Sym2 and *nod-3* are independent but closely linked genes influencing nodule development in pea

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Gene *sym2* confers strain-specific nodulation in a few primitive pea accessions from the Middle East. It was initially described as a recessive gene responsible for partial resistance of cv. 'Afghanistan' to European strains of *Rhizobium leguminosarum* bv. *vicia* (2 8). Resistance to nodulation, conferred by *sym2,* is not complete and depends on genetic and environmental factors. Gene *sym2* can act as recessive or semidominant depending on the strain of rhizobium used, and at higher temperatures nodulation occurs with higher efficiency (9). It was recently shown that *Sym-1,* which originally was described as a dominant gene responsible for temperature-dependent nodulation of cv. 'Iran' (7, 9), is an allele of *sym2* (6).

Recessive mutation *nod-3,* induced in cv. 'Rondo', is characterized by an excessive number of nodules even in the presence of nitrate (3) and by shortened lateral roots (10). The mutant phenotype is not strain-specific (4); however, *nod-3* appears to map very near *sym2.* Gene *sym2* was localized at 17 cM from marker *d* (anthocyanin ring at the base of stipules) (13) and at 25 cM from locus *Idh* (isocitrate dehydrogenase) (12) which is about 10 cM from *d.* Our data indicate that *nod-3* and *Idh* are separated by about 20 cM (11). In addition, fine mapping experiments demonstrated that these two genes map close to the same RAPD marker (our unpublished data). In order to determine unambiguously whether *nod-3* is a phenotypically different allele of *sym2* or a different locus we attempted to identify the double mutant phenotype in progeny of crosses between the two mutants.

Parental material included lines Nod3 (homozygous for allele *nod-3* in a 'Rondo' genetic background) and BC-sym2 (a near-isogenic line produced as described by Kneen et al. (5) by six repeated backcrosses of line 'Afghanistan' to cv. 'Sparkle' and a final self-pollination), and a single F_2 plant, heterozygous for *sym2* and derived from a cross between BC-sym2 and multiple-marker line JI73. In our experiments, when plants were grown in vermiculite at 18-20°C and inoculated with *R. leguminosarum* strain 128C53, line BC-sym2 formed no nodules (non-nodulating), the Nod3 line displayed 300 ± 70 nodules (hypernodulating), and lines with normal nodulation ('Rondo', 'Sparkle') displayed 50 to 100 nodules per plant.

In the cross between lines BC-sym2 and Nod3, the F_1 hybrids formed from 2 to 40 nodules per plant (low nodule number), indicating that in this cross gene *sym2* behaved as incompletely dominant. Similar results have been observed in a cross between line 'Afghanistan' and cv. 'Sparkle' tested under similar conditions (5). The F_2 progeny of the cross BC-sym2 x Nod3 segregated as follows:

8 hypernodulating : 18 low nodulating : 18 non-nodulating : 6 normal.

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The eight hypernodulating plants also all displayed shortened roots, similar to the Nod3 line. We postulate that these represented *nod-3/nod-3* genotypes. Thirty-six out of fifty F_2 plants formed a few or no nodules, presumably due to the semidominant effect of gene *sym2*. The F_2 plants with normal nodulation were suspected to be recombinant between genes *sym2* and *nod-3*. In the F_3 progeny of three of these plants with normal phenotype, normal and hypernodulating plants segregated in a 3:1 ratio. These parental F_2 plants thus possessed the genotype *Sym2, nod-3/Sym2, Nod-3*. Progeny of the other three plants consisted of non-nodulating, low-nodulating and normally nodulating plants in proportion 1:2:1, consistent with a genotype of *sym2, Nod-3 / Sym2, Nod-3*.

From these data we conclude that *sym2* and *nod-3* are different loci. The apparent recombination frequency between these genes was about 25%, much higher than predicted based on earlier results. However, the uncertainty in scoring *sym2* as semidominant and the small size of the population compelled us to investigate another segregating population, which produced recombinants between *sym2* and *nod-3* with a double mutant phenotype.

This second cross (between mutant line Nod3 and the F_2 plant heterozygous for *sym2* and derived from a cross between BC-sym2 and multiple-marker line JI73) produced nine F_1 hybrids, which all displayed normal nodule number. Five of these plants produced F_2 progeny that segregated only for *nod-3* and therefore had genotype *Sym2, nod-3/Sym2, Nod-3*. The remaining four plants produced F_2 progeny that included non-nodulating phenotypes (Table 1), suggesting that those four F_1 hybrids were heterozygous for both *sym2* and *nod-3* and that the two mutants complemented each other in these hybrids.

Table 1. Phenotypic segregation in an F_2 population derived from *Sym2, nod-3/sym2, Nod-3* hybrids.

Phenotype	Number of F_2 plants
Lateral roots normal; non nodulating	31
Lateral roots normal; low nodulating	25
Lateral roots normal; normal nodulation	39
Lateral roots short; hypernodulation	31
Lateral roots short; few nodules	3

In this cross the proportion of plants with normal root morphology and normal nodulation was high and did not seem to represent a recombinant class. Ten plants of this category were subjected to progeny analysis and, in contrast to the results from the first cross, all were heterozygous at both *sym2* and *nod-3,* resembling the initial F_1 hybrids. Thus, in this second cross, the semidominant effect of *sym2* was less pronounced and the class of heterozygotes for *sym2* consisted of plants with nodule number ranging from relatively few (5-10) to that expected for a wild type.

Three plants with a novel phenotype were found among the F_2 population. These plants were characterized by a low number of nodules and shortened roots. The progeny of these plants consisted of 1/4 hypemodulating, 1/2 low nodulating and 1/4 nonnodulating plants, all having the same altered root morphology. When two of these plants with the novel phenotype were crossed with the parental line Nod3, all eight F_1 hybrids had an excessive number of nodules and shortened roots. These data indicate that the recombinants were homozygous for mutation *nod-3* and heterozygous for gene *sym2 (sym2, nod-3/Sym2, nod-3),* further confirming that *syml* and *nod-3* are different loci.

The expected probabilities of progeny phenotypes in the second cross, according to the epistatic model (1), were 0.25 non-nodulating, 0.5 low-nodulating, $(1-\mathbf{r})^2/4$ hypernodulating, and $(2\mathbf{r} - \mathbf{r}^2)/4$ hypernodulating plants with short roots, where **r** is the recombination frequency between the *sym2* and *nod-3* loci. Solution of the likelihood equation

$$\text{HYPER} \times 2/(\mathbf{r}-1) + \text{RECOMB} \times 2\,(1-\mathbf{r})/(2\mathbf{r}-\mathbf{r}^2) = 0$$

gave an estimated **r** of 0.05 ± 0.05, when HYPER and RECOMB were replaced by the observed counts of these classes, 31 and 3, respectively.

The data obtained in this study clearly demonstrate that *sym2* and *nod-3* are different loci. Although the estimates of genetic distance between the two loci varied in two different crosses we analysed, our data in combination with previous mapping results (6, 11, 12, 13) suggest that these two genes influencing the same developmental process are closely linked. The occurrence of recombinants with few nodules but shortened roots shows that hypernodulation and short roots in the *nod-3* mutant are regulated separately, and that in the nodulation process, *sym2* is epistatic to *nod-3*. The determinants of semidominant versus recessive expression of *sym2* have yet to be elucidated but appear to partially involve the genotype at other loci affecting nodule development.

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