common fatty acid moiety in LCOs structure in *Rhizobium* membrane. The interaction between rhizobia and plants is very specific due to the type of LCOs structure [8]. Glazebrook *et al*. [59] found that a *bacA* gene is an integral inner membrane protein with seven domains plays essential role in bacteroid development during symbiosis stage. Gene *tts* is a cluster of genes belong to type III secretion system that was found to be affecting nodulation capacity in *Glycin max* [60]. While *VirB*, a gene like tumor structure in *Agrobacterium tumefaciens* and belongs to type IV secretion system that was found to be involved in nodulation by *Mesorhizobium loti* strain R7A [61]. Gene *acds* is a gene characterized and identified as promoting nodule formation in bea plants by modulating the ethylene levels in the roots of plants during the early stages of nodule development [62]. Canonical *nodABC* genes and typical lipochito-oligosaccharidic Nod factors are not required for symbiosis in photosynthetic *Bradyrhizobium*. Mutational analyses in this gene indicated that these unique rhizobia use an alternative pathway to initiate symbioses, where a purine derivative may play a key role in triggering nodule formation [45]. *RosR* regulates genes of diverse function, including those involved in polysaccharide production and in carbohydrate metabolism and those in a region containing sequence similarity to virC1 and virD3 from *Agrobacterium tumefaciens*. *RosR* gene directly or indirectly influences expression of diverse genes in *R. etli*, some of which affect the cell surface and nodulation competitiveness [63]. Inositol catabolic pathway (*iol*), is and its proper regulation are important nutritional or signaling factors in the *S. meliloti*-alfalfa symbiosis [64]. Trifolitoxin (*tfx*) is a gene that increases nodulation competitiveness of *R. etli* strains to occupy high number of nodules on common bean roots [65]. Rhizobine catabolism (*moc*) is a gene that found to be in the bacteroids of nodulated legumes. In competition study [66] they noticed that in the presence of a rhizopine producing strain the strain that can catabolize the rhizopine occupies a higher percentage of the nodule occupancy. During the infection and nodule development process several plant genes are expressed. Depending on the time point of activation, these genes are called early or late nodulin genes. *enod2*, *enod12* and *enod40* are well characterized as early nodulin genes while leghemoglobin and uricase are example of late nodulin genes with known functions. Both of the two types of nodulin genes are playing pivotal role in nodule development. The early nodulin are playing role in signaling communication during infection process, but the late ones are promoting the conversion of bacteria into bacteroids and assist the settlement of bacteria inside nodules [67].

Lectins are involved in *Rhizobium*-symbiotic interaction in two locations, first lectins have been found in the tip of root hairs [68] they suggesting that lectins contribute in the infection process and second in the nodule mature. *Rj2* and *Rfg1* are allelic genes encoding a member of the Toll-interleukin receptor/nucleotidebinding site/leucine-rich repeat (TIR-NBS-LRR) class of plant resistance (R) proteins, can control genotype-specific infection and nodulation [69]. The involvement of host R genes in the control of genotype-specific infection and nodulation reveals a common recognition mechanism underlying symbiotic and pathogenic host bacterial interactions. The knOx gene coding for cytokinin hormone

is playing essential role in nodule development and organogenesis [70]. Nukui *et al*. [71] found that ACC gene coding for aminocyclopropane 1- carboxylase is involoved in *Rhizobium* symbiosis and nodule senescence, as well as, Xi *et al.* [72] who found that genes involoved in macronutrient degradation and remobilization are greatly upregulated during nodule development in the esn1 mutant, confirming the role of ESN1 in nodule senescence and symbiotic nitrogen fixation. Suzaki *et al.* [73] noted that LHK1 coding for lotus histidine kinase is playing a common role in nodule intiation and primordium. Cre1 gene coding for cytokinin in *S. meliloti* allowing crosstalk between plant cytokinins and bacterial Nod factors signals [74]. MtNIN functions downstream of the early nodulation factor (NF) signaling pathway to coordinate and regulate the correct temporal and spatial formation of root nodules [75]. Root-derived CLE glycopeptides that can control nodulation by direct binding to HAR1 receptor kinase [76]. Sanchez *et al.* [77] noted that both of nap encoding periplasmic nitrate reductase and nos encoding N_2O reductase play important role in nodule regulation. Microscopic analysis of the symbiotic phenotypes of the ANU843 wild type and CelC2 knockout mutant derivative revealed that this enzyme fulfils an essential role in the primary infection process required for development of the canonical nitrogen-fixing *R. leguminosarum* sv. *trifolii*-white clover symbiosis [78]. Another genes MtRAR1 noted by Radzman *et al.* [79] that playing role and increasing root nodule formation.

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