SELECTION OF NODULATION RESISTANT MUTANTS OF PEA

Jacobsen, E. Dept. of Genetics, University of Groningen
Haren, The Netherlands

Legumes are capable of fixing with nodules containing bacteriods of <u>Rhizobium</u>, a symbiosis which is affected by several genes of both partners. Moreover, natural variants and mutants that negatively affect nodulation and/or N_2 fixation have been found in both partners. In pea, natural variation with respect to nodulation has been described (1,2) and recently nodulation resistance has been found in material after mutagenic treatment (3). In the present contribution, the successful selection of other nodulation resistant mutants is reported. Selection of these mutants was carried out in M2 families which had been used before for the isolation of nitrate reductase deficient mutants (4) and a mutant, nod-3, highly nodulating in the presence of nitrate (5).

Seeds were germinated in moistened vermiculite, and after one week, seedlings were transferred to aerated nitrogen-free liquid medium inoculated with <u>Rhizobium leguminosarurn</u> strain PF2. Sixty seedlings were grown per plastic container, including 10 plants of the parent variety 'Rondo'. The latter were included to verify the effectiveness of the infection. Eighteen days after sowing, Rondo seedlings were clearly nodulated. Twenty-eight days after sowing, M_2 seedlings were screened for nodulation. Plants with well-developed roots but without nodules, from containers in which all 10 control plants had nodulated well, were transferred to aerated nitrogen containing liquid medium for seed multiplication. In all, 20 plants were selected from among 250 families screened. A few of the M3 progenies showed nodulation resistance in all plants tested. Of these, lines and were investigated more extensively. Nodulation of M3 and M4 plants and of F1 and F2 plants after crossing with Rondo is shown in Table 1. The results indicate that recessively inherited mutations are involved. These results support the experiences of LaRue (3; personal communication), who showed that nodulation resistance can be found in M_2 material in relatively high frequencies.

Table 1. Nodulation of nodulation resistant lines K5 and K9 in the M3 and M4 generation, and in the F1 and F2 after crossing with Rondo after inoculation with R. <u>leguminosarum</u> strain PF2.

Generation	Nodulation of K_5	Nodulation of K_9
M_3	–	–
M_4	–	–
F_1	+	+
F_2	+ (15)	+ (37)
	– (9)	– (14)

Mutants other than those affecting nodulation were also recovered from these crosses. In the generation of mutant K5 x Rondo, another recessively inherited character, viz a weak root development, segregated independently of the nodulation resistance. In the F2 generation of K9. x Rondo, two other mutations were isolated, viz narrow leaves and dwarfness.

The nodulation resistance of mutant K5 has been tested for strain specificity. In Table 2, nodulation results of Rondo, after inoculation with strains PF2, F13 and Him (kindly supplied by Dr. Lie, Wageningen, the Netherlands) are given; the data indicate that the nodulation resistance of K5 is not restricted to R. leguminosarum strain PF2 and show that sometimes nodules appear on the lateral roots of mutant K5. This observation indicates that this mutation strongly inhibits but does not preclude nodulation. Preliminary observations on root hairs showed that after inoculation with Rhizobium root hair curling takes place and infection threads are present, suggesting that in line K5, the block in the development of nodules probably occurs after or at a late stage of the infection process.

Table 2. Nodulation of Rondo and mutant K5 with Rhizobium leguminosarum strain PF2, F13 or Him.

Genotype	Nodule number on		Total nodule weight (g)	Strain
	Taproot	Lateral roots		
Rondo	> 16.3	197.8	0.112	PF_2
K_5	0	5.8	0.001	PF_2
Rondo	25.6	84.8	0.185	Him
K_5	0	3.0	0.001	Him
Rondo	>150	>150	0.518	F_{13}
K_5	0	9.2	0.004	F_{13}

1. Lie, T. A., D. Hille, R. Lambers and A. Houwers. 1975. In: Nutman, P. S. (ed.) Symbiotic Nitrogen Fixation in Plants. JBP 7 Cambridge University Press, London p. 319-333.
2. Holl, F. B. 1975. Euphytica 24:767-770.
3. Kneen, B. E., and T. A. LaRue. In: C. Veeger and W. E. Newton (eds.). Advances in Nitrogen Fixation Research. Martinns Nijhoff/Dr. W. Junk, Publishers, The Hague, Boston, Lancaster and PUDOC, Wageningen, p. 599.
4. Feenstra, W. J., and E. Jacobsen. 1980. Theor. Appl. Genet. 58:39-42.
5. Jacobsen, E., and H. Nijdam. 1983. PNL 15:31-32.