A new mutant allele of the symbiotic gene *sym*40 of pea (*Pisum sativum* L.): dynamics of arbuscular mycorrhiza development

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Introduction

Pea (*Pisum sativum* L.) forms symbioses with soil bacteria *Rhizobium leguminosarum bv. viciae* and arbuscular mycorrhizal (AM) fungi belonging to the phylum Glomeromycota (Schüssler et al., 2001), and is one of the actively used model species for studying beneficial plant-microbe interactions. Research on these types of symbioses is critical because there exists a high degree of genetic integration between the partners and because these plant-microbe systems are very important in agriculture.

The mutation in the gene *sym*40, SGEFix$^-$-1, was characterized previously revealing a change of dynamics in arbuscule development and turnover when compared to the wild type line SGE (2). The present study was focused on studying AM development in a new mutant, SGEFix$^-$-6, in the same gene.

Materials and methods

Plant material. Pea (*Pisum sativum* L.) lines, SGE (4), SGEFix$^-$-1 (sym40) (2) and SGEFix$^-$-6 (sym40) (9) were obtained from the ARRIAM collection (Saint Petersburg, Russia; www.arriam.spb.ru/eng/lab9/collections/sge.html).

Plant growth conditions and inoculation technique. Pea seeds were surface-sterilized, scarified and germinated at room temperature under sterile conditions. Three-day seedlings were planted into a nurse-plant inoculation system (NPIS) (6) modified with a chive (*Allium schoenop rasumh*) as the nurse plant. The plants were grown in the growth chamber under following conditions: day/night, 16h/8h, temperature, 21° C, relative air humidity, 75%, light intensity, 300 μmol $m^{-2}s^{-1}$. The potting mix was a sterile mixture of equal parts (v/v) of quartz sand and expanded clay, containing Ca_3PO_4 (1 g per kg of the mixture). The plants were watered once a week with ½-strength Hoagland's solution (Hoagland & Arnon, 1938) without phosphates.

Arbuscular-mycorrhizal fungus. An isolate of *Glomus intraradices* Schenck and Smith CIAM8 (ARRIAM, Saint-Petersburg, Russia; deposited in The International Bank for the Glomeromycota as BEG144) was used for inoculation. The isolate demonstrates high effectiveness in symbiosis with most of agricultural plants (5).

Measurements and analysis of the results. Pea plants were collected six, eight, and 11 days after planting into NPIS, as well as at the following stages: 1) early flowering and 2) almost mature but not dry first pod because both mutant lines differ from the wild type in timing of plant development (1). Whole root systems were used for analysis of AM formation. The roots or the root pieces were collected from 9-15 plants per treatment. Ink staining for visualization of fungal structures in the roots was performed according to Vierheilig et al. (10). AM development in the roots was estimated as described in Trouvelot et al. (8) by two parameters: intensity of mycorrhizal colonization in the root system (M%), and arbuscule abundance in mycorrhizal root fragments (a%), using a standard light microscope. Data presented were obtained from two independent experiments.

Results

Wild type line SGE. The wild type line SGE displayed a gradual increase in number of appressoria on the surface of the root during plant growth in the period of growth in NPIS from six to 11 days (Fig. 1). The

dynamics of arbuscule development and turnover was similar to that described before in Jacobi et al. (2) (Fig. 2, 3).

Mutant SGEFix⁻-1. Many more appressoria were formed on the roots of the mutant line SGEFix⁻-1 (*sym*40) than on those of SGE or SGEFix⁻-6 (*sym*40) (Fig. 1). Plants of the mutant line SGEFix⁻-1 showed an intensity of colonization close to that of the wild type at the early time points (Fig. 2) and high speed of arbuscule development accompanied with their fast turnover (Fig. 3) as was described previously (2).

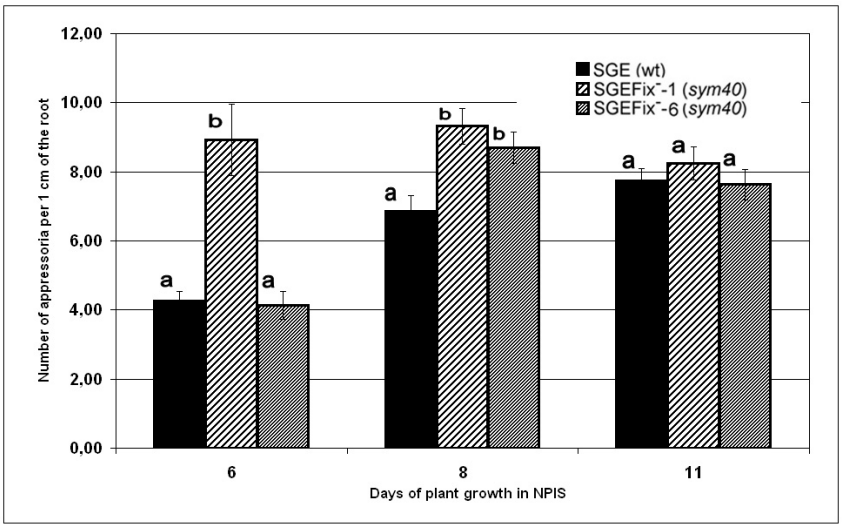

Fig. 1. Number of appressoria per 1 cm of the root in pea symbiotic mutants SGEFix⁻-1 (sym40), SGEFix⁻-6 (sym40) and the wild-type line SGE. Standard errors show variance of mean values at certain time points. Values marked with the same letters do not statistically significant differ at P≥0.95 at certain time points.

Mutant SGEFix⁻-6. In contrast to SGE, by eight days of growth in NPIS the mutant line SGEFix⁻-6 *(sym*40*)*, exhibited a similar high number of appressoria as SGEFix⁻-1 (*sym*40) (Fig. 1). Plants of SGEFix⁻-6 (*sym*40) differed from the other two lines by the reduced speed of mycorrhization (Fig. 2). Although the speed of arbuscule development in the roots of line SGEFix⁻-6 *(sym*40*)* is slightly slower in comparison with the other mutant line, the process of arbuscule turnover in this line was similar to that of the line SGEFix⁻-1 (*sym*40) (Fig. 3).

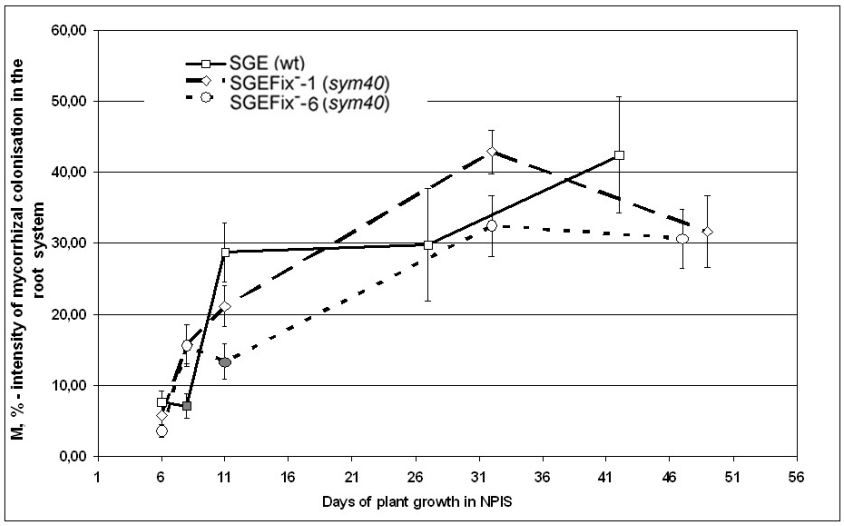

Fig. 2. Intensity of mycorrhizal colonization in the root system (M%) in pea symbiotic mutants SGEFix⁻-1 (sym40), SGEFix⁻-6 (sym40) and the wild-type line SGE. Standard errors show variance of mean values at certain time points. Values marked with the same color (white, gray) do not differ at P≥0.95 at certain time points.

Discussion

The difference between the wild type line SGE and the mutant line SGEFix⁻-1 (*sym*40) in number of appressoria in process of AM development (at six and eight days of plant growth in NPIS) has been shown for the first time. In contrast, the mutant line SGEFix⁻-6 (*sym*40) at six days of growth in NPIS did not differ significantly from the wild type line SGE, whereas at eight days the two mutant lines did not differ from each other, but they both differed from the wild type line. Intensity of mycorrhizal colonization (M%) in the root system of the both mutant lines was similar until eight days of plant growth in NPIS, but after eight days statistically significant differences between these lines existed. All the lines differed from each other in dynamics of arbuscule development and turnover, especially at the early time

points. However, arbuscule abundance of the both mutant lines was identically changed after 11 days of plant growth in NPIS.

Thus, two independently obtained allelic mutations characterized in the present study differ by phenotypic manifestation, especially at the early time points. Consequently, we may suppose that we deal with mutations in different structural domains of the gene *sym*40. Perhaps, new independently obtained mutants in this gene will have new phenotypic manifestations.

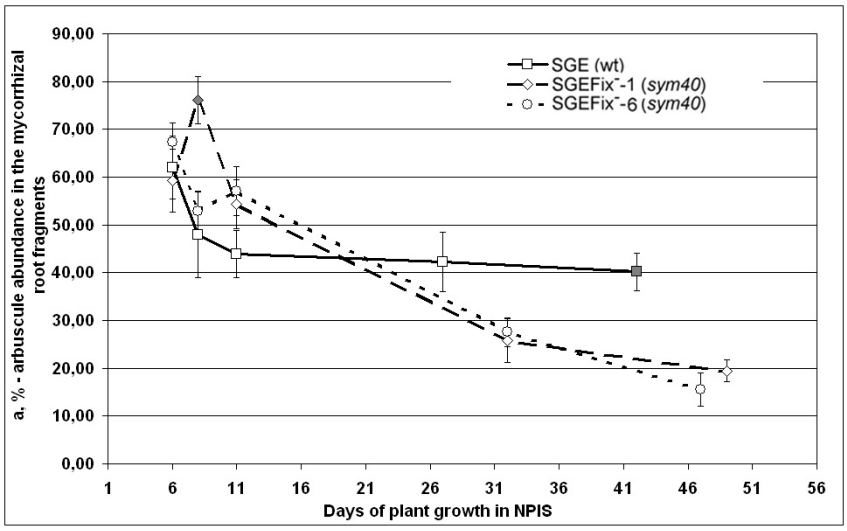

Fig. 3. Arbuscule abundance in the mycorrhizal root fragments (a%) in pea symbiotic mutants SGEFix⁻-1 (sym40), SGEFix⁻-6 (sym40) and the wild-type line SGE. Standard errors show variance of mean values at certain time points. Values marked with different colors (white, gray) differ at P≥0.95 at certain time points.

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