Grafting between model legumes demonstrates roles for roots and shoots in determining nodule type and host/rhizobia specificity

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Abstract

Previous grafting experiments have demonstrated that legume shoots play a critical role in symbiotic development of nitrogen-fixing root nodules by regulating nodule number. Here, reciprocal grafting experiments between the model legumes *Lotus japonicus* and *Medicago truncatula* were carried out to investigate the role of the shoot in the host-specificity of legume–rhizobia symbiosis and nodule type. *Lotus japonicus* is nodulated by *Mesorhizobium loti* and makes determinate nodules, whereas *M. truncatula* is nodulated by *Sinorhizobium meliloti* and makes indeterminate nodules. When inoculated with *M. loti*, *L. japonicus* roots grafted on *M. truncatula* shoots produced determinate nodules identical in appearance to those produced on *L. japonicus* self-grafted roots. Moreover, the hypernodulation phenotype of *L. japonicus* hart-1 roots grafted on wild-type *M. truncatula* shoots was restored to wild type when nodulated with *M. loti*. Thus, *L. japonicus* shoots appeared to be interchangeable with *M. truncatula* shoots in the *L. japonicus* root/*M. loti* symbiosis. However, *M. truncatula* roots grafted on *L. japonicus* shoots failed to induce nodules after inoculation with *S. meliloti* or a mixture of *S. meliloti* and *M. loti*. Instead, only early responses to *S. meliloti* such as root hair tip swelling and deformation, plus induction of the early nodulation reporter gene *MtENOD11:GUS* were observed. The results indicate that the *L. japonicus* shoot does not support normal symbiosis between the *M. truncatula* root and its microsymbiont *S. meliloti*, suggesting that an unidentified shoot-derived factor may be required for symbiotic progression in indeterminate nodules.

Introduction

Legumes develop symbiotic associations with gram-negative bacteria of the family Rhizobiaceae (collectively called rhizobia). The symbiosis involves the formation of a novel plant organ called a nodule wherein differentiated rhizobia fix atmospheric dinitrogen, benefiting the plant. Most well-characterized legume–rhizobia symbioses have a stringent host-specificity in that only closely related legumes can be nodulated by a particular rhizobial strain (Young et al., 2003). Plant and rhizobial genes involved in host-specificity have been identified. The earliest recognition events involve the bacterial response to flavonoids produced by the legume roots, resulting in the activation of rhizobial *NOD* genes. The *NOD* gene products participate in the synthesis of nod factors, lipo-oligosaccharide signalling molecules that trigger many of the host’s early responses (Limpens and Bisseling, 2003). LysM-like receptor kinases are likely to be involved in the recognition of bacterial *Nod* factors by the host plant (Limpens et al., 2003; Radutoiu et al., 2003). Calcium influx and spiking in root hair cells, possibly mediated by the *DMI1* and *DMI3* gene products in *Medicago truncatula*, have been identified as requirements for the transduction of further downstream signals that lead to the formation of nodules and symbiotic nitrogen fixation (Riely et al., 2004). In spite of the discovery of many plant genes involved at the early interaction in legume–rhizobia symbiosis, details of host specificity at later steps of symbiosis are still poorly understood.

Key words: Host specificity, *Lotus, Medicago*, Nod factor, nodule type, root, shoot.
The shoot plays an important role in legume-rhizobia symbiosis, not only as the source of photosynthetic carbon to support the nodules, but also as a source of signals to the root. In grafting and split-root experiments, signals from the shoot have been implicated in the systemic regulation of nodule numbers (Kossak and Bohlool, 1984; Delves et al., 1986). HARI in Lotus japonicus and its equivalent NARK in Glycine max L. (soybean) encode leucine-rich repeat (LRR) receptor kinases (Krussel et al., 2002; Searle et al., 2003). These genes have been shown to regulate a shoot signal essential for controlling root nodule numbers, although the molecular nature of the signal transported to the roots is still unknown. Also unknown is whether shoots regulate legume-rhizobia host specificity or other aspects of nodule development.

Legume nodules have been classified as determinate or indeterminate depending on the presence of a transient or a persistent meristem, respectively (Hirsch, 1992). Examples of determinate nodule-forming legumes include soybean and close relatives in the Phaseoleae, as well as Lotus spp., all of which produce globular nodules. By contrast, indeterminate nodules of determinate nodule-forming legumes include soybean and close relatives in the Phaseoleae, as well as Lotus spp., all of which produce globular nodules. By contrast, indeterminate nodules of determinate nodule-forming legumes include Pisum (peas), Lens (lentils), Medicago (medics, alfalfa), and Trifolium (clovers). Indeterminate nodules have an apical meristem, resulting in a cylindrical nodule shape. Currently, it is not known whether the shoot has any role in determining nodule type.

Just as some shoot signals control numbers of nodules on the root, it is possible that there are shoot signals controlling host-specificity, nodule type, and other aspects of legume-rhizobia symbiosis. A grafting experiment involving shoots and roots of legume plants differing in host-specificity and nodule type can help understand the roles of the shoot in these processes. Here results from grafting experiments involving the model legumes L. japonicus (referred to as Lj hereafter) and M. truncatula (referred to as Mt hereafter) are described. Lj is nodulated by its microsymbiont Mesorhizobium loti and makes determinate nodules (Szczyslawski et al., 1998). By contrast, Mt is nodulated by Sinorhizobium meliloti and makes indeterminate nodules (Cook, 1999). Here it is shown that wild-type Mt shoots can support determinate nodule formation on Lj roots in association with M. loti. Moreover, the Mt shoot can rectify the hypernodulation phenotype of the har1-1 (hypernodulation and aberrant root) Lj mutant root. However, the Lj shoot was not observed to support nodule formation on Mt roots in association with S. meliloti or a mixture of S. meliloti and M. loti. These results support a role for the root in determining the determinate nature of nodule development, although factors governing indeterminate nodule formation are still unknown.

Materials and methods

Plant materials and growth conditions

Lj ecotype Gifu B129, the Lj supernodulation mutant har1-1 (Wopereis et al., 2000), Mt ecotype A17, and an Mt transgenic line expressing MtENOD11:gusA fusion (Chabaud et al., 2002) were used for experiments described herein. Seed was treated with concentrated sulphuric acid for 5–7 min with gentle agitation, and washed five times with sterile water under a sterile hood. For Lj, seed was germinated in sterile Petri dishes with wet filter papers at room temperature in the dark for 2 d. For Mt, after washing, the seed was vernalized at 4 °C for about 24 h with five or six changes of chilled water. The seed was then transferred to sterile Petri dishes and germinated upside down in the dark at room temperature for about 24 h.

Grafting

Germianted seeds were transferred to 1.2% agar slopes with 1/2 Gamborg's B5 salts (Gamborg, 1970), and the plates were incubated vertically at 22 °C with a 16 h day and 8 h night cycle for 7 d. Shoots were severed at the hypocotyl and kept in 1/2× B5 salts with 1/2× B5 vitamins before use in grafting. For intergeneric grafting, stocks and scions (referred to as roots and shoots, respectively, hereafter) of Mt were prepared by making a vertical incision with a sharp scalpel in the hypocotyls. By contrast, Lj shoots and roots were prepared by a slant cut of the hypocotyls. Since Mt roots and shoots were thicker than Lj roots and shoots of the same age, the vertical incision was always made in Mt roots and shoots in intergeneric grafting. For self-grafting, the root was given a vertical incision, whereas the shoot was given a slant cut. The shoot was inserted into the root and secured in place using a thin strip of autoclave tape. The plates were incubated vertically and a successful graft was identified by the presence of fresh root growth in about 5 weeks. The plants were then transferred to a mixture of vermiculite and perlite (3:1) and grown in black plastic pots enclosed in Saran Wrap to maintain humidity. The Saran Wrap was removed gradually over 1 week.

Nodulation

Plants were inoculated with an appropriate rhizobial strain(s) at the time of transferring the successful grafted plants (5 weeks after grafting) to a vermiculite and perlite (3:1) mixture. Plants with an Lj root were inoculated with M. loti carrying a constitutive lacZ gene (Schauer et al., 1998), and those with an Mt root were inoculated with S. meliloti constitutively expressing a lacZ gene (Penmetsa and Cook, 1997). Mt roots grafted on Lj shoots did not nodulate with S. meliloti, and therefore some of these plants were also inoculated with a mixture of S. meliloti and M. loti. Nodulation was observed and counted 3 weeks after inoculation in 8–13 grafted plants. Means and 95% confidence intervals of the mean were calculated for nodule number per plant for each grafting type.

Microscopy

Rhizobia in the root were detected using X-Gal (5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside) staining as described (Bovin et al., 1990) with a modification as follows. Three-week-old nodules from grafted plants were fixed in 0.1 M HEPES (N-(2-hydroxyethyl)piperazine-N'-2-ethane sulphonic acid), pH 7.5, with 0.25 M glutaraldehyde, under three cycles of vacuum and vent with 30 s each, and for 1 h further at atmospheric pressure. The nodules were washed three times for 10 min each with 0.1 M HEPES. Hand sections of the fixed nodules were stained in X-Gal overnight at 37 °C, washed twice for 10 min each in 0.1 M HEPES with 0.25 M glutaraldehyde, pH 7.5, and mounted on glass slides in 50% glycerol. Mt roots with MtENOD11:gusA were stained with GUS assay buffer as described by Jefferson (1987) 1 d after inoculation with S. meliloti. Stained roots were mounted on glass slides in 50% glycerol. Sections were observed under a Nikon microscope (DIAPHOT 200), and photographed using a Nikon E4500 digital camera. Sections of 10 nodules from different plants of each grafting type were observed.
Results

Success of intergeneric grafting between Lj and Mt

A successful grafting event was identified by new root growth 3–5 weeks after grafting (Fig. 1A). In successful grafts, a strong graft joint was observed at 6 weeks after grafting (Fig. 1B) along with new shoot growth (Fig. 1C, D). A success rate of over 30% was obtained in Lj self-grafting (46 out of 150 attempted). A success rate of about 10% was observed for intergeneric grafting of Mt shoots to Lj roots (13 out of 120 attempted). A low success rate was obtained when grafting Lj shoots to Mt roots (<5%, 6 out of 130), and in Mt self-grafting (<8%, 13 out of 170 tried). Mt roots were found to be more difficult to handle than Lj roots since the former could be easily damaged due to the soft nature of the tissues.

In successful grafts, seedlings grew well up to 7 weeks after grafting. In intergeneric grafts, however, shoots later developed symptoms of stress. Lj shoots grafted on Mt roots developed chlorotic leaves about 7 weeks after grafting (Fig. 2A, B) in all grafted plants. The shoot tip remained stunted with a sickly appearance. The lower leaves rolled inward, and developed purple pigmentation. The upper leaves made a narrow angle with the stem compared with Lj self-grafted shoots (Fig. 2A). Mt roots with Lj shoots started degrading 8 weeks after grafting (Fig. 2C). Shoot appearance of all Mt shoots grafted on Lj roots differed from Mt shoots grafted on their own roots 8 weeks after grafting (Fig. 2D, E). The leaf lamina was reduced in Mt shoots grafted on Lj roots and had interveinal chlorosis resembling micronutrient deficiency symptoms (Fig. 2F, G). However, the root growth was normal, similar to that of Lj grafted on its own root (Fig. 1C). For these reasons, analysis of a possible role in symbiotic development was assayed at 8 weeks after grafting.

Lj roots grafted on Mt shoots form determinate nodules after inoculation with M. loti

Lj roots grafted on Mt shoots were able to be nodulated by M. loti NZP 2235 carrying a constitutive lacZ gene, similar to Lj self-grafted roots (Fig. 3A, B). A total of 25 plants remained stunted with a sickly appearance. The lower leaves rolled inward, and developed purple pigmentation. The upper leaves made a narrow angle with the stem compared with Lj self-grafted shoots (Fig. 2A). Mt roots with Lj shoots started degrading 8 weeks after grafting (Fig. 2C). Shoot appearance of all Mt shoots grafted on Lj roots differed from Mt shoots grafted on their own roots 8 weeks after grafting (Fig. 2D, E). The leaf lamina was reduced in Mt shoots grafted on Lj roots and had interveinal chlorosis resembling micronutrient deficiency symptoms (Fig. 2F, G). However, the root growth was normal, similar to that of Lj grafted on its own root (Fig. 1C). For these reasons, analysis of a possible role in symbiotic development was assayed at 8 weeks after grafting.

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Fig. 1. Reciprocal grafting between Lj and Mt. (A) A successful graft between an Mt shoot and an Lj root 5 weeks after grafting. New root growth indicates the success of grafting. Arrows mark the point where new growth starts. (B) A successful graft union (arrow) between an Mt shoot and an Lj root 6 weeks after grafting. (C, D) Successfully grafted plants between Mt shoot and Lj root (C), and Lj shoot and Mt root (D) 6 weeks after grafting.

Fig. 2. Shoot and root phenotypes of self-grafted plants and plants with intergeneric grafts 8 weeks after grafting. (A) An Lj shoot with an Lj root. (B) An Lj shoot with an Mt root. (C) A part of an Mt root with an Lj shoot. Arrows indicate the sites of root disintegration. (D) An Mt shoot with an Mt root. (E) An Mt shoot with an Lj root. (F) A trifoliate leaf from (D). (G) A trifoliate leaf from (E).
with Mt shoots and Lj roots (both wild type and har1-1 combined) were inoculated, and all were observed to be nodulated. Nodules formed on Lj roots with Mt shoots were spherical in shape, and so were judged to be of determinate infection by rhizobia, longitudinal nodule sections were observed in either case (Fig. 4A). By contrast, S. meliloti always induced indeterminate nodules on Mt roots grafted on Mt shoots (Fig. 4B). Root hair curling was observed on Mt roots grafted under Mt shoots after inoculation with S. meliloti which was absent in uninoculated roots (Fig. 4C, D). However, root hair deformation and swelling were observed only occasionally on Mt roots grafted on Lj shoots (Fig. 4E). A strain of S. meliloti carrying a constitutive lacZ reporter gene was used to observe infection thread formation and root hair deformation microscopically. No infection thread or nodule primordium was found in the six Lj/Mt roots examined, while in Mt/Mt roots healthy nodules, numerous infection threads, and nodule primordia were observed 3 weeks after inoculation (data not shown).

MtENOD11 is an early nodulin gene, encoding a putative extracellular matrix protein that is transcribed during early symbiotic events. Mt transgenic plants with an MtENOD11:GUS construct have been used previously to detect responses to arbuscular mycorrhizal fungi, rhizobia, or isolated Nod factors (Journet et al., 2001; Charron et al., 2004). To aid the evaluation of early symbiotic responses in the intergeneric grafts that failed to form nodules, Mt transgenic plants with an MtENOD11:GUS construct as root and Lj as shoot were used. In uninoculated plants, MtENOD11:GUS expression was restricted to the root tip, as observed by blue staining (Fig. 4F). Following inoculation with S. meliloti, MtENOD11:GUS was strongly induced in all six MtENOD11:GUS roots grafted on Lj shoots in root tips, with the product from β-glucuronidase activity accumulating in the root meristem, epidermis, and in loci within the root cortex (Fig. 4G) similar to inoculated root in MtENOD11:GUS plants (Fig. 4H).

Fig. 3. Nodulation of grafted plants. (A) An Lj shoot grafted on its own root. (B) An Mt shoot grafted on an Lj root. (C) An Mt shoot grafted on an Mt root. (A) and (B) were inoculated with M. loti, and (C) was inoculated with S. meliloti. Both strains carry a constitutively expressed lacZ gene. (D), (E), and (F) present nodules from roots in (A), (B), and (C), respectively. (G), (H), and (I) represent the sections of nodules in the plane of a cross-section through the parent root in (A), (B), and (C), respectively. Blue staining in the sections indicates the presence of rhizobia due to X-Gal staining. Iz=invasion zone, nc=nodule cortex, IZ=invasion zone, m=nodule meristem. Bars represent 300 μm in (G) and (H), and 100 μm in (I).
har1-1 roots. Figure 5 depicts the nodulation and root phenotypes of a har1-1 Lj shoot on a har1-1 Lj root (Fig. 5A), a wild-type Lj shoot on a har1-1 Lj root (Fig. 5B), an Mt A17 shoot on a har1-1 Lj root (Fig. 5C), and nodule numbers per plant in different grafting combinations (Fig. 5D). The numbers of nodules formed on roots of different grafting combinations was counted 3 weeks after inoculation and 8 weeks after grafting. Nodule numbers per root did not differ significantly in LjWT/LjWT, MtWT/LjWT, LjWT/har1-1, and MtWT/har1-1. However, there were significantly more nodules per root in Ljhar1-1/Ljhar1-1 and Ljhar1-1/LjWT than the rest. Ljhar1-1/LjWT had more nodules than Ljhar1-1/Ljhar1-1, likely due to the larger root system of the former than the latter. Thus, the data show that wild-type Mt shoots were able to restore the hypernodulation phenotype of har1-1 roots of Lj to wild-type level. Similarly, the short bushy root phenotype of har1-1 was also restored to wild type (Fig. 5A–C) by wild-type Mt shoots.

**Discussion**

Grafting is an important tool for studying the effects of shoot and root genotype on plant growth and development and their interactions with other organisms. Grafting has been used extensively to study the effects of shoots on autoregulation of nodulation of legume roots interacting with rhizobia (Delves et al., 1986, 1987; Sheng and Harper, 1997). Grafting experiments have been used successfully to investigate nodulation in the actinorhizal rosaceous species.
Purshia tridentata and P. glandulosa (Kyle and Righetti, 1996). Kyle and Righetti (1996) were able to show that both P. tridentata and P. glandulosa roots nodulated normally when grafted on P. tridentata shoots. However, both P. tridentata and P. glandulosa are nodulated by the same actinomycete strains of *Frankia* sp. (Righetti and Munns, 1980). Thus, the effect of the shoot on host specificity could not be assessed. Similarly, Sheng and Harper (1997) demonstrated that roots of the legume *Lablab purpureus* grafted on soybean shoots produced normal nodules, indicating that the shoot of both species is interchangeable for nodulation. Again, both soybean and lablab are nodulated by *Bradyrhizobium japonicum* and make determinate nodules. Therefore, it was not possible to determine the role of shoots in host specificity and nodule type.

The model legumes *Mt* and *Lj* differ in both host/rhizobial specificity and in nodule type, and are both widely used in symbiotic research. *Mt* makes indeterminate nodules and is nodulated by *S. meliloti*, whereas *Lj* makes determinate nodules and is nodulated by *M. loti*. Here, it is demonstrated that a successful intergeneric graft can be made between *Mt* and *Lj*, and that *Lj* roots can form similar determinate nodules when grafted either to *Mt* shoots or their own shoots. This indicates that the root genotype determines the determine nature of nodules on *Lj* roots. Moreover, it is speculated that either the shoot does not play a role in regulating meristematic persistence in nodule development, or that *Mt* can produce a shoot factor(s) that can adequately substitute for the native *Lj* factor(s).

Fernández-Lopez et al. (1998) reported the formation of indeterminate nodules in the stem-nodulating legume *Sesbania rostrata* that normally formed determinate nodules. In the presence of inhibitors of ethylene action, they showed that indeterminate nodules were formed. By contrast, the presence of an ethylene precursor caused the formation of determinate nodules. Thus, nodule type in *S. rostrata* appeared to be controlled by environmental conditions such as the presence of ethylene and not the shoot. Ethylene insensitivity leads to hypermodulation of the root in *Mt* and *Lj* (Penmetsa and Cook, 1997; Nukui et al., 2000). However, a change in nodule structure has not been reported either in the ethylene-insensitive *Lj* (Nukui et al., 2004) or in *Mt* with exogenous ethylene precursor application (Penmetsa and Cook, 1997).

It has been shown here, for the first time, that the shoot does not control the legume—rhizobia specificity in the case of *Lj* root/*M. loti* interaction, because host/endophyte specificity of the interaction is unaffected by the presence of the *Mt* shoot. Therefore, root-derived signals are sufficient to account for host specificity. This result is similar to results from grafting experiments involving plants interacting with pathogenic micro-organisms. Pavlou et al. (2002) reported that root and stem rot of cucumber (caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum*) could be controlled by grafting susceptible scions on resistant root-stocks, implying that the susceptible (compatible) interaction between the fungus and the susceptible root is determined solely by the root.

Isolated roots of the determinate nodule-forming species *Phaseolus vulgaris* (*L*.), *G. soja* (*L*.), as well as *Trifolium* spp., which form indeterminate nodules, have been successfully nodulated with their respective rhizobial symbionts when the roots were fed organic compounds through the root base, resembling a natural, intact plant (Raggio et al., 1959, 1965). Compounds furnished to the root by basal feeding that were required for efficient nodulation included sucrose, glycine, thiamine, pyridoxin, nicotinic acid, and inositol. In some cases, nitrate fed via the root base also enhanced nodulation. These results indicate that the shoot may act only as a nutrient source in the establishment of legume—rhizobia symbiosis. It is also possible that these small molecules may act as specific developmental signals as has been seen for sugars in other plant systems (Rolland et al., 2002).

In the present experiments, there was no root hair curling, infection, or nodulation of *Mt* roots grafted on *Lj* shoots when inoculated with *S. meliloti* or *M. loti*, indicating the inability of the root to nodulate in the presence of the *Lj* shoot. One possible explanation for the lack of nodulation response in *Mt* roots with *Lj* shoots would be the unhealthy shoot and degrading root observed 7 and 8 weeks after grafting, respectively, suggesting that a failure of the graft union might be responsible for the failure to nodulate. However, roots were inoculated 5 weeks after grafting, and plants looked healthy until 2 weeks after inoculation. Root hair curling, infection, and initiation of primordia all occur within 3 d of inoculation in wild type. Therefore, the lack of nodulation response in *Mt* roots grafted on *Lj* shoot is unlikely to be due to the bad health of grafted plants. Moreover, because there was some deformation of root hairs and *Mt* *ENOD11* induction in *Mt* roots with *Lj* shoots, it is likely that *S. meliloti* Nod factor(s) was recognized by Nod factor receptor(s) expressed in *Mt* roots. *Mt* non-nodulating mutants *nspl* and *nspl* (nodulation signalling pathway) show root hair deformation upon Nod factor treatment or *S. meliloti* inoculation, similar to that observed in *Mt* roots grafted on *Lj* shoots (Catoira et al., 2000; Oldroyd and Long, 2003; Mitra et al., 2004). Mutation in both *NSP1* and *NSP2* causes a reduced root hair deformation compared with wild type. Furthermore, *NSP1* mutants are able to induce *Mt* *ENOD11* expression, albeit at a reduced level (Catoira et al., 2000; Oldroyd and Long, 2003). However, *NSP2* mutants are completely blocked for *Mt* *ENOD11* induction (Oldroyd and Long, 2003). Therefore, the block to rhizobial infection of the grafted *Mt* root probably occurs downstream of rhizobial Nod factor recognition, and close to *NSP* gene activity. At this point, it is difficult to speculate on the reason for this infection block in *Mt* roots due to *Lj* shoots. Determinate nodulation has been proposed to have evolved from the indeterminate
type several times independently (Doyle, 1994). It is possible that during evolution Lj (a determinate nodulator) gained shoot factor(s) that are incompatible with Mt (an indeterminate nodulator) and S. meliloti interaction. Alternatively, a required small molecule signal that is supplied by Mt shoots may be absent in Lj shoots.

The HAR1 (HYPERNODULATION ABERRANT ROOT1) gene in Lj encodes a transmembrane LRR-type receptor-like kinase similar to the CLAVATA1 (CLV1) receptor kinase gene in Arabidopsis (Krussel et al., 2002; Nishimura et al., 2002). Hypernodulation is controlled by the shoot, and grafting of a har1-1 root on a wild-type Lj shoot restores the wild-type nodulation and root phenotype. It has been shown here that wild-type Mt shoots, when grafted on har1-1 roots, formed a number of nodules similar to that of wild-type Lj shoot grafted on har1-1 root. Similarly, the short and bushy root phenotype of har1-1 root was also restored to wild type by wild-type Mt shoots. This indicates that a gene functionally similar to HAR1 exists in Mt that complements the mutant phenotype. An Mt mutant called sunn (super-numerary nodulation), which is phenotypically similar to the Lj har1-1, has been reported in Mt (Penmetsa et al., 2003). As with har1-1, the sunn root is hypernodulated by S. meliloti and is controlled by the shoot, as revealed by grafting experiments. Recently, SUNN from Mt was cloned, and is predicted to encode an LRR receptor kinase orthologous to HAR1 in Lj that is expressed in roots, stems, and flowers of nodulated plants (J Frugoli, unpublished results).

In summary, using grafting experiments, it was shown that determinate nodulation in Lj is not dependent on its shoot for host specificity and nodule type. By contrast, the Lj shoot does not permit nodulation of the Mt root with its microsymbiont S. meliloti. A possible shoot factor that is required for nodule primordium initiation and later symbiotic development in indeterminate nodules needs further investigation. Since Lj and Mt are both model legumes being extensively used for research, the successful grafting between the two species can be used as a tool to complement the studies of both model legumes.

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References


