Mycorrhiza (2000) 10:101–106

ORIGINAL PAPER

P.C. Shrihari · K. Sakamoto · K. Inubushi · S. Akao

Interaction between supernodulating or non-nodulating mutants of soybean and two arbuscular mycorrhizal fungi

Accepted: 7 July 2000

Abstract Twelve nodulation mutants (seven non-nodulating and five supernodulating) of soybean [*Glycine max* (L.) Mirr.] were screened for arbuscular mycorrhizal colonization in the presence of either *Glomus etunicatum* Becker and Gerdemann or *Gigaspora margarita* Becker and Hall. The cultivars showed variation in colonization parameters. The two supernodulating mutants En6500 and NOD1–3 had higher frequencies of colonization with 2.5–4.5 times higher arbuscular abundance than the respective wild types. The enhanced mycorrhization resulted in significant enhancement of P uptake by En6500. The non-nodulating mutants showed decreases in mycorrhizal parameters. Mutants En1282 and Harosoy^{-} exhibited aborted infection after formation of typical appressorium-like structures at some sites. However, none of these had the non-mycorrhizal phenotype. Growth and nutrient-uptake parameters should be considered while studying plant mutants for mycorrhization.

Keywords Arbuscular mycorrhiza · Mycorrhizal inoculation effect · Nodulation mutants · Soybean · Supernodulation

Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with more than 80% of plant species examined, including many crop plants. Mycorrhizal plants are known to absorb more nutrients and acquire disease resistance (Srivastava et al. 1996). Successful symbiosis involves a series of steps from host recognition to the formation of functional arbuscules, with a complex molecular dialogue between the symbiotic partners. Despite the complexity and obligate symbiotic nature of the fungus, significant attempts have been made in the recent past to unravel the secrets of the AM symbiosis by developing mycorrhiza-defective plant mutants and molecular cytological techniques (Gianinazzi-Pearson et al. 1995; Gianinazzi-Pearson 1996).A range of host-encoded proteins, including defense-related enzymes, are induced during mycorrhization (Gianinazzi-Pearson et al. 1999). This indicates that plant genes controlling fungal proliferation in roots are activated in response to fungal infection. The discovery of genetic resistance to AM formation in some spontaneous or chemically induced non-nodulating mutants of pea (*Pisum sativum* L.) and fababean (*Vicia faba* L.) opened a new dimension in identifying the host genes involved in endomycorrhizal symbiosis (Duc et al. 1989; Gianinazzi-Pearson et al. 1991). In these mutants, fungal growth is arrested either at the stage of appressorium formation (Myc^{-1}) or after development of intraradical defective hypha before arbuscule differentiation (Myc^{-2}). This suggests a common mechanism in plant control over some step(s) in endomycorrhiza and nodule symbiosis. T-DNA insertion mutagenesis of *Lotus japonicus* yielded mutants at loci *sym2*, *sym3* and *sym4* defective for nodulation and mycorrhizal colonization (Wegel et al. 1998). Other studies provided further evidence of the genetic relationship between nodulation and mycorrhization (Peterson and Bradbury 1999). Contrary to these findings, Wyss et al. (1990a) reported that a Nod^{-} mutant of soybean [*Glycine max* (L.) Merr. cv. Bragg.] was colonized by *Glomus mosseae* to the same extent as wild-type (Nod^{+}) soybean. However, it has been proposed that the grid-line intersection assessment method (Giovannetti and Mosse 1980) used by these researchers resulted in overestimation of root colonization and did not distinguish between fungal structures formed (Bradbury et al.1991).

P.C. Shrihari · K. Sakamoto (✉) · K. Inubushi
Faculty of Horticulture, Chiba University, Matsudo, Chiba, 271-8510, Japan
e-mail: sakamoto@midori.h.chiba-u.ac.jp
Fax: +81-47-3088817

S. Akao
Department of Plant Physiology, National Institute of Agrobiological Resources, Tsukuba, Ibaraki, 305-8602, Japan

Supernodulating (Nod^{++}) mutants of soybean have been isolated from several cultivars following chemical mutagenesis (Jacobsen and Feentsra 1984; Carroll et al. 1985). These lines exhibit profuse nodulation compared with the parent lines and some are nitrate tolerant. These are also considered to have a higher agronomic potential and may be interesting models to study endomycorrhizal association in relation to phosphate.

In this present study, we screened various non-nodulating and supernodulating mutants of soybean [*Glycine max* (L.) Merr.] for mycorrhization to examine the correlation between extent of nodulation and degree of mycorrhiza formation. Among the supernodulating mutants screened in this study, En6500 nodulates at high nitrate concentrations (Francisco et al. 1992), while NOD mutant lines show partial nitrate tolerance. Certain Nod$^-$ mutants of legumes form nodules in the presence of different *Rhizobium* populations (Lie and Timmermans 1983). Therefore, we used two fungal inocula, *Gigaspora margarita* Becker and Hall and *Glomus etunicatum* Becker and Gerdemann, to test whether the variation, if any, in mycorrhization phenotype among the nodulation mutants is horizontal. The method proposed by Trouvelot et al. (1986) was followed to estimate colonization parameters, including both the amount of colonization and arbuscular abundance. In most of the earlier works, only the colonization parameters of Nod$^-$ mutants were studied (Duc et al. 1989; Gianinazzi-Pearson et al. 1991; Bradbury et al.1991, 1993a). We also examined mycorrhizal inoculation effects on P uptake and dry weight of plants as well as mycorrhizal parameters in selected non-nodulating and supernodulating mutants. We further hoped to identify mutants showing normal mycorrhizal colonization but inefficient in P uptake (Myc$^+$eff$^-$).

Materials and methods

Plant material

Soybean lines of seven non-nodulating and five supernodulating mutants were screened for mycorrhization (Table 1). Of these, En1282 and En6500 were induced by ethyl methanesulfonate (EMS) mutagenesis of cv. Enrei. All the nodulation mutants (NOD1–3, NOD3–7, NOD4, NOD2–4 and NN5) of cv. Williams were received from Prof. James Harper, University of Illinois, USA. The supernodulating lines NOD1–3, NOD3–7 and NOD4 and the non-nodulating line NN5 were obtained after mutation with *N*-nitroso-*N*-methylurea. A further supernodulating mutant NOD2–4 was obtained by EMS treatment. All other nodulation mutants were received from the National Agriculture Research Center and National Institute of Agrobiological Resources, Tsukuba, Japan. Of these, T201 was a natural mutant of T202 obtained at the US Regional Soybean Laboratory in 1947. A62–2 and To 1–0 resulted from the combination of T201 × A62–1 and T201 × Toiku106, respectively. The isogenic lines of Clark$^-$ and Harosoy$^-$ were developed from a backcross of Clark and Harosoy to the donor parent T201.

AM inocula

Pot cultures of *Glomus etunicatum* Becker and Gerdemann were obtained after mass multiplying under soybean grown in steam-sterilized sand. Plants were grown for 75 days and the above-ground portion was removed. Sand in the pot containing chopped roots and mycorrhizal propagules was air-dried under shade and stored at 4 °C. The inoculum of *Gigaspora margarita* Becker and Hall was obtained from the Central Glass Co. Ltd., Japan. Spores of *Glomus etunicatum* and *Gigaspora margarita* were extracted by wet sieving and decanting (Gerdemann and Nicolson 1963).

Table 1 Development of mycorrhizal colonization in the roots of various nodulation mutants of soybean inoculated with *Gigaspora margarita.* The results are the means of four replicates. Values within a column with the same letters do not differ significantly at $P = 0.05$

Host plant genotype	Nodulation phenotype	Frequency of colonization (F%)	Intensity of colonization (M%)	Arbuscular abundance (A%)
Enrei	Nod+	83.9 ae	36.4 f	14.3 cde
En1282	Nod-	72.2 bcd	23.0 ade	1.6 f
En6500	Nod++	96.2 f	49.7 g	36.1 g
Clark+	Nod+	83.2 abe	25.6 ab	10.2 abce
Clark–	Nod-	79.8 acd	24.6 a	9.1 abc
Harosoy+	Nod+	80.8 abcd	27.8 ab	17.6 d
Harosoy–	Nod-	69.5 d	15.3 c	1.4 f
A62–1	Nod+	84.0 ae	33.2 bf	15.7 de
A62–2	Nod-	71.6 cd	23.6 ad	8.9 ab
T202	Nod+	82.8 ab	25.2 ab	16.0 d
T201	Nod-	81.3 abc	23.6 ad	16.6 d
To 1–1	Nod+	77.2 abcd	29.3 abf	12.4 bcde
To 1–0	Nod-	87.0 ae	28.0 ab	7.0 a
Williams	Nod+	79.7 abcd	16.0 ce	7.1 a
NN5	Nod-	78.7 abcd	15.4 c	6.2 ab
Nod4	Nod++	80.8 abcd	15.3 c	7.8 ab
Nod1–3	Nod++	91.9 ef	49.8 g	31.7 g
Nod2–4	Nod++	83.3 abe	24.0 d	8.9 abc
Nod3–7	Nod++	81.3 abc	17.2 cde	9.4 abc

Screening experiment

Pots of 1-l capacity were filled with a steam-sterilized sand and soil (1:1) mixture. The soil used was an Andosol (pH 6.04, Olsen's P 0.06 mg kg^{-1} soil, total N 2.2 g kg^{-1} soil). The sand-soil mixture in each pot was amended with N (9 mg), P (39.4 mg), K (59.9 mg) and Mg (18.6 mg) in the form of $(NH_4)_2SO_4$, KH_2PO_4 and $MgSO_4$. After sowing seeds of nodulation mutants and the respective wild types, pots were kept in the glasshouse under natural conditions (day temperature 26–31 °C, night temperature 16–21 °C, day length 14–16 h and humidity 80–85%) and watered regularly. Six days after sowing, 100 spores of either *Glomus etunicatum* or *Gigaspora margarita* were inoculated to each pot. Four replicates were maintained for each treatment. After 7 weeks, plants were uprooted carefully and the roots were washed with tap water. Root samples (<2 mm diameter) representing the whole root system were collected and stained with trypan blue (Phillips and Hayman 1970). After staining, 90 root bits were selected randomly and observed under the light microscope to determine the frequency of mycorrhizal colonization (F%), relative infection intensity (M%) and relative arbuscule formation in the whole root system (A%) (Trouvelot et al.1986). The percent data were arcsine transformed and analyzed by Tukey's test (WinSTAT version 3.1) at $P = 0.05$.

Mycorrhizal inoculation effect

Based on the results of the screening experiment, the two supernodulating mutants En6500 and NOD1-3 showing the highest my-

corrhizal parameters were examined for mycorrhizal inoculation effect (MIE) on dry weight and P uptake. Of the Nod$^-$ mutants, NN5 and T-201, which had the same level of colonization as their wild types (Williams and T-202), and En1282 and Harosoy$^-$, with the lowest arbuscular abundance, were also included. Plants were grown in the presence or absence of *Gigaspora margarita* as described in the screening experiment, except that the pots used were of 3-l capacity. Inoculated and uninoculated pots were kept in pairs and three replicates were maintained for each treatment. Plants were uprooted after 9 weeks and the roots were washed and plant weight recorded after drying at 70 °C for 24 h. MIE on plant dry weight was calculated from:

$$\text{MIE on plant dry weight (\%)} = \frac{\text{Dry weight of inoculated plant} - \text{Dry weight of control plant}}{\text{Dry weight of control plant}} \times 100$$

Plant P was estimated using the Kitson and Mellon method (Chapman and Pratt 1961). MIE on P uptake was calculated by considering plant P uptake in inoculated and uninoculated control plants, which were maintained in pairs. The percent data were arcsine transformed and analyzed by Tukey's test at $P = 0.05$.

Results

Typical AM extra-metrical (hypha, auxiliary spores and chlamydospores) and intraradical (hypha and arbuscules) structures were produced by *Gigaspora margarita* mainly in the outer cortical cells. However, the colonization by *Glomus etunicatum* also spread to the internal cortical cells, with the production of typical hypha, globular vesicles and arbuscules. The colonization parameters varied between the different wild types.

Table 2 Development of mycorrhizal colonization in the roots of various nodulation mutants of soybean inoculated with *Glomus etunicatum.* The results are the means of four replicates. Values within a column with the same letters do not differ significantly at $P = 0.05$

Host plant genotype	Frequency of colonization (F%)	Intensity of colonization (M%)	Arbuscular abundance (A%)
Enrei	88.5 bcd	38.1 e	12.1 bcde
En1282	83.4 abcd	31.9 bcde	2.3 fg
En6500	97.6 f	66.8 k	47.2 I
Clark+	83.2 abcd	23.2 abhi	9.4 abce
Clark–	77.2 a	20.5 ghi	7.1 ab
Harosoy+	83.0 abc	34.2 de	15.9 de
Harosoy–	63.4 e	12.1 f	1.3 f
A62-1	81.1 abc	30.7 abcde	16.3 d
A62-2	74.0 ae	24.1abchi	9.0 abc
T-202	81.5 abc	29.6 abcde	18.1 d
T-201	79.2 ab	27.6 abcdi	17.1 d
To 1-1	81.8 abc	32.2 abcde	14.2 cde
To 1-0	89.5 cd	32.0 cde	5.4 ag
Williams	79.8 ab	14.9 fg	6.3 ab
NN5	78.7 ab	13.7 bcde	6.0 a
NOD4	80.8 abc	16.6 fgh	9.3 abc
NOD1-3	91.9 d	50.6 j	34.8 h
NOD2-4	83.3 abcd	23.0 ahi	7.8 ab
NOD3-7	81.3 abc	14.5 fg	8.3 abc

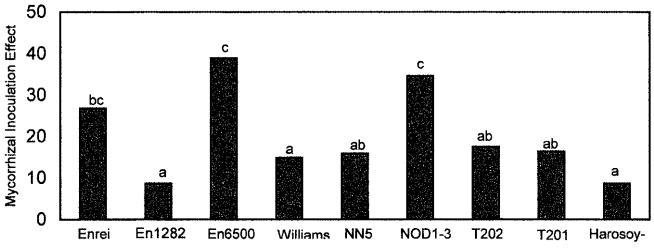

Fig. 1 Mycorrhizal inoculation effect of *Gigaspora margarita* on the plant dry weight of soybean mutants and wild types. The results represent the means of three replicates. *Bars* with the same letter are not significantly different at $P = 0.05$

The supernodulating mutants En6500 and NOD1–3 had significantly higher F%, M% and A% than their wild types (Tables 1,2). Heavy colonization was found in these two mutants throughout the observed root length with an abundant of vesicles and intraradical hypha. In particular, the arbuscular abundance was 2.5–4.5 times higher than in the respective wild types. *Gigaspora margarita* formed arbuscules even in the internal cortical cells of NOD1–3, while *Glomus etunicatum* formed slightly larger vesicles in both En6500 and NOD1–3. These mutants are referred to as Myc^{++} in analogy to the Nod^{++} for supernodulation. Further study revealed that only NOD1–3 had a significantly higher MIE on plant dry weight than its wild type (Fig. 1). The MIE on P uptake by *Gigaspora margarita* was enhanced significantly in En6500 (Fig. 2). None of the other supernodulating mutants showed a significant increase in mycorrhizal colonization.

All the Nod$^-$ mutants developed typical mycorrhizal structures, including well-differentiated arbuscules and vesicles. En1282 and Harosoy$^-$ had the lowest colonization parameters. The hypha that entered the roots of En1282 and Harosoy$^-$ did not proliferate after formation of the typical appressoria at many places. The size and shape of the appressoria varied throughout the root length. In En1282, the appressoria differentiated into intraradical hypha without forming arbuscules at some sites. However, well-differentiated vesicles and arbuscules were also found in En1282 and Harosoy$^-$ roots to a considerable extent. These mutants showed a

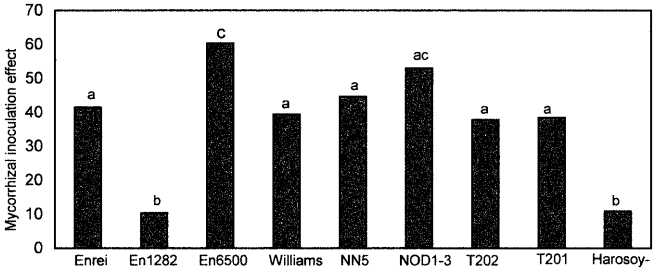

Fig. 2 Mycorrhizal inoculation effect of *Gigaspora margarita* on the uptake of P by soybean mutants and wild types. The results represent the means of three replicates. *Bars* with the same letter are not significantly different at $P = 0.05$

negative response to inoculation with *Gigaspora margarita* in terms of MIE on P uptake and plant dry weight (Figs. 1,2). All the Nod$^-$ mutants, except To 1–0, had lower colonization parameters. However, Clark$^-$, T201 and NN5 did not differ significantly from their wild types. The latter two mutants were on a par with T202 and Williams, respectively, in MIE on dry weight and P uptake. The non-nodulating mutant To 1–0 had a higher frequency of colonization with a lower arbuscular abundance than its wild type.

Discussion

Although AM fungi are ubiquitous in nature, plant genotypes show an inter- or intra-specific preference for a particular endophyte (Peterson and Bradbury 1999). Significant differences between plant genotypes in the formation of various intraradical structures have also been reported (Toth et al. 1984; Bradbury et al. 1993a). In our study, variation in colonization parameters was observed between the cultivars screened. The AM efficiency of wheat plants has been reported to be governed by the host chromosomal genes with a cytoplasmic effect (Manske 1994).

In our study, the two mutants En6500 and NOD1–3 had a 2.5–4.5 times higher A% than their respective wild types. However, MIE on P uptake was not enhanced significantly in NOD1–3 when compared to its wild type. This suggests that the arbuscules formed in this mutant are not all efficiently involved in P nutrition. The significant increase in MIE on plant dry weight of NOD1–3 may be due to higher uptake of nutrients other than P. Wyss et al. (1990b) did not find enhanced mycorrhization in a nitrate-tolerant supernodulating soybean mutant of cv. Bragg. On the other hand, an excess of available P reduced the mycorrhizal colonization of these mutants, which indicates that the nitrate control of nodulation and the P control of mycorrhization were mediated by different mechanisms. It is worth noting that these researchers measured mycorrhizal root length and did not consider other mycorrhizal parameters such as arbuscular abundance. Previous results from grafting experiments showed that the inability of either Nod$^-$ mutants to nodulate (Duc and Messager 1989) or Myc$^-$ lines to show mycorrhizal colonization (Vierheilig and Piche 1996) was governed by the root genotype. In contrast, the character of supernodulation in various mutants, including En6500, was reported to be determined by shoot factors (Delves et al. 1987; Hamaguchi et al. 1992). Therefore, we suggest that Myc^{++} is a shoot-controlled character. Grafting of Myc^{++} mutant and normal wild type (Myc$^+$) would help define this phenomenon. Furthermore, there is evidence for the expression of symbiosis-related genes during endomycorrhizal development with the synthesis of new proteins (endomycorrhizins) that appear to be host encoded (Dumas et al. 1989). A similar investigation of supernodulation mutants showing high colonization parameters might possibly reveal the induction of new proteins not expressed or expressed at a lower level in the wild type. The nodulation process in soybeans is autoregulated (Pierce and Bauer 1983). Supernodulating mutants are considered to lose or show a reduced ability to control the rhizobial infection process that ultimately leads to the formation of more nodules. Similarly during AM symbiosis, the flow of carbon from the host (Anderson 1988) and a plant defense mechanism (Gianinazzi-Pearson et al. 1996) may regulate proliferation of the fungus. The Myc^{++} mutants reported in our study may differ from their wild types in the autoregulation of the colonization process. Notably, NOD3–7, which shares a common point mutation in the single locus *rj7* with NOD1–3 and En6500 (Tri et al. 1996), did not show enhanced mycorrhization.

We found that none of the Nod$^-$ mutants was Myc$^-$. Furthermore, two of the mutants, T-201 and NN5, did not differ significantly either in colonization parameters or in MIE on P uptake from their respective wild types. Other researchers (Kawai and Yamamoto 1986; Heckman and Angle 1987; Wyss et al. 1990a) also reported normal colonization in Nod$^-$ mutants of soybean. Further, the Myc$^-$ phenotype does not always coincide with either the Nod$^-$ fix$^-$ or Nod$^+$ fix$^-$ characters (Duc et al.1989; Bradbury et al. 1993a; Balaji et al. 1994). However, considering similarities between nodulation and mycorrhization in molecular and cellular events, there is growing evidence that common elements are involved in the process of infection during both types of symbiosis (Gollotte et al. 1996). In the work of Wyss et al. (1990a), symbiosis-specific polypeptides induced in the endomycorrhizal roots of Nod$^-$ mutants and the wild type of soybean were reported to be immunologically crossreactive with nodulins. This indicates that symbiosis-specific polypeptides, possibly identical with nodulins, may be induced during mycorrhization. Furthermore, Gianinazzi-Pearson et al. (1991) reported that the expression of Myc$^-$ and Nod$^-$could not be dissociated in pea and appeared to be the result of pleiotropic effects of a single gene. Plant growth conditions such as light intensity may govern the ability of Nod$^-$ mutant to exhibit the Myc$^-$ phenotype (Bradbury et al. 1993b). Mutation of plant genes involved in common physiological processes such as sugar synthesis or transport may result in such unstable mutations (Gianinazzi-Pearson 1996).

In En1282 and Harosoy$^-$, fungal growth was arrested after the formation of irregular appressoria at many sites and arbuscular formation was reduced drastically. The wild-type alleles of this mutated loci from En1282 and Harosoy$^-$ may be involved in down-regulation of defense-related responses or the production of plant susceptibility factors during successful AM colonization (Gianinazzi-Pearson 1996). Duc et al. (1989) attributed the formation of arbuscules at very low frequency in Myc$^-$ roots to breakdown of resistance to mycorrhizal colonization. We did not classify En1282

and Harosoy^{-} as Myc^{-} because not only were arbuscules formed to a considerable extent but these Nod^{-} mutants also showed an MIE on dry weight and P uptake (Figs. 1,2). We did not find any mycorrhizal-inefficient mutants (Myc^{+}eff^{-}).

Our study stresses the need to screen further Nod^{-} mutants for mycorrhization in other legumes where the Myc^{-} character has not yet been reported. Obtaining Myc^{-} mutants in non-legumes (Barker et al. 1998) has helped identify and clone plant genes responsible for endomycorrhizal symbiosis other than those present in legumes. Consideration of growth and nutrient uptake parameters while studying mycorrhization of plant mutants is important for gathering meaningful information. Further study of the P-tolerance and the physiological basis of the enhanced mycorrhization in NOD 1–3 and En6500 may deepen our understanding of the host-fungi relationship.

Acknowledgements We thank Dr. Gianinazzi-Pearson, Laboratoire de Phytoparasitologie, INRA-CNRS, France for providing full information on the Trouvelot method. We are also grateful to Mr. Takahashi, National Agricultural Research Center, Tsukuba, Japan and Professor J. A. Harper, University of Illinois, USA for providing seed material and to the Central Glass Co. Ltd., Tokyo, Japan for the inoculum of *Gigaspora margarita*.

References

Anderson AJ (1988) Mycorrhiza-host specificity and recognition. Phytopathology 78:375–378

Balaji B, Ba AM, LaRue TA, Tepfer D, Piche Y (1994) *Pisum sativum* mutants insensitive to nodulation are also insensitive to invasion in vitro by the mycorrhizal fungus *Gigaspora margarita*. Plant Sci 102:195–203

Barker SJ, Stummer B, Gao L, Dispain I, O'Connor PJ, Smith SE (1998) A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterization. Plant J 15:791–797

Bradbury SM, Peterson RL, Bowley SR (1991) Interaction between three alfalfa nodulation genotypes and *Glomus* species. New Phytol 119:115–120

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

Bradbury SM, Peterson RL, Bowley SR (1993b) Colonization of three alfalfa (*Medicago sativa* L.) nodulation genotypes by indigenous vesicular-arbuscular mycorrhizal fungi from soil. Symbiosis 15:207–215

Carroll BJ, McNeil DL, Gresshoff PM (1985) A supernodulation and nitrate-tolerant symbiotic (*nts*) soybean mutant. Plant Physiol 78:34–40

Chapman HD, Pratt PF (1961) Methods of analysis for soils, plants and water. University of California, Riverside, pp 161–174

Delves A, Higgins A, Gresshoff PM (1987) Supernodulation in interspecific grafts between *Glycine max* (soybean) and *Glycine soja*. J Plant Physiol 128:473–478

Duc G, Messanger A (1989) Mutagenesis of pea (*Pisum sativum* L.) and the isolation of mutants for nodulation and nitrogen fixation. Plant Sci 60:207–213

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

Dumas E, Gianinazzi-Pearson V, Gianinazzi S (1989) Production of new soluble proteins during VA endomycorrhiza formation. Agric Ecosyst Environ 29:111–114

Francisco PB Jr, Akao S, Kokubun M (1992) Irradiance and nitrate effects on growth and symbiotic parameters of supernodulating and nitrate-tolerant soybean mutant En6500 and its parent cultivar Enrei. J Plant Physiol 140:453–459

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244

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

Gianinazzi-Pearson V, Gianinazzi S, Guillemin JP, Trouvelot A, Duc G (1991) Genetic and cellular analysis of resistance to vesicular arbuscular (VA) mycorrhizal fungi in pea mutants. In: Hennecke H, Verma DPS (eds) Advances in molecular genetics of plant microbe interactions. Kluwer, Dordrecht, The Netherlands, pp 336–342

Gianinazzi-Pearson V, Gollotte A, Lherminer J, Tisserant B, Franken P, Dumas-Gaudot E, Lemoine M, Tuinen D, Gianinazzi S (1995) Cellular and molecular approaches in the characterization of symbiotic events in functional arbuscular mycorrhizal associations. Can J Bot 73:526–532

Gianinazzi-Pearson V, Dumas-Gaudot E, Gollotte A, Tahiri-Alaoui A, Gianinazzi S (1996) Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. New Phytol 133:45–57

Gianinazzi-Pearson V, Dumas-Gaudot E, Gianinazzi S (1999) Proteins and protein activities in endomycorrhizal symbioses. In: Varma A, Hock B (eds) Mycorrhiza structure, function, molecular biology and biotechnology, 2nd edn. Springer, Berlin Heidelberg New York, pp 255–272

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Gollote A, Lemoine M, Gianinazzi-Pearson V (1996) Morphological integration and cellular compatibility between endomycorrhizal symbionts. In: Mukerji KG (ed) Concept of mycorrhizal research [Lieth H (ed) Handbook of vegetation science, vol 19/2]. Kluwer, Dordrecht, The Netherlands, pp 91–112

Hamaguchi H, Kokubun M, Akao S (1992) Shoot control nodulation is modified by the root in the supernodulating soybean mutant En6500 and its wild type parent cultivar Enrei. Soil Sci Plant Nutr 38:771–774

Heckman JR, Angle JS (1987) Variation between soybean cultivars in vesicular arbuscular mycorrhiza fungi colonization. Agron J 79:428–430

Jacobsen E, Feenstra WJ (1984) A new pea mutant with efficient nodulation in the presence of nitrate. Plant Sci Lett 33:337–344

Kawai Y, Yamamoto Y (1986) Increase in the formation and nitrogen fixation of soybean nodules by vesicular-arbuscular mycorrhiza. Plant Cell Physiol 27:399–405

Lie TA, Timmermans PCJM (1983) Host genetic control of nitrogen fixation in the legume-*Rhizobium* symbiosis: complication in genetic analysis due to maternal effects. Plant Soil 75:449–453

Manske GGB (1994) Genetical analysis of the efficiency of VA mycorrhiza with spring wheat. Agric Ecosystem Environ 29:273–280

Peterson RL, Bradbury SM (1999) Use of plant mutants, intraspecific variants and non-hosts in studying mycorrhiza formation and function. In: Varma A, Hock B (eds) Mycorrhiza structure, function, molecular biology and biotechnology, 2nd edn. Springer, Berlin Heidelberg New York, pp 153–176

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Pierce M, Bauer WD (1983) A rapid regulatory response governing nodulation in soybean. Plant Physiol 73:286–290

Srivastava D, Kapoor R, Srivastava SK, Mukerji KG (1996) Vesicular arbuscular mycorrhiza – an overview. In: Mukerji KG (ed) Concept of mycorrhizal research [Lieth H (ed) Hand book of vegetation science, vol 19/2]. Kluwer, Dordrecht, The Netherlands, pp 1–40

Toth R, Page T, Castleberry R (1984) Differences in the mycorrhizal colonization of maize selections for high and low ear leaf phosphorus. Crop Sci 24:994–996

Tri TD, Nickell CD, Harper JE (1996) Genetic and allelism analyses of hyper-nodulation soybean mutants from two genetic backgrounds. Crop Sci 36:1153–1158

Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) Mesure du taux de Mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiological and genetical aspects of mycorrhizae. INRA, Paris, pp 217–221

Vierheilig H, Piche Y (1996) Graft between peas forming the arbuscular mycorrhizal symbiosis (Myc^+) and pea mutants resistance to AM fungi (Myc^-) show the same colonization characteristics as ungrafted plants. Plant Physiol 147:762–764

Wegel E, Schauser L, Sandal N, Stougaard J, Parniske M (1998) Mycorrhiza mutants of *Lotus japonicus* define genetically independent steps during symbiotic infection. Mol Plant-Microbe Interact 11:933–936

Wyss P, Mellor RB, Wiemken A (1990a) Vesicular arbuscular mycorrhizas of wild type soybean and non-nodulating mutants with *Glomus mosseae* contain symbiosis specific polypeptides (mycorrhizins), immunologically cross-reactive with nodulins. Planta 182:22–26

Wyss P, Robert B, Mellor RB, Wiemken A (1990b) Mutants of soybean (*Glycine max*) unable to suppress nodulation in presence of nitrate retain the ability to suppress mycorrhization in presence of phosphate. J Plant Physiol 136:507–509