**Review**

**Rj (rj) genes involved in nitrogen-fixing root nodule formation in soybean**

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It has long been known that formation of symbiotic root nodules in soybean (*Glycine max* (L.) Merr.) is controlled by several host genes referred to as *Rj* (*rj*) genes, but molecular cloning of these genes has been hampered by soybean’s complicated genome structure and large genome size. Progress in molecular identification of legume genes involved in root nodule symbiosis has been mostly achieved by using two model legumes, *Lotus japonicus* and *Medicago truncatula*, that have relatively simple and small genomes and are capable of molecular transfection. However, recent development of resources for soybean molecular genetic research, such as genome sequencing, large EST databases, and high-density linkage maps, have enabled us to isolate several *Rj* genes. This progress has been achieved in connection with systematic utilization of the information obtained from molecular genetics of the model legumes. In this review, we summarize the current status of knowledge of host-controlled nodulation in soybean based on information from recent studies on *Rj* genes, and discuss the future research prospects.

**Key Words:** soybean, root nodule symbiosis, *Rj* (*rj*) gene, host-controlled nodulation, host-restriction of nodulation, autoregulation of nodulation, model legume.

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**Introduction**

Leguminous plants can establish a nitrogen-fixing symbiosis with soil bacteria, collectively termed rhizobia, in a unique organ, the root nodule. Nodule formation and accommodation of endosymbiotic rhizobia inside nodules are strictly controlled by host plant genes. Recent progress in molecular genetics using two model legume plants, *Lotus japonicus* and *Medicago truncatula*, has enabled identification of a number of host genes essential for symbiotic nodule formation (for recent reviews, see Kouchi et al. 2010, Murray 2011, Oldroyd and Downie 2008).

In soybean (*Glycine max* (L.) Merr.), one of the most important legume crops in the world, the genetic loci, namely *Rj* (*s*) or *rj* (*s*), have been identified as controlling nodulation traits upon inoculation with compatible *Bradyrhizobium* and *Ensifer/Sinorhizobium* species. Some alleles of these genes have come from natural variation while others were identified by artificially induced mutations. We classified *Rj* and/or *rj* genotypes into three categories as summarized in Table 1. Recessive alleles at three loci, *rj1*, *rj5* and *rj6*, result in non-nodulation phenotypes (Pracht et al. 1993, Williams and Lynch 1954). Another recessive locus, known as *rj7* or *nts1* (nitrate-tolerant symbiosis 1) was identified by EMS (ethyl methane sulfonate)-induced mutagenesis and causes a so-called ‘hypernodulation’ phenotype, i.e., the formation of an unusually large number of nodules (Akao and Kouchi 1992, Carroll et al. 1985a, 1985b, Harper and Nickell 1995). In addition to these recessive genes, the dominant alleles, *Rj2*, *Rj3*, *Rj4* and *Rfg1* are known to have unique features that restrict nodulation with specific strains (or serogroups) of *Bradyrhizobium* or *Ensifer/Sinorhizobium*. The *Rj2*, *Rj3*, *Rj4* and *Rfg1* genotypes exclude nodulation with certain strains of *B. japonicum*, *B. elkanii* and *E. fredii*, respectively. Represented by *B. japonicum* USDA122, *B. elkanii* USDA33, *B. elkanii* USDA61 and *E. fredii/S. fredii* USDA257, respectively (Caldwell 1966, Caldwell et al. 1966, Tres 1995, Vest 1970, Vest and Caldwell 1972, Weiser et al. 1990).

Genetic loci that control root nodule symbiosis of soybean were first identified in the 1950s, but molecular cloning of genes for those loci was not successful until a few years ago. This is due in large part to the fact that soybean was not amenable to map-based cloning because of its large genome size and genome complexity. However, establishment of the resources for genomics studies of the model legumes such as *L. japonicus* (Sato and Tabata 2006, Sato et al. 2008), followed by vast progress in the molecular genetic study of host...
genes involved in root nodule symbiosis in these model legumes, enabled great advances in the molecular identification of soybean recessive rj genes. For instance, rj7 (ntsI) was isolated as an ortholog of LjHARI (L. japonicus HYPERNODULATION ABERRANT ROOT FORMATION 1), of which defects in L. japonicus result in a hypernodulation phenotype similar to those in soybean rj7 (ntsI) mutants (Nishimura et al. 2002a, see also Searle et al. 2003). The syntenic relationships between the soybean and L. japonicus genomes also made it feasible to isolate LjHARI by transfer of molecular marker information from soybean (Nishimura et al. 2002a). Furthermore, the recent acceleration of soybean genomics through efforts such as the whole-genome sequencing project (Schmutz et al. 2010, http://soybase.org/) is enabling the positional cloning of symbiotic genes from soybean, as demonstrated by the molecular cloning of Rj2 and Rfg1 (Yang et al. 2010).

In this review, we summarize recent discoveries related to the genes involved in root nodule symbiosis in soybean and outline prospects for the future study. In addition, we briefly describe progress in gene identification using L. japonicus as a model legume in relation with soybean recessive rj genes, because it is of critical importance to transfer the knowledge obtained from model legumes to agriculturally important legume crops.

**Non-nodulation mutants and the corresponding genes**

Symbiotic interactions of legume plants and Rhizobium bacteria exhibit strict species-species specificity; individual Rhizobium species infect only a limited range of host legume species. This specificity is determined by the structures of lipochitin oligosaccharide signal molecules, termed ‘Nod factors’ (NFs), which are secreted from rhizobia. NFs activate a series of signaling cascades in host legume roots that lead to rhizobial infection and trigger nodule organogenesis (Cullimore et al. 2001). Putative NF receptors in legumes belong to a family of LysM-RLKs (lysin-motif receptor-like kinases) that have a common structure of a single transmembrane domain with an extracellular lysin motif (LysM) receptor domain and an intracellular Ser/Thr kinase domain. In L. japonicus, two genes encoding LysM-RLK, NFR1 and NFR5 (NOD-FACTOR RECEPTOR KINASE 1 and 5), have been identified based on studies of non-nodulation mutants (Madsen et al. 2003, Radutoiu et al. 2003); the two proteins encoded by these genes are believed to form a receptor complex (Radutoiu et al. 2007). In M. truncatula, LYK3 (LysM DOMAIN-CONTAINING RECEPTOR-LIKE KINASE 3) and NFP (NOD FACTOR PERCEPTION) have been identified; the former is thought to be an ortholog of LjNFR1 and the latter an ortholog of LjNFR5 (Arrighi et al. 2006, Limpens et al. 2003, Smit et al. 2007). The structures and combinations of the extracellular LysM domains are thought to be crucial for recognition of specific structures of NFs.

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**Table 1. Soybean genes controlling nodule formation**

| Genotype and nodulation phenotype | Restricted strain | Origin | Linkage group | Gene | Gene product | Possible function | Legume ortholog | Regulatory organ | References |
|----------------------------------|------------------|--------|---------------|------|--------------|------------------|----------------|----------------|-----------|
| **Recessive gene and non-nodulation phenotype** | | | | | | | | | |
| rj1 | all | USDA257 | McCall, Williams 82, Hill, Jack | 2 (D1b) | GmNFR1α | LysM-Ser/Thr-RLK | NFR receptor | LjNFR1, MtLYK3, PsSYM37 | root | 1, 2, 3 |
| rj5, rj6 | all | USDA122, Is-1 | Hardee, CNS, IAC-2, Bonminori | 11 (B1), 1 (D1a) | GmNFR5α, GmNFR5β | LysM-Ser/Thr-RLK | NFR receptor | LjNFR5, MnNFP, PsSYM10 | root | 4, 5, 6 |

| **Recessive gene and hypernodulation phenotype** | | | | | | | | | |
| rj7 | all | USDA122, Is-1 | Hardee, CNS, IAC-2, Bonminori | 12 (H) | NTSI/| LRR-Ser/Thr-RLK | AON | LjHARI, MsSUNN, PsSYM29 | shoot | 7, 8, 9, 10 |

| Dominant gene and restriction nodulation phenotype | | | | | | | | | |
| Rfg1 | E. fredii/S. fredit | USDA257 | McCall, Williams 82, Hill, Jack | 16 (J) | Rfg1 | TIR-NBS-LRR | R protein | unknown | root | 11 |
| B. japonicum | USDA122, Is-1 | USDA122, Is-1 | Hardee, CNS, IAC-2, Bonminori | 16 (J) | Rj2 | TIR-NBS-LRR | R protein | unknown | root | 11 |
| B. elkanii | USDA122, USDA33 | USDA122, USDA33 | D-51, Hardee, CNS, IAC-2, Bonminori | unknown | unknown | unknown | unknown | unknown | root |
| B. elkanii | USDA61 | USDA61 | Hill, Amsoy 71, Dunfield, Akiengokou, Fukuyutaka | unknown | unknown | unknown | unknown | unknown | root |

1, Radutoiu et al. (2003); 2, Limpens et al. (2003); 3, Indrasumunar et al. (2011); 4, Madsen et al. (2003); 5, Arrighi et al. (2006); 6, Indrasumunar et al. (2010); 7, Nishimura et al. (2002a); 8, Kruse et al. (2002); 9, Searle et al. (2003); 10, Schnabel et al. (2005); 11, Yang et al. (2010)
secreted from *Rhizobium* species, making them compatible with individual host legumes (Radutoiu et al. 2007), whereas the intracellular kinase domains are functionally well conserved across legume species (Nakagawa et al. 2011). Most of the loss-of-function mutants of these putative NF receptors exhibit neither rhizobial infection nor cortical cell division (CCD) leading to formation of nodule primordia (Radutoiu et al. 2003). Similar LysM-RLK genes, PsSYM37 (*Pisum sativum* SYMBIOSIS 37) orthogonal to *LjNFR1* and PsSYM10 orthogonal to *LjNFR5* were identified from pea as the genes of which mutation cause a non-nodulation phenotype (Madsen et al. 2003, Zhukov et al. 2008).

A non-nodulation soybean mutant was first identified as *rj1* genotype, which was found in natural population (Weber 1966a, 1966b, Williams and Lynch 1954). An EMS-induced mutant, *nod49*, from soybean cultivar (cv.) Bragg was shown to be allelic to *rj1* (Carroll et al. 1986). The *rj1* non-nodulation trait is monogenic and recessive, and the causal gene was very recently discovered to be an ortholog of *LjNFR1* (Indrasumunar et al. 2011). Despite the absence of normal infection-related events such as root hair deformation, curling, and infection thread formation, the *rj1* genotype occasionally shows subepidermal CCD upon rhizobial inoculation. Indrasumunar et al. (2011) cloned two LysM-RLK genes, *GmNFR1α* and *GmNFR1β*, which share high similarity in their genomic sequences. They found frameshift mutations in *GmNFR1α* leading to truncated proteins in both the *nod49* mutant and the *rj1* mutant. *GmNFR1β* in these mutant lines appeared to be functionally intact, though it contains a deletion in the sixth intron. Since the level of expression of *GmNFR1β* was very low in these mutants and their parental cultivars compared to the level of expression of *GmNFR1α* in these lines, *GmNFR1α* was assumed to play the more critical role in NF perception in soybean (Indrasumunar et al. 2011). Indeed, a nonsense mutation in *GmNFR1β* found within genotype PI437.654 caused no defect in nodulation upon rhizobial inoculation. However, the possibility that the functional *GmNFR1α* gene in PI437.654 could complement the mutation in *GmNFR1β* cannot be excluded. We have isolated three non-nodulation mutants from cv. Enrei, all of which have mutations in *GmNFR1α*, and found that the original and wild-type cv. Enrei has a natural mutation in *GmNFR1β*. Genetic analyses using crosses of these mutant lines with wild-type cv. Moshi-dou 503 showed that the mutations in both *GmNFR1α* and *GmNFR1β* were needed to display the non-nodulation phenotype in the F$_2$ populations (Hayashi et al. unpublished data). This result indicates that *GmNFR1α* and *GmNFR1β* are functionally redundant. Leaky phenotypes of the *nod49/rj1* mutants, such as occurrence of subepidermal CCDS or infrequent but successful nodulation when inoculated with a high titer of *Bradyrhizobium* (Indrasumunar and Gresshoff 2011), may be due to the functional redundancy between *GmNFR1α* and *GmNFR1β*. Interestingly, over-expression of *GmNFR1α* in soybean was shown to result in a significant increase in nodule number and plant nitrogen content (Indrasumunar et al. 2011), suggesting that higher expression of *GmNFR1α* would be a possible target in breeding efforts for enhanced symbiotic nitrogen fixation.

The other known non-nodulation loci, *rj5* and *rj6* were identified from the mutants *nod139* from cv. Bragg (Mathews et al. 1989), and *nn5* from cv. Williams (Pracht et al. 1993). The mutation of *nn5* is known to be allelic to *nod139*, and these mutants show neither rhizobial infection events nor CCD. The soybean genome contains two *LjNFR5* orthologs, *GmNFR5α* and *GmNFR5β*. In both *nod139* and *nn5*, nonsense mutations were found in the kinase domain of *GmNFR5α*, whereas *GmNFR5β* was inactive in both wild-type cultivars (Bragg and Williams) due to a common ancestral retroelement insertion (Indrasumunar et al. 2010). In other soybean genotypes, however, both *GmNFR5α* and *GmNFR5β* were functional, and these two duplicated loci correspond to the dominant wild-type alleles *Rj5* and *Rj6* reported previously (Pracht et al. 1993). Indeed, transformation of *nod139* or *nn5* with wild-type *GmNFR5β* completely suppressed the non-nodulation phenotype of these mutants, indicating that *GmNFR5α* and *GmNFR5β* are functionally redundant (Indrasumunar et al. 2010).

The putative NF receptors *GmNFR1α*, *GmNFR5α* and *GmNFR5β*, show very high similarity (>90%) in amino acid sequences in their kinase domain. However, cross-complementation experiments of *GmNFR1α* mutants (*nod49* and *rj1*) with wild-type *GmNFR5α* and *GmNFR5β*, and of *GmNFR5* mutants (*nod139* and *nn5*) with wild-type *GmNFR1α*, showed no complementation of the non-nodulation phenotype each other (Indrasumunar et al. 2010), suggesting that *GmNFR1α* and *GmNFR5αβ* comprise a receptor complex as well as the *LjNFR1* and *LjNFR5* receptor complex supposed in *L. japonicus* (Radutoiu et al. 2007), but are not functionally redundant. *GmNFR5α* and *GmNFR5β*, like *LjNFR5*, appeared to lack an activation loop that is essential for kinase activity, and thus *GmNFR1α* very likely plays a key role in signal transmission to downstream symbiotic signaling pathways. A model for putative the Nod factor receptor complex, *GmNFR1* and *GmNFR5*, in soybean is represented in Fig. 1A.

Progress in molecular genetics studies of model legumes in the past decade has revealed a number of the plant genes involved in early steps of signal transduction leading to rhizobial infection and nodule formation. They include genes involved in a ‘common symbiotic pathway’ (CSP) required for both root nodule and arbuscular mycorrhizal symbioses, and in nodulation-specific pathways (Kouchi et al. 2010, Murray 2011). Homeologs and/or paralogs of these genes can be found in a soybean genome database (Indrasumunar et al. 2010, see also Schmutz et al. 2010). However, soybean mutants for those genes lying downstream of NF receptors have not been isolated.

**Hypernodulation mutants and the corresponding genes**

Hypernodulation mutants were first isolated from soybean
lines that showed a large excess of nodule numbers even under high concentrations of soil nitrate, which inhibits nodule formation in wild-type plants, and this trait was shown to be controlled by a single recessive gene, \textit{rj7 (nts1)} (Akao and Kouchi 1992, Carroll et al. 1985a, 1985b, Gremaud and Harper 1989). The nitrogen-fixing root nodules consume a large amount of energy (photosynthates) from the host plants, and hence excessive nodulation results in retardation of plant growth. To avoid inappropriately excessive nodulation and keep the balance of symbiosis, legume plants have developed a negative feedback regulatory system of nodulation that is called AON (autoregulation of nodulation). Defects in AON result in the hypernodulation phenotype. The \textit{nts1} mutant phenotype is controlled by the shoot genotype as demonstrated by reciprocal grafting (Caetano-annolles and Gresshoff 1991, Nishimura et al. 2002a), showing that AON is controlled systemically through long-distance signalling between shoots and roots. The AON gene, \textit{LjHAR1}, was first cloned from the \textit{L. japonicus} hypernodulation mutant, \textit{har1} (Krusell et al. 2002, Nishimura et al. 2002a). Subsequently, soybean \textit{NTS1/GmNARK} (\textit{G. max NODULE AUTOREGULATION RECEPTOR KINASE}) was proven to be an ortholog of \textit{LjHAR1} and the causal gene of the \textit{nts1} mutants (Nishimura et al. 2002a, Searle et al. 2003). In general, hypernodulation mutants display retarded shoot growth because of formation of too many nodules. However, it is noteworthy that a hypernodulation soybean cultivar, Sakukei 4 (present name is Kanto 100), shows a high yielding ability and does not display unfavourable phenotypes in terms of plant growth, especially in the fields with low nitrogen fertility (Takahashi et al. 2003). Sakukei 4 was bred using a hypernodulation mutant, \textit{en6500} from cv. Enrei, and has a nonsense mutation in \textit{NTS1/GmNARK} (Arai et al. 2005). During the breeding process of Sakukei 4, the occurrence of natural

Fig. 1. Schematic representation of Rj proteins involved in nitrogen-fixing root nodule formation in soybean. (A) The putative Nod factor receptor complex, Rj1/GmNFR1 and Rj5/Rj6/GmNFR5. Binding of Nod factors to extracellular LysM domains of the complex and subsequent transducing the signal through the intracellular kinase of Rj1/GmNFR1 to downstream signalling cascades leads to rhizobial infection and nodule organogenesis. (B) The AON signalling mediated by the putative Rj7/NTS1/GmNARK and CLV2-like protein complex. Recognition of root-derived signal(s) produced in response to NF perception by extracellular LRR domains of the complex in shoots results in production of the AON signal(s) through the intracellular kinase of Rj7/NTS1/GmNARK. The AON signal(s) inhibits formation of new nodules. (C) A TIR-NBS-LRR class of plant resistance (R) protein, Rj2/Rfg1, involved in host-restriction of nodulation with specific rhizobial strains. Determination of symbiotic specificity might be achieved in the manner of ETI responses.
crossing with cv. Tamahomare was detected by parentage analysis using soybean SSR (simple sequence repeat) markers (Yamamoto et al. 2004). Sakukei 4 sometimes displayed higher yielding ability than the recurrent parent, cv. Enrei, whereas the yield was significantly lower than that of the accidental pollen parent, cv. Tamahomare (Shimamura et al. 2007). Therefore, it is still obscure as to whether the hypernodulation genotype contributes to improve the soybean yield in the breeding programs.

**NTS1/GmNARK and LjHAR1** each encode a leucine-rich repeat Ser/Thr receptor-like kinase (LRR-RLK) that is highly homologous to *Arabidopsis* CLAVATA1 (CLV1, Clark et al. 1997). CLV1 interacts with CLV2, forming a complex that recognizes signalling peptide(s); this complex is involved in regulation of the cell fate in the shoot and floral apical meristems through cell-cell communication (DeYoung and Clark 2001). In legumes, an LRR-RLK that has close resemblance to CLV1 regulates nodule development systemically, by organ-organ communication. Orthologs of NTS1/GmNARK and LjHAR1 were also cloned from hypernodulation mutants of pea (PsSYM29) and of *M. truncatula* (MtSUNN, *M. truncatula* SUPER NUMERIC NODULES) (Krusell et al. 2002, Schnabel et al. 2005).

AON is triggered by root-derived signals produced in response to NF perception. The root-derived signals are perceived by the CLV1-like LRR-RLK in the shoots; in turn, the shoot-derived signals are transported to the roots in a negative feedback system that inhibits further nodule formation (reviewed in Magori and Kawaguchi 2009). AON signalling mediated by the putative NTS1/GmNARK and CLV2-like protein complex is presented in Fig. 1B. The root-derived signals are most likely CLE (CLAVATA3/ESR-related) peptides that are produced upon NF perception and/or nitrate treatment. The genes encoding CLE peptides involved in AON have been cloned, for the first time, from *L. japonicus*; LjCLE-RS1 (*L. japonicus* CLE Root Signal 1) was induced in response to NFs and LjCLE-RS2 was induced in response to either NF or nitrate treatment, and their constitutive expression strongly inhibited nodulation in an LjHAR1-dependent manner (Okamoto et al. 2009). In soybean, three candidate CLE peptide-encoding genes were recently identified (Reid et al. 2011). In contrast, molecular identification of shoot-derived signals involved in AON is still in a preliminary stage. Recent studies have indicated the presence of low-molecular-weight substances in the leaf extracts of wild-type soybean, but not in those of hypernodulation mutants, and these substances show down-regulating activity of nodulation (Kenjo et al. 2010, Lin et al. 2010, Yamaya and Arima 2010a, 2010b). Some other loci and/or genes related to hypernodulation phenotypes that are regulated by shoot or root genotype have been documented in *L. japonicus* (Magori et al. 2009, Miyazawa et al. 2010, Nishimura et al. 2002b, Oka-Kira et al. 2005, Yoshida et al. 2010), but the corresponding mutants have not been found in soybean.

**Genes involved in restriction of nodulation with specific strains of Bradyrhizobium and Ensifer/Sinorhizobium bacteria**

Soybeans normally establish a nitrogen-fixing symbiosis with such strains as *B. japonicum*, *B. elkanii*, *B. liaoningense*, *B. yuanmingense*, *E. fredii*, *S. fredii*, *Rhizobium tropici*, *R. oryzae*, and *Mesorhizobium tianshanense*. It has been well documented that some soybean genotypes differentially restrict nodulation with specific strains (or serogroups) of Bradyrhizobium or *Ensifer/Sinorhizobium* (Cregan and Keyser 1986, Cregan et al. 1989a, 1989b, Devine and Kuykendall 1996, Ferrey et al. 1994, Keyser and Cregan 1987, Weiser et al. 1990). This phenomenon has been attracting interest from the view of agricultural practice. Inoculation with *Bradyrhizobium* strains that have efficient nitrogen fixation activity has often been unsuccessful in the field condition, because of competition with less effective indigenous *Bradyrhizobium* or *Ensifer/Sinorhizobium* strains in the soil. Identification of genes that exclude or substantially reduce nodulation with the ineffective indigenous strains, and then elucidation of the molecular mechanisms for the host-restriction of nodulation might provide insight into improving the efficiency of symbiotic nitrogen fixation in soybean by application of inoculants.

Soybean genotypes that restrict nodulation with specific rhizobial strains have been designated R2, R3, R4, and Rfg1, each of which behaves as a single dominant gene. Soybean cultivar Hardee and its parental line CNS were found to restrict nodulation with *B. japonicum* strain USDA122 (Caldwell 1966, Caldwell et al. 1966). The nodulation-restriction phenotype is controlled by Rj2 that is located within a cluster of resistance gene analogues (RGAs), including the resistance genes, Rmd-c (powdery mildew) and Rps2 (*Phytophthora* stem and root rot), on linkage group 16(J) (Kanazin et al. 1996). Recently, Yang et al. (2010) cloned the Rj2 gene, which encodes a member of the Toll-interleukin receptor/nucleotide-binding site/leucine-rich repeat (TIR-NBS-LRR) class of plant resistance (R) proteins against microbial pathogens by a manner of effector-triggered immune (ETI) responses. Rj2 is allelic to Rfg1, a gene that prevents effective nodulation with certain fast-growing *E. fredii*, *S. fredii* strains such as USDA257 (Trese 1995). Interestingly, only seven amino acid substitutions in the NBS and LRR domains of the Rj2/Rfg1 gene products define the genetic basis of specificity differences between these two genotypes. A schematic representation of the R protein, Rj2/Rfg1, is shown in Fig. 1C. Furthermore, Hardee and CNS were found to nodulate ineffectively with *B. japonicum* strain 33 (whose present classification is *B. elkanii* USDA33) due to the presence of Rj3 (Vest 1970). Rj3 behaves as a single dominant gene, but it has not been cloned yet.

Vest and Caldwell (1972) identified the Rj4 genotype in cultivars Hill and Amsoy 71. Rj4 restricts nodulation with the *B. japonicum* serogroup 61 (whose present classification is *B. elkanii* USDA61) and also protects the host plant from...
nodulation by many strains of *Bradyrhizobium* strains. These strains produce rhizobitoxine, a compound that induces chlorosis in the host plant, and are relatively inefficient symbionts for soybean (Devine et al. 1988). The *Rj4* genotype is common in *Glycine max*, the putative wild progenitor of the domesticated soybean (*Glycine max*), and is most frequently found in cultivars from Southeast Asia but less frequently in cultivars from North Asia (Devine and Kuykendall 1996). Like *Rj3*, *Rj4* has not been cloned; thus, the details of the host-controlled restriction of nodulation by these dominant genes have yet to be unraveled.

**Characterization of the rhizobial community with respect to host *Rj* genotypes**

By using host-restriction of nodulation determined by *Rj* genotypes, *Bradyrhizobium* strains indigenous to a soybean field in Japan were classified into three nodulation types *A*, *B* and *C* (Ishizuka et al. 1991). *Bradyrhizobium* strains which were compatible with any *Rj* genotypes were classified as nodulation type *A*, whereas the strains incompatible with *Rj* genotypes were classified as type *B*, and the strains incompatible with *Rj4* cultivars were classified as type *C*. When soybean cultivars of *Rj* genotypes were grown in the same field, the nodules formed on non-*Rj* (*rj2rj3rj4*), *Rj4* and *Rj2Rj3* soybeans were occupied preferentially by type *A*, type *B* and type *C* strains, respectively, indicating that the indigenous *Bradyrhizobium* strains in the soil display preferences for nodulation on compatible *Rj* genotypes (Minami et al. 2009, Saeki et al. 2000, 2005, 2008). These observations raise the possibility that the *Rj2Rj3Rj4* lines would be more suitable than the single *Rj* genotype to eliminate infection with those indigenous *Bradyrhizobium* and expected to be applicable for nodulation by type-A inoculants with high nitrogen-fixing ability. Indeed, the *Rj2Rj3Rj4* lines generated by crossing the cultivars IAC-2 (*Rj2Rj3*) and Hill (*Rj4*) (Ishizuka et al. 1993, Yamakawa et al. 1999) showed much higher nodule occupancy with a serogroup represented by *B. japonicum* USDA110, which belongs to type *A*, than did single *Rj* genotypes (Yamakawa et al. 2003). A more recent study on *Rj* genotype-specific nodule occupancy with indigenous *Bradyrhizobium* strains that were clustered based on polymorphism of the 16S–23S rDNA internal transcribed spacer region demonstrated preferential nodulation with *B. japonicum* USDA110-cluster strains on the *Rj2Rj3Rj4* genotype (Minami et al. 2009).

The bacterial genes involved in soybean *Rj* genotype-specific nodulation and/or nodulation preference are still largely unknown. Some candidate genes, mostly related to rhizobial cell surface structures, were identified from *B. japonicum* strain Is1-1 by Tn5 mutagenesis; these genes might be responsible for incompatibility with *Rj2*-genotype soybean (Tsurumaru et al. 2008). It has been also proposed that rhizobial surface polysaccharides and proteins called NOPs (nodule outer proteins), which are secreted through the type III secretion system (T3SS) of rhizobia, determine intra-species host range (Deakin and Broughton 2009, D’Haeze and Holsters 2004). Cultivar Hill (*Rj4*) was nodulated normally by type III secretion gene cluster (*tts*) mutants of *B. elkanii* USDA61, a strain that is incompatible with *Rj4*-genotype soybean (Okazaki et al. 2009), suggesting the involvement of T3SS in determining host-specificity through a gene-for-gene interaction mechanism similar to that found in pathogenic plant-microbe interactions. The fact that *Rj2* encodes a TIR-NBS-LRR protein (Yang et al. 2010), one of a class of R proteins involved in plant resistance against microbial pathogens which is achieved by a manner of ETI responses, strongly supports the role of bacterial components secreted through T3SS in the host *Rj* genotype-specific incompatibility of nodulation.

**Future prospects**

During the past decade, the isolation and functional characterization of a number of host genes essential for symbiotic nodule formation has been achieved by using model legume plants. Homologs of those symbiotic genes have been found by searches of soybean genome sequences and collections of EST (expressed sequence tag) sequences, whereas only a few genes, *i.e.*, two NF receptor components (*GmNFR1* and *GmNFR5*) and an AON gene (*NTS1/GmNARK*), were identified based on mutant phenotypes in soybean. This is mainly due to the allotetraploid nature of soybean genome, which in the past had made it difficult to investigate the symbiotic genes of soybean in great detail. However, this disadvantageous situation is now being overcome rapidly by the availability of resources for soybean genome research. Two independent draft genome sequences have been released (Kim et al. 2010, Lam et al. 2010, Schmutz et al. 2010, http://soybase.org/) and another soybean genome sequencing project is now in progress for Japanese cultivar Enrei. A huge EST collection of more than 390,000 sequences is now available (http://www.ncbi.nlm.nih.gov/dbEST/) together with approximately 40,000 full-length cDNA clones (Umezawa et al. 2008). Genetic linkage maps have been developed for various combinations of soybean cultivars and now contain more than 1,000 DNA markers (Choi et al. 2007, Hwang et al. 2009, Hyten et al. 2010, Song et al. 2004, 2010, Xia et al. 2007), and several BAC (bacterial artificial chromosome) libraries have been constructed (Meksem et al. 2000, Tomkins et al. 1999, Wu et al. 2004, Xia et al. 2005). In addition, hairy-root and stable transformation techniques for soybean have been developed (Kereszt et al. 2007, Kita et al. 2007, Yamada et al. 2010). Therefore, more extensive studies by forward-genetics approaches are now expected for root nodule symbiosis in soybean, in connection with the information obtained from the model legumes.

The study of bacterial strain-specific restriction of nodulation by dominant *Rj* alleles has a long history. Since particular *Rj* genotypes can exclude nodulation with indigenous
Bradyrhizobium strains that belong to specific clusters, they are expected to have practical importance in agriculture for improving the efficiency of inoculation with desirable B. japonicum strains that exhibit effective nitrogen fixation activity. In this regard, however, the molecular mechanisms underlying the host specificity or affinity between host cultivars and rhizobial strains, nodulation preference or competitiveness, and survivability of indigenous rhizobia should be studied in greater detail, because the population dynamics of rhizobia in the field has been shown to be quite complicated (Minami et al. 2009, Saeki et al. 2000, 2005, 2008). Elucidation of the molecular basis of the Rf genotypes is providing new insights into the mechanisms that govern affinity with rhizobia or fine-tune host-microsymbiont interactions, as suggested recently by the cloning of Rf2/Rfg1 (Yang et al. 2010). In addition, molecular cloning of another dominant gene of host-restricted nodulation, Rf4, is currently in progress in our laboratory.

Following the construction of the genetic linkage maps and development of a number of recombinant inbred line (RIL) populations, quantitative trait locus (QTL) mapping has been performed for various agronomically important traits of soybean such as plant developmental and reproductive characteristics, disease resistance, seed quality, and nutritional traits (Harada and Xia 2004, Zhang et al. 2004). To our knowledge, however, no such effort has been made in regard to nitrogen fixation ability, despite the fact that both nitrogen fixation activity and the ratio of nitrogen fixed from the atmosphere to the total nitrogen accumulation in plants have been shown to vary significantly within soybean cultivars (Hungria and Bohler 2000, Nohara et al. 2006). Among major legume crops, soybean is the most highly dependent on atmospheric nitrogen fixed in the nodules, and symbiotic nitrogen fixation is of critical importance in seed productivity. It is necessary for soybean breeding programs to put much more emphasis on the traits related to symbiotic nitrogen fixation, and the recent advances in soybean genomics described here are expected to contribute to continuing progress in this area.

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