Symbiosis, Inventiveness by Recruitment?[1]

Krzysztof Szczyglowski* and Lisa Amyot

Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, 1391 Sandford Street, London, Ontario N5V 4T3, Canada

LEGUMES, A UNIQUE FLOWERING PLANT FAMILY

With one notable exception, namely the genus *Parasponia* in the elm family, the ability to form nitrogen-fixing symbiosis with gram-negative soil bacteria known as rhizobia is restricted to the legume family, Leguminosae. It has been well established that initiation of successful nodular symbiosis requires strict compatibility between rhizobial-secreted Nod factors and a perception machinery of the plant host roots (Albrecht et al., 1999). Nod factors comprise a heterogeneous group of morphogenic lipochitooligosaccharides with a major role as determinants of host specificity (Spaink, 2000). Nanomolar concentrations of purified Nod factors can mimic bacterial infections to a certain extent by inducing several root cellular responses that are characteristic of compatible interaction between the host plant and symbiotic bacteria. Early plant responses to Nod factors, including activation of a subset of plant specific genes called *e*arly *nod*ulins (ENODs), calcium spiking, root hair curling, pre-infection thread formation, and induction and organogenesis of nodule primordia (NP), have been extensively studied and constitute the subject of several recent reviews (for example, Geurts and Bisseling, 2002). Curiously, while the biology of nitrogen-fixing root nodules has been broadly investigated, we still do not understand what unique evolutionary event predisposed legume plants and *Parasponia* to form nodular symbiosis with rhizobia. In an attempt to address this question, we analyze here three specific examples demonstrating that this symbiosis may have recruited existing plant regulatory programs during its evolution.

The fact that there are homologs of ENOD genes in non-legumes suggests that development of nodular symbiosis involved the harnessing of genetic functions from existing developmental pathways. Albrecht et al. (1999) discussed genetic and corroborating molecular observations that support the notion that legume-*Rhizobium* symbiosis may have evolved, in part, from a pre-existing pathway(s) that regulates

the more widespread (pertaining to more than 80% of extant plant species) and ancient phosphate-acquiring symbiosis of plant roots with fungi, termed arbuscular mycorrhiza (AM). In the first section of this *Update*, we discuss recently characterized genes that may represent "footprints" of such a pathway(s). That a number of nodulin genes are also expressed in non-symbiotic plant tissues suggests that genes involved in non-symbiotic pathways may also have been recruited during the evolution of nodular symbiosis. We highlight this point in the second section of the *Update* by discussing similarities between early rhizobial infections and events occurring during flower pollination. The final section addresses early nodulation events on a broader, organismal, level. Here, we consider a long-range signaling mechanism, termed autoregulation of nodulation, and its relationship with other regulatory pathways that coordinate plant growth and development. While these three examples refer to a broad spectrum of early developmental events during legume-*Rhizobium* interactions, they emphasize that the evolution of nodular symbiosis probably involved the recruitment of several different plant developmental programs.

FUNGAL FOOTPRINTS IN THE NODULATION PROGRAM

One of the most exciting discoveries in the area of symbiotic plant-microbe interactions has been the identification of common genetic determinants underlying AM and *Rhizobium* symbioses (Duc et al., 1989). To what extent this observation reflects commonality of the mechanisms used by legumes to establish both types of symbioses remains to be clarified. Nevertheless, the initial molecular and genetic observations support a hypothesis that the mechanism underlying the compatible response of legume roots to rhizobial infections may have evolved in part from a pre-existing pathway that regulates the more ancient AM symbiosis (Albrecht et al., 1999).

The orthologous genes *LjSYMRK* and *NORK*, recently characterized from *Lotus japonicus* (Stracke et al., 2002) and alfalfa (*Medicago sativa*) (Endre et al., 2002), respectively, may represent footprints of such pathway(s). These genes were identified in genetic screens for plant mutants resistant to both rhizobial (Nod⁻ or non-nodulation phenotype) and AM (Myc⁻ or non-mycorrhizal phenotype) infections and were subsequently cloned and used to characterize the

orthologous *sym19* and *dmi2* loci from pea (*Pisum sativum*) and barrel medic (*Medicago truncatula*), respectively. *SYMRK/NORK* are predicted to encode receptor kinases that are highly similar with respect to their structure and predicted function. The extracellular receptor ectodomains of SYMRK/NORK contain three Leu-rich repeats (LRR) presumed to mediate protein-protein interactions, but the nature of a protein ligand(s) is presently unknown. An apparent strict requirement for SYMRK/NORK function in AM symbiosis indicates their probable widespread occurrence in the plant kingdom. In agreement with this prediction, a survey of available protein sequences and expressed sequenced tags (ESTs) revealed the presence of proteins homologous to the ectodomain of NORK in a variety of dicotyledonous and monocotyledonous plants, and also in Gymnosperms (Endre et al., 2002). A recent analysis of EST records for land plants suggests that sequences homologous to the plant RLK/Pelle family of receptor kinases, to which SYMRK/NORK belong, existed before diversification of land plant lineages (Shiu and Bleecker, 2001). In this context, and considering the ancient origin of AM symbiosis, it is possible that SYMRK/NORK reflects an extant form of an ancestral receptor(s). Such hypothetical receptor(s) could have functioned in an ancient pathway that facilitated symbioses of the earliest plants with fungi during their expansion to a terrestrial environment (Pirozynski and Malloch, 1975). With the knowledge of SYMRK/NORK sequences, a search for such an ancestral receptor and its ligand can now be initiated.

Sequencing of the entire Arabidopsis genome has revealed 417 receptor-like kinase (RLK) sequences, with over half of them containing LRR-type ectodomains. Plant RLKs have been implicated in diverse developmental processes, including meristem development mediated by CLV1, self-incompatibility via SRKs, perception of flagelline by FLS2, and race-specific resistance by Xa21, to name a few (Becraft, 2002 and refs. therein). The SYMRK/NORK RLKs are predicted to function in the Nod factor-dependent transduction pathway and in the signaling processes that underlie AM symbiosis. SYMRK/NORK function is required early on during symbiotic interaction for root hair curling, and *nork* mutants were found to be incapable of performing calcium spiking, one of the earliest root hair responses to Nod factors and rhizobial infection. In wild-type plants, root hair curling sets the stage for initiation of intracellular infection, which is blocked in *symrk/nork* mutants. The Myc⁻ phenotype of *symrk/nork* mutants also involves an early block at or inside the root epidermis that prevents infection and colonization of roots by AM fungi (Endre et al., 2002; Stracke et al., 2002), and a common requirement for calcium spiking in both mycorrhizal and nodulation signaling has been postulated (Walker et al., 2000).

In contrast to legume-*Rhizobium* symbiosis, signaling events during AM symbiosis are poorly understood and the nature of a hypothetical AM factor(s) that could activate SYMRK/NORK-dependent signaling is unknown. By analogy to Nod factors, chitin-like molecules derived from the fungal cell wall represent good candidates for such factors. However, whether SYMRK/NORK receptor kinase represents a component of a perception apparatus or works downstream from a symbiont-specific perception mechanism remains an open question. Considering that pharmacological studies implicate heterotrimeric G-proteins and phosphoinositide (PI)-mediated signaling as downstream elements of the Nod factor transduction pathway (Pingret et al., 1998), it will be interesting to see if SYMRK/NORK work as G-protein-coupled receptors.

In addition to SYMRK/NORK, several other elements of the Nod factor transduction pathway required for both symbioses have been identified. In pea, for example, at least three genetically defined symbiotic loci, Sym8, Sym9 and Sym30, have been implicated in early stages of both symbioses, and this observation has now been extended to other legume species, including two model legumes, *L. japonicus* and *M. truncatula* (Marsh and Schultze, 2001). The observation that Nod factors can activate transcription of the *M. truncatula* early nodulin gene *MtENOD12* in transgenic rice (*Oryza sativa*) suggests that at least part of the perception/transduction mechanism may operate in non-legume plants, and that the genetically defined Nod⁻ Myc⁻ loci may underlie the elements of the mechanism that are common for legume and non-legume plants (Reddy et al., 1998). This conclusion is further supported by the ability of *Rhizobium* to nodulate a non-legume plant, *Parasponia andersonii*, in a process that requires Nod factor-dependent signaling (Scott and Bender, 1990).

Characterization of the remaining loci required for both symbioses will undoubtedly unveil the signaling mechanisms involved and define to what extent the species-specific nodular symbiosis has "learned" from the evolutionarily much older AM symbiosis of plant and fungi. New molecular data indicate the presence of common plant genetic elements underlying parasitic nematodes and rhizobial endosymbioses (Koltai et al., 2001). Considering the postulated involvement of Nod⁻ Myc⁻ loci in the regulation of plant defense responses (Gianinazzi-Pearson, 1996), it will be interesting to see whether these loci also have specific roles during plant response to parasitic endosymbionts. Parallel insight into symbiont-specific plant functions may provide the long-awaited answer to the question regarding the unique evolutionary event(s) that predisposed legume plants and *Parasponia* to form nitrogen-fixing nodular symbiosis with rhizobia. Transposon-tag-based cloning of the *L. japonicus nin* gene (Schauser et al., 1999) and its ortholog from pea (corresponding to *sym35* allele;

Borisov et al., 2003) offers the first defined molecular example of such a symbiont (*Rhizobium*)-specific plant function.

NODULES: MADS ABOUT FLOWERS

A number of nodulin genes are also expressed in non-symbiotic plant tissues. Have these genes been recruited from pre-existing pathways to operate in an analogous fashion in nodules? While this question remains largely unanswered, we may gain important insight into the evolution and symbiotic role of such genes by examining their involvement in non-symbiotic plant processes. For example, several nodulin genes have elevated expression levels in floral tissues or are homologous to floral-specific genes. Do these genes have similar roles in nodules and flowers? A growing body of evidence appears to indicate that plant plasma membrane-derived infection structures called infection threads (ITs), which contain the rhizobial symbiont, elongate toward nodule primordia (NP) using a mechanism resembling polar tip growth of pollen tubes elongating toward ovules.

Initiation of ITs requires strict compatibility between Nod factors and host-specific root hair receptor(s) (Geurts and Bisseling, 2002). As discussed in the previous section, RLKs with extracellular LRRs have been implicated in successful Nod factor signaling in root hairs (SYMRK/NORK). Analogously, pollen tubes are initiated upon successful signaling between the stigma and mature pollen grains, and pollen-expressed RLKs with extracellular LRRs have been implicated in compatible pollinations (Becraft, 2002 and refs. therein). Once initiated, polar tip growth of ITs and pollen tubes is directionally guided toward NP and ovules, respectively, and in the case of ITs, a specific alignment of cytoplasmic strands (phragmosomes) in activated cortical cells has been postulated to predetermine the orientation and position of IT development (Brewin, 1991).

Interestingly, a number of observations indicate that the tip growth mechanism in pollen tubes and ITs may involve similar key regulators. Small G-proteins have emerged as "master signaling switches" of polar tip growth in pollen (Yang, 2002 and refs. therein). Given that the small G-protein Rab2 is highly expressed in mature pollen in tobacco (*Nicotiana tabacum*) and Arabidopsis, it is tempting to speculate that elevated levels of *Rab2* transcripts in *L. japonicus* nodules (Borg et al., 1997) are associated with polar tip growth of ITs, especially considering that dominant negative mutations in the tobacco *NtRab2* gene inhibit pollen tube elongation (Cheung et al., 2002). Since a major component of G-protein-mediated polar tip growth of pollen tubes is PI signaling, it is also intriguing that transcripts of the *L. japonicus* gene *LjPLPIV*, which encodes a phosphatidylinositol transfer protein, were detected in flowers and infected cells of nodules (Ka-

pranov et al., 2001). Importantly, recent analysis of *LjPLPIV* promoter activity has demonstrated that its floral expression is pollen specific.

MADS-box genes have also been implicated in the pollen tube tip growth mechanism. These genes, known as "master regulators" of flower development, are now emerging as important regulators of other plant processes, including nodular symbiosis (Zucchero et al., 2001). Interestingly, the nodule-specific alfalfa gene *nmhC5* belongs to the same MADS-box clade as the "late" pollen-expressed genes *DEFH125* and *ZmMADS2* from *Antirrhinum majus* and maize (*Zea mays*), respectively. While the role(s) of *nmhC5* has yet to be established, genes belonging to the same MADS-box clade are considered to be related functionally (Theissen et al., 2000); therefore, comparison with its non-symbiotic clade members should provide clues to *nmhC5* function(s) during nodule development. The DEFH125 protein localizes to mature pollen and also to transmitting tract cells in the style after pollination, indicating a role in pollen tube elongation and guidance (Zachgo et al., 1997). A specific role for *ZmMADS2* in pollen tube elongation has been implicated by in situ RNA hybridization, which demonstrated that *ZmMADS2* transcripts are translocated into pollen tubes in a tip-focused gradient (Heuer et al., 2000). Given the predicted participation of *DEFH125* and *ZmMADS2* in pollen tube tip growth and that *nmhC5* expression begins in the nodule invasion zone (Heard et al., 1997), it is tempting to speculate that *nmhC5* is involved in regulating tip growth of ITs. Interestingly, the other identified symbiotically expressed alfalfa MADS-box genes, *nmh7* and *ngl9*, are only expressed in flowers and infected cells of nodules; however, their function remains unknown (Zucchero et al., 2001). It is important to point out that nodules have probably recruited several non-symbiotic plant mechanisms and that polar tip growth represents just one example of a developmental process that may have been recruited during the evolution of nodular symbiosis from a pre-existing plant pathway.

SHOOTS "COUNT" ON NODULES

In response to rhizobia and Nod factor signaling the cell cycle in the susceptible zone of the root pericycle and cortex of the host plant is reactivated giving rise to cell division clusters termed NP. A subset of NP cells is targeted by ITs, a process which leads to colonization of plant cells by symbiotic bacteria and formation of the nitrogen-fixing cells of the nodules. The host plant regulates the number of successful infection events and differentiation of NP via a locally operating mechanism(s) involving the plant hormone ethylene and a systemically operating mechanism(s) that involves a mobile signal(s) of an as yet unknown nature (Penmetsa et al., 2003). Differentiation of NP has been linked to a systemically

operating root-shoot regulatory mechanism known as autoregulation of nodulation (AON). AON constitutes a long-distance signaling process where early cellular events associated with formation of NP are communicated and transformed in the shoot into a regulatory feedback response. This shoot-derived response usually restricts proliferation of new nodule tissues, thus maintaining homeostasis of the symbiotic interaction (Gresshoff, 1993 and refs. therein). Although poorly understood on the molecular level, the available data seem to suggest that AON may have evolved from pre-existing growth-related signaling mechanisms possibly integrating root development with events in the plant shoot.

Plant mutants defective in AON form an excessive number of nodules (Nod^{++} or hypernodulation), a phenotype often associated with additional (pleiotropic) alterations in shoot and/or root growth. In *L. japonicus*, for example, homozygous mutations in the Har1 locus block AON, which results in hypernodulation. *har1* mutants also have abnormal root development when grown in the absence of symbiotic bacteria (Wopereis et al., 2000; Kawaguchi et al., 2002). Data from grafting experiments indicate that the hypernodulation and altered root phenotypes of *har1* mutants are shoot-genotype dependent (Krusell et al., 2002), suggesting a common role for *Har1* in long-distance shoot regulation of root development and autoregulation of nodule organogenesis. The existence of overlapping regulatory functions for nodulation (AON) and root development is also suggested by the inferior root growth phenotypes observed for several independent hypernodulating mutants of different legume species, including the *nod3* mutant in pea. Unlike all other AON-defective mutants characterized thus far, the hypernodulation and altered root characteristics of the *nod3* mutant are not under shoot genotype control, but are dictated by the root genotype of the mutant plant (Postma et al., 1988). Nevertheless, both *har1* and *nod3* mutants share similar features of modified root architecture such as diminished root length and increased lateral root formation (Postma et al., 1988; Wopereis et al., 2000).

An overlap between developmental processes associated with lateral root formation and nodulation is supported by the recent work of Mathesius et al. (2000), who show that mature cortical cells of white clover that are activated during lateral root development, recapitulate some of the early responses associated with nodule formation and can be "hijacked" by rhizobia to form the so-called lateral root associated nodules. Interestingly, a homozygous mutation in the *L. japonicus LjBzf* gene (corresponding to Ljsym77 locus; Kawaguchi et al., 2002), which encodes a homolog of the Arabidopsis bZIP transcriptional factor Hy5, enhances nodulation and exerts a pleiotropic effect on root, shoot, and hypocotyl growth. *Hy5* has a role in photomorphogenesis as

well as in the regulation of lateral root development in Arabidopsis (Hardtke et al., 2000), whereas *LjBzf* regulates nodule organogenesis in *L. japonicus* (Nishimura et al., 2002a). Mathesius et al. (2000) hypothesize that the morphogenic process of nodule formation evolved from a developmental pathway activated during lateral root organogenesis. This hypothesis can be extended by suggesting that AON evolved from regulatory mechanisms that control root growth and differentiation of lateral root primordia.

Regulation of proliferation and growth of lateral roots constitutes an important part of the mechanism used by plants to accommodate changes in the surrounding soil environment. Local and long-range shoot-root regulatory pathways were shown to be involved in this regulation, and auxin and NO_3^- represent two major internal and external factors, respectively, with important signaling roles in root branching (Forde, 2002). NO_3^- also represents a major environmental factor that regulates nodulation. It is thus significant that almost all hypernodulating mutants described thus far, including *L. japonicus har1* and pea *nod3*, have nodulation phenotypes that are at least partially insensitive to normally inhibitory concentrations of nitrate fertilizer (Nts or nitrate-tolerant nodulation phenotype). The reverse is also true, i.e. plant mutants selected for their Nts phenotype (e.g. nts mutants of soybean [*Glycine max*], Carroll et al., 1985) were found to be defective in AON. The existence of an overlap between auxin and NO_3^- signaling pathways in the mechanism that regulates lateral root growth has been suggested (Forde, 2002), and early events of both lateral root formation and nodulation have recently been shown to have common requirements for the plant hormone auxin (de Billy et al., 2001). Clearly, the AON and NO_3^- signaling pathways must also intersect while mediating the regulation of nodule proliferation. This intersection is predicted to involve both root and shoot localized functions, and recent cloning experiments provide, for the first time, an insight into the molecular nature of the shoot components involved.

L. japonicus Har1 (Krusell et al., 2002; Nishimura et al., 2002b) and *GmNARK* from soybean (corresponding to soybean Nts locus and identical to *GmCLV1B* cDNA; Searle et al., 2002; Nishimura et al., 2002b) were map-based cloned and characterized. The *Har1* gene sequence was subsequently used to identify its presumed ortholog, *PsSym29*, from pea (Krusell et al., 2002). All three genes encode highly similar LRR-type RLKs, and we refer to them hereafter as nodule autoregulation receptor kinases (NARKs), following nomenclature proposed for the soybean NTS locus (*GmNARK*, Searle et al., 2002). Of the receptor kinases in Arabidopsis, NARKs are most similar to the CLAVATA 1 protein (CLV1; Clark et al., 1997). However, while CLV1 Ser/Thr receptor kinase restricts proliferation of stem cells through a short-distance feedback loop in Arabidopsis shoot apices, NARKs

mediate a long-range shoot-to-root AON mechanism. The recessive *clv1* mutants are characterized by an enlarged stem cell population resulting in fasciation of the shoot. Interestingly, this phenotype was not reported for *har1*, *sym29*, and *nts* mutants but was described in association with the hypernodulating phenotype of pea mutant *sym28* (Sagan and Duc, 1996). Thus, the Sym28 locus may represent a function common to AON and the mechanism(s) that specifies differentiation and/or proliferation of shoot apical meristems.

The function of NARKs in AON and other signaling pathways can now be addressed. The major challenge will be to determine how NARKs receive and convert the spectrum of external and internal cues from the roots into a shoot-derived response that controls early stages of nodule development. The other issue will be to understand the extent of cross talk between different growth-related signaling events and AON and how they converge to establish harmonious plant development, including symbiotic development. Interestingly, NARKs may also regulate plant-fungi interactions since roots of *L. japonicus* and pea plants carrying homozygous mutations in Har1 and Sym29 loci, respectively, are colonized more intensively by AM fungi (Myc^{++} or hypermycorrhization phenotype) than their wild-type counterparts (Morandi et al., 2000; Solaiman et al., 2000). Thus, NARKs may be another link between the two symbiotic interactions.

CONCLUDING REMARKS

While discussing the origin of land plants, Pirozynski and Malloch (1975) concluded, "land plants never had an independence (from fungi); for if they had, they could never have colonized the land". The fossil records support this hypothesis (Redecker et al., 2000), suggesting that plants have resolved the intricacies of the mechanism(s) for selective recognition of beneficial versus harmful microorganisms long before nodular symbiosis evolved. Taking advantage of at least some of the elements of this pre-existing mechanism(s) was likely one of the events leading to establishment of legume-*Rhizobium* symbiosis. It appears though that nodules "have recruited" a number of other key plant regulatory programs and/or elements of these programs for their own development. As more pieces are added to the nodulation puzzle, a common picture with non-symbiotic aspects of plant development emerges. Thus, the ability to form nodular symbiosis (nodulation) may be not that different from non-nodulation after all; yet there must be something "special" to allow for nodular symbiosis of legumes with nitrogen-fixing rhizobia to occur (see Hirsch et al., 2001). Uncovering this special feature(s) of legumes remains an exciting and challenging task, and new genomic tools, especially those being developed in association with model le-

gumes, should greatly facilitate this effort (Stacey and VandenBosch, 2003). Recently initiated genomic projects aimed at obtaining the sequences of the entire genome of two model legumes, *L. japonicus* and *M. truncatula*, will provide the foundation for performing genome-wide comparative analyses. Information regarding the progress of the model legume genome sequencing projects can be found at the following URL locations: http://www.kazusa.or.jp/lotus and http://www.genome.ou.edu/medicago_table.html. The prospect of being able to make genome-wide comparisons between legumes and non-legumes, such as Arabidopsis, is especially attractive since the latter is unable to form symbiotic associations with mycorrhizal fungi or with rhizobia.

LITERATURE CITED

Albrecht C, Geurts R, Bisseling T (1999) Legume nodulation and mycorrhizae formation; two extremes in host specificity meet. EMBO J **18**: 281–288

Becraft PW (2002) Receptor kinase signalling in plant development. Annu Rev Cell Dev Biol **18**: 163–192

Borg S, Grandstrup B, Jensen TJ, Poulsen C (1997) Identification of new protein species among 33 different small GTP-binding proteins encoded by cDNAs from *Lotus japonicus*, and expression of corresponding mRNAs in developing root nodules. Plant J **11**: 237–250

Borisov AY, Madsen LH, Tsyganov VE, Umehara Y, Voroshilova VA, Batagov AO, Sandal N, Frederiksen A, Schauser L, Ellis N et al. (2003) The *Sym35* gene for root nodule development in *Pisum sativum* is an orthologue of *Nin* from *Lotus japonicus*. Plant Physiol **131**: 1009–1017

Brewin NJ (1991) Development of the legume root nodule. Annu Rev Cell Biol **7**: 191–226

Carroll BJ, McNeil DL, Gresshoff PM (1985) 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

Cheung AY, Chen CY, Glaven RH, de Graaf BH, Vidali L, Hepler PK, Wu HM (2002) Rab2 GTPase regulates vesicle trafficking between the endoplasmic reticulum and the Golgi bodies and is important to pollen tube growth. Plant Cell **14**: 945–962

Clark SE, Williams RW, Meyerowitz EM (1997) The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. Cell **89**: 575–585

de Billy F, Grosjean C, May S, Bennett M, Cullimore JV (2001) Expression studies on *Aux1*-like genes in *Medicago truncatula* suggest that auxin is required at two steps in early nodule development. Mol Plant Microbe Interact **14**: 267–277

Duc G, Trouvelot A, Gianinazzi-Pearson V, Gianinazzi S (1989) First report of non-mycorrhizal plant mutants (Myc$^-$) obtained in pea (*Pisum sativum* L.) and fababean (*Vicia faba* L.). Plant Sci **60**: 215–222

Endre G, Kereszt A, Kevei Z, Mihacea S, Kalo P, Kiss GB (2002) A receptor kinase gene regulating symbiotic nodule development. Nature **417**: 962–966

Forde BG (2002) Local and long-range signalling pathways regulating plant responses to nitrate. Annu Rev Plant Biol **53**: 203–224

Geurts R, Bisseling T (2002) *Rhizobium* Nod factor perception and signalling. Plant Cell **14**: S239–S249

Gianinazzi-Pearson V (1996) Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. Plant Cell **8:** 1871–1883

Gresshoff PM (1993) Molecular genetic analysis of nodulation genes in soybean. Plant Breed Rev **11:** 275–318

Hardtke CS, Gohda K, Osterlund MT, Oyama T, Okada K, Deng XW (2000) HY5 stability and activity in *Arabidopsis* is regulated by phosphorylation in its COP1 binding domain. EMBO J **19:** 4997–5006

Heard J, Caspi M, Dunn K (1997) Evolutionary diversity of symbiotically induced nodule MADS box genes: characterization of *nmhC5*, a member of a novel subfamily. Mol Plant Microbe Interact **10:** 665–676

Heuer S, Lörz H, Dresselhaus T (2000) The MADS box gene *ZmMADS2* is specifically expressed in maize pollen and during maize pollen tube growth. Sex Plant Reprod **13:** 21–27

Hirsch AM, Lum MR, Downie JA (2001) What makes the Rhizobia-legume symbiosis so special? Plant Physiol **127:** 1484–1492

Kapranov P, Routt SM, Bankaitis VA, de Bruijn FJ, Szczyglowski K (2001) Nodule-specific regulation of phosphatidylinositol transfer protein expression in *Lotus japonicus*. Plant Cell **13:** 1369–1382

Kawaguchi M, Imaizumi-Anraku H, Koiwa H, Niwa S, Ikuta A, Syono K, Akao S (2002) Root, root hair, and symbiotic mutants of the model legume *Lotus japonicus*. Mol Plant Microbe Interact **15:** 17–26

Koltai H, Dhandaydham M, Opperman C, Thomas J, Bird D (2001) Overlapping plant signal transduction pathways induced by a parasitic nematode and a rhizobial endosymbiont. Mol Plant Microbe Interact **14:** 1168–1177

Krusell L, Madsen LH, Sato S, Aubert G, Genua A, Szczyglowski K, Duc G, Kaneko T, Tabata S, de Bruijn F et al. (2002) Shoot control of root development and nodulation is mediated by a receptor-like kinase. Nature **420:** 422–426

Marsh JF, Schultze M (2001) Analysis of arbuscular mycorrhizas using symbiosis-defective plant mutants. New Phytol **150:** 525–532

Mathesius U, Weinman JJ, Rolfe BG, Djordjevic MA (2000) Rhizobia can induce nodules in white clover by "hijacking" mature cortical cells activated during lateral root development. Mol Plant Microbe Interact **13:** 170–182

Morandi D, Sagan M, Prado-Vivant E, Duc G (2000) Influence of genes determining supernodulation on root colonization by the mycorrhizal fungus *Glomus mosseae* in *Pisum sativum* and *Medicago truncatula* mutants. Mycorrhiza **10:** 37–42

Nishimura R, Hayashi M, Wu GJ, Kouchi H, Imaizumi-Anraku H, Murakami Y, Kawasaki S, Akao S, Ohmori M, Nagasawa M et al. (2002b) HAR1 mediates systemic regulation of symbiotic organ development. Nature **420:** 426–429

Nishimura R, Ohmori M, Fujita H, Kawaguchi M (2002a) 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

Penmetsa RV, Frugoli JA, Smith L, Long S, Cook DR (2003) Dual genetic pathways controlling nodule number in *Medicago truncatula*. Plant Physiol **131:** 998–1008

Pingret JL, Journet EP, Barker DG (1998) *Rhizobium* Nod factor signalling: evidence for a G protein-mediated transduction mechanism. Plant Cell **10:** 659–672

Pirozynski KA, Malloch DW (1975) The origin of land plants: a matter of mycotrophism. BioSystems **6:** 153–164

Postma JG, Jacobsen E, Feenstra WJ (1988) Three pea mutants with an altered nodulation studied by genetic analysis and grafting. J Plant Physiol **132:** 424–430

Reddy PM, Ladha JK, Ramos MC, Maillet F, Hernandez RJ, Torrizo B, Olivia NP, Datta SK, Datta K (1998) Rhizobial lipochitooligosaccharide nodulation factors activate expression of the legume early nodulation gene *ENOD12* in rice. Plant J **14:** 693–702

Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science **289:** 1920–1921

Sagan M, Duc G (1996) *Sym28* and *Sym29*, two new genes involved in regulation of nodulation in pea (*Pisum sativum* L.). Symbiosis **20:** 229–245

Schauser L, Roussis A, Stiller J, Stougaard J (1999) A plant regulator controlling development of symbiotic root nodules. Nature **402:** 191–195

Scott KF, Bender GL (1990) The *Parasponia-Bradyrhizobium* symbiosis. *In* Gresshoff PM, ed, The Molecular Biology of Symbiotic Nitrogen Fixation. CRC Press, Boca Raton, FL, pp 231–251

Searle IR, Men AE, Laniya TS, Buzas DM, Iturbe-Ormaetxe I, Gresshoff PM (2003) Long distance signalling in nodulation directed by a CLAVATA1-like receptor kinase. Science **299:** 109–112

Shiu SH, Bleecker AB (2001) Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. Proc Natl Acad Sci USA **98:** 10763–10768

Solaiman MZ, Senoo K, Kawaguchi M, Imaizumi-Anraku H, Akao S, Tanaka A, Obata H (2000) Characterization of mycorrhizas formed by *Glomus* sp. on roots of hypernodulating mutants of *Lotus japonicus*. J Plant Res **113:** 443–448

Spaink HP (2000) Root nodulation and infection factors produced by rhizobial bacteria. Annu Rev Microbiol **54:** 257–288

Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Szczyglowski K, Parniske M (2002) A plant receptor-like kinase required for both bacterial and fungal symbiosis. Nature **417:** 959–962

Theissen G, Becker A, Rosa AD, Kanno A, Kim JT, Münster T, Winter KU, Saedler H (2000) A short history of MADS-box genes in plants. Plant Mol Biol **42:** 115–149

Stacey G, VandenBosch K (2003) Summaries of legume genomics projects from around the globe: community resources for crops and models. Plant Physiol **131:** 840–865

Walker SA, Viprey V, Downie JA (2000) Dissection of nodulation signaling using pea mutants defective for calcium spiking induced by Nod factors and chitin oligomers. Proc Natl Acad Sci USA **97:** 13413–13418

Wopereis J, Pajuelo E, Dazzo FB, Jiang QY, Gresshoff PM, de Bruijn FJ, Stougaard J, Szczyglowski K (2000) Short root mutant of *Lotus japonicus* with a dramatically altered symbiotic phenotype. Plant J **23:** 97–114

Yang Z (2002) Small GTPases: versatile signalling switches in plants. Plant Cell **14:** S375–S388

Zachgo S, Saedler H, Schwarz-Sommer Z (1997) Pollen-specific expression of *DEFH125*, a MADS-box transcription factor in *Antirrhinum* with unusual features. Plant J **11:** 1043–1050

Zucchero JC, Caspi M, Dunn K (2001) *ngl9*: a third MADS box gene expressed in alfalfa root nodules. Mol Plant Microbe Interact **14:** 1463–1467