
Review

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

Masaki Hayashi^{*1}, Yuichi Saeki², Michiyo Haga³, Kyuya Harada¹, Hiroshi Kouchi¹ and Yosuke Umehara¹

¹

²

<sup>3

---</sup>

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 have 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.

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/S. fredii*, represented by *B. japonicum* USDA122, *B. elkanii* USDA33, *B. elkanii* USDA61 and *E. fredii/S. fredii* USDA257, respectively (Caldwell 1966, Caldwell *et al.* 1966, Trese 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

Communicated by T. Anai

Received June 30, 2011. Accepted August 18, 2011.

*Corresponding author (e-mail: twrees@affrc.go.jp)

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									
<i>rj1</i>	all	<i>rj1, nod49, T201, A62-2, To-1-0</i>	2 (D1b)	<i>GmNFR1α</i>	LysM-Ser/Thr-RLK	NF receptor	<i>LjNFR1, MtLYK3, PsSYM37</i>	root	1, 2, 3
<i>rj5, rj6</i>	all	<i>nm5, nod139</i>	11 (B1), 1 (D1a)	<i>GmNFR5α, GmNFR5β</i>	LysM-Ser/Thr-RLK	NF receptor	<i>LjNFR5, MtNFP, PsSYM10</i>	root	4, 5, 6
Recessive gene and hypernodulation phenotype									
<i>rj7</i>	all	<i>nts1, nod1-3, nod3-7, en6500</i>	12 (H)	<i>NTS1/GmNARK</i>	LRR-Ser/Thr-RLK	AON	<i>LjHAR1, MtSUNN, PsSYM29</i>	shoot	7, 8, 9, 10
Dominant gene and restriction nodulation phenotype									
<i>Rfg1</i>	<i>E. fredii/S. fredii</i> USDA257	McCall, Williams Hill, Jack	82, 16 (J)	<i>Rfg1</i>	TIR-NBS-LRR	R protein	unknown	root	11
<i>Rj2</i>	<i>B. japonicum</i> USDA122, Is-1	Hardee, CNS, Bonminori	IAC-2, 16 (J)	<i>Rj2</i>	TIR-NBS-LRR	R protein	unknown	root	11
<i>Rj3</i>	<i>B. elkanii</i> USDA33	D-51, Hardee, CNS, IAC-2, Bonminori	unknown	unknown	unknown	unknown	unknown	root	
<i>Rj4</i>	<i>B. japonicum</i> Is-34, <i>B. elkanii</i> USDA61	Is-34, Hill, Amsoy 71, Dunfield, Akisengoku, 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, Krusell *et al.* (2002); 9, Searle *et al.* (2003); 10, Schnabel *et al.* (2005); 11, Yang *et al.* (2010)

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* (*nts1*) was isolated as an ortholog of *LjHAR1* (*L. japonicus* HYPERNODULATION ABERRANT ROOT FORMATION 1), of which defects in *L. japonicus* result in a hypernodulation phenotype similar to those in soybean *rj7* (*nts1*) 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 *LjHAR1* 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

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) orthologous to *LjNFR1* and *PsSYM10* orthologous 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, *GmNFR1 α* and *GmNFR1 β* , which share high similarity in their genomic sequences. They found frameshift mutations in *GmNFR1 α* leading to truncated proteins in both the *nod49* mutant and the *rj1* mutant. *GmNFR1 β* in these mutant lines appeared to be functionally intact, though it contains a deletion in the sixth intron. Since the level of expression of *GmNFR1 β* was very low in these mutants and their parental cultivars compared to the level of expression of *GmNFR1 α* in these lines, *GmNFR1 α* was assumed to play the more critical role in NF perception in soybean (Indrasumunar *et al.* 2011). Indeed, a nonsense mutation in *GmNFR1 β* found within genotype PI437.654 caused no defect in nodulation upon rhizobial inoculation. However, the possibility that the functional *GmNFR1 α* gene in PI437.654 could complement the mutation in *GmNFR1 β* cannot be excluded. We have isolated three non-nodulation mutants from cv. Enrei, all of which have mutations in *GmNFR1 α* , and found that the original and wild-type cv. Enrei has a natural mutation in *GmNFR1 β* . Genetic analyses using crosses of these mutant lines with wild-type cv. Moshi-dou Gong 503 showed that the mutations in both *GmNFR1 α* and *GmNFR1 β* were needed to display the non-nodulation phenotype in the F₂ populations (Hayashi *et al.* unpublished data). This result indicates that *GmNFR1 α* and *GmNFR1 β* 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 *GmNFR1 α* and *GmNFR1 β* . Interestingly, over-expression of *GmNFR1 α* 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 *GmNFR1 α* 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, *GmNFR5 α* and *GmNFR5 β* . In both *nod139* and *nn5*, nonsense mutations were found in the kinase domain of *GmNFR5 α* , whereas *GmNFR5 β* 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 *GmNFR5 α* and *GmNFR5 β* 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 *GmNFR5 β* completely suppressed the non-nodulation phenotype of these mutants, indicating that *GmNFR5 α* and *GmNFR5 β* are functionally redundant (Indrasumunar *et al.* 2010).

The putative NF receptors *GmNFR1 α* , *GmNFR5 α* and *GmNFR5 β* , show very high similarity (>90%) in amino acid sequences in their kinase domain. However, cross-complementation experiments of *GmNFR1 α* mutants (*nod49* and *rj1*) with wild-type *GmNFR5 α* and *GmNFR5 β* , and of *GmNFR5* mutants (*nod139* and *nn5*) with wild-type *GmNFR1 α* , showed no complementation of the non-nodulation phenotype each other (Indrasumunar *et al.* 2010), suggesting that *GmNFR1 α* and *GmNFR5 α/β* 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. *GmNFR5 α* and *GmNFR5 β* , like *LjNFR5*, appeared to lack an activation loop that is essential for kinase activity, and thus *GmNFR1 α* 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

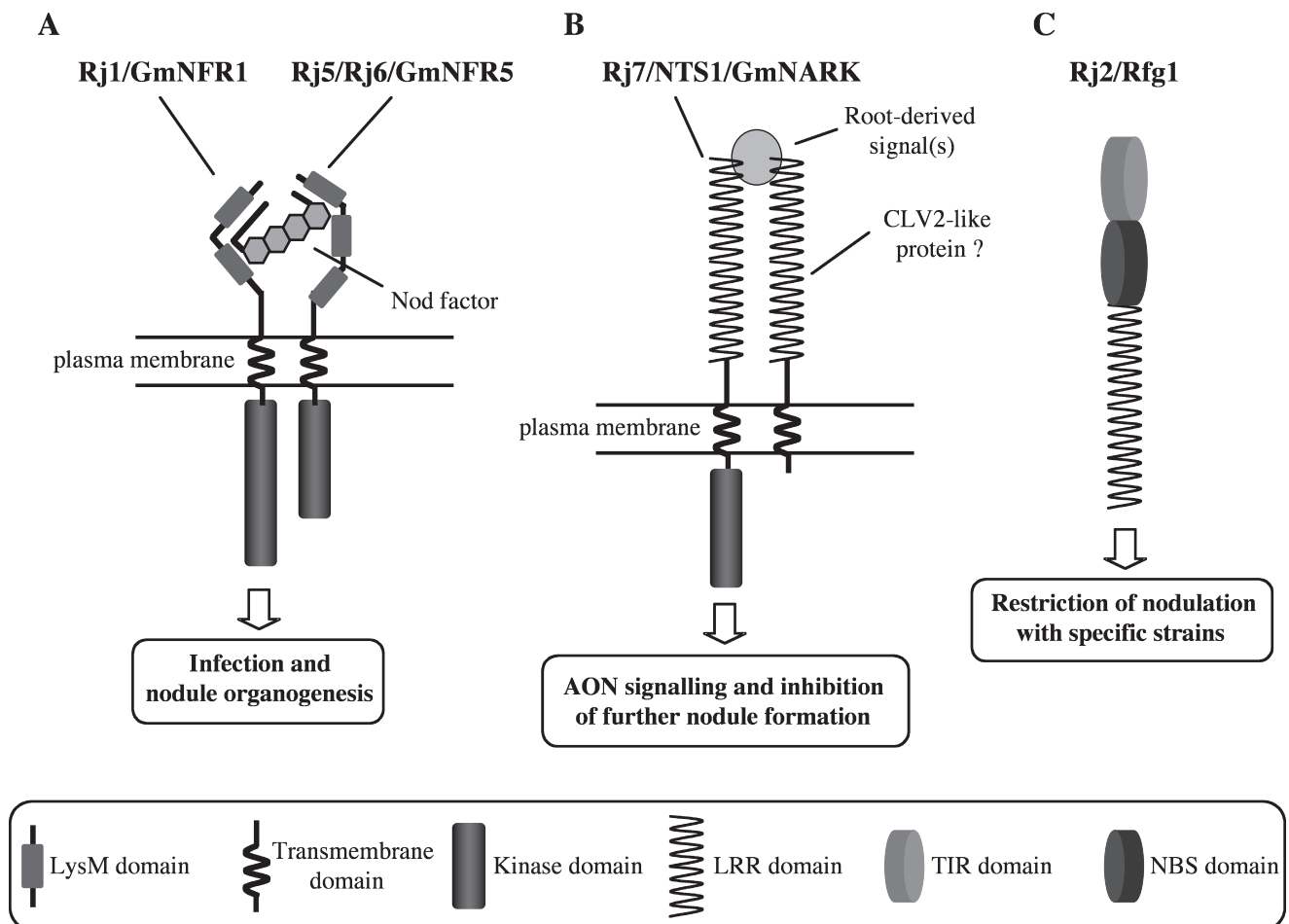


Fig. 1. Schematic representation of Rj proteins involved in nitrogen-fixing root nodule formation in soybean. (A) The putative Nod factor receptor complex, Rj1/GmNFR1 and Rj5/Rj6/GmNFR5. Binding of Nod factors to extracellular LysM domains of the complex and subsequent transducing the signal through 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.

lines that showed a large excess of nodule numbers even under high concentrations of soil nitrate, which inhibits nodulation in wild-type plants, and this trait was shown to be controlled by a single recessive gene, *ry7* (*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 *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 sig-

nalling between shoots and roots. The AON gene, *LjHAR1*, was first cloned from the *L. japonicus* hypernodulation mutant, *har1* (Krusell *et al.* 2002, Nishimura *et al.* 2002a). Subsequently, soybean *NTS1/GmNARK* (*G. max* NODULE AUTOREGULATION RECEPTOR KINASE) was proven to be an ortholog of *LjHAR1* and the causal gene of the *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, Sakukey 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). Sakukey 4 was bred using a hypernodulation mutant, *en6500* from cv. Enrei, and has a nonsense mutation in *NTS1/GmNARK* (Arai *et al.* 2005). During the breeding process of Sakukey 4, the occurrence of natural

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 *Rj2*, *Rj3*, *Rj4* 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 *B. elkanii*. 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 soja*, 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 *Rj2Rj3* cultivars were classified as type B, and the strains incompatible with *Rj4* cultivars were classified as type C. When soybean cultivars of various *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 rather 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 Is-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 (nodulation outer proteins), which are secreted

through the type III secretion system (T3SS) of rhizobia, determine intra-species host range (Deakin and Broughton 2009, D'Haese and Holsters 2004). Cultivar Hill (*Rj4*) was nodulated normally by type III secretion gene cluster (*ttt*) 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. Homeologs 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 *Rj* 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 *Rj2/Rfg1* (Yang *et al.* 2010). In addition, molecular cloning of another dominant gene of host-restricted nodulation, *Rj4*, 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.

Acknowledgments

We acknowledge financial support from the Ministry of Agriculture, Forestry, and Fisheries of Japan [Genomics for Agricultural Innovation SOY2001].

Literature Cited

- Akao, S. and H. Kouchi (1992) A supernodulating mutant isolated from soybean cultivar Enrei. *Soil Sci. Plant Nutr.* 38: 183–187.
- Arai, M., M. Hayashi, M. Takahashi, S. Shimada and K. Harada (2005) Expression and sequence analysis of systemic regulation gene for symbiosis, *NTS1/GmNARK* in supernodulation soybean cultivar, Sakukei 4. *Breed. Sci.* 55: 147–152.
- Arrighi, J.F., A. Barre, B.B. Amor, A. Bersoult, L.C. Soriano, R. Mirabella, F. de Carvalho-Niebel, E.P. Journet, M. Ghéardi, T. Huguet *et al.* (2006) The *Medicago truncatula* lysine motif-receptor-like kinase gene family includes *NFP* and new nodule-expressed genes. *Plant Physiol.* 142: 265–279.
- Caetano-Anollés, G. and P.M. Gresshoff (1991) Plant genetic control of nodulation. *Annu. Rev. Microbiol.* 45: 345–382.
- Caldwell, B.E. (1966) Inheritance of a strain-specific ineffective nodulation in soybeans. *Crop Sci.* 6: 427–428.
- Caldwell, B.E., K. Hinson and H.W. Johnson (1966) A strain-specific ineffective nodulation reaction in the soybean *Glycine max* L. Merrill. *Crop Sci.* 6: 495–496.
- Carroll, B.J., D.L. McNeil and P.M. Gresshoff (1985a) Isolation and properties of soybean (*Glycine max* (L.) Merr.) mutants that nodulate in the presence of high nitrate concentrations. *Proc. Natl. Acad. Sci. USA* 82: 4162–4166.
- Carroll, B.J., D.L. McNeil and P.M. Gresshoff (1985b) A supernodulation and nitrate-tolerant symbiotic (*nts*) soybean mutant. *Plant Physiol.* 78: 34–40.
- Carroll, B.J., D.L. McNeil and P.M. Gresshoff (1986) Mutagenesis of soybean (*Glycine max* (L.) Merr.) and the isolation of non-nodulating mutants. *Plant Sci.* 47: 109–119.
- Choi, I.Y., D.L. Hyten, L.K. Matukumalli, Q. Song, J.M. Chaky, C.V. Quigley, K. Chase, K.G. Lark, R.S. Reiter, M.S. Yoon *et al.* (2007) A soybean transcript map: Gene distribution, haplotype and single-nucleotide polymorphism analysis. *Genetics* 176: 685–696.
- Clark, S.E., R.W. Williams and E.M. Meyerowitz (1997) The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. *Cell* 89: 575–585.
- Cregan, P.B. and H.H. Keyser (1986) Host restriction of nodulation by *Bradyrhizobium japonicum* strain USDA123 in soybean. *Crop Sci.* 26: 911–916.
- Cregan, P.B., H.H. Keyser and M.J. Sadowsky (1989a) A soybean genotype that restricts nodulation of a previously unrestricted isolate of *Bradyrhizobium japonicum* serocluster 123. *Crop Sci.* 29: 307–312.
- Cregan, P.B., H.H. Keyser and M.J. Sadowsky (1989b) Host plant effects on nodulation and competitiveness of the *Bradyrhizobium japonicum* serotype strains constituting serocluster 123. *Appl. Environ. Microbiol.* 55: 2532–2536.
- Cullimore, J.V., R. Ranjeva and J.J. Bono (2001) Perception of lipochitoooligosaccharidic Nod factors in legumes. *Trends Plant Sci.* 6: 24–30.
- Deakin, W.J. and W.J. Broughton (2009) Symbiotic use of pathogenic strategies: Rhizobial protein secretion systems. *Nat. Rev. Microbiol.* 7: 312–320.
- Devine, T.E., L.D. Kuykendall and J.J. O'Neill (1988) DNA homology group and the identity of bradyrhizobial strains producing rhizobitoxine-induced foliar chlorosis on soybean. *Crop Sci.* 28: 939–941.
- Devine, T.E. and L.D. Kuykendall (1996) Host Genetic control of symbiosis in soybean (*Glycine max* L.). *Plant Soil* 186: 173–187.
- DeYoung, B.J. and S.E. Clark (2001) Signaling through the *CLAVATA1* receptor complex. *Plant Mol. Biol.* 46: 505–513.
- D'Haese, W. and M. Holsters (2004) Surface polysaccharides enable bacteria to evade plant immunity. *Trends Microbiol.* 12: 555–561.
- Ferrey, M.L., P.H. Graham and M.P. Russelle (1994) Nodulation efficiency of *Bradyrhizobium japonicum* strains with genotypes of soybean varying in the ability to resist nodulation. *Can. J. Microbiol.* 40: 456–460.
- Gremaud, M.F. and J.E. Harper (1989) Selection and initial characterization of partially nitrate tolerant nodulation mutants of soybean. *Plant Physiol.* 89: 169–173.
- Harada, K. and Z. Xia (2004) Soybean genomics: efforts to reveal the

- complex genome. *Breed. Sci.* 54: 215–224.
- Harper, J.E. and C.D. Nickell (1995) Genetic analysis of nonnodulating soybean mutants in a hypernodulated background. *Soybean Genet. Newsl.* 22: 185–190.
- Hungria, M. and T.R.J. Bohrer (2000) Variability of nodulation and dinitrogen fixation capacity among soybean cultivars. *Biol. Fertil. Soils* 31: 45–52.
- Hwang, T.Y., T. Sayama, M. Takahashi, Y. Takada, Y. Nakamoto, H. Funatsuki, H. Hisano, S. Sasamoto, S. Sato, S. Tabata *et al.* (2009) High-density integrated linkage map based on SSR markers in soybean. *DNA Res.* 16: 213–225.
- Hyten, D.L., I.Y. Choi, Q. Song, J.E. Specht, T.E. Carter, R.C. Shoemaker, E.Y. Hwang, L.K. Matukumalli and P.B. Cregan (2010) A high density integrated genetic linkage map of soybean and the development of 1536 universal soy linkage panel for quantitative trait locus mapping. *Crop Sci.* 50: 960–968.
- Indrasumunar, A., A. Kereszt, I. Searle, M. Miyagi, D. Li, C.D.T. Nguyen, A. Men, B.J. Carroll and P.M. Gresshoff (2010) Inactivation of duplicated Nod factor receptor 5 (NFR5) genes in recessive loss-of-function non-nodulation mutants of allotetraploid soybean (*Glycine max* L. Merr.). *Plant Cell Physiol.* 51: 201–214.
- Indrasumunar, A. and P.M. Gresshoff (2011) Duplicated nod-factor receptor 5 (NFR5) genes are mutated in soybean (*Glycine max* L. Merr.). *Plant Signal. Behav.* 5: 535–536.
- Indrasumunar, A., I. Searle, M.H. Lin, A. Kereszt, A. Men, B.J. Carroll and P.M. Gresshoff (2011) Nodulation factor receptor kinase 1 α controls nodule organ number in soybean (*Glycine max* L. Merr.). *Plant J.* 65: 39–50.
- Ishizuka, J., Y. Suemasu and K. Mizogami (1991) Preference of *Rj*-soybean cultivars for *Bradyrhizobium japonicum* for nodulation. *Soil Sci. Plant Nutr.* 37: 15–21.
- Ishizuka, J., S.D. Kim, A.K.M.A. Hussain and T. Yamakawa (1993) Soybean preference for *Bradyrhizobium japonicum* for nodulation—Isolation of *Rj₂Rj₄*-lines from the cross of soybean cvs. IAC-2 (*Rj₂*) and Hill (*Rj₄*). *Soil Sci. Plant Nutr.* 39: 79–86.
- Kanazin, V., L.F. Marek and R.C. Shoemaker (1996) Resistance gene analogs are conserved and clustered in soybean. *Proc. Natl. Acad. Sci. USA* 93: 11746–11750.
- Kenjo, T., H. Yamaya and Y. Arima (2010) Shoot-synthesized nodulation-restricting substances of wild-type soybean present in two different high-performance liquid chromatography peaks of the ethanol-soluble medium-polarity fraction. *Soil Sci. Plant Nutr.* 56: 399–406.
- Kereszt, A., D. Li, A. Indrasumunar, C.D.T. Nguyen, S. Nontachaiyapoom, M. Kinkema and P.M. Gresshoff (2007) *Agrobacterium rhizogenes*-mediated transformation of soybean to study root biology. *Nat. Protoc.* 2: 948–952.
- Keyser, H.H. and P.B. Cregan (1987) Nodulation and competition for nodulation of selected soybean genotypes among *Bradyrhizobium japonicum* serogroup123 isolates. *Appl. Environ. Microbiol.* 53: 2631–2635.
- Kim, M.Y., S. Lee, K. Van, T.H. Kim, S.C. Jeong, I.Y. Choid, D.S. Kim, Y.S. Lee, D. Park, J. Ma *et al.* (2010) Whole-genome sequencing and intensive analysis of the undomesticated soybean (*Glycine soja* Sieb. and Zucc.) genome. *Proc. Natl. Acad. Sci. USA* 107: 22032–22037.
- Kita, Y., K. Nishizawa, M. Takahashi, M. Kitayama and M. Ishimoto (2007) Genetic improvement of the somatic embryogenesis and regeneration in soybean and transformation of the improved breeding lines. *Plant Cell Rep.* 26: 439–447.
- Kouchi, H., H. Imaizumi-Anraku, M. Hayashi, T. Hakoyama, T. Nakagawa, Y. Umehara, N. Suganuma and M. Kawaguchi (2010) How many peas in a pod? Legume genes responsible for mutualistic symbioses underground. *Plant Cell Physiol.* 51: 1381–1397.
- Krusell, L., L.H. Madsen, S. Sato, G. Aubert, A. Genua, K. Szczyglowski, G. Duc, T. Kaneko, S. Tabata, F. de Bruijn *et al.* (2002) Shoot control of root development and nodulation is mediated by a receptor kinase. *Nature* 420: 422–425.
- Lam, H.M., X. Xu, X. Liu, W. Chen, G. Yang, F.L. Wong, M.W. Li, W. He, N. Qin, B. Wang *et al.* (2010) Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. *Nature Genet.* 42: 1053–1059.
- Limpens, E., C. Franken, P. Smit, J. Willemsse, T. Bisseling and R. Geurts (2003) LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. *Science* 302: 630–633.
- Lin, Y.H., B.J. Ferguson, A. Kereszt and P.M. Gresshoff (2010) Suppression of hypernodulation in soybean by a leaf-extracted, NARK- and Nod factor-dependent, low molecular mass fraction. *New Phytol.* 185: 1074–1086.
- Madsen, E.B., L.H. Madsen, S. Radutoiu, M. Olbryt, M. Rakwalska, K. Szczyglowski, S. Sato, T. Kaneko, S. Tabata, N. Sandal *et al.* (2003) A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. *Nature* 425: 637–640.
- Magori, S. and M. Kawaguchi (2009) Long-distance control of nodulation: Molecules and models. *Mol. Cells* 27: 129–134.
- Magori, S., E. Oka-Kira, S. Shibata, Y. Umehara, H. Kouchi, Y. Hase, A. Tanaka, S. Sato, S. Tabata and M. Kawaguchi (2009) *TOO MUCH LOVE*, a root regulator associated with the long-distance control of nodulation in *Lotus japonicus*. *Mol. Plant Microbe Interact.* 22: 259–268.
- Mathews, A., B.J. Carroll and P.M. Gresshoff (1989) A new nonnodulation gene in soybean. *J. Hered.* 80: 357–360.
- Meksem, K., K.K. Zobrist, E. Ruben, D. Hyten, T. Quanzhou, H.B. Zhang and D.A. Lightfoot (2000) Two large-insert soybean genomic libraries constructed in a binary vector: applications in chromosome walking and genome wide physical mapping. *Theor. Appl. Genet.* 101: 747–755.
- Minami, M., T. Yamakawa, A. Yamamoto, S. Akao and Y. Saeki (2009) Estimation of nodulation tendency among *Rj*-genotype soybeans using the bacterial community isolated from an Andosol. *Soil Sci. Plant Nutr.* 55: 65–72.
- Miyazawa, H., E. Oka-Kira, N. Sato, H. Takahashi, G.J. Wu, S. Sato, M. Hayashi, S. Betsuyaku, M. Nakazono, S. Tabata *et al.* (2010) The receptor-like kinase KLAVER mediates systemic regulation of nodulation and non-symbiotic shoot development in *Lotus japonicus*. *Development* 137: 4317–4325.
- Murray, J.D. (2011) Invasion by invitation: rhizobial infection in legumes. *Mol. Plant Microbe Interact.* 24: 631–639.
- Nakagawa, T., H. Kaku, Y. Shimoda, A. Sugiyama, M. Shimamura, K. Takanashi, K. Yazaki, T. Aoki, N. Shibuya and H. Kouchi (2011) From defense to symbiosis: limited alterations in the kinase domain of LysM receptor-like kinases are crucial for evolution of legume-*Rhizobium* symbiosis. *Plant J.* 65: 169–180.
- Nishimura, R., M. Hayashi, G.J. Wu, H. Kouchi, H. Imaizumi-Anraku, Y. Murakami, S. Kawasaki, S. Akao, M. Ohmori, M. Nagasawa *et al.* (2002a) HAR1 mediates systemic regulation of symbiotic organ development. *Nature* 420: 426–429.
- Nishimura, R., M. Ohmori, H. Fujita and M. Kawaguchi (2002b) A *Lotus* basic leucine zipper protein with a RING-finger motif negatively regulates the developmental program of nodulation. *Proc. Natl. Acad. Sci. USA* 99: 15206–15210.
- Nohara, T., N. Nakayama, T. Nakamura, M. Takahashi, S. Maruyama,

- J. Arihara and S. Shimada (2006) Cultivar differences of nitrogen fixation capacity and its contribution to nitrogen accumulation in soybean grown in the field with a high soil nitrate level. *Jpn. J. Crop Sci.* 75: 350–359.
- Oka-Kira, E., K. Tateno, K. Miura, T. Haga, M. Hayashi, K. Harada, S. Sato, S. Tabata, N. Shikazono, A. Tanaka *et al.* (2005) *klavier* (*klv*), a novel hypernodulation mutant of *Lotus japonicus* affected in vascular tissue organization and floral induction. *Plant J.* 44: 505–515.
- Okamoto, S., E. Ohnishi, S. Sato, H. Takahashi, M. Nakazono, S. Tabata and M. Kawaguchi (2009) Nod factor/nitrate-induced *CLE* genes that drive HAR1-mediated systemic regulation of nodulation. *Plant Cell Physiol.* 50: 67–77.
- Okazaki, S., S. Zehner, J. Hempel, K. Lang and M. Göttfert (2009) Genetic organization and functional analysis of the type III secretion system of *Bradyrhizobium elkanii*. *FEMS Microbiol. Lett.* 295: 88–95.
- Oldroyd, G.E.D. and J.A. Downie (2008) Coordinating nodule morphogenesis with rhizobial infection in legumes.
- Pracht, J.E., C.D. Nickell and J.E. Harper (1993) Genes controlling nodulation in soybean: *Rj5* and *Rj6*. *Crop Sci.* 33: 711–713.
- Radutoiu, S., L.H. Madsen, E.B. Madsen, H.H. Felle, Y. Umehara, M. Grönlund, S. Sato, Y. Nakamura, S. Tabata, N. Sandal *et al.* (2003) Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* 425: 585–592.
- Radutoiu, S., L.H. Madsen, E.B. Madsen, A. Jurkiewicz, E. Fukai, E.M. Quistgaard, A.S. Albrektsen, E.K. James, S. Thirup and J. Stougaard (2007) LysM domains mediate lipochitin-oligosaccharide recognition and *Nfr* genes extend the symbiotic host range. *EMBO J.* 26: 3923–3935.
- Reid, D.E., B.J. Ferguson and P.M. Gresshoff (2011) Inoculation- and nitrate-induced CLE peptides of soybean control NARK-dependent nodule formation. *Mol. Plant Microbe Interact.* 24: 606–618.
- Saeki, Y., I. Akagi, H. Takaki and Y. Nagatomo (2000) Diversity of indigenous *Bradyrhizobium* strains isolated from three different *Rj*-soybean cultivars in terms of randomly amplified polymorphic DNA and intrinsic antibiotic resistance. *Soil Sci. Plant Nutr.* 46: 917–926.
- Saeki, Y., A. Kaneko, T. Hara, K. Suzuki, T. Yamakawa, M.T. Nguyen, Y. Nagatomo and S. Akao (2005) Phylogenetic analysis of soybean-nodulating rhizobia isolated from alkaline soils in Vietnam. *Soil Sci. Plant Nutr.* 51: 1043–1052.
- Saeki, Y., M. Minami, A. Yamamoto and S. Akao (2008) Estimation of the bacterial community diversity of soybean-nodulating bradyrhizobia isolated from *Rj*-genotype soybeans. *Soil Sci. Plant Nutr.* 54: 718–724.
- Sato, S. and S. Tabata (2006) *Lotus japonicus* as a platform for legume research. *Curr. Opin. Plant Biol.* 9: 128–132.
- Sato, S., Y. Nakamura, T. Kaneko, E. Asamizu, T. Kato, M. Nakao, S. Sasamoto, A. Watanabe, A. Ono, K. Kawashima *et al.* (2008) Genome structure of the legume, *Lotus japonicus*. *DNA Res.* 15: 227–239.
- Schmutz, J., S.B. Cannon, J. Schlueter, J. Ma, T. Mitros, W. Nelson, D.L. Hyten, Q. Song, J.J. Thelen, J. Cheng *et al.* (2010) Genome sequence of the palaeopolyploid soybean. *Nature* 463: 178–183.
- Schnabel, E., E.P. Jourmet, F. de Carvalho-Niebel, G. Duc and J. Frugoli (2005) The *Medicago truncatula* *SUNN* gene encodes a CLV1-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Mol. Biol.* 58: 809–822.
- Searle, I.R., A.E. Men, T.S. Laniya, D.M. Buzas, I. Iturbe-Ormaetxe, B.J. Carroll and P.M. Gresshoff (2003) Long-distance signaling in nodulation directed by a CLAVATA1-like receptor kinase. *Science* 299: 109–112.
- Shimamura, S., M. Takahashi, T. Nakamura, N. Nakayama, R. Yamamoto, Y.H. Kim and S. Shimada (2007) Comparison of productivity among supernodulating soybean cultivar ‘Sakukei 4’ and wild type cultivars ‘Enrei’ and ‘Tamahomare’ under field conditions. *Jpn. J. Crop Sci.* 76: 548–554.
- Smit, P., E. Limpens, R. Geurts, E. Fedorova, E. Dolgikh, C. Gough and T. Bisseling (2007) Medicago LYK3, an entry receptor in rhizobial nodulation factor signalling. *Plant Physiol.* 145: 183–191.
- Song, Q.J., L.F. Marek, R.C. Shoemaker, K.G. Lark, V.C. Concibido, X. Delannay, J.E. Specht and P.B. Cregan (2004) A new integrated genetic linkage map of the soybean. *Theor. Appl. Genet.* 109: 122–128.
- Song, Q.J., G. Jia, Y. Zhu, E. Hwang, D.L. Hyten, P.B. Cregan, D.M. Grant and R. Nelson (2010) Abundance of SSR motifs and development of candidate polymorphic SSR markers (BARCSOYSSR_1.0) in soybean. *Crop Sci.* 50: 1950–1960.
- Takahashi, M., J. Arihara, N. Nakayama and M. Kokubun (2003) Characteristics of growth and yield formation in the improved genotype of supernodulating soybean (*Glycine max* L. Merr.). *Plant Prod. Sci.* 6: 112–118.
- Tomkins, J.P., R. Mahalingam, H. Smith, J.L. Goicoechea, H.T. Knap and R.A. Wing (1999) A bacterial artificial chromosome library for soybean PI 437654 and identification of clones associated with cyst nematode resistance. *Plant Mol. Biol.* 41: 25–32.
- Trese, A.T. (1995) A single dominant gene in McCall soybean prevents effective nodulation with *Rhizobium fredii* USDA257. *Euphytica* 81: 279–282.
- Tsurumaru, H., T. Yamakawa, M. Tanaka and M. Sakai (2008) Tn5 mutants of *Bradyrhizobium japonicum* Is-1 with altered compatibility with *Rj2*-soybean cultivars. *Soil Sci. Plant Nutr.* 54: 197–203.
- Umehara, T., T. Sakurai, Y. Totoki, A. Toyoda, M. Seki, A. Ishiwata, K. Akiyama, A. Kurotani, T. Yoshida, K. Mochida *et al.* (2008) Sequencing and analysis of approximately 40000 soybean cDNA clones from a full-length-enriched cDNA library. *DNA Res.* 15: 333–346.
- Vest, G. (1970) *Rj3*—A gene conditioning ineffective nodulation in soybean. *Crop Sci.* 10: 34–35.
- Vest, G. and B.E. Caldwell (1972) *Rj4*—A gene conditioning ineffective nodulation in soybean. *Crop Sci.* 12: 692–693.
- Weber, C.R. (1966a) Nodulating and nonnodulating soybean isoline: I. Agronomic and chemical attributes. *Agron. J.* 58: 43–46.
- Weber, C.R. (1966b) Nodulating and non-nodulating soybean isolines: II. Response to applied nitrogen and modified soil conditions. *Agron. J.* 58: 46–49.
- Weiser, G.V., H.D. Skipper and A.G. Wollum (1990) Exclusion of inefficient *Bradyrhizobium japonicum* serogroups by soybean genotypes. *Plant Soil* 121: 99–105.
- Williams, L.F. and D.L. Lynch (1954) Inheritance of a non-nodulation character in the soybean. *Agron. J.* 46: 28–29.
- Wu, C.C., P. Nimmakayala, F.A. Santos, R., C. Scheuring, K. Meksem, D.A. Lightfoot and H.B. Zhang (2004) Construction and characterization of a soybean bacterial artificial chromosome library and use of multiple complementary libraries for genome physical mapping. *Theor. Appl. Genet.* 109: 1041–1050.
- Xia, Z., H. Sato, S. Watanabe, S. Kawasaki and K. Harada (2005) Construction and characterization of a BAC library of soybean. *Euphytica* 141: 129–137.
- Xia, Z., Y. Tsubokura, M. Hoshi, M. Hanawa, C. Yano, K. Okamura, T.A. Ahmed, T. Anai, S. Watanabe, M. Hayashi *et al.* (2007) An integrated high-density linkage map of soybean with RFLP, SSR,

- STS, and AFLP markers using a single F2 population. *DNA Res.* 14: 257–269.
- Yamada, T., S. Watanabe, M. Arai, K. Harada and K. Kitamura (2010) Cotyledonary node pre-wounding with a micro-brush increased frequency of *Agrobacterium*-mediated transformation in soybean. *Plant Biotech.* 27: 217–220.
- Yamakawa, T., M. Eriguchi, A.K.M.A. Hussain and J. Ishizuka (1999) Soybean preference for *Bradyrhizobium japonicum* for nodulation—Nodulation by *Rj₂Rj₃Rj₄*-genotypes isolated from the progenies of a cross between soybean cvs. IAC-2 (*Rj₂Rj₃*) and Hill (*Rj₄*). *Soil Sci. Plant Nutr.* 45: 461–469.
- Yamakawa, T., A.K.M.A. Hussain and J. Ishizuka (2003) Soybean preference for *Bradyrhizobium japonicum* for nodulation—Occupation of serogroup USDA110 in nodules of soybean plants harboring various *Rj*-genes grown in a field. *Soil Sci. Plant Nutr.* 49: 835–841.
- Yamamoto, R., R. Takahashi, K. Harada, M. Takahashi and S. Shimada (2004) Parentage analysis of supernodulating soybean cultivar “Sakukei 4”. *Breed. Res.* 6: 149–151.
- Yamaya, H. and Y. Arima (2010a) Evidence that a shoot-derived substance is involved in regulation of the super-nodulation trait in soybean. *Soil Sci. Plant Nutr.* 56: 115–122.
- Yamaya, H. and Y. Arima (2010b) Shoot-synthesized nodulation-restricting substances are present in the medium-polarity fraction of shoot extracts from wild-type soybean plants. *Soil Sci. Plant Nutr.* 56: 418–421.
- Yang, S., F. Tang, M. Gao, H.B. Krishnan and H. Zhu (2010) *R* gene-controlled host specificity in the legume–rhizobia symbiosis. *Proc. Natl. Acad. Sci. USA* 107: 18735–18740.
- Yoshida, C., S. Funayama-Noguchi and M. Kawaguchi (2010) *plenty*, a novel hypernodulation mutant in *Lotus japonicus*. *Plant Cell Physiol.* 51: 1425–1435.
- Zhang, W.K., Y.J. Wang, G.Z. Luo, J.S. Zhang, C.Y. He, X.L. Wu, J.Y. Gai and S.Y. Chen (2004) QTL mapping of ten agronomic traits on the soybean (*Glycine max* L. Merr.) genetic map and their association with EST markers. *Theor. Appl. Genet.* 108: 1131–1139.
- Zhukov, V., S. Radutoiu, L.H. Madsen, T. Rychagova, E. Ovchinnikova, A. Borisov, I. Tikhonovich and J. Stougaard (2008) The pea *Sym37* receptor kinase gene controls infection-thread initiation and nodule development. *Mol. Plant Microbe Interact.* 21: 1600–1608.