Pea mutant line Sprint-2Nod-3 represents a new mutant allele of pea symbiotic gene *sym*19

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Pea (*Pisum sativum* L.) remains an important model object for studying genetics of symbiotic systems, despite the fact that it has a large genome and a relatively low capability for genetic transformation. A large collection of well-characterized symbiotic mutants with unique phenotypic manifestation has been obtained (1).

Here we describe the mutant Sprint-2Nod$^-$-3 obtained in the background of laboratory line Sprint-2 after EMS mutagenesis more than 15 years ago. The line Sprint-2Nod$^-$-3 is derived from M_2 plant that had gray and green nodules and was deficient in nitrogen fixation (Fix$^-$ phenotype). Four plants of the next generation (M_3) also had gray and green nodules, but all the progenies in M_4 totally lacked nodules. The line was finally phenotypically characterized as Nod$^-$ mutant forming specific deformations of root hairs resembling drumsticks by shape (Fig. 1). This phenotype is specific for pea mutants in *sym*8 and *sym*19 genes (2, 3). Allelism tests with pea lines carrying mutations in "early" symbiotic genes *sym*8, *sym*9, *sym*10 and *sym*19 demonstrated that the line Sprint-2Nod$^-$-3 is a mutant of *sym*19.

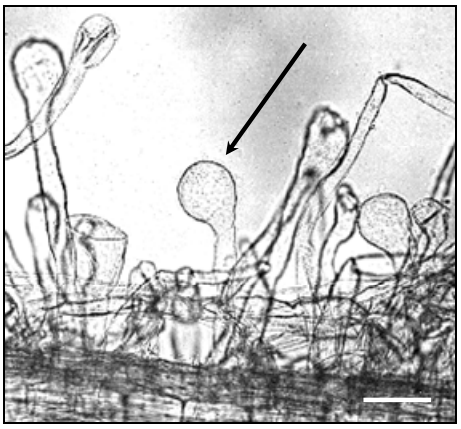

Fig. 1. A characteristic deformation of root hair tips in Sprint-2Nod-3 mutant (sym19). Arrow points to deformed root hair tip. Scale bar 0.05 mm

How could a Fix$^-$-mutant turn out to be a Nod$^-$-phenotype? The explanation we could propose is the following. The initial M_2 plant (Fix$^-$) was grown in summer under "outdoor" conditions, where the temperature varied from 10°C to 25°C. Next winter M_3 plants (Fix$^-$) were grown in a greenhouse where the temperature also varied from 10°C to 25°C. All the following generations were cultivated in the climatic chamber (Vötsch Industrietechnik VB 1014, Germany) under strictly controlled temperature conditions (21 ± 1°C) and demonstrated Nod$^-$-phenotype. Lowering the night temperature to 15°C is usually enough for manifestation of temperature-sensitive phenotype in pea symbiotic mutants. Therefore, we suggest that Sprint-2Nod$^-$-3 is an example of temperature-sensitive mutation of *sym*19.

The fact that the mutation blocks the development of symbiosis at a definite stage does not mean that this gene "works" only at this stage. Mutations in the gene *dmi2* of *Medicago truncatula* Gaertn. lead to inability in developing infection process (4, 5), but experiments of Limpens et al. (6), knocking out *DMI*2 by inducible RNA interference, demonstrated that activity of *DMI*2 is also essential for symbiosome formation at late stages of symbiosis development. The gene in pea that corresponds to *DMI*2 in *M. truncatula* (also to NORK in *Medicago sativa* L. and *SymRK* in *Lotus japonicus* (Regel.) K.Larsen) is *Sym*19 (7, 8). The presence of the mutation in pea *sym*19 that could cause Nod$^-$ or Fix$^-$ phenotype depending on growth conditions is good evidence of the role of this gene on early and late stages of symbiosis development.

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