Mycorrhiza (2003) 13:9–16
DOI 10.1007/s00572-002-0189-2

ORIGINAL PAPER

Lidia M. Jacobi · Lidia A. Zubkova
Ekaterina M. Barmicheva · Viktor E. Tsyganov
Alexey Y. Borisov · Igor A. Tikhonovich

Effect of mutations in the pea genes *Sym33* and *Sym40*

II. Dynamics of arbuscule development and turnover

Received: 13 November 2000 / Accepted: 6 June 2002 / Published online: 17 July 2002

Abstract Two symbiotic pea (*Pisum sativum* L.) mutants SGEFix$^-$-1 (*sym40*) and SGEFix$^-$-2 (*sym33*) with abnormalities in infection thread formation in symbiotic root nodules were characterised with respect to dynamics of arbuscule development at 15°C and 24°C. Mutation of *sym33* decreased mycorrhiza colonisation at both temperatures and delayed arbuscule development at 15°C, whereas mutation of *sym40* accelerated mycorrhiza colonisation and arbuscule senescence at 24°C. The differences between the mutants and the wild-type were more pronounced at 24°C, a temperature close to the optimum for pea growth. The results demonstrate that both pea genes are important in the control of arbuscular mycorrhiza development and can be considered necessary for the tripartite symbiosis in pea.

Keywords Plant–microbe interactions · *Pisum sativum* L. · Pea symbiotic genes · Arbuscule development · Tripartite symbiosis

Introduction

Pea (*Pisum sativum* L.) forms symbioses with the soil bacteria *Rhizobium leguminosarum* bv. *viciae* and arbuscular mycorrhiza (AM) fungi belonging to the order Glomales. The AM symbiosis is supposed to date back 400–500 million years (Pirozynski and Dalpé 1989; Remy et al. 1994; Redecker et al. 2000), whereas the nitrogen-fixing symbiosis (NFS) of legumes is thought to be much younger (Sprent 1994). It has been hypothesised that parts of the cell programmes in the latter type of endosymbiosis have evolved from those of AM (Gianinazzi-Pearson 1997) and several types of evidence exist for common genetic and molecular bases of the two symbioses.

Firstly, a group of symbiotic mutants in legumes are impaired in the development of both types of symbioses (Gianinazzi-Pearson 1996; Harrison 1999; Marsh and Schultz 2001). The mutations determine either Nod$^-$ or Fix$^-$ phenotypes in nodulation and several phenotypes of AM development: Pen$^-$ (no penetration of root epidermis), Coi$^-$ (absence of cortex invasion), Ici$^-$ (absence of inner cortex invasion), Arb$^-$ (no arbuscules) and Ard$^-$ (abnormal arbuscule development) (reviewed in Marsh and Schultze 2001). To date, six genes have been shown to control the development of both symbioses in pea (*Pisum sativum* L.): *sym8* (Kolycheva et al. 1993; Balaji et al. 1994), *sym9* (Balaji et al. 1994), *sym19*, *sym30*, *sym36* (Gianinazzi-Pearson 1996; gene symbol *sym36* assigned by G. Duc and M. Sagan, personal communication), and *brz* (Resendes et al. 2001). All these genes, when mutated, determine a Nod$^-$ phenotype in the symbiosis with *Rhizobium*, whilst such mutated genes determine two phenotypes with respect to AM fungi. With mutation of *sym8*, *sym9*, *sym19* or *sym30* (Pen$^-$ phenotype or Myc^{-1} according to Gianinazzi-Pearson 1996), AM development is blocked after appressorium formation (Duc et al. 1989), whereas with *sym36* (Ard$^-$ phenotype or Myc^{-2} according to Gianinazzi-Pearson 1996) arbuscule development is reduced to a few stumpy branches (Gianinazzi-Pearson 1996).

Secondly, it was shown that some plant macromolecules are induced in a similar fashion during development of the two symbioses. For example, some plant proteins and glycoproteins present in the symbiosome of nodules are also found at the AM symbiotic interface (Gianinazzi-Pearson et al. 1991, 1996; Perotto et al. 1994; Gollotte et al. 1995; Balestrini et al. 1999), and some nodulins (Van Kammen 1984) are expressed during establishment of both NFS and AM (Wyss et al. 1990; Gianinazzi-Pearson 1996; Frühling et al. 1997; Van Rhijn et al. 1997; Albrecht et al. 1998; Journet et al. 2001).

L.M. Jacobi · L.A. Zubkova · V.E. Tsyganov · A.Y. Borisov (✉)
I.A. Tikhonovich
All-Russia Research Institute for Agricultural Microbiology,
Podbelsky chaussee 3, St.-Petersburg, Pushkin 8, 196608, Russia
e-mail: rifam@mail.rcom.ru
Tel.: +7-812-4704392, Fax: +7-812-4704362

E.M. Barmicheva
V.L. Komarov's Institute of Botany, Professor Popov's str. 2,
197022 St.-Petersburg, Russia

We have shown recently that another pea gene, *Sym33*, is involved in AM formation. Although mutation of this gene decreases mycorrhizal colonisation, it does not affect AM function in the mutated (SGEFix$^-$ -2) pea line (Jacobi et al. 2002). The aim of the present study was a more detailed comparative analysis of arbuscule development during AM colonisation in mutants SGEFix$^-$-2 (*sym33*) and SGEFix$^-$-1 (*sym40*); the latter shows no change in the AM phenotype (Jacobi et al. 2002). Both mutants have abnormal formation and functioning of infection threads in the NFS with *Rhizobium* (Tsyganov et al. 1998b) and have been shown to be leaky with two types of nodules formed on their roots. Mutant SGEFix$^-$-1 (*sym40*) forms white nodules with hypertrophied infection droplets and abnormal endocytosis of bacteria, as well as very rare pink nodules without distinct morphological abnormalities (Tsyganov et al. 1998b). SGEFix$^-$-2 (*sym33*) forms white nodules with "locked" infection threads and no endocytosis of bacteria, as well as rare pinkish nodules in which bacterial endocytosis occurs but bacteroids do not undergo complete morphologically pronounced differentiation (Tsyganov et al. 1998b). The leakiness of both mutant phenotypes depends to some extent on the temperature during plant growth (Tsyganov et al. 1998a). The present study was, therefore, carried out at two different temperatures.

Materials and methods

Plant material

Two symbiotic pea mutants SGEFix$^-$-1 (*sym40*) and SGEFix$^-$-2 (*sym33*), defective for NFS formation (Tsyganov et al. 1994, 1998b) and the wild-type laboratory line SGE (Kosterin and Rozov 1993) were used in the study. The mutant lines have monogenic mutations (Tsyganov et al. 1998b; Jacobi et al. 2002).

Seeds of each line were selected within a narrow weight range (99–100 mg) for experiments, surface sterilised with concentrated sulphuric acid for 20 min by shaking at room temperature and washed with distilled water. One seed was planted per pot with 500 g of sterilised soil/sand mixture (Jacobi et al. 2002).

Mycorrhizal fungus and *Rhizobium* inoculation

Glomus intraradices Schenck and Smith isolate CIAM8 (Muromtsev et al. 1989) from the Collection of the All-Russia Institute for Agricultural Microbiology (registered in the European Bank of Glomales as isolate BEG144) was used for inoculation as described previously (Jacobi et al. 2002). All plants were also inoculated with a commercial strain of *Rhizobium leguminosarum* bv. *viciae* CIAM 1026 (Safronova and Novikova 1996) from the collection of the All-Russia Research Institute for Agricultural Microbiology (water suspension, 10^8–10^9 bacterial cells per seed).

Growth conditions and measurements

Plants were grown in a growth chamber HPS2000 (Heraeus Vötch, Germany) (day/night 16/8 h, relative humidity of 75%, photon irradiance of 490 E/m^2 per s) at 15°C (temperature lower than optimal for pea growth) or 24°C (a temperature close to optimal for pea growth).

Since both mutant lines differ from the wild type in timing of plant development (Jacobi et al. 2002), plants of all genotypes were collected for analysis at the following stages: (1) few leaves, (2) early flowering, and (3) almost mature but not dry first pod.

Mycorrhizal colonisation of the mutants and the wild type was estimated as described by Trouvelot et al. (1986) for two parameters: M% intensity of mycorrhizal colonisation in the root system and a% arbuscule abundance in mycorrhizal root fragments.

Transmission electron microscopy

Specimens (1-cm root pieces thoroughly washed with water) were fixed for 3 h with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. After rinsing 20 min in the same buffer, samples were post-fixed for 1.5 h with 1% osmium tetroxide (same buffer) and rinsed three times for 20 min in this buffer. After dehydration by transfer through an alcohol series, the specimens were embedded in an Epon-Araldite resin mixture. Sections (0.05–0.09 μm) were cut using an LKBIII ultramicrotome, stained for 20 min with uranyl acetate and 5 min with lead citrate (Reynolds 1963) and examined with an Hitachi H-300 electron microscope at 70 kV.

Statistical analysis

Roots or root pieces supposed to be mycorrhizal were collected from 4–5 plants per treatment for analysis. Whole root systems were used for estimation of mycorrhizal colonisation, except for those pieces removed for the transmission electron microscopy (TEM) study.

The number of cells containing arbuscules examined by TEM per treatment were: first time point, SGE 297, SGEFix$^-$-1 (*sym40*) 216, SGEFix$^-$-2 (*sym33*) 180, and second time point, SGE 180, SGEFix$^-$-1 (*sym40*) 288, SGEFix$^-$-2 (*sym33*) 324. The proportion (%) of cell area occupied by fungal structures and plant cell cytoplasm was estimated by morphometry, i.e. by comparing the portion of cell area profile occupied by the arbuscules.

The data were calculated by standard methods of variance analyses and comparison of two groups of data and two distributions. SigmaStat for Windows version 2.3, SPSS Inc. was used for statistical analysis and processing of the data.

Results

Dynamics of mycorrhizal colonisation in the roots of mutants

All pea lines grew more slowly at 15°C than 24°C and had decreased mycorrhizal colonisation. Mycorrhizal colonisation of wild-type SGE at 15°C decreased by 20–40% for the different parameters measured compared with 24°C (a% and M%, Figs. 1, 2, 3, 4). At 24°C, this line was characterised by a high level of colonisation (Figs. 2, 4) similar to that observed earlier (Jacobi et al. 2002). At the first time point, all parameters of mycorrhizal colonisation increased in SGEFix$^-$-1 (*sym40*) at 15°C compared with the wild-type. However, at the second and third time points, differences between these two lines were not significant (Figs. 1, 3). At the first time point at 15°C, SGEFix$^-$-2 (*sym33*) did not differ from the wild type except for m% (intensity of mycorrhizal colonisation in mycorrhizal root fragments; data not shown) and a% (Fig. 3), which were significantly lower. At the second and third time points, almost all parameters describing mycorrhizal colonisation in the roots of this line were significantly reduced relative to the wild-type (e.g. M%, Fig. 1) and the rate of decrease corresponded to that found in a previous study (Jacobi et al. 2002). The only exception was at the second time point,

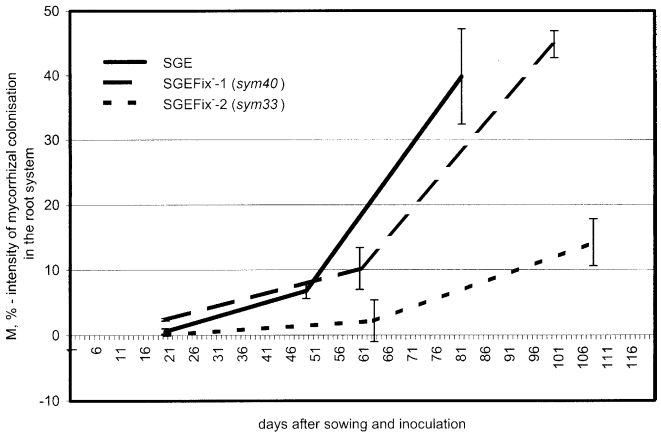


Fig. 1 Intensity of mycorrhizal colonisation in the root system (*M%*, according to Trouvelot et al. 1986) in pea symbiotic mutants SGEFix⁻-1 (*sym40*), SGEFix⁻-2 (*sym33*) and the wild-type line SGE grown at 15°C. Standard errors show variance of mean values at certain time points. At the first time point, SGEFix⁻-1 (*sym40*) has a statistically significant increase in this parameter compared with the other two lines ($P \geq 0.95$), whereas SGE and SGEFix⁻-2 (*sym33*) do not differ from each other. At the second time point, the values of all lines are not statistically different. At the third time point, the mean value for SGEFix⁻-2 (*sym33*) differs from those of the other lines ($P \geq 0.95$), whereas SGEFix⁻-1 (*sym40*) and SGE are not statistically different

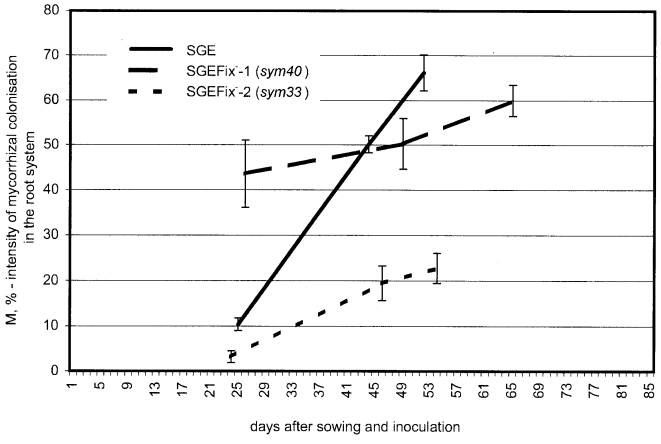


Fig. 2 Intensity of mycorrhizal colonisation in the root system (*M%*, according to Trouvelot et al. 1986) in SGEFix⁻-1 (*sym40*), SGEFix⁻-2 (*sym33*) and SGE grown at 24°C. Standard errors show variance of mean values at certain time points. All lines differ from each other at the first time point ($P \geq 0.95$). At the second and third time points, the mean values for SGEFix⁻-2 (*sym33*) differ from those of the other lines ($P \geq 0.95$), whereas values for SGEFix⁻-1 (*sym40*) and SGE are not statistically different

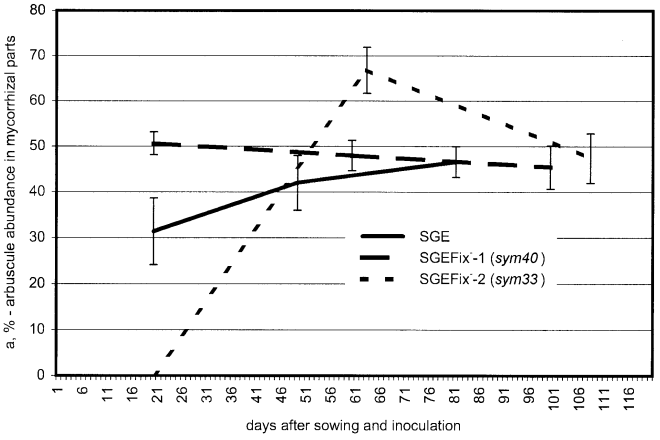


Fig. 3 Arbuscule abundance in mycorrhizal root fragments (*a%*, according to Trouvelot et al. 1986) in SGEFix⁻-1 (*sym40*), SGEFix⁻-2 (*sym33*) and SGE grown at 15°C. Standard errors show variance of mean values at certain time points. All lines differ from each other at the first time point ($P \geq 0.95$). At the second time point, the mean value for SGEFix⁻-2 (*sym33*) differs from those of the other two lines ($P \geq 0.95$), which are not statistically different. At the third time point, the values for all lines are not significantly different

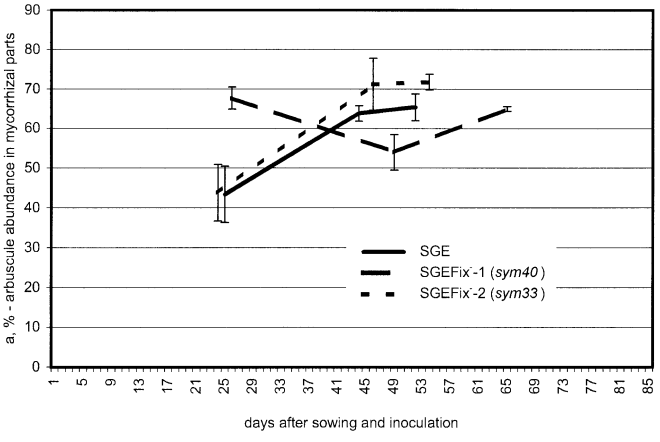


Fig. 4 Arbuscule abundance in mycorrhizal root fragments (*a%*, according to Trouvelot et al. 1986) in SGEFix⁻-1 (*sym40*), SGEFix⁻-2 (*sym33*) and SGE grown at 24°C. Standard errors show variance of mean values at certain time points. Only the mean value of SGEFix⁻-1 (*sym40*) at the first time point and that of SGEFix⁻-2 (*sym33*) at the third time point differ from others at corresponding time points ($P \geq 0.95$). Values at all other time points are not statistically different

where a% was significantly increased by about 20% compared with the wild type (Fig. 3). This was also consistent with data obtained earlier and can be considered as an indication that the mycorrhiza in the roots of this mutant is functional (Jacobi et al. 2002).

When plants were grown at 24°C, mycorrhizal colonisation in SGEFix⁻-1 (*sym40*) was again significantly higher at the first time point than in the other lines; this was true of all parameters (e.g. M% and a%, Figs. 2, 4) except m%, which appeared to be higher but not significantly (data not shown). Again, the decreased mycorrhizal colonisation of SGEFix⁻-2 (*sym33*) observed earlier (Jacobi et al. 2002) and in the experiments at 15°C was confirmed (Figs. 1, 2, 3). However, at 24°C, colonisation was the same as that of the wild-type SGE at all stages of plant growth (Fig. 4). This result is different to that obtained earlier by Jacobi et al. (2002), who found a% to have increased in the roots of SGEFix⁻-2 (*sym33*).

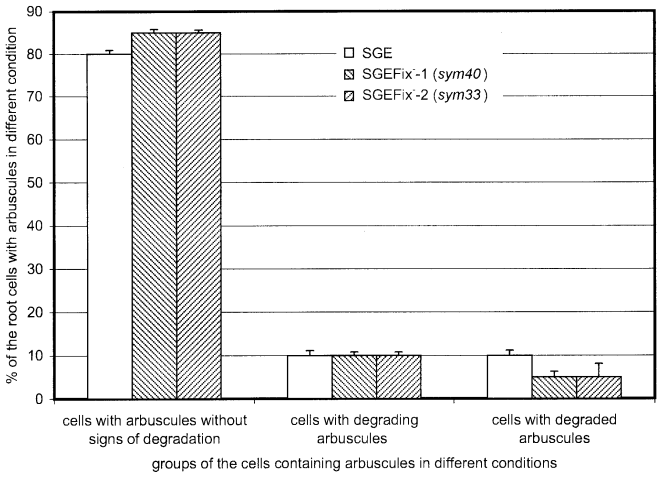


Fig. 5 Proportion of cells containing arbuscules in SGEFix⁻-1 (*sym40*), SGEFix⁻-2 (*sym33*) and SGE grown at 24°C in different conditions at the first time point. The distributions corresponding to the different lines do not differ significantly

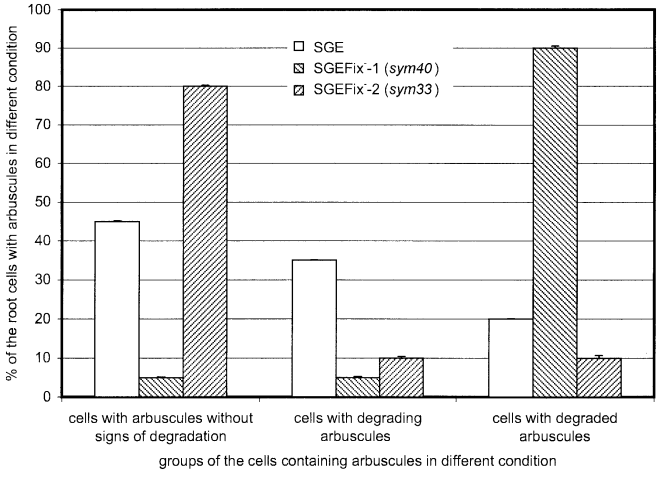


Fig. 6 Proportion of cells containing arbuscules in SGEFix⁻-1 (*sym40*), SGEFix⁻-2 (*sym33*) and SGE grown at 24°C in different conditions at the second time point. The distributions corresponding to different lines differ from each other significantly, $P \geq 0.95$

Pattern of arbuscule development in root cells

The pattern of arbuscule development was analysed in AM-inoculated roots of plants grown at 24°C and collected at the two first time points, when differences between lines were maximal with respect to mycorrhizal colonisation (Figs. 5, 6, 7). In all the pea lines, arbuscules differed in rate of maturation and degradation, which coincides with the ephemeral nature of these structures (Alexander et al. 1988). Cells could be subdivided into three groups: (1) cells with arbuscules without signs of degradation (Fig. 7A–C), (2) cells with degrading arbuscules (Fig. 7D) and (3) cells with degraded arbuscules (Fig. 7E), and the relative frequencies of each cell type calculated.

Table 1 Percent of root cell content occupied by arbuscule structures in pea wild-type line SGE and the symbiotic mutants SGEFix⁻-1 (*sym40*) and SGEFix⁻-2 (*sym33*), respectively, at two different stages of plant development at 24°C

Pea lines	First time-point	Second time-point
SGE (wild-type)	46.1±11.80*	30.2±12.44
SGEFix⁻-1 (*sym40*)	33.4±13.87	15.2±4.44**
SGEFix⁻-2 (*sym33*)	36.1±8.36	34.9±9.28

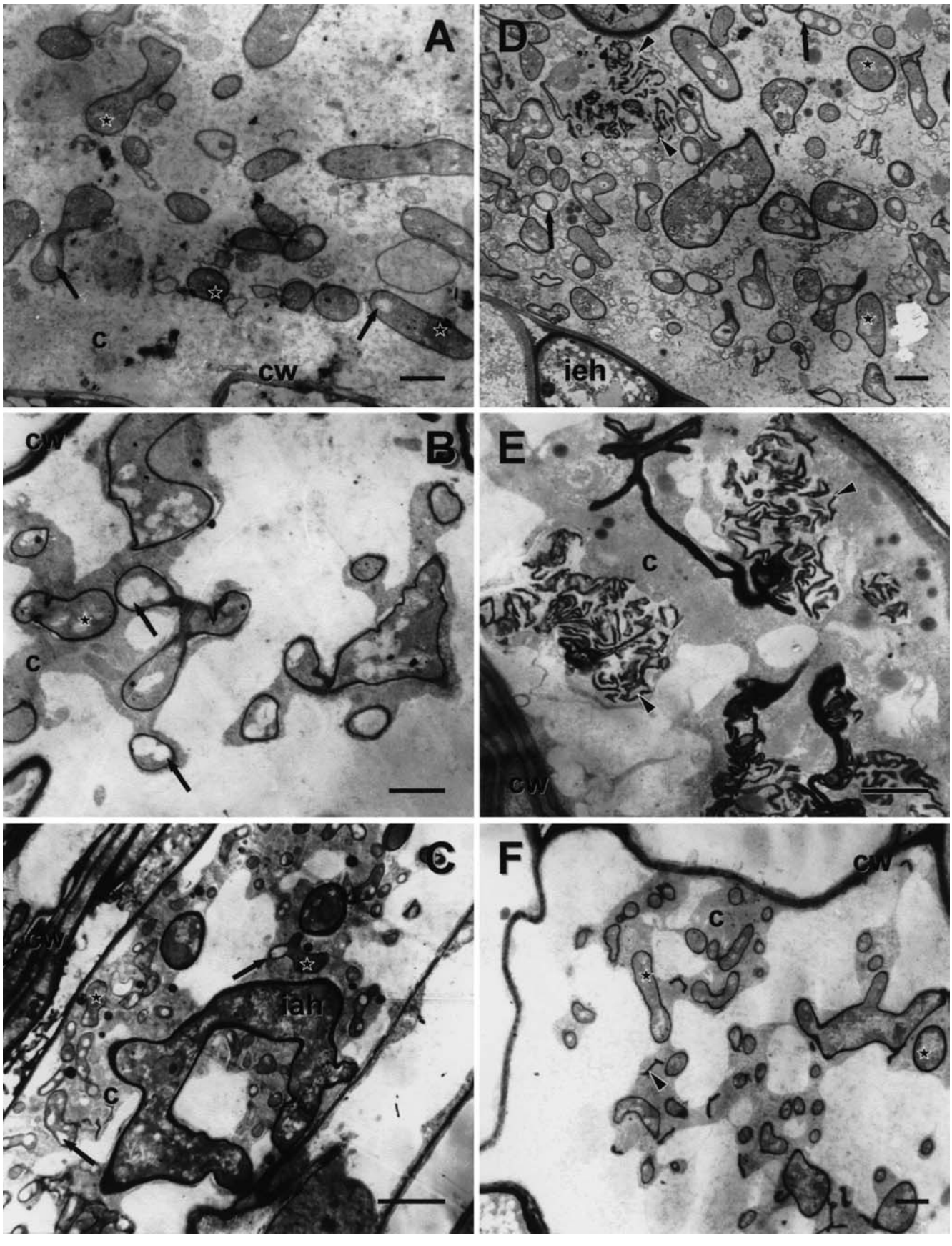
*, **Mean value significantly differs from all others at $P \geq 0.95$ and $P \geq 0.99$, respectively

At the first time point, roots of all three pea genotypes contained mainly cells of type 1 and there was no significant difference between them in the distribution of cells into different groups (Fig. 5). At the second time point, roots of the wild-type contained cells belonging to all of the defined groups but cells containing arbuscules without signs of degradation still prevailed (Fig. 6). The situation was quite different in roots of SGEFix⁻-1 (*sym40*), where the majority of cells contained arbuscules in an almost fully-degraded state (Figs. 6, 7E). In contrast, the large majority of cells in roots of SGEFix⁻-2 (*sym33*) contained arbuscules without signs of degradation (Figs. 6, 7F). At this time point, the distribution of arbuscule-containing cells amongst the different groups differed significantly ($P \geq 0.99$) between the three pea lines.

In addition, the extent to which arbuscules developed in root cells, i.e. the area of arbuscule profile relative to whole plant cell area, differed between the pea lines. At the first time point, the wild type had the most-developed arbuscules, with about 50% of the plant cell occupied by arbuscule structures (Table 1). Arbuscules in the mutant lines were less developed and did not differ between the lines (Table 1). At the second time point, SGEFix⁻-1 (*sym40*) was characterised by decreased arbuscule development in root cells and two times less of the plant cell content was occupied by arbuscules, compared with the wild-type and SGEFix⁻-2 (*sym33*). The latter lines did not differ from each other (Table 1).

The rate of vacuolation of arbuscules also differed between the pea lines. At the first time point, the majority of root cells of the wild-type contained arbuscules in a slightly vacuolated state (Fig. 7A), whilst arbuscules in the root cells of SGEFix⁻-1 (*sym40*) and SGEFix⁻-2 (*sym33*) were highly vacuolated (Fig. 7B, C). At the second time point, the number of highly vacuolated arbuscules increased in the wild-type (Fig. 7D), whereas arbuscules in cells of SGEFix⁻-2 (*sym33*) were less vacuo-

Fig. 7 Ultrastructure of plant root cells containing arbuscules in SGEFix⁻-1 (*sym40*) (**B**, **E**), SGEFix⁻-2 (*sym33*) (**C**, **F**) and SGE (**A**, **D**) grown at 24°C at two time points (time point I, A–C, time point II, D–F). Functional (*stars*) and degrading (*arrowheads*) parts of arbuscules, vacuoles in arbuscule parts (*arrows*), intercellular hypha (*ieh*) and intracellular hyphae (*iah*), cytoplasm (*c*) and cell walls (*cw*) are seen; *bar* 2 μm ▶


A
c
cw
D
ieh
cw
B
c
E
c
cw
C
cw
ieh
c
F
cw
c

lated (Fig. 7F). Almost all arbuscules were fully degraded in the roots of SGEFix⁻-1 (*sym40*) at this time point (Fig. 7E).

Discussion

Two pea mutants, SGEFix⁻-1 (*sym40*) and SGEFix⁻-2 (*sym33*), with different abnormalities in infection thread formation in NFS were characterised with respect to the dynamics of mycorrhizal colonisation and arbuscule development at two different temperatures and in the presence of nodule bacteria. At a temperature of 24°C, close to optimal for pea growth (21–23°C), overall AM development in both the wild-type and the mutants was higher than at 15°C. At both temperatures, mycorrhizal colonisation of roots of the mutant SGEFix⁻-1 (*sym40*) was higher than that of SGEFix⁻-2 (*sym33*), which was characterised by delayed/decreased colonisation. Considering these results at 24°C, it is necessary to take into account the stage of plant development at the time points at which material was collected for analysis. Although both pea mutants have delayed flowering and pod ripening, this is more striking in SGEFix⁻-1 (*sym40*) (Jacobi et al. 2002). SGEFix⁻-1 (*sym40*) has a slower rate of plant development but an accelerated mycorrhizal colonisation and increased rate of arbuscule degradation than the wild-type. SGEFix⁻-2 (*sym33*) is more similar to the wild-type in its growth (Jacobi et al. 2002) but has decreased mycorrhizal colonisation and a much slower rate of arbuscule development. This conclusion is further supported by the proportion of root cells occupied by arbuscules, where the wild-type had more fully developed arbuscules in root cells than either mutant line at the first time point. In contrast, SGEFix⁻-1 (*sym40*) had less-developed arbuscules than the other lines at the second time point. These observations may reflect differences in the functional state and/or the rate of development of arbuscules in the pea lines.

The defects in AM development in the pea mutants studied here cannot be fitted to any of the phenotypic codes previously proposed to describe defective AM development in mutated plants. It is, therefore, proposed to introduce the code Rmd (rate of mycorrhizal development) to describe the extent and rate of AM development. In line with this, the mutant SGEFix⁻-1 (*sym40*) has an Rmd⁺⁺ phenotype, whereas SGEFix⁻-2 (*sym33*) has the phenotype Rmd⁻. The nodulation pattern in these two pea mutants has been described earlier (Tsyganov et al. 1998b) as "decreased (as compared to the wild-type line) number of ineffective nodules" for SGEFix⁻-2 (*sym33*) and "increased (as compared to the wild-type line) number of small ineffective nodules" for SGEFix⁻-1 (*sym40*) (data not shown). The AM phenotypes of the rhizobia-inoculated mutant lines used in this study were for the most part similar to those described in the previous study without rhizobial inoculation (Jacobi et al. 2002), which suggests that nodulation does not affect the mutant phenotypes in terms of AM development. However, biomass accumulation was affected; shoot biomass accumulation was lower than wild-type in both pea mutants (data not shown), which is in contrast to data from the same plants without rhizobial inoculation (Jacobi et al. 2002). This may be explained by the ineffective nodulation of the mutant plants, nodule formation being an energy-consuming process (Postma et al. 1988).

Some parallels can be drawn when comparing the pea mutant phenotypes with respect to NFS and AM. SGEFix⁻-2 (*sym33*) is characterised by (1) delayed and decreased numbers of infection initials during nodulation (V.A. Voroshilova, V.E. Tsyganov, A.Y. Borisov, I.A. Tikhonovich, unpublished results), decreased number of nodules (Tsyganov et al. 1998b) and reduced mycorrhizal colonisation (Jacobi et al. 2002; this study), (2) altered endocytosis of bacteria (very rare nodule cells with endocytosed bacteria, Tsyganov et al. 1998b) and delayed AM and arbuscule development (Jacobi et al. 2002 and this study). SGEFix⁻-1 (*sym40*) is characterised by (1) an increased number of accelerated infection initials during nodulation (V.A. Voroshilova, V.E. Tsyganov, A.Y. Borisov, I.A. Tikhonovich, unpublished results), increased number of nodules (Tsyganov et al. 1998b) and accelerated mycorrhizal colonisation (this study), (2) hypertrophied infection droplet formation and premature degradation of nodule symbiotic compartments (Tsyganov et al. 1998b) and increased rate of arbuscule formation and degradation (this study). These relations between NFS and AM phenotypes confirm those found for Nod⁻ (non-nodulating) and Nod⁺⁺ (supernodulating) mutants of *Medicago truncatula* Gaertn., pea and soybean (*Glycine max* (L.) Merr.) (Duc et al. 1989; Catoira et al. 2000; Morandi et al. 2000; Shrihari et al. 2000). The ability or rate of nodulation relates to some extent to the ability or rate of AM development. Analysis of other mutants blocked at late stages of NFS development should give more insight into phenomena common to the two root symbioses.

In conclusion, the present study has shown that the two pea symbiotic genes *Sym33* and *Sym40* are implicated in establishment of both nitrogen-fixing nodules and AM. They are, therefore, common to the two endosymbiotic systems, adding to the evidence that the legume plant symbiotic genetic system jointly controls development of a tripartite symbiosis. In addition, both genes (Tsyganov et al. 1998b) are involved not only in the formation of root symbioses but also in the plant life cycle and whole plant development (Jacobi et al. 2002). Since both SGEFix⁻-1 (*sym40*) and SGEFix⁻-2 (*sym33*) mutants have leaky phenotypes which depend on growth conditions (Tsyganov et al. 1998a), it seems likely that these genes are involved in some kind of hormonal regulation of plant and symbiosis development (Tsyganov et al. 1999; Jacobi et al. 2002).

Acknowledgements This work was supported by the Russian Academy of Agricultural Sciences, European programme INCO-COPERNICUS (IC15CT-98–0116), Russian State Research Programmes "Biodiversity" and "Phytobiotechnology", the Russian Foundation for Basic Research (97–04–50033, 01–04–48580), a grant of the President of Russia (02–15–99408) and US CRDF

award (ST-012-0). The authors are very grateful to L.E. Dvoryaninova for her excellent technical assistance. Many thanks to F. Guinel for critical reading of the manuscript. Special thanks to V. Gianinazzi-Pearson and D. van Tuinen for taxonomical identification of the arbuscular mycorrhizal fungus used in this study.

References

Albrecht C, Geurts R, Lapeyrie F, Bisseling T (1998) Endomycorrhizae and rhizobial Nod factors both require SYM8 to induce the expression of the early nodulin genes *PsENOD5* and *PsENOD12A*. Plant J 15:605–614

Alexander T, Meier R, Toth R, Weber HC (1988) Dynamics of arbuscule development and degeneration in mycorrhizas of *Triticum aestivum* L. and *Avena sativa* L. with references to *Zea mays* L. New Phytol 110:363–370

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

Balestrini R, Perotto S, Gasverde E, Dahiya P, Guldmann LL, Brewin NJ, Bonfante P (1999) Transcription of a gene encoding a lectin-like glycoprotein is induced in roots cells harboring arbuscular mycorrhizal fungi in *Pisum sativum.* Mol Plant Microbe Interact 12:785–791

Catoira R, Galera C, De Billy F, Penmetsa RV, Journet EP, Raillet F, Rosenberg C, Cook D, Gough C, Denarié J (2000) Four genes of *Medicago truncatula* controlling components of a Nod factor transduction pathway. Plant Cell 12:1647–1665

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

Frühling M, Roussel H, Gianinazzi-Pearson V, Pühler A, Perlik A (1997) The *Vicia faba* leghemoglobin gene *VfLb29* is induced in root nodules and in roots colonized by the arbuscular mycorrhizal fungus *Glomus fasciculatum*. Mol Plant-Microbe Interact 10:124–131

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 (1997) Have common plant systems co-evolved in fungal and bacterial root symbioses? In: Legocki A, Bothe H, Pühler A (eds) Biological fixation of nitrogen for ecology and sustainable agriculture. Springer, Berlin Heidelberg New York, pp 321–324

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

Gianinazzi-Pearson V, Gollotte A, Cordier C, Gianinazzi S (1996) Root defence responses in relation to cell and tissue invasion by symbiotic microorganisms: cytological investigations. In: Nicole M, Gianinazzi-Pearson V (eds) Histology, ultrastructure and molecular cytology of plant–microorganism interactions. Kluwer, Dordrecht, pp 127–191

Gollotte A, Gianinazzi-Pearson V, Gianinazzi S (1995) Immunodetection of infection thread glycoprotein and arabinogalactan protein in wild-type *Pisum sativum* L. or an isogenic mycorrhiza resistant mutant interacting with *Glomus mosseae*. Symbiosis 18:69–85

Harrison MJ (1999) Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. Annu Rev Plant Physiol Plant Mol Biol 50:361–389

Jacobi LM, Petrova OS, Tsyganov VE, Borisov AY, Tikhonovich IA (2002) Effect of mutations in the pea genes *Sym33* and *Sym40*. I. Arbuscular mycorrhiza formation and function. Mycorrhiza (this issue)

Journet E, El Gachtouli N, Vernoud V, Billy F, de Pichon M, Dedieu A, Arnould C, Morandi D, Barker D, Gianinazzi-Pearson V (2001) *Medicago truncatula* ENOD11: a novel RPRP-encoding early nodulin gene expressed during mycorrhization in arbuscule-containing cells. Mol Plant Microbe Interact 14:737–748

Kolycheva AN, Jacobi LM, Borisov AY, Filatov AA, Tikhonovich IA, Muromtsev GS (1993) Pea gene *sym8* affects symbiosis both with *Rhizobium* and with endomycorrhizal fungi. Pisum Genet 25:22

Kosterin OE, Rozov SM (1993) Mapping of the new mutation *blb* and the problem of integrity of linkage group I. Pisum Genet 25:27–31

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

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

Muromtsev GS, Marshunova GA, Jacobi LM (1989) USSR Inventor's Certificate no. 1501509

Perotto S, Brewin NJ, Bonfante P (1994) Colonization of pea roots by the mycorrhizal fungus *Glomus versiforme* and by *Rhizobium* bacteria: immunological comparison using monoclonal antibodies as probes for plant cell surface components. Mol Plant Microbe Interact 7:91–98

Pirozynski KA, Dalpé Y (1989) Geological history of the *Glomaceae*, with particular reference to mycorrhizal symbiosis. Symbiosis 7:1–36

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

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

Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred million-years-old vesicular-arbuscular mycorrhizae. Proc Natl Acad Sci USA 91:11841–11843

Resendes CM, Geil RD, Guinel FC (2001) Mycorrhizal development in a low nodulating pea mutant. New Phytol 150: 563–572

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17: 208–212

Safronova VI, Novikova NI (1996) Comparison of two methods for root nodule bacteria preservation: lyophilization and liquid nitrogen freezing. J Microbiol Methods 24:231–237

Shrihari PC, Sakamoto K, Inubushi K, Akao S (2000) Interaction between supernodulating or non-nodulating mutants of soybean and two arbuscular mycorrhizal fungi. Mycorrhiza 10:101–106

Sprent JL (1994) Evolution and diversity in the legume-*Rhizobium* symbiosis: chaos theory? Plant Soil 161:1–10

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

Tsyganov VE, Borisov AY, Rozov SM, Tikhonovich IA (1994) New symbiotic mutants of pea obtained after mutagenesis of laboratory line SGE. Pisum Genet 26:36–37

Tsyganov VE, Borisov AY, Kukalev AS, Voroshilova VA, Tikhonovich IA (1998a) Pea (*Pisum sativum* L.) genetic control over infection thread formation and functioning. In: Wijffelman C, Wijffjes A, Lugtenberg B (eds) Abstracts of Third European Nitrogen Fixation Conference, Leiden, p 122

Tsyganov VE, Morzhina EV, Stefanov SY, Borisov AY, Lebsky VK, Tikhonovich IA (1998b) New pea (*Pisum sativum* L.) genes *sym33* and *sym40* control infection thread formation and root nodule function. Mol Gen Genet 256:491–503

Tsyganov VE, Batagov AO, Voroshilova VA, Borisov AY, Tikhonovich IA (1999) Pea (*Pisum sativum* L.) gene *Sym33* can play a role in ethylene-dependent regulation of nodulation. In: Pedrosa FO, Hungria M, Yates MG, Newton WE (eds) Nitrogen fixation: from molecules to crop productivity. Kluwer, Dordrecht, p 262

Van Kammen A (1984) Suggested nomenclature for plant genes involved in nodulation and symbiosis. Plant Mol Biol Rep 2:43–45

Van Rhijn P, Fang Y, Glili S, Shaul O, Atzmon N, Wininger S, Eshed Y, Lum M, Li Y, Fujishige N, Kapulnik Y, Hirsch M (1997) Expression of early nodulin genes in alfalfa mycorrhizae indicates that signal transduction pathways used in forming arbuscular mycorrhizae and *Rhizobium-induced* nodules may be conserved. Proc Natl Acad Sci USA 94:5467–5472

Wyss P, Mellor RB, Wiemken A (1990) 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