GENES REPORTED TO AFFECT SYMBIOTIC NITROGEN FIXATION BY PEAS

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Pisum sativum has many desirable features which make it suitable for research in symbiotic nitrogen fixation. Compared to other legume hosts, peas are genetically and physiologically well characterized. They are specifically nodulated by their nitrogen-fixing symbiont *Rhizobium leguminosarum*, and some lines exhibit further specificity for certain strains of the bacteria. There are now nine plant genes reportedly involved in nodulation on peas.

Three genes are reported to condition the number of nodules. Gelin and Blixt (2) crossed 'Parvus', a line with a relatively high number of nodules, or "Parvus low", a line with about half the number of nodules, with the low nodulating line 1127P. The Parvus x 1127P progeny varied from 0 to >70 nodules per plant on a continuum well beyond the parental type. With no discrete classes, divisions were made based on how the data would best fit monogenic or dihybrid genetic ratios. It was concluded that high nodule number in Parvus was determined by two recessive gene pairs, nod-1 and nod-2 (= no and nod). Jacobsen and Feenstra (5) mutagenized 'Rondo' and obtained a mutant with higher than normal nodule numbers. Unlike the lines described by Gelin and Blixt, the mutant is highly nodulated even in the presence of 15 mM KNO_3. The mutant gene is tentatively designated nod-3, although tests for allelism with nod-1 and nod-2 are yet to be done.

Nodulation is sensitive to environmental factors, but only one pertinent gene has been described. Lie (8) found that the wild variety 'Iran' did not nodulate with temperature sensitive strains of R. *leguminosarum* at 20C, but a brief exposure to 26C permitted formation of normal nodules. Lie reported that the temperature dependent nodulation resistance in Iran is dominant (11); the gene was designated sym-1 (4).

The variety 'Afghanistan' (9,10) is representative of a small class of wild peas with *Rhizobium* strain specificity (6,16,17). These peas are nodulated by some Middle East strains of R. *leguminosarum*, typified by strain TOM, but form few or no nodules with strains from temperate soils. Lie and his coworkers (13) found that R. *leguminosarum* from European soils competitively inhibited the formation of nodules by strain TOM, though the European strains themselves are not infective. The infectivity of strain TOM is associated with a transmissible plasmid (1).

Holl (3) crossed Lie's nodulation-resistant Afghanistan with nodulating 'Trapper'. With commercial inoculant, the F2 segregation fit a 3:1 ratio, suggesting that non-nodulation is conditioned by homozygous recessive alleles (sym-2 sym-2). Lie, et al. (11) claimed that nodulation resistance was dominant based on analysis of F2 and backcross progeny of Afghanistan x Rondo crosses tested with R. *leguminosarum* strain PRE in hydroponics. Nodule numbers were both temperature and strain dependent (11).

Ohlendorf (14) presented evidence for two partially dominant genes, A and B, controlling nodulation resistance. The two genes were found in different lines from Afghanistan; it is not known if they are similar to Lie's Afghanistan line. In both the F1 and F2 generations of crosses between completely resistant "Afgh I" and occasionally low (<4) nodulating "Afgh III", approximately 15% of the plants had higher (4-25) nodule counts than the parental lines. When either line was crossed with 'Bottnia' (35-102), the F. plants varied from 0-30 nodules and F2 populations exhibited a wide and continuous range of nodulation (0-105) which was attributed to the segregation of a third gene, C, for nodule number. If the F2 populations are arbitrarily divided into two nodulation classes, <30:>30, the data fit a dihybrid 13:3 ratio. However, nodulation patterns of F3 progeny of selected F2 plants did not confirm the existence of all the expected genotypes.

At the Boyce Thompson Institute, reciprocal crosses between an inbred line of Lie's Afghanistan (which has no, or rarely, a few (<5) nodules) and 'Sparkle' (20-90 nodules) yield F1 plants with 5-50 nodules when grown in vermiculite inoculated with R. *leguminosarum* 128C53. Nodule number on F1 plants tends to be lower when Afghanistan is the female parent. F2 populations show continuous variation for nodulation with 20-27% nonnods, 10-30% 1-9, 25-50% 10-50, and 10-30% >50 nodules. The segregation for presence versus absence of nodules fit that expected for two alleles at a single locus (sym-2). How to delineate classes within the non-parental intermediates is unclear. We hoped to clarify the segregation ratios by repeatedly backcrossing selected non-nodulating plants to 'Sparkle' and eliminating variation due to genes modifying nodule numbers. With rhizobial strain 128C53, Afghanistan x Sparkle BC4F1 plants all noduled in the Sparkle parental range. BC4F2 populations still included 20-40% non-parental type intermediate nodulating plants and 5-25% nonnods. Selection against genes for low nodule number and for strain specific nodulation resistance will require testing each plant with 2 strains of R. *leguminosarum* - one infective, eg. TOM, to select for high number of nodules and one to which Afghanistan is nodulation resistant, eg. 128C53. Afghanistan x Sparkle F2 populations scored with R. *leguminosarum* strain TOM all nodulated with a range of 10-90 nodules. Testcrosses of F1 plants to both parents were made. With strain 128C53, 42% of the progeny from F1's x Afghanistan and reciprocals were nonnod, while the remaining 58% nodulated in the range of the F1's. Backcrossing F1's to Sparkle yielded nodulating plants with wide variation in nodule number.

We have investigated the genetics of nodulation resistance by analyzing for linkage between non-nodulation and isozyme loci which segregated In F2 progeny of Sparkle x Afghanistan and reciprocal crosses as well as F plants testcrossed to Afghanistan.

A correlation was observed (Table 1) with the isocitrate dehydrogenase (IDH) phenotype, which is specified by the locus *Idh* on chromosome 1 (15). The Afghanistan parent was homozygous for the "fast" allele at *Idh* and Sparkle was homozygous for the "slow" allele. Of the 23 F2 individuals resistant to nodulation, 15 were homozygous for the "fast' allele at *Idh*. In the testcross progeny, 9 out of 12 nonnodulating plants were "fast", a significant deviation from random assortment. No correlation was observed between low nodule number (1-5) and the IDH "fast" phenotype, indicating that the sym-2 gene acts primarily as an on-off switch for nodulation by temperate strains of R. leguminosarum.

Table 1. Correlation of isocitrate dehydrogenase alleles and nonnodulation.

	Number of plants	IDH Phenotype	Nodules absent	Nodules present
AxS F_2 1687-2-	36	Slow	1	6
		Het	1	17
		Fast	9	2
			11	25
SxA F_2 1696-3-	56	Slow	0	17
		Het	6	25
		Fast	6	2
			12	44
(SxA F_1 1696-X)A	38	Het	3	21
		Fast	9	5
			12	26

Holl (3) reported that F_2 seedlings from crosses of Afghanistan and 'Trapper' included segregants which nodulated but did not fix nitrogen. The gene pair conditioning the nonfix character was designated sym-3 8ym-3. Lie (pers. comm., 1983) has discovered another wild pea with strain specific nodulation (sym-4 sym-4). Afghanistan also has a gene pair (sym-6 sym-6) conditioning partially ineffective nodules with R. leguminosarum strain F13 (12).

We have mutagenized Sparkle with EMS and obtained a stable mutant resistant to nodulation by all rhizobial strains tested, including strains infective on Afghanistan (7). In crosses between Sparkle and the mutant, F1 plants nodulate like Sparkle and the F2 progeny segregate 3 nodulated : 1 nodulation resistant (nonnod or <10). Testcrosses of F1 plants to the mutant segregate 1:1. When inoculated with R. leguminosarum 128C53, Afghanistan x mutant F1 plants are nodulating (10-50) and F2 progeny include nodulating (>10) recombinants. With the TOM strain, 25% of the F2 progeny have nodulation resistance inherited from the mutant line. The differences in strain specificity and genetic data is evidence that the mutant gene is nonallelic with Afghanistan. This mutant has been designated sym-5.

Analysis of such pea mutants defective in nodule formation will help elucidate the role of the host in symbiotic nitrogen fixation.

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