Isolation of pea (*Pisum sativum*) mutants impaired in arbuscular mycorrhiza development, using a direct screening

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Arbuscular mycorrhiza (AM) is an ancient symbiotic system formed between 80-90% of terrestrial plants and fungi belonging to the phylum *Glomeromycota*. It plays a positive role in plant development due to improved acquisition of minerals (mainly phosphorus) and water. This beneficial plant-microbe system has a potential for use in sustainable agriculture. However, the molecular-genetic basis of this symbiosis is insufficiently studied primarily due to obligatory symbiotic nature of AM-fungi. Until recently, the only approach used for identification of legume genes controlling AM development was based on the fact that legumes have a common genetic system responsible both for nitrogen-fixing symbiosis (NFS) and AM. However this approach does not allow identifying AM-specific genes. In particular, in pea (*Pisum sativum* L.) eight common symbiotic genes have been identified using the plant mutants impaired in NFS development (for review see 2). AM-specific pea genes have not been revealed to date. Only recently, the first three AM-specific legume mutants were identified in *Medicago truncatula* using direct screening of a mutagenized plant population (4).

In order to identify similar AM-specific genes in pea, the direct screening of an M_2 population of laboratory line SGE (3) after ethyl-methyl-sulfonate mutagenesis was initiated. A "nurse plant" inoculation system (5) was specially modified for pea plants and the local conditions, and this system was shown to have several advantages in comparison with conventional methods of inoculation with AM-fungi. By using the system, more than 700 plants of 193 families were analyzed and 78 putative mutants (PMs) having altered AM phenotypes were revealed. Thirty-four of them manifested *rmc*$^-$ phenotype (reduced mycorrhizal colonization); twenty-three displayed *pen*$^-$ (lack of penetration); twelve showed *arb*$^-$ (lack or reduced amount of arbuscules) and nine were *myc*$^{++}$ (hypermycorrhizic). Frequency of chlorophyll mutations was about 1.5%.

The seeds of 90% of the chosen plants have been collected and the progeny of PMs have been propagated. Tests for stability of the revealed phenotypes are now in progress. The progeny of the first plant, the mutant phenotype of which has been confirmed, is involved in genetic analysis. Also the progeny of twelve PMs was involved in analysis of symbiotic root nodule formation. The majority of the plants had normal root nodules. The progeny of a single PM displayed a Nod$^-$ phenotype, in that it formed "drumstick" root hairs. It has been shown that this trait is inherited monogenic and recessively. This genotype has been involved in allelism tests with different pea mutants in *sym*8 (1) and *sym*19 (6) genes, having similar nodulation phenotype.

Thus, the first PMs presumably defective in AM development were isolated in a direct screen. One of the PMs is probably impaired in the "common" symbiotic gene for both AM and nodule formation. The fact that a majority of the plants impaired in AM formation had normal nodulation phenotype, indicates, that mutations have occurred in genes specific to AM development. The genetic analysis of the mutants isolated and confirmed will permit the identification of new pea genes controlling the development of AM but not NFS.

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