Plant and Soil **245:** 147–162, 2002.

Breeding for better symbiosis

Z. Rengel
*Soil Science and Plant Nutrition, Faculty of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia**

Key words: associative N_2 fixation, dinitrogen fixation, genotype, mycorrhiza, nodulation, rhizobia, root exudation, screening, selection, symbiosis

Abstract

The present review gives a critical assessment of the literature dealing with symbiosis between rhizobia and legumes and between AM fungi and most plants. Associative N_2 fixation (even though strictly speaking not a symbiotic relationship) does have some characteristics of symbiosis due to mutualistic dependence and usefulness of the relationship, and is therefore covered in this review. Nodulation in the rhizobia–legume symbiosis may be limited by an insufficient amount of the *nod*-gene inducers released from seed and/or roots. However, there is genotypic variation in the germplasm of legume species in all components of the signalling pathway, suggesting a prospect for improving nodulation by selecting and/or transforming legume genotypes for increased exudation of flavonoids and other signalling compounds. Deciphering chromosomal location as well as cloning *nod*, *nif* and other genes important in nodulation and N_2 fixation will allow manipulation of the presence and expression of these genes to enhance the symbiotic relationship. Increased efficacy of symbiotic N_2 fixation can be achieved by selecting not only the best host genotypes but by selecting the best combination of host genotype and nodule bacteria. As flavonoids exuded by legume seedlings may not only be *nod*-gene inducers, but also stimulants for hyphal growth of the AM fungi, selecting and/or transforming plants to increase exudation of these flavonoids may result in a double benefit for mycorrhizal legumes. Mutants unable to sustain mycorrhizal colonisation are instrumental in understanding the colonisation process, which may ultimately pay off in breeding for the more effective symbiosis. In conclusion, targeted efforts to breed genotypes for improved N_2 fixation and mycorrhizal symbiosis will bring benefits in increased yields of crops under a wide range of environmental conditions and will contribute toward sustainability of agricultural ecosystems in which soil-plant-microbe interactions will be better exploited.

Introduction

Two most important types of symbioses are N_2 fixation and acquisition of P and other nutrients by mycorrhizae. They will be discussed with particular reference to signalling pathways involved and genotypic differences in the capacity of symbionts to sustain the symbiosis, especially under unfavourable environmental conditions.

Dinitrogen fixation provides more N to the agricultural ecosystems worldwide than the total amount of fertiliser N applied. However, a significant potential exists to further improve symbiotic and associative N_2 fixation by breeding genotypes with a greater capacity to sustain these interactions with bacteria. Since taxonomy of root and stem nodule bacteria has been expanding recently (six genera have been distinguished so far: *Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium, Azorhizobium* and *Allorhizobium*), the generic term 'rhizobium' will be used in this article to refer to members of these six genera.

Better N_2-fixing symbiosis may be brought about by manipulating both rhizobia and plant hosts and by eventually creating an artificial rhizosphere. An important aim is also to improve the symbiotic relationship in suboptimal environmental situations related to soil-borne or environmental stress. However, it is still unclear whether attempts to increase the symbi-

* FAX No.: +61-8-9380-1050; Tel.: +61-8-9380-2557; E-mail: zrengel@agric.uwa.edu.au

otic capacity where symbiosis is already fully functional ('overexpression', supernodulation, etc.) will be beneficial in terms of increasing crop yields.

Symbiotic or even mutualistic relationships involving rhizobia depend on chemical signals between the two organisms. These signals define the rhizobia–host specificity in the relationship. Selecting for the optimal combination of the rhizobium and the host usually results in more effective symbiosis and better growth of the host plant.

Many crop plants may form symbiotic associations with mycorrhizae and thus gain access to soil pools of P that are unavailable to non-mycorrhizal plants (e.g., Smith et al., 1994). This is especially important in soils where P-sorption capacity is high, resulting in poor agronomic efficiency of P fertilisers applied. On P-sorbing soils, only 10–20% of applied P fertilisers may be utilised by crops in the year of application, while a major portion of applied fertilisers becomes fixed in soils and unavailable to non-mycorrhizal crops (cf. Zhang and Rengel, 1999). Mycorrhizal hyphae are thinner than roots, and they create a greater P concentration gradient between the bulk soil and the hyphal surface, thus allowing more substantial movement of P toward hyphae and greater uptake across the membrane.

This review will concentrate on genetics and breeding related to N_2 fixation via symbiotic rhizobia and associative diazotroph bacteria as well as to AM symbiotic relationship. Ectomycorrhiza will not be covered here, and readers are referred to papers by Godbold et al. (1998) and Tagu and Martin (1996) for more information on that topic.

Associative N_2 fixation

Associative diazotroph bacteria are more numerous in the rhizosphere than in the bulk soil, indicating dependence on the organic compounds exuded by plant roots (Natsvaladze et al., 1992). Therefore, associative N_2 fixation, even though strictly speaking not a symbiotic relationship, does have some characteristics of such a relationship due to mutualistic dependence of, and usefulness to, the partners in the relationship. Indeed, recent evidence suggests that N_2 fixation in some grasses (e.g., sugarcane) may be symbiotic (James, 2000).

Under certain conditions, biological N_2 fixation via associative bacteria may contribute up to 30% of N needs of wheat and up to 150 kg N/ha per year in sugar cane fields in Brazil. It has been suggested that Brazilian cereal and sugar cane genotypes have inadvertently been selected for efficient utilisation of N fixed by associative diazotrophs (Dobereiner, 1997), thus significantly decreasing or completely eliminating the need for N fertilisation of these crops. Single or dual inoculation of wheat with *Azotobacter chroococcum*, *Azospirillum brasilense* and *Streptomyces mutabilis* in sterile soil allowed these microorganisms to establish well in the rhizosphere and to increase N content both in soil and in the wheat shoots (El-Shanshoury, 1995).

Associative N_2 fixation can provide between 10 and 80 kg N/ha per cropping season in rice depending on the cultural practices and the rice variety grown (Rao et al., 1998). Rice genetic factors contributing to efficient associative N_2 fixation in the rhizosphere have been identified and can be used in the breeding programme. Four putative QTL loci all showed a recessive gene action. Individual markers explained between 8 and 16% of the total phenotypic variation (Wu et al., 1995).

Differences among cereal species as well as among genotypes within cereal species in their capacity to support associative N_2 fixation have been clearly established (Jagnow, 1990). In rice, appropriate combinations of auxin action, *Acetobacter diazotrophicus* and rice genotype are needed for the relationship to be effective: some plants grew for up to 12 months on N-free medium getting N from the associative fixation (Rolfe et al., 1995). Further research on designing suitable screening methods to select and breed crop cultivars with desirable responses to beneficial diazotroph bacteria is warranted.

Rice genotypes have differential ability to support associative N_2 fixation, with the percentage of N derived from air (Ndfa) ranging from 1.5 to 21% in various genotypes (Shrestha and Ladha, 1996). Maize genotypes also showed differences in the capacity to support associative N_2 fixation, with some genotypes having similar yield when inoculated with diazotroph *Azospirillum* bacteria and when fertilised with 100 kg N/ha (Garcia de Salamone et al., 1997). Wheat genotypes also differed in the degree of support of associative *Azospirillum* bacteria in the rhizosphere and the rhizoplane (Ramanathan et al., 1997; Schloter and Hartmann, 1998).

The effectiveness of associative N_2 fixation may be diminished under environmental stress. Under saline conditions, differential tolerance of Azospirillum strains to salt stress resulted in the significant interac-

tion between plant genotypes (finger millet, *Eleusine coracana*) and *Azospirillum brasilense* strains (Rai, 1991a).

Genes important in initial steps of Azospirillum attachment to roots (chemotaxis and adhesion) are being deciphered (Moens et al., 1997). However, chemotaxis in Azospirillum may be a general response rather than a specific host-dependent one (Fedi et al., 1992). Nevertheless, increased knowledge about genes mediating associative N_2 fixation will be instrumental in breeding crop plants for more effective utilisation of N from associative fixation. New insights into the process of associative N_2 fixation can also be gained from studies employing genetically engineered bacteria and cyanobacteria. Nitrogenase-derepressed mutant of *Anabaena variabilis* inoculated onto wheat roots resulted in high nitrogenase activity in the growing zones of young roots and increased wheat yield (Spiller et al., 1993).

There is a possibility that the rhizosphere competence of associative diazotrophs may be improved by increasing the capacity to degrade polygalacturonic acid (pectin) by overexpressing the polygalacturonase (pectinase) gene as it was shown that the rhizosphere competence, at least in a rhizobacterium *Burkholderia pickettii* MSP3Rif, depends on the activity of that particular enzyme (Ikeda et al., 1998). Another exciting strategy for enhancing growth of associative N_2-fixing microorganisms is to engineer plants to exude into the rhizosphere compounds specifically catabolised by the targeted associative N_2 fixers (see O'Connell et al., 1996). This strategy is based on the rhizopine concept (Rossbach et al., 1994; Murphy et al., 1995; Vlassak and Vanderleyden, 1997).

In addition to the associative diazotrophs in the rhizosphere of non-legume plants, these non-legumes may benefit from endophytic N_2-fixing bacteria. For example, N_2-fixation-competent endophytic strains of *R. leguminosarum* bv. *trifolii* significantly enhanced shoot and root growth of rice in growth chamber experiments (albeit no evidence of N_2 fixation was found), and increased grain yield as well as agronomic fertilizer N-use efficiency of hybrid rice in a field inoculation experiment conducted in the Nile Delta, Egypt (Yannai et al., 1997). These endophytic strains multiplied and moved within root tissue, indicating that they possess necessary genes to colonise and get established in rice roots and promote rice growth (Prayitno et al., 1999). However, possible N_2 fixation by these strains in rice roots is yet to be confirmed. Other possible causes of improved growth caused by endophytic bacteria may be through production of plant growth regulators and/or chelation of nutrients that have poor availability to plants. Further work on clarifying mechanisms underlying positive contribution of endophytic bacteria to plant growth is eagerly awaited.

The nodulation process in the rhizobia-legume symbiosis

Inheritance of the process of nodulation may be relatively simple. Recent discovery of non-fixing mutants with altered patterns of nodulation has revealed that genes involved in the control of the nodulation process are recessive and may not be too numerous (Tsyganov et al., 1998). Duc (1995) found that faba bean mutants unable to nodulate, as well as those with ineffective nodulation and supernodulation, had mutations of single recessive genes. However, grafting experiments showed that supernodulating mutant was influenced by the shoot genotype, while the non-nodulating and ineffective nodulation mutants were influenced by the root genotype, indicating that the regulation of gene expression may be more complicated.

A common translocatable factor facilitating hypernodulation in various legume species appears to exist in their shoots (e.g., in soybean, mung bean, *Vigna radiata*, and hyacinth bean, *Lablab purpureus*, Harper et al., 1997). Isolation and characterisation of such a factor may prove instrumental in increasing efficiency of the nodulation and thus ultimately of the N_2 fixation process.

Soybean genotypes with loci that restrict nodulation by native ineffective strains allow nodulation only by inoculant superior N_2-fixing strains of specific serogroups (Keyser and Cregan, 1987). However, more recent work shows that the host–rhizobia relationship is more complicated (Lohrke et al., 1995), indicating that better understanding of molecular mechanisms involved in selectivity of the nodulation process is needed. Work with other plant species (e.g., clover and *R. leguminosarum* bv. *trifolii*, Rolfe et al., 1995, and references therein) also indicates that positive and negative control mechanisms in the nodulation process are quite complex.

Lipo-chitooligosaccharide nodulation factors (Nod factors) produced by rhizobia determine the host range. These factors play a pivotal role in the molecular signal exchange, infection and induction of symbiotic developmental responses in legumes leading to

the formation of a nodule in which rhizobia carry out N_2 fixation (Reddy et al., 1998). Subnanomolar concentrations of membrane lipo-chitooligosaccharides from *R. leguminosarum* bv. *trifolii* induce root hair deformation that leads to nodulation on white clover roots (Orgambide et al., 1996). There is fairly strong rhizobia–legume specificity because the same compounds could not induce any changes in lucerne, and more than two orders of magnitude greater concentration was required to induce a measurable response in vetch.

Lucerne genotypes capable of exuding *nod*-gene inducers into the rhizosphere were nodulated by *R. meliloti* to a larger extent and had a better growth than genotypes that lacked the capacity to exude *nod*-gene inducers. An addition of root exudates from highly-nodulating genotypes significantly improved nodulation of otherwise poorly nodulating genotypes (Hernandez et al., 1995). Root exudates turned on the proline dehydrogenase gene in *R. meliloti* during root invasion and nodule formation (Jimenez-Zurdo et al., 1997).

Initial nodulation may be limited by an insufficient amount of the *nod*-gene inducers released from seed, as shown for bean *Phaseolus vulgaris* (Hungria and Phillips, 1993) and lucerne (Hernandez et al., 1995). Isogenic bean genotypes differing in the quantitative and qualitative composition of flavonoid released from seed also differed in nodulation, with a positive relationship between the amount of flavonoids and nodulation (Hungria and Phillips, 1993). Testing a range of lucerne genotypes also detected the variability in the flavonoid exudation (Phillips et al., 1995). These results suggest a prospect of improving nodulation by appropriately selecting legume genotypes, or altering them through transformation and breeding, for increased exudation of flavonoids.

In considering improvement of the symbiotic relationships by changing the host plant to increase exudation from roots, the extra carbon cost of such exudation will need to be counterbalanced with potential benefits that such a change in root exudation may give in a particular set of environmental conditions. Indeed, it appears that selection of genotypes in breeding programmes resulted in a decreased capacity to exude organic compounds into the rhizosphere (e.g., in rice, Waschutza et al., 1992). To minimise extra carbon costs, only increased exudation of targeted compounds that enhance symbiosis should be pursued in selection and breeding.

Rhizobial mutants (e.g., Tn5 mutants with decreased production of acidic exopolysaccharides, Tn5 mutants of *R. fredii* USDA257 with increased nodulation capacity, etc.) are important in elucidating processes and characterising products required for efficient symbiosis (e.g., Eisenschenk et al., 1994; Heron et al., 1989). This knowledge will also be important in manipulating rhizobial inoculum to improve symbiosis. However, an assessment of the genotypic variation among a large number of *Sinorhizobium meliloti* strains isolated from nodules developed on various lucerne genotypes showed that the host genotype had a major influence on the shaping of the genetic structure of nodulating bacterial strains (Paffetti et al., 1998).

Transgenic crops will provide excellent material for studying various aspects of the symbiotic processes and will thus prove valuable in breeding for more effective symbioses. For example, transgenic clover *Trifolium repens* (Rolfe et al., 1997) has been used to study genetic programmes activated during nodule initiation and development.

Isogenic lines, with more than 95% similarity in the genetic background and a difference only in genes of interest, represent an excellent plant material for studying relevant physiological processes. Shelp and colleagues (1998) have produced a range of isogenic pea lines differing in production of effective nodules with *R. leguminosarum* bv. *viceae* and tolerance of nodulation to nitrate. These lines should be used in future studies of nodulation and N_2 fixation.

Signalling pathways in the rhizobia-legume symbiosis

There are three distinct parts in the signalling process involved in the rhizobia–legume symbiosis. First, flavonoids in plant root exudates induce *nod* genes in rhizobia and mediate the nodulation process. Secondly, the product of the regulatory rhizobial *nodD* gene induced transcription of rhizobial structural *nod*, *nol* and *noc* genes which code for extracellular lipochitooligosaccharides, protein Nod O, and other extracellular Nod factors. Thirdly, endogenous plant hormones, as well as factors produced by rhizobia, are important mediators of nodule initiation. It should, however, be emphasised that the current knowledge of the signalling process involved in the rhizobia–legume symbiosis is somewhat fragmented and hampered by the complexity of the plant–soil–microbe interactions

that exist in agricultural ecosystems (Van Rhijn and Vanderleyen, 1995; Vlassak and Vanderleyen, 1997).

Nod factors are lipo-chitooligosaccharides varying in the oligosaccharide chain length, the nature of the fatty acids and substitutions on the oligosaccharide. Allelic and non-allelic genes coding for Nod factors have co-evolved together, while the host plant divergence ensures variability in Nod factors between different rhizobial strains and the specificity of the rhizobia–host relationship. Nod factor genes are located on the transmissible plasmids and/or 'symbiosis islands', which are symbiotic regions associated with movable elements, thus allowing for easy horizontal gene transfer (Mergaert et al., 1997). Rhizobia have a range of *nodD* genes producing NodD factors to cater for a variety of hosts (e.g., *R. meliloti* has three *nodD* genes; Honma et al., 1990). Functional analysis of *nodD* alleles is a useful way of ascertaining the differences between various NodD products (Van Rhijn et al., 1994).

A large body of literature has been accumulated on the flavonoid signalling pathway in the *nod* gene induction (e.g., Bolanos-Vasquez and Werner, 1997; Janczarek et al., 1997; Maxwell et al., 1989; Zuanazzi et al., 1998). It appears that expression of *nod* genes in various species of symbiotic N_2-fixing rhizobia may be increased by a number of flavonoid components (e.g., aldonic acids, Gagnon and Ibrahim, 1998; 4′,7-dihydroxyflavone and 4,4′-dihydroxy-2′-methoxychalcone, McKhann et al., 1997), even though different flavonoids may also suppress the *nod* gene expression (Zuanazzi et al., 1998). These flavonoid compounds appear to be synthesised predominantly in roots (Armero et al., 1994). It is interesting that specific Nod factors of *B. japonicum* increase flavonoid exudation by soybean roots (Schmidt et al., 1994), indicating the mutualistic character of the signalling pathway.

Transconjugants of rhizobial isolates having nodABC–lacZ fusion are frequently used for studying activation of the *nod* genes by the flavonoid/root exudate compounds by monitoring β-galactosidase activity (e.g., Bolanos-Vasquez and Werner, 1997; Dakora et al., 1993; Janczarek et al., 1997; Maxwell et al., 1989; Pandya et al., 1998). The potential host range of the rhizobial isolates (for example in the case of cowpea miscellany *Rhizobium* spp.; Pandya et al., 1998) can be determined in such a way.

Flavonoid exudation by the host seed/seedlings can also be regulated by the nutritional status of the growing medium: increasing N supply decreases exudation of phenolics (e.g., isoflavonoid and aglycone) (Wojtaszek et al., 1993). Since these isoflavones may serve as signalling molecules in the rhizobial–legume symbiosis, it is obvious that N supply may act as an additional control mechanism in such exudation.

Specificity of the *Rhizobium*-legume symbiosis is also governed by specific genes on the bacterial chromosome that code for proteins involved in recognition and uptake of specific signal molecules present in root exudates. *R. tropici* contains *teu* genes (*t*ropici *e*xudate *u*ptake) activated by root exudates of *Phaseolus vulgaris* and *Macroptilium artropurpureum* as hosts for *R. tropici*, but not by root exudates of other non-host legumes (Rosenblueth et al., 1998). Localisation of *nod*, *nif* and other genes important in development of the symbiotic relationship (e.g., on the large plasmid in the slow-growing cowpea *Rhizobium* spp. S2; Pandya and Desai, 1998) will create new avenues in using molecular biology tools to manipulate the presence and expression of these genes to enhance the symbiotic relationship.

Not only symbiotic colonisation (infection stress), but other types of stresses may induce root exudation of similar compounds (e.g., genistenin and a range of isoflavonoids) (Gagnon and Ibrahim, 1997), indicating some non-specificity in the rhizobia–host signalling sequence and suggesting that transformation of non-host plants to produce compounds that act as inducers in the effective symbiotic relationship may be quite feasible. It should be emphasised that isoflavonoids also act as phytoalexins (Dakora et al., 1993), indicating some similarity in response to pathogenic infection and infection with rhizobia. However, the level of phytoalexin production caused by *B. japonicum* in soybean roots was considerably lower than that caused by pathogenic infection by *Phytophthora megasperma* f.sp. *glycinea* (Schmidt et al., 1992).

There is genotypic variation in the germplasm of legume species in all components of the signalling pathway. Such a statement is based on the literature in which testing of only a limited number of genotypes has been reported (frequently only two, e.g., Soejima et al., 1992) and would therefore need to be ascertained on a large number of genotypes. Improved understanding of the signalling process will allow improving the symbiosis by more targeted breeding efforts. There is generally a gene(s)-for-gene interaction between rhizobia and the host. The genotypic specificity of the nodulation process depends on such an interaction. For example, McCall genotype of soybean cannot be nodulated when inoculated with *R. fredii*

USDA257 that nodulates other soybean genotypes tested (Trese, 1995). Even though host-genotype-specific flavonoid signals have not been found, it was clear that the strain- and host-genotype-specific interactions are characterised by unique patterns of signal release and response (Pueppke et al., 1998). Genotypic variation in flavonoids, betaines and other nod-gene inducers indicates a potential for genotype selection for increased exudation of these beneficial, but sometimes limiting, compounds (see Phillips et al., 1995).

Different flavonoids are exuded by germinating lucerne seeds (3′,4′,5,7-substituted flavonoids) compared with roots (5-deoxy molecules) (Hartwig et al., 1991). These compounds apparently have a different function, with 5-deoxy molecules being more effective in stimulating *R. meliloti* growth, and the other types being more effective as *nod*-gene inducers. These results pose another level of difficulty in breeding for better symbiosis because different metabolic pathways obviously predominate during different stages in the plant growth cycle.

Competitiveness of rhizobial strains

Under most soil conditions, odds are stacked against the inoculant strain. These strains have a limited gene pool for adapting to local conditions as opposed to indigenous strains which are well adjusted to prevailing conditions at the site and, moreover, can adapt to just about any change in conditions (see Vlassak and Vanderleyden, 1997). It is therefore of utmost importance that inoculant rhizobia have strong nodulation effectiveness for the host being grown as well as that inoculant strains are well adapted to the relevant soil conditions.

Better N_2 fixation can be achieved by selecting superior rhizobia. However, selection of these rhizobia would need to take into consideration not only their N_2-fixing capacity, but also competitive ability against native rhizobia which are frequently ineffective in N_2 fixation. Superior N_2-fixing strains have to outcompete native rhizobia and occupy a significant proportion of the nodules. For this to be achieved, rhizobia have to be selected under natural conditions in competition with the native rhizobia.

Better rhizobial symbiosis can be achieved by more effective rhizobia that will establish well in the soil and the rhizosphere (saprophytic competence), will cause greater nodulation, occupy a greater proportion of nodules, and have greater activity of nitrogenase and associated enzymes. More effective rhizobial strains can be selected from native populations (Howieson, 1995) or constructed in various ways (for references see Vlassak and Vanderleyden, 1997). However, achieving a large occupancy of nodules by these effective rhizobia represents one of the most significant practical problems. Mass inoculation does not always enhance the nodule occupancy (Kuykendall, 1989), and the amount of inoculum needed to overwhelm the ineffective native rhizobia is frequently excessive and uneconomical (Vlassak and Vanderleyden, 1997). However, the number of inoculant bacteria in comparison to the native rhizobia is not the most important parameter of competitiveness because other more qualitative criteria play a part (e.g., rhizobia mobility may influence their capacity to nodulate lateral roots in addition to the crown where the frequency of nodulation is the greatest due to inoculum most frequently being applied to seed, McLoughlin et al., 1990).

Competitiveness of introduced rhizobia strains can be increased if they are engineered to produce compounds that are inhibitory to *nod* gene expression in native rhizobia. For example, a repressor of the *nod* gene expression, NolA, was identified in USDA110; *nolA* gene transferred to *B. japonicum* serocluster 123 resulted in depression of Nod factor production (Dockendorff et al., 1994; Vlassak and Vanderleyden, 1997).

In contrast to *nod* genes and surface determinants, the role of an increasing number of genes and gene products that determine strain competitiveness has been elucidated (e.g., genes controlling catabolism of specific legume secondary metabolites like homoserine and trigonelline (Boivin et al., 1991), genes regulating nodule formation efficiency in *R. meliloti* (Sanjuan and Olivares, 1991), etc. — for further examples see Vlassak and Vanderleyden, 1997). These genes and gene products increase competitiveness as they allow nutritional utilisation of specific legume-produced substrates. However, even a larger number of genes and gene products remain to be characterised.

Competitiveness may also be increased by transferring the rhizopine *moc* and *mos* genes to good N_2 fixers (Vlassak and Vanderleyden, 1997). In the presence of rhizopine-producing bacteria, the rhizobia strains that are capable of catabolising rhizopines occupy a higher percentage of nodules (Murphy et al., 1995).

The specific compatibility between *nodX* gene carried on the symbiotic plasmid and *sym-2* gene of pea cv. Afghanistan (this cultivar could be nodulated only by strains containing *nodX* gene) has been recognised as offering the capacity to increase competitiveness of desired rhizobia–host combination and exclude all other combinations. Therefore, the *sym-2* gene was crossed into other pea cultivars and *nodX* was transferred into good N_2 fixers (Fobert et al., 1991), establishing superbly competitive host-rhizobia combination.

Rhizobia strains have been genetically modified to increase their competitiveness (e.g., Toro et al., 1998). Genes conferring capacity to produce trifolitoxin, a compound with bactericidal and/or bacteriostatic effects on other bacteria (see Vlassak and Vanderleyden, 1997), have been transferred to the effective nodulator *R. leguminosarum* bv. *trifolii* strain, which showed increased competitiveness for nodulation of clover when compared with the trifolitoxin-sensitive strain (Leung et al., 1994).

Expression of some genes in *Bradyrhizobium japonicum* was induced by exposure to soil extract as opposed to the basal medium (Bhagwat and Keister, 1992). The strain that was a better nodulator had more genes expressed; the two genes cloned by a subtractive RNA hybridisation from the better nodulating strain increased the competitive nodulation capacity in the otherwise poorly nodulating strain.

Genotypic differences in symbiotic N_2 fixation

A continuous and coordinated selection of the most effective combinations of host and microbial symbionts is a prerequisite for profitable and sustainable agricultural systems. Different legume genotypes differ in efficacy of symbiotic N_2 fixation they can support (e.g., white clover, *Trifolium repens*, Ledgard, 1989; faba bean, Caba et al., 1993; soybean, Ofosu Budu et al., 1993; Song et al., 1993; De Chueire and Hungria, 1997; mung bean, *Vigna radiata*, Espiritu et al., 1993; common bean, *Phaseolus vulgaris*, Hardarson et al., 1993; pea, Evans et al., 1995; Fesenko et al., 1995; groundnut, Bell et al., 1994, Ibrahim et al., 1995; lucerne, Hernandez et al., 1995; chickpea, Sattar et al., 1995; tree *Gliricidia sepium*, Sanginga et al., 1992) as well as in the response of N_2 fixation to fertiliser N addition (e.g., faba bean, Caba et al., 1993; soybean, Song et al., 1993; Zhang et al., 1997; pea, Evans et al., 1995). Genotypic differences in activities of glutamine synthetase (glutamate-ammonia ligase) and glutamate synthase (GOGAT) in nodules were related to genotypic differences in symbiotic N_2 fixation efficiency, at least in faba bean (Caba et al., 1993). Therefore, screening for increased activities of these enzymes might be used in a breeding programme to increase symbiotic efficiency of faba bean genotypes.

A number of other symbiotic characteristics can be considered in breeding for better symbiosis. For example, chickpea genotypes differed in the total number and weight of nodules per plant as well as the weight of individual nodules (Dangaria et al., 1994). Other criteria related to the functioning of nodules may also be considered. In pea cultivars, N accumulation in shoots is a more suitable criterion than the shoot mass for selection of either highly effective or highly competitive *R. leguminosarum* bv. *viceae* strains in the pot experiments (Fesenko et al., 1995).

Over the course of evolution, rhizobial strains developed that could nodulate only one or, at best, a limited number of legume species. Various rhizobia strains also differ in N_2 fixation capacity; it is therefore possible to select for more efficient strains (Hartmann et al., 1998; Hungria et al., 1998). As the effectiveness of the nodulation and the N_2-fixation process varies widely in different rhizobia–host combinations, it would be worthwhile to identify the highly effective rhizobial strains, followed by identifying responsible genes that regulate high effectiveness of the symbiosis. These genes could then be transferred into commercial strains of rhizobia to increase efficiency of N_2 fixation or their host range. In addition, there is generally a strong host genotype $\times$ rhizobia strain interaction that determines the efficiency of the nodulation and N_2 fixation processes (Luna and Planchon, 1995). However, in bean genotypes of Mezo-American and Andean origin tested with 10 genetically diverse bean rhizobia, no host $\times$ rhizobia interaction was detected (Buttery et al., 1997).

It would be particularly important to develop genotypes that maintain high efficiency of N_2 fixation in the presence of small or moderate levels of nitrate. Otherwise, nitrate produced from mineralisation of the organic matter may not be taken up by legumes (Blumenthal and Russelle, 1996), but instead would leach into the lower horizons or ground water (see Anderson et al., 1998) and/or may hinder the symbiotic process. This is especially important as recently released, high-yielding cultivars may contain high N concentrations in the harvestable product, thus removing large amounts of N from the field. Genotypic variability

exists among host genotypes in the degree of tolerance of the N_2-fixation process to nitrate (Raffin and Roumet, 1994). Additional genotypic variability and tolerance to nitrate can be introduced by mutagenesis (e.g., in soybean, Neo et al., 1996). Also, there is a genetic variability within nodule bacteria germplasm in tolerance of the N_2 fixation process to nitrate (e.g., Leung et al., 1994; Nour et al., 1994), which needs to be taken into account when creating a breeding programme for better symbioses. Such genotypic variability clearly indicates a potential for greatly increasing efficacy of symbiotic N_2 fixation by selecting the best combination of nitrate-tolerant plant genotype and rhizobia.

Screening genotypes for nodulation and N_2 fixation in hydroponics under controlled environmental conditions needs to be calibrated against field performance of standard cultivars because nodulation of, for example, bean genotypes may be different between controlled conditions and the field, even when growth in the two environments appears similar (Hernandez et al., 1993). Early screening of soybean genotypes for nodulation and N_2 fixation (20–60 days after planting) in both glasshouse and field may generate useful parameters for breeding programmes aimed at improving efficiency of the legume-rhizobia symbiosis (Pazdernik et al., 1997).

Wild genotypes of crop plants may serve as a source of genes for improved and more effective N_2 fixation (Andriolo et al., 1994). Similarly, host mutants (e.g., white sweetclover, *Melilotus alba*, Utrup et al., 1993), unable to achieve complete and functional symbiosis with rhizobia, should be studied more to increase our understanding of the symbiotic process.

Adaptation of legume-rhizobia symbiosis to unfavourable environments

Nodulation of a range of pasture and crop legumes (e.g., white clover, subclover, lucerne, pea, cowpea, bean, etc.) is reduced in acid soils, mainly because of sensitivity of early nodulation events, such as attachment, root hair curling and initiation of infection thread formation (for references see Vlassak and Vanderleyden, 1997). Problems with *nod* gene expression, more notable in acid-sensitive than in acid-tolerant rhizobial strains, may account for these deleterious acidity-related effects (McKay and Djordjevic, 1993). It is also important to mention that legume hosts grown under conditions of environmental stresses differ in the quantity and quality of *nod*-gene inducers found in their root exudates (e.g., Raghuwanshi et al., 1994).

Low P (Mullen et al., 1988) and low Ca (Munns, 1970), the soil properties associated with acidic soils, deleteriously affect the nodulation process independently of pH. Rhizobial strains with relatively higher capacity to nodulate their hosts under low P (Leung and Bottomley, 1987) or in acidic soils (Howieson, 1995) have been isolated. These strains are essential for good symbiosis under such adverse conditions that are prevalent in many tropical soils farmed by subsistence farmers who are unable to invest substantial funds required to alleviate soil acidity.

Considerable efforts over the years have been directed toward selecting the optimal combination of rhizobial inoculum and the legume genotype for acidic soils where high H^+, Al or Mn may limit the effectiveness of some rhizobia–host combinations (e.g., in lucerne, Munns, 1970; white clover, Wood et al., 1984; chickpea, Rai, 1991b; *Lens culinaris*, Rai, 1992). In general, slow-growing bradyrhizobia are more acid-tolerant than fast-growing rhizobia (Cooper et al., 1985). The mechanisms behind such a difference have yet to be elucidated. Nevertheless, screening of rhizobia for tolerance to acidic conditions in the laboratory did result in strains that prove more effective in acid soils under field conditions (e.g., using acid-tolerant *R. meliloti* increased the area under pasture in Australia by at least 350 000 ha; Howieson et al., 1988). However, there are also examples of poor correlation between tolerance to acidity in the laboratory and in the field (e.g., Gemell and Roughley, 1993).

When grown in the acidic environments, some acid-tolerant Medicago species (*M. murex, M. polymorpha*) can nodulate better than others (*M. truncatula, M. littoralis*) (Howieson et al., 1992) because these acid-tolerant species maintained the composition of root exudates suitable for inducing *nod* genes in *R. meliloti* and thus fostering nodulation. *Biserulla pelecinus*, a pasture legume adapted to acidic soils, has a strain of nodule bacteria that is different from other known symbiotic N_2-fixing bacteria, which apparently represents an adaptation to acidic soils (Howieson et al., 1995).

Eight characteristics required in an acid-tolerant pasture legume symbiosis for use in ley-farming have been described by Howieson (1995). Among others, the capacity of rhizobia (preferably of the genus *Bradyrhizobium*) to regulate internal pH and thus

maintain saprophytic function at low pH were emphasised together with stability of the nodABC gene products as well as surface polysaccharides and proteins at acidic pH. The capacity of host roots to produce pH-stable organic acids for linkage with rhizobial cell surface structures is also important.

Large differences exist among rhizobial strains in tolerance to Al or Mn toxicity (for references see Vlassak and Vanderleyden, 1997), with generally a poor correlation between tolerance to acidity and tolerance to Al or Mn. However, strains showing tolerance to Al in laboratory conditions also performed better in Al-toxic soils (e.g., Graham et al., 1982).

Al-tolerant wheat genotypes exuded a greater amount of organic acid anions into the rhizosphere than Al-sensitive genotypes exposed to Al toxicity (for the review see Rengel, 1996). As organic acid anions are a good substrate for *Azospirillum brasilense*, these associative diazotroph fixers contributed considerably more fixed nitrogen to the Al-tolerant than to the Al-sensitive wheat genotype (Christiansen-Weniger et al., 1992).

Tn5-induced mutants varying in acid tolerance have been instrumental in isolating several acid-tolerant chromosome-located loci. Some of the acid-tolerant loci can be transferred into acid-sensitive rhizobia to increase their tolerance to soil acidity (Tiwari et al., 1992).

Tolerance of the symbiotic relationship was tested under saline stress (e.g., in faba bean, Cordovilla et al., 1995; chickpea, Zurayk et al., 1998; soybean, Abd-Alla et al., 1998), low temperature (e.g., in faba bean, Fyson and Sprent, 1982; sainfoin, Prevost and Bromfield, 1991; soybean, Zhang et al., 1997), drought (e.g., in subclover, Leung and Bottomley, 1994; bean, Castellanos et al., 1996; soybean, Serraj and Sinclair, 1998) and waterlogging (e.g., in *Trifolium semipilosum*, Lupwayi et al., 1997). Genotypic differences in tolerance of the legume-rhizobia symbiosis to these various stresses have been documented.

Symbiotic N_2 fixation in non-legume hosts

While there are several avenues for increasing the effectiveness of the naturally occurring symbiotic relationships (e.g *Rhizobium*–legume), it appears quite difficult at present to create conditions conducive to other symbioses (e.g., *Rhizobium*–rice) (Ladha et al., 1997; Reddy et al., 1997). Among some notable problems, rice root exudates did not induce expression of the *nod* genes in various rhizobial species, and neither the wild-type *Rhizobium* nor purified lipochitooligosaccharide Nod factors induced root hair deformation or true nodulation on rice roots (Reddy et al., 1997). However, continued improvement in our understanding of the physiological and molecular basis of the *Rhizobium*–legume symbiosis may allow transfer and expression of required genes into non-host species, thus improving chances that the viable symbiosis between rhizobia and (otherwise) non-host plants can be achieved. Indeed, early results on transforming rice with the *nodulin* gene (see below) indicate that a rice line may soon be developed that can fix nitrogen from the atmosphere. Results with rice root endophytes fixing atmospheric N_2 are also encouraging (e.g., Hurek et al., 1999).

When the early nodulin gene *ENOD12* from *Medicago truncatula* was transferred into rice, the gene was expressed in root cortical parenchyma upon induction by rhizobial lipo-chitooligosaccharide nodulation factors (Reddy et al., 1998). These results have indicated that rice possesses at least a part of the signal transduction pathway required for legume root nodulation. Further work has shown that species from genera *Oryza* as well as from a range of other grasses (including maize and sugarcane) possess homologues of *ENOD* early nodulation genes (Reddy et al., 1999). Moreover, rice *ENOD40* promoter activity is essentially the same as that of soybean *ENOD40*, indicating a similar role of these genes in the differentiation and/or function of nodular vascular bundles (Kouchi et al., 1999).

Symbiosis between plants and arbuscular mycorrhizal fungi

Mycorrhizal symbiosis is sensitive to the soil P status. If soils are fertilized with P, mycorrhizal symbiosis becomes ineffective (Smith et al., 1994), but also unnecessary as plants can access sufficient P. However, there are at least two situations when an improvement in mycorrhizal symbiosis may be worth considering:

(1) P-sorbing soils where only a small proportion (10-20%) of fertilizer P is available to plants, while most of fertilizer P is sorbed and complexed in soils (cf. Zhang and Rengel, 1999); and

(2) Low-input farming, mostly in developing countries, where little or no P fertilizer is used because of prohibitive costs.

Efficiency of AM symbiosis can be evaluated by considering the benefit:cost ratio, with the benefit represented by the difference in shoot dry matter between mycorrhizal and non-mycorrhizal plants under stress conditions and cost represented by the same difference under optimal conditions (Al-Karaki, 1998). Various host genotypes can be evaluated in such a way to optimise AM symbiosis under a range of environmental conditions.

There is genetic variability in the AM colonisation capacity of various genotypes of host species (e.g., bell pepper and tomato, Nemec and Datnoff, 1993; barley, Boyetchko and Tewari, 1995; grapevine, Karagiannidis et al., 1995). Wide variability also exists in populations of mycorrhizal fungi in their hyphal growth and thus competitive ability (De la Bastide et al., 1995). In addition, the genotype of the fungus is important because some degree of host specificity exists in AM fungi (Boyetschko and Tewari, 1995).

Wheat genotypes differ in the capacity to sustain AM colonisation, with the yield responses varying from zero to positive or negative values (Xavier and Germida, 1998). In durum wheat genotypes the benefit arising from the AM symbiosis is not proportional to the extent of the root colonisation because various genotypes have a various degree of dependence on mycorrhiza (Al-Karaki and Al-Raddad, 1997). Similar results were obtained for barley genotypes varying in P efficiency (Baon et al., 1993).

Using differential RNA display, Martin-Laurent et al. (1997) cloned one of the plant genes involved in early events leading to the successful colonisation of pea roots by *Glomus moseae*. Expression of that gene was independent of the rhizobial bacteria. Knowledge on genetics of the colonisation process will be instrumental in designing screening procedures and molecular markers to breed genotypes for more efficient AM symbiosis. Mutants unable to sustain mycorrhizal colonisation (e.g., in pea, Balaji et al., 1995; tomato, Barker et al., 1998) are going to be important in increasing such knowledge.

Mutants resistant to AM colonisation were introduced in pea and *Medicago truncatula* (for references see Gianinazzi-Pearson, 1996). The Myc^- mutation is recessive, genetically stable and controlled by the same single gene as Nod^- (Duc et al., 1989). The product of the wild-type alleles of Myc^- mutated loci may be involved in the biosynthesis of a plant susceptibility factor that negatively regulates the defence response (Gianinazzi-Pearson, 1996). So, without this susceptibility factor, plant root cells of Myc^- mutants have thick, reinforced cell walls loaded with defence-related molecules, thus preventing AM colonisation of such cells. In contrast to Myc^- mutation, Myc^{2-} loci may be involved in metabolic specialisation of the AM-containing cells (Gianinazzi-Pearson et al., 1995).

Clearly, it would be too dangerous to try to enhance the symbiosis by down-regulating plant defence response as susceptibility to the pathogen attack may increase. Even though Myc^- and Nod^- mutants described so far do not appear to suffer from increased susceptibility to the pathogen attack, indicating considerable specificity in the infection pattern and localisation of changes in plant defence responses of AM fungi and rhizobia as opposed to pathogens. In contrast, the fact that Myc^- mutants are also Nod^- mutants (at least in pea) indicate that there are common mechanisms regulating the plant–microbe interactions in the two symbioses (Gianinazzi-Pearson, 1996), thus raising the possibility that breeding efforts to improve one symbiosis may fortuitously result in improvements to the other.

A number of genes involved in AM or rhizobial symbioses have been cloned (see Gianinazzi-Pearson, 1996; Murphy et al., 1997). However, it would be fair to say that genes known so far represent just a tip of the iceberg of what is involved in the effective symbioses. With the expanding number of genes cloned and with insights into transcription regulation and product function, we should see that knowledge translated into breeding for better symbioses.

AM signalling

Hydrophobic components in the root exudates of P-deficient onion facilitate appressorium growth and mycorrhizal colonisation of onion plants (Tawaraya et al., 1998). Flavonoid compounds in root exudates of carrot seedlings stimulate hyphal growth of the AM fungus *Gigaspora margarita* (Poulin et al., 1993). In contrast, root exudates from pea inhibited hyphal growth of the same fungus (Balaji et al., 1995).

Polyamines may be signalling factors exuded by host roots and allowing colonisation by the AM fungi, with a positive correlation between the polyamine chain length and the fungal development (El-Ghachtouli et al., 1995). Non-host plants appear rather to lack factors necessary for stimulation of the AM colonisation than to have inhibitory factors (cf. Becard and Piche, 1990). Therefore, introduction of these stimulatory factors into non-host plants via genetic

transformation to study possibilities of extending the AM host range appears to be an appealing possibility. Flavonoids that elicit rhizobial *nod* genes also stimulate mycorrhizal colonisation (Phillips and Tsai, 1992), indicating commonality of at least some mechanisms involved in plant-microbe interactions in the two symbioses. In addition, nodulating genotype of lucerne developed AM mycorrhizae with genera *Glomus* and *Gigaspora*, but the non-nodulating and the poorly nodulating genotype did not, as the aborted appressoria could not penetrate the cortical cells of these genotypes (Bradbury et al., 1993).

As flavonoids exuded by legume seedlings represent *nod*-gene inducers (see above) and may also be stimulants for hyphal growth of the AM fungi (Kape et al., 1992; Phillips et al., 1995; Poulin et al., 1993), there is a possibility that needs to be explored about a potential stimulating effect of several common flavonoids for a range of AM and rhizobial hosts because selecting and/or transforming plants to increase exudation of these flavonoids may result in a double benefit for mycorrhizal legumes. Preliminary research has shown that flavonones and flavones that induce *nod* gene expression in rhizobia also stimulate spore germination and hyphal growth of the AM fungus *Gigaspora margarita*, probably through facilitating the contact between the AM symbionts (Gianinazzi-Pearson et al., 1989). In contrast, a mutation causing a change in the morphology of root hairs in pea plants resulted in inability to form nodules with Rhizobium or to become colonised by AM hyphae (Borisov et al., 1994).

Tripartite symbioses

Improvements in the symbiotic relationship between rhizobia and legumes may come from introducing the third partner: *Azospirillum brasilense* increased exudation of the flavonoid *nod* gene inducers into the bean rhizosphere, thus increasing nodulation by rhizobial inoculum (Burdman et al., 1996). Similarly, there was a positive interaction among cassava, diazotrophic *Azospirillum* and the AM *Glomus clarum* fungus, with root exudates from cassava stimulating mycorrhizal growth (Balota et al., 1995). N_2 fixation was enhanced by AM colonisation in comparison to non-mycorrhizal lucerne plants (Toro et al., 1998).

In the tripartite symbiosis (AM + nodule bacteria + groundnut as a host plant species), there was a significant genotype effect (Ibrahim et al., 1995), indicating a genetic variability in host capacity to sustain effective symbiosis with AM and nodule bacteria. Therefore, including AM inoculum together with the rhizobial inoculum into the groundnut breeding programme should be considered.

Helper bacteria

Helper bacteria may be involved in facilitating rhizobia nodulation of non-host plants (e.g., in canola plants infected with *B. japonicum* the presence of bacteria *Erwinia carotovora* that produce cell wall-degrading enzymes cellulase and pectinase allowed nodule formation; Katsunori et al., 1995). Experiments like that appear to have dubious practical significance (as *E. carotovora* causes plant death if present in higher numbers), but the direction of research appears promising if combinations of the non-host plant, rhizobia and helper bacteria can be found that will be suitable.

Mycorrhization helper bacteria show specificity in their action, i.e. they may support only a single host-symbiont interaction (Duponnois et al., 1993). P-solubilising bacteria (e.g., *Bacillus subtilis* or *Enterobacter* sp.) may act as mycorrhization helper bacteria when inoculated together with *Glomus intraradices* on lucerne. These P-solubilising bacteria released P from rock phosphate or indigenous plant-unavailable P sources in soils, while external AM mycelium took up that released P (Toro et al., 1997, 1998).

Conclusions

Advances in the basic knowledge about processes and gene products involved in plant–microbe interactions in the N_2 fixation and AM symbioses will be instrumental in improving these relationships. Molecular biology together with screening genotypes and classical breeding techniques will increase productivity of symbioses and eventually result in increased maximum economic yields of crop plants. Utilisation of N_2 fixation and AM symbioses described here will be extremely important in increasing the world food supplies required to feed the rapidly increasing population. Such an importance is emphasised by the fact that the area of good agricultural land is shrinking, thus forcing agriculture onto more marginal land where nutrient availability may be jeopardised by soil

chemical and physical conditions. Moreover, the expansion in food production will be required, especially in developing nations because they will experience the greatest increase in population, yet they lack resources to supply sufficient fertilizers to allow adequate crop nutrition. Thus, increasing capacity of plants to scavenge nutrients from soil as well as fix N_2 from air will be of immense importance.

References

Abd-Alla M H, Vuong T D and Harper J E 1998 Genotypic differences in dinitrogen fixation response to NaCl stress in intact and grafted soybean. Crop Sci. 38, 72–77.

Al-Karaki G N 1998 Benefit, cost and water-use efficiency of arbuscular mycorrhizal durum wheat grown under drought stress. Mycorrhiza 8, 41–45.

Al-Karaki G N and Al-Raddad A 1997 Drought stress and VA mycorrhizal fungi effects on growth and nutrient uptake of two wheat genotypes differing in drought resistance. Crop Res. (Hisar) 13, 245–257.

Anderson G C, Fillery I R P, Dunin F X, Dolling P and Asseng S 1998 Nitrogen and water flows under pasture-wheat and lupin-wheat rotations in deep sands in Western Australia. 2. Drainage and nitrate leaching. Austr. J. Agric. Res. 49, 345–361.

Andriolo J, Pereira P A A and Henson R A 1994 Variability among wild *Phaseolus vulgaris* lines for traits related to biological N_2 fixation. Pesquisa Agropecuaria Brasileira 29, 831–837.

Armero J, Lopez-Valbuena R, Jorrin J and Tena M 1994 Contribution of different plant organs to the accumulation and secretion of phenolic compounds in glutathione-treated chickpea (*Cicer arietinum*) seedlings. Acta Hortic. 381, 506–509.

Balaji B, Poulin M J, Vierheilig H and Piche Y 1995 Responses of an arbuscular mycorrhizal fungus, *Gigaspora margarita*, to exudates and volatiles from the Ri T-DNA-transformed roots of nonmycorrhizal and mycorrhizal mutants of *Pisum sativum* L. Sparkle. Exp. Mycol. 19, 275–283.

Balota E L, Lopes E S, Hungria M and Dobereiner J 1995 Interactions and physiological effects of diazotrophic bacteria and arbuscular mycorrhizas on cassava. Pesquisa Agropecuaria Brasileira 30, 1335–1345.

Baon J B, Smith S E and Alston A M 1993 Mycorrhizal responses of barley cultivars differing in P efficiency. Plant Soil 157, 97–105.

Barker S J, Stummer B, Gao L, Dispain I, O'Connor P J and Smith S E 1998 A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. Plant J. 15, 791–797.

Becard G and Piche Y 1990 Physiological factors determining vesicular-arbuscular mycorrhizal formation in host and nonhost Ri T-DNA transformed roots. Can. J. Bot. 68, 1260–1264.

Bell M J, Wright G C, Suryantini and Peoples M B 1994 The N_2-fixing capacity of peanut cultivars with differing assimilate partitioning characteristics. Aust. J. Agric. Res. 45, 1455–1468.

Bhagwat A A and Keister D L 1992 Identification and cloning of *Bradyrhizobium japonicum* genes expressed strain selectively in soil and rhizosphere. Appl. Environ. Microbiol. 58, 1490–1495.

Blumenthal J M and Russelle M P 1996 Subsoil nitrate uptake and symbiotic dinitrogen fixation by alfalfa. Agron. J. 88, 909–915.

Boivin C, Barran L R, Malpica C A and Rosenberg C 1991 Genetic analysis of a region of the *Rhizobium meliloti* pSym plasmid specifying catabolism of trigonelline, a secondary metabolite present in legumes. J. Bacteriol. 173, 2809–2817.

Bolanos-Vasquez M C and Werner D 1997 Effects of *Rhizobium tropici*, *R. etli*, and *R. leguminosarum* bv. *phaseoli* on *nod* gene-inducing flavonoids in root exudates of *Phaseolus vulgaris*. Mol. Plant-Microbe Interact. 10, 339–346.

Borisov A Y, Rozov S M, Tsyganov V E, Kulikova O A, Kolycheva A N, Yakobi L M, Ovtsyna A O and Tikhonovich I A 1994 Identification of symbiosis genes in pea (*Pisum sativum* L.) by means of induced mutagenesis. Genetika (Moskva) 30, 1484–1494.

Boyetchko S M and Tewari J P 1995 Susceptibility of barley cultivars to vesicular-arbuscular mycorrhizal fungi. Can. J. Plant Sci. 75, 269–275.

Bradbury S M, Peterson R L and Bowley S R 1993 Further evidence for a correlation between nodulation genotypes in alfalfa (*Medicago sativa* L.) and mycorrhiza formation. New Phytol. 124, 665–673.

Burdman S, Volpin H, Kigel J, Kapulnik Y and Okon Y 1996 Promotion of *nod* gene inducers and nodulation in common bean (*Phaseolus vulgaris*) roots inoculated with *Azospirillum brasilense* Cd. Appl. Environ. Microbiol. 62, 3030–3033.

Buttery B R, Park S J, Van Berkum P 1997 Effects of common bean (*Phaseolus vulgaris* L.) cultivar and rhizobium strain on plant growth, seed yield and nitrogen content. Can. J. Plant Sci. 77, 347–351.

Caba J M, Lluch C and Ligero F 1993 Genotypic differences in nitrogen assimilation in *Vicia faba*: effect of nitrate. Plant Soil 151, 167–174.

Castellanos J Z, Pena-Cabriales J J and Acosta-Gallegos J A 1996 ^{15}N-determined dinitrogen fixation capacity of common bean (*Phaseolus vulgaris*) cultivars under water stress. J. Agric. Sci. 126, 327–333.

Christiansen-Weniger C, Groneman A F and Van Veen J A 1992 Associative N_2 fixation and root exudation of organic acids from wheat cultivars of different aluminium tolerance. Plant Soil 139, 167–174.

Cooper J E, Wood M and Bjourson A J 1985 Nodulation of *Lotus peduncululatus* in acid rooting solution by fast- and slow-growing rhizobia. Soil Biol. Biochem. 17, 487–492.

Cordovilla M P, Ligero F, Lluch C and Cordovilla M P 1995 Influence of host genotypes on growth, symbiotic performance and nitrogen assimilation in faba bean (*Vicia faba* L.) under salt stress. Plant Soil 172, 289–297.

Dakora F D, Joseph C M and Phillips D A 1993 Common bean root exudates contain elevated levels of daidzein and coumestrol in response to *Rhizobium* inoculation. Mol. Plant-Microbe Interact. 6, 665–668.

Dangaria C J, Parameshwarappa R, Salimath P M and Annigeri B S 1994 Genetic divergence for nodulating characters in chickpea. Legume Research 17, 32–36.

De Chueire L M and Hungria M 1997 N_2-fixation ability of Brazilian soybean cultivars with *Sinorhizobium fredii* and *Sinorhizobium xinjiangensis*. Plant Soil 196, 1–5.

De la Bastide P Y, Kropp B R and Piche Y 1995 Population structure and mycelial phenotypic variability of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Maire) Orton. Mycorrhiza 5, 371–379.

Dobereiner J 1997 Biological nitrogen fixation in the tropics: social and economic contributions. Soil Biol. Biochem. 29, 771–774.

Dockendorff T C, Sanjuan J, Grob P and Stacey G 1994 NolA represses *nod* gene expression in *Bradyrhizobium japonicum*. Mol. Plant-Microbe Interact. 7, 596–602.

Duc G 1995 Mutagenesis of faba bean (*Vicia faba* L.) and the identification of five different genes controlling no nodulation,

ineffective nodulation or supernodulation. Euphytica 83, 147–152.

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

Duponnois R, Garbaye J, Bouchard D and Churin J L 1993 The fungus-specificity of mycorrhization helper bacteria (MHBs) used as an alternative to soil fumigation for ectomycorrhizal inoculation of bare-root Douglas-fir planting stocks with *Laccaria laccata*. Plant Soil 157, 257–262.

Eisenschenk L, Diebold R, Perez-Lesher J, Peterson A C, Peters N K and Noel K D 1994 Inhibition of *Rhizobium etli* polysaccharide mutants by *Phaseolus vulgaris* root compounds. Appl. Environ. Microbiol. 60, 3315–3322.

El-Ghachtouli N, Paynot M, Morandi D, Martin-Tanguy J and Gianinazzi S 1995 The effect of polyamines on endomycorrhizal infection of wild-type *Pisum sativum*, cv. Frisson (nod+myc+) and two mutants (nod-myc+ and nod-myc-). Mycorrhiza 5, 189–192.

El-Shanshoury A R 1995 Interactions of *Azotobacter chroococcum*,*Azospirillum brasilense* and *Streptomyces mutabilis*, in relation to their effect on wheat development. J. Agron. Crop Sci. 175, 119–127.

Espiritu B M, Lales E H and Palacpac N Q 1993 Interaction effects of Bradyrhizobium strain and cultivar in mungbean (*Vigna radiata* (L.) Wilczek). Philippine J. Biotechnol. 4, 61–68.

Evans J, Chalk P M and O'Connor G E 1995 Potential for increasing N_2 fixation of field pea through soil management and genotype. Biol. Agric. Hortic. 12, 97–112.

Fedi S, Montaini P and Favilli F 1992 Chemotactic response of *Azospirillum* toward root exudates of C_3 and C_4 plants. Symbiosis 13, 101–105.

Fesenko A N, Provorov N A, Orlova I F, Orlov V P and Simarov B V 1995 Selection of *Rhizobium leguminosarum* bv. *viceae* strains for inoculation of *Pisum sativum* L. cultivars: analysis of symbiotic efficiency and nodulation competitiveness. Plant Soil 172, 189–198.

Fobert P R, Roy N, Nash J H E and Iyer V N 1991 Procedure for obtaining efficient root nodulation of a pea cultivar by a desired *Rhizobium* strain and preempting nodulation by other strains. Appl. Environ. Microbiol. 57, 1590–1594.

Fyson A and Sprent J I 1982 The development of primary root nodules on *Vicia faba* L. grown at two temperatures. Ann. Bot. 50, 681–692.

Gagnon H and Ibrahim R K 1997 Effects of various elicitors on the accumulation and secretion of isoflavonoids in white lupin. Phytochemistry 44, 1463–1467.

Gagnon H and Ibrahim R K 1998 Aldonic acids: a novel family of nod gene inducers of *Mesorhizobium loti*, *Rhizobium lupini*, and *Sinorhizobium meliloti*. Mol. Plant-Microbe Interact. 11, 988–998.

Garcia de Salamone I E, Dobereiner J, Urquiaga S and Boddey R M 1997 Biological nitrogen fixation in *Azospirillum* strain–maize genotype associations as evaluated by the ^{15}N isotope dilution technique. Biol. Fertil. Soils 23, 249–256.

Gemell L G and Roughley R J 1993 Field evaluation in acid soils of strains of *Rhizobium leguminosarum* bv. *trifolii* selected for their tolerance or sensitivity to acid soil factors in agar medium. Soil Biol. Biochem. 25, 1447–1452.

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

Gianinazzi-Pearson V, Branzanti B and Gianinazzi S 1989 In vitro enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. Symbiosis 7, 243–255.

Gianinazzi-Pearson V, Gollote A, Lherminier J, Tisserant B, Franken P, Dumas-Gaudot E, Lemoine M C, Van Tuinen D and Gianinazzi S 1995 Cellular and molecular approaches in the characterization of symbiotic events in functional arbuscular mycorrhizal associations. Can. J. Bot. 73, S526–S532.

Godbold D L, Jentschke G, Wintr S and Marschner P 1998 Ectomycorrhizas and amelioration of metal stress in forest trees. Chemosphere 36, 757–762.

Graham P H, Viteri S E, Mackie F, Vargas A T and Palacios A 1982 Variation in acid soil tolerance among strains of *Rhiozobium phaseoli*. Field Crops Res. 5, 121–128.

Hardarson G, Bliss F A, Cigales-Rivero M R, Henson R A, Kipe-Nolt J A, Longeri L, Manrique A, Pena-Cabriales J J, Pereira P A A, Sanabria C A and Tsai S M 1993 Genotypic variation in biological nitrogen fixation by common bean. Plant Soil 152, 59–70.

Harper J E, Corrigan K A, Barbera A C and Abd-Alla M H 1997 Hypernodulation of soybean, mung bean, and hyacinth bean is controlled by a common shoot signal. Crop Sci. 37, 1242–1246.

Hartmann A, Giraud J J and Catroux G 1998 Genotypic diversity of *Sinorhizobium* (formerly *Rhizobium*) *meliloti* strains isolated directly from a soil and from nodules of alfalfa (*Medicago sativa*) grown in the same soil. FEMS Microbiol. Ecol. 25, 107–116.

Hartwig U A, Joseph C M and Phillips D A 1991 Flavonoids released naturally from alfalfa seeds enhance growth rate of *Rhizobium meliloti*. Plant Physiol. 95, 797–803.

Hernandez G, Vasquez H, Toscano V, Sanchez M, Penate-T, Franchi-Alfaro A, Mendez N and Drevon J J 1993 Nodulation and growth of common bean (*Phaseolus vulgaris* L.) cultivars in hydroponic culture and in the field. Trop. Agric. 70, 230–234.

Hernandez G, Ramirez M, Suarez R and Fuentes S I 1995 Root exuded *nod*-gene inducing signals limit the nodulation capacity of different alfalfa varieties with *Rhizobium meliloti*. Plant Cell Rep. 14, 626–629.

Heron D S, Ersek T, Krishnan H B and Pueppke S G 1989 Nodulation mutants of *Rhizobium fredii* USDA257. Mol. Plant-Microbe Interact. 2, 4–10.

Honma M A, Asomaning M and Ausubel F 1990 *Rhizobium meliloti nodD* genes mediate host-specific activation of *nodABC*. J. Bacteriol. 172, 901–911.

Howieson J G 1995 Characteristics of an ideotype acid tolerant pasture legume symbiosis in Mediterranean agriculture. Plant Soil 171, 71–76.

Howieson J G, Ewing M A and D'Anuono M F 1988 Selection for acid tolerance in *Rhizobium meliloti*. Plant Soil 105, 179–188.

Howieson J G, Robson A D and Abbott L K 1992 Acid-tolerant species of Medicago produce root exudates at low pH which induce the expression of nodulation genes in *Rhizobium meliloti*. Aust. J. Plant Physiol. 19, 287–296.

Howieson J G, Loi A and Carr S J 1995 *Biserrula pelecinus* L. — a legume pasture species with potential for acid, duplex soils which is nodulated by unique root-nodule bacteria. Aust. J. Agric. Res. 46, 997–1009.

Hungria M and Phillips D A 1993 Effects of a seed color mutation on rhizobial *nod*-gene-inducing flavonoids and nodulation in common bean. Mol. Plant-Microbe Interact. 6, 418–422.

Hungria M, Boddey L H, Santos M A and Vargas M A T 1998 Nitrogen fixation capacity and nodule occupancy by *Bradyrhizobium japonicum* and *B. elkanii* strains. Biol. Fertil. Soils 27, 393–399.

Hurek T, Tan Z, Egener T, Engelhard M, Gyaneshwar P, Ladha J K and Reinhold-Hurek B 1999 Novel nitrogen-fixing bacteria associated with the root interior of rice. *In* Proceedings of the Meeting

of the Third Working Group on Biological Nitrogen Fixation in Rice, August 9–12, 1999. IRRI, Manila, Philippines.

Ibrahim K K, Arunachalam V, Rao P S K and Tilak K V B R 1995 Seasonal response of groundnut genotypes to arbuscular mycorrhiza – *Bradyrhizobium* inoculation. Microbiol. Res. 150, 218–224.

Ikeda K, Toyota K and Kimura M 1998 Role of extracellular pectinases in the rhizoplane competence of a rhizobacterium *Burkholderia pickettii* MSP3Rif. Soil Biol. Biochem. 30, 323–329.

Jagnow G 1990 Differences between cereal crop cultivars in root-associated nitrogen fixation, possible causes of variable yield response to seed inoculation. Plant Soil 123, 255–259.

James E K 2000 Nitrogen fixation in endophytic and associative symbiosis. Field Crops Res. 65, 197–209.

Janczarek M, Urbanik-Sypniewska T and Skorupska A 1997 Effect of authentic flavonoids and the exudate of clover roots on growth rate and inducing ability of *nod* genes of *Rhizobium leguminosarum* bv. *trifolii*. Microbiol. Res. 152, 93–98.

Jimenez-Zurdo J I, Garcia-Rodriguez F M and Toro N 1997 The *Rhizobium meliloti putA* gene: its role in the establishment of the symbiotic interaction with alfalfa. Mol. Microbiol. 23, 85–93.

Kape R, Wex K, Parniske M, Gorge E, Wetzel A and Werner D 1992 Legume root metabolites and VA-mycorrhiza development. J. Plant Physiol. 141, 54–60.

Karagiannidis N, Nikolaou N and Mattheou A 1995 Influence of three VA-mycorrhiza species on the growth and nutrient uptake of three grapevine rootstocks and one table grape cultivar. Vitis 34, 85–89.

Katsunori I, Makie K, Perigio F B Jr and Shoichiro A 1995 Induction of nodule-like structures on oilseed rape by inoculation with rhizobia in the presence of helper bacteria. Soil Sci. Plant Nutr. 41, 313–320.

Keyser H H and Cregan P B 1987 Nodulation and competition for nodulation of selected soybean genotypes among *Bradyrhizobium japonicum* serogroup 123 isolates. Appl. Environ. Microbiol. 53, 2631–2635.

Kouchi H, Takane K, So R B, Ladha J K and Reddy P M 1999 Rice ENOD40: isolation and expression analysis in rice and transgenic soybean root nodules. Plant J. 18, 121–129.

Kuykendall L D 1989 Influence of *Glycine max* nodulation on the persistence in soil of a genetically marked *Bradyrhizobium japonicum* strain. Plant Soil 116, 275–277.

Ladha J K, De Bruijn F J and Malik K A (eds.) 1997 Opportunities for Biological Nitrogen Fixation in Rice and Other Non-Legumes. Kluwer Academic Publishers, Dodrecht.

Ledgard S F 1989 Nitrogen fixation and transfer to associated grasses by white clover cultivars under dairy cow grazing. *In* Proceedings of the XVI International Grassland Congress, 4–11 October 1989, Nice, France. pp. 169–170.

Leung K and Bottomley P J 1987 Influence of phosphate on the growth and nodulation characteristics of *Rhizobium trifolii*. Appl. Environ. Microbiol. 53, 2098–2105.

Leung K and Bottomley P J 1994 Growth and nodulation characteristics of subclover (*Trifolium subterraneum* L.) and *Rhizobium leguminosarum* bv. *trifolii* at different soil water potentials. Soil Biol. Biochem. 26, 805–812.

Leung K, Strain S R, De Bruijn F J and Bottomley P J 1994 Genotypic and phenotypic comparisons of chromosomal types within an indigenous soil population of *Rhizobium leguminosarum* bv. *trifolii*. Appl. Environ. Microbiol. 60, 416–426.

Lohrke S M, Orf J H, Martinez-Romero E and Sadowsky M J 1995 Host-controlled restriction of nodulation by *Bradyrhizobium japonicum* strains in serogroup 110. Appl. Environ. Microbiol. 61, 2378–2383.

Luna R and Planchon C 1995 Genotype × *Bradyrhizobium japonicum* strain interactions in dinitrogen fixation and agronomic traits of soybean (*Glycine max* L. Merr.). Euphytica 86, 127–134.

Lupwayi N Z, Haque I and Holl F B 1997 Strain-specific response of *Trifolium semipilosum* to inoculation with *Rhizobium* and the significance of waterlogging in Vertisols. J. Agric. Sci. 129, 439–446.

Martin-Laurent F, Van Tuinen D, Dumas-Gaudot E, Gianinazzi-Pearson V, Gianinazzi S and Franken P 1997 Differential display analysis of RNA accumulation in arbuscular mycorrhiza of pea and isolation of a novel symbiosis-regulated plant gene. Mol. Gen. Genet. 256, 37–44.

Maxwell C A, Hartwig U A, Joseph C M and Phillips D A 1989 A chalcone and two related flavonoids released from alfalfa roots induce *nod* genes of *Rhizobium meliloti*. Plant Physiol. 91, 842–847.

McKay I A and Djordjevic M A 1993 Production and excretion of Nod metabolites by *Rhizobium leguminosarum* bv. *trifolii* are disrupted by the same environmental factors that reduce nodulation in the field. Appl. Environ. Microbiol. 59, 3385–3392.

McKhann H I, Paiva N L, Dixon R A and Hirsch A M 1997 Chalcone synthase transcripts are detected in alfalfa root hairs following inoculation with wild-type *Rhizobium meliloti*. Mol. Plant-Microbe Interact. 10, 50–58.

McLoughlin T J, Alt S G and Merlo P A 1990 Persistence of introduced *Bradyrhizobium japonicum* strains in forming nodules in subsequent years after inoculation in Wisconsin soils. Can. J. Microbiol. 36, 794–800.

Mergaert P, Van Montagu M and Holsters M 1997 Molecular mechanisms of Nod factor diversity. Mol. Microbiol. 25, 811–817.

Moens S, Bastelaere E V, Broek A V, Lambrecht M, Keijers V, Revers L F, Passaglia L M P, Schrank I S and Vanderleyden J 1997 *Azospirillum* genes involved in chemotaxis and adhesion to plant roots. *In* Biological Fixation of Nitrogen for Ecology and Sustainable Agriculture. A Legocki, H Bothe and A Puhler (Eds.). pp. 123–127. NATO Advanced Research Workshop, Poznan, Poland.

Mullen M D, Israel D W and Wollum A G I I 1988 Effects of *Bradyrhizobium japonicum* and soybean (*Glycine max* (L.) Merr.) phosphorus nutrition on nodulation and dinitrogen fixation. Appl. Environ. Microbiol. 54, 2387–2392.

Munns N D 1970 Nodulation of *Medicago sativa* in solution culture. V. Calcium and pH requirements during infection. Plant Soil 32, 90–102.

Murphy P J, Wexler W, Grzemski W, Rao J P and Gordon D 1995 Rhizopines — their role in symbiosis and competition. Soil Biol. Biochem. 27, 525–529.

Murphy P J, Langridge P and Smith S 1997 Cloning plant genes differentially expressed during colonisation of *Hordeum vulgare* (L.) roots by the vesicular arbuscular-mycorrhizal fungus *Glomus intraradices* Schenck & Smith. New Phytol. 135, 291–301.

Natsvaladze M Y, Nitse L K and Sakharova S N 1992 Free-living and associative diazotrophs in the rhizocoenosis of tea. Izvestiya Timiryazevskoi Sel'skokhozyaistvennoi Akademii 2, 95–102.

Nemec S and Datnoff L 1993 Pepper and tomato cultivar responses to inoculation with *Glomus intraradices*. Adv. Hortic. Sci. 7, 161–164.

Neo H H, Hunt S and Layzell D B 1996 Can genotypes of soybean (*Glycine max*) selected for nitrate tolerance provide good 'models' for studying the mechanism of nitrate inhibition of nitrogenase activity? Physiol. Plant. 98, 653–660.

Nour S M, Cleyet-Marel J C, Beck D, Effosse A and Fernandes M P 1994 Genotypic and phenotypic diversity of Rhizobium isolated from chickpea (*Cicer arietinum* L.). Can. J. Microbiol. 40, 345–354.

O'Connell K P, Goodman R M and Handelsman J 1996 Engineering the rhizosphere: expressing a bias. Trends Biotechnol. 14, 83–88.

Ofosu Budu K G, Fujita K, Gamo T and Akao S 1993 Dinitrogen fixation and nitrogen release from roots of soybean cultivar Bragg and its mutants nts1116 and nts1007. Soil Sci. Plant Nutr. 39, 497–506.

Orgambide G G, Philip-Hollingsworth S, Mateos P F, Hollingsworth R I and Dazzo F B 1996 Subnanomolar concentrations of membrane chitolipooligosaccharides from *Rhizobium leguminosarum* biovar *trifolii* are fully capable of eliciting symbiosis-related responses on white clover. Plant Soil 186, 93–98.

Paffetti D, Daguin F, Fancelli S, Gnocchi S, Lippi F, Scotti C and Bazzicalupo M 1998 Influence of plant genotype on the selection of nodulating *Sinorhizobium meliloti* strains by *Medicago sativa*. Antonie van Leeuwenhoek 73, 3–8.

Pandya S and Desai A 1998 Localization of nod, nif, and acidic exopolysaccharide determinants on a large plasmid in slow-growing cowpea *Rhizobium* sp. S2. Curr. Microbiol. 36, 36–40.

Pandya S, Uchil P, Subramanian M and Desai A 1998 Determination of host specificity of cowpea miscellany Rhizobium spp. by nodABC-lacZ fusion. Curr. Microbiol. 36, 361–364.

Pazdernik D L, Graham P H and Orf J H 1997 Variation in the pattern of nitrogen accumulation and distribution in soybean. Crop Sci. 37, 1482–1486.

Phillips D A and Tsai S M 1992 Flavonoids as plant signals to rhizosphere microbes. Mycorrhiza 1, 55–58.

Phillips D A, Wery J, Joseph C M, Jones A D and Teuber L R 1995 Release of flavonoids and betaines from seeds of seven *Medicago* species. Crop Sci. 35, 805–808.

Poulin M J, Bel-Rhlid R, Piche Y and Chenevert R 1993 Flavonoids released by carrot (*Daucus carota*) seedlings stimulate hyphal development of vesicular-arbuscular mycorrhizal fungi in the presence of optimal CO_2 enrichment. J. Chem. Ecol. 19, 2317–2327.

Prayitno J, Stefaniak J, McIver J, Weinman J J, Dazzo F B, Ladha J K, Barraquio W, Yanni Y G and Rolfe B G 1999 Interactions of rice seedlings with bacteria isolated from rice roots. Aust. J. Plant Physiol. 26, 521–535.

Prevost D and Bromfield E S P 1991 Effect of low root temperature on symbiotic nitrogen fixation and competitive nodulation of *Onobrychis viciifolia* (sainfoin) by strains of arctic and temperate rhizobia. Biol. Fertil. Soil 12, 161–164.

Pueppke S G, Bolanos-Vasquez M C, Werner D, Bec-Ferte M P, Prome J C and Krishnan H B 1998 Release of flavonoids by the soybean cultivars McCall and Peking and their perception as signals by the nitrogen-fixing symbiont *Sinorhizobium fredii*. Plant Physiol. 117, 599–608.

Raffin A and Roumet P 1994 Shoot-root control of nitrate tolerance of N_2 fixation in spontaneously tolerant soybean lines: reciprocal grafting experiments. Agronomie 14, 473–480.

Raghuwanshi A, Dudeja S S and Khurana A L 1994 Effect of temperature on flavonoid production in pigeonpea (*Cajanus cajan* (L.) Millsp) in relation to nodulation. Biol. Fertil. Soils 17, 314–316.

Rai R 1991a Strain-specific salt tolerance and chemotaxis of *Azospirillum brasilense* and their associative N-fixation with finger millet in saline calcareous soil. Plant Soil 137, 55–59.

Rai R 1991b Effects of soil acidity factors on interaction of chickpea (*Cicer arietinum* L.) genotypes and Rhizobium strains: symbiotic N-fixation, grain quality and grain yield in acid soils. *In* Plant-Soil Interactions at Low pH. R J Wright, V C Baligar and R P Murrmann (Eds.). pp. 619–631. Kluwer Academic Publishers, Dordrecht.

Rai R 1992 Effect of acidity factors on aspects of symbiotic N_2 fixation of *Lens culinaris* in acid soils. J. Gen. Appl. Microbiol. 38, 391–406.

Ramanathan N, Ramamurthy R and Balasubramanian V 1997 Nitrogenase activity and *Azospirillum* population in the rhizosphere of rice cultivars. Indian J. Microbiol. 37, 211–212.

Rao V R, Ramakrishnan B, Adhya T K, Kanungo P K and Nayak D N 1998 Review: current status and future prospects of associative nitrogen fixation in rice. World J. Microbiol. Biotechnol. 14, 621–633.

Reddy P M, Aggarwal R K, Ramos M C, Ladha J K, Brar D S and Kouchi H 1999 Widespread occurrence of the homologues of the early nodulin (ENOD) genes in *Oryza* species and related grasses. Biochem. Biophys. Res. Commun. 258, 148–154.

Reddy P M, Ladha J K, So R B, Hernandez R J, Ramos M C, Angeles O R, Dazzo F B and De Bruijn F J 1997 Rhizobial communication with rice roots: induction of phenotypic changes, mode of invasion and extent of colonization. Plant Soil 194, 81–98.

Reddy P M, Ladha J K, Ramos M C, Maillet F, Hernandez R J, Torrizo L B, Oliva N P, Datta S K and Datta K 1998 Rhizobial lipochitooligosaccharide nodulation factors activate expression of the legume early nodulin gene *ENOD12* in rice. Plant J. 14, 693–702.

Rolfe B G, Djordjevic M A, Weinman J J, McIver J, Gärtner E, Chen C, Creaser E H, Britt K, Lawson C G R, De Boer M H, McKay I A, Shoobridge M V, De Majnik J, Pittock C, Broderick K and Delbridge T 1995 Molecular genetic analysis of subterranean clover-microbe interactions. Soil Biol. Biochem. 27, 485–490.

Rolfe B G, Djordjevic M A, Weinman J J, Mathesius U, Pittock C, Gärtner E, Ride K M, Dong Z M, McCully M and McIver J 1997 Root morphogenesis in legumes and cereals and the effect of bacterial inoculation on root development. Plant Soil 194, 131–144.

Rosenblueth M, Hynes M F and Martinez-Romero E 1998 *Rhizobium tropici teu* genes involved in specific uptake of *Phaseolus vulgaris* bean-exudate compounds. Mol. Gen. Genet. 258, 587–598.

Rossbach S, Kulpa D A, Rossbach U and De Bruijn F J 1994 Molecular and genetic characterization of the rhizopine catabolism (*mocABRC*) genes of *Rhizobium meliloti* L5-30. Mol. Gen. Genet. 245, 11–24.

Sanginga N, Danso S K A and Bowen G D 1992 Variation in growth, sources of nitrogen and N-use efficiency by provenances of *Gliricidia sepium*. Soil Biol. Biochem. 24, 1021–1026.

Sanjuan J and Olivares J 1991 Nifa-NtrA regulatory system activates transcription of *nfe*, a gene locus involved in nodulation competitiveness of *Rhizobium meliloti*. Arch. Microbiol. 155, 543–548.

Sattar M A, Quader M A and Danso S K A 1995 Nodulation, N_2 fixation and yield of chickpea as influenced by host cultivar and *Bradyrhizobium* strain differences. Soil Biol. Biochem. 27, 725–727.

Schloter M and Hartmann A 1998 Endophytic and surface colonization of wheat roots (*Triticum aestivum*) by different *Azospirillum brasilense* strains studied with strain-specific monoclonal antibodies. Symbiosis 25, 159–179.

Schmidt P E, Parniske M and Werner D 1992 Production of the phytoalexin glyceollin I by soybean roots in response to symbiotic and pathogenic infection. Bot. Acta 105, 18–25.

Schmidt P E, Broughton W J and Werner D 1994 Nod factors of *Bradyrhizobium japonicum* and *Rhizobium sp*. NGR234 induce flavonoid accumulation in soybean root exudate. Mol. Plant-Microbe Interact. 7, 384–390.

Serraj R and Sinclair T R 1998 Soybean cultivar variability for nodule formation and growth under drought. Plant Soil 202, 159–166.

Shelp B J, Kaiser B N and Deschesne A M 1998 Registration of five near-isogenic genetic stocks of 'Juneau' pea with altered nodulation and nitrate reductase deficiency: A317I, nod3I, A317nod3I, E135I, and R25I. Crop Sci. 38, 554.

Shrestha R K and Ladha J K 1996 Genotypic variation in promotion of rice dinitrogen fixation as determined by nitrogen-15 dilution. Soil Sci. Soc. Am. J. 60, 1815–1821.

Smith S E, Gianinazzi-Pearson V, Koide R and Cairney J W G 1994 Nutrient transport in mycorrhizas: structure, physiology and consequences for efficiency of the symbiosis. Plant Soil 159, 103–113.

Soejima H, Sugiyama T and Ishihara K 1992 Changes in cytokinin activities and mass spectrometric analysis of cytokinins in root exudates of rice plants (*Oryza sativa* L.). Comparison between cultivars Nipponbare and Akenohoshi. Plant Physiol. 100, 1724–1729.

Song L, Carroll B J, Gresshoff P M and Herridge D F 1993 Field assessment of supernodulating genotypes of soybean for yield, N_2 fixation and benefit to subsequent crops. Soil Biol. Biochem. 27, 563–569.

Spiller H, Stallings W J, Woods T and Gunasekaran M 1993 Requirement for direct association of ammonia-excreting *Anabaena variabilis* mutant (SA-1) with roots for maximal growth and yield of wheat. Appl. Microbiol. Biotechnol. 40, 557–566.

Tagu D and Martin F 1996 Molecular analysis of cell wall proteins expressed during the early steps of ectomycorrhiza development. New Phytol. 133, 73–85.

Tawaraya K, Hashimoto K and Wagatsuma T 1998 Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonisation by the arbuscular mycorrhizal fungus *Gigaspora margarita*. Mycorrhiza 8, 67–70.

Tiwari R P, Reeve W G, Glenn A R 1992 Mutations conferring acid sensitivity in the acid-tolerant strains *Rhizobium meliloti* WSM419 and *Rhizobium leguminosarum* biovar *viciae* WSM710. FEMS Microbiol. Lett. 100, 107–112.

Toro M, Azcon R and Barea J M 1997 Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability and nutrient cycling. Appl. Environ. Microbiol. 63, 4408–4412.

Toro M, Azcon R and Barea J M 1998 The use of isotopic dilution techniques to evaluate the interactive effects of *Rhizobium* genotype, mycorrhizal fungi, phosphate-solubilizing rhizobacteria and rock phosphate on nitrogen and phosphorus acquisition by *Medicago sativa*. New Phytol. 138, 265–273.

Trese A T 1995 A single dominant gene in McCall soybean prevents effective nodulation with *Rhizobium fredii* USDA257. Euphytica 81, 279–282.

Tsyganov V E, Morzhina E V, Stefanov S Y, Borisov A Y, Lebsky V K and Tikhonovich I A 1998 The pea (*Pisum sativum* L.) genes *sym33* and *sym40* control infection thread formation and root nodule function. Mol. Gen. Genet. 295, 491–503.

Utrup L J, Cary A J and Norris J H 1993 Five nodulation mutants of white sweetclover (*Melilotus alba* Desr.) exhibit distinct phenotypes blocked at root hair curling, infection thread development, and nodule organogenesis. Plant Physiol. 103, 925–932.

Van Rhijn P and Vanderleyen J 1995 The *Rhizobium*-plant symbiosis. Microbiol. Rev. 59, 124-142.

Van Rhijn P, Desair J, Vlassak K and Vanderleyden J 1994 Functional analysis of *nodD* genes of *Rhizobium tropici* CIAT899. Mol. Plant-Microbe Interact. 7, 666–677.

Vlassak K M and Vanderleyden J 1997 Factors influencing nodule occupancy by inoculant rhizobia. Crit. Rev. Plant Sci. 16, 163–229.

Waschutza S, Hofmann N, Niemann E G and Fendrik I 1992 Investigations on root exudates of Korean rice. Symbiosis 13, 181–189.

Wood M, Cooper J E and Holding A J 1984 Soil acidity factors and nodulation of *Trifolium repens*. Plant Soil 78, 367–79.

Wojtaszek P, Stobiecki M and Gulewicz K 1993 Role of nitrogen and plant growth regulators in the exudation and accumulation of isoflavonoids by roots of intact white lupin (*Lupinus albus* L.) plants. J. Plant Physiol. 142, 689–694.

Wu P, Zhang G, Ladha J K, McCouch S R and Huang N 1995 Molecular-marker-facilitated investigation on the ability to stimulate N_2 fixation in the rhizosphere by irrigated rice plants. Theor. Appl. Genet. 91, 1177–1183.

Xavier L J C and Germida J J 1998 Response of spring wheat cultivars to *Glomus clarum* NT4 in a P-deficient soil containing arbuscular mycorrhizal fungi. Can. J. Soil Sci. 78, 481–484.

Yanni Y G, Rizk R Y, Corich V, Squartini A, Ninke K, Philip-Hollingsworth S, Orgambide G, De Bruijn F, Stoltzfus J, Buckley D, Schmidt T M, Mateos P F, Ladha J K and Dazzo F B 1997 Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. Plant Soil 194, 99–114.

Zhang X-K and Rengel Z 1999 Gradients of pH and ammonium and phosphorus concentration between the banded fertilizer and wheat roots. Aust. J. Agric. Res. 50, 365-373.

Zhang F, Dijak M, Smith D L, Lin J, Walsh K, Voldeng H, Macdowell F and Layzell D B 1997 Nitrogen fixation and nitrate metabolism for growth of six diverse soybean (*Glycine max* (L.) Merr.) genotypes under low temperature stress. Environ. Exp. Bot. 38, 49–60.

Zuanazzi J A S, Clergeot P H, Quirion J C, Husson H P, Kondorosi A and Ratet P 1998 Production of *Sinorhizobium meliloti* nod gene activator and repressor flavonoids from *Medicago sativa* roots. Mol. Plant-Microbe Interact. 11, 784–794.

Zurayk R, Adlan M, Baalbaki R and Saxena M C 1998 Interactive effects of salinity and biological nitrogen fixation on chickpea (*Cicer arietinum* L.) growth. J. Agron. Crop Sci. 180, 249–258.