

Symbiotic gene *Sym33* is located on linkage group I

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In pea 40 genes controlling pea-*Rhizobium* symbiosis have been identified up to now using genetical approaches (1). Nineteen of these have been localized on the genetic map (2, 7, 9, 14). Most of these genes control early nodule developmental stages (6, 12). Only three symbiotic genes controlling late nodule developmental stages, *sym13*, *sym27* and *sym31*, have been mapped to date (5, 8, 9). In addition, the late symbiotic gene *sym26* was linked with a DNA marker found in an unclassified linkage group (13). Some of these genes are also involved in the interaction of the pea host with arbuscular mycorrhizal fungi (1).

In this study we localized late symbiotic gene *sym33*, which have been identified in mutant SGEFix⁻2. It has been shown that gene *sym33* controls the endocytosis of bacteria into host-cell cytoplasm from infection droplets and differentiation of infection threads in young nodule tissue (11). Mutation in the gene *sym33* also influences mycorrhiza development, decreasing mycorrhizal colonization of roots and delaying arbuscule development at low temperature (3, 4).

For the mapping of *Sym33* we first crossed the mutant SGEFix⁻2 with the line NGB1238. Segregation at locus *Sym33* was analyzed in F₃ plants to identify F₂ plants homozygous and heterozygous by wild type and mutant alleles of *Sym33*. Joint segregation analysis showed barely significant linkage between *Sym33* and

Table 1. Joint segregation data in the F₂ populations of crosses 1) NGB1238 (*d*) x SGEFix-2 (*sym33*), 2) SGEFix-2 (*sym33*) x NGB1515 (*d*, *l*), 3) NGB2715 (*d*, *l*, *af*) x SGEFix-2 (*sym33*), 4) (Wt-10584 (*aero*, *l*) x SGEFix-2 (*sym33*)).

Cross	Gene pairs	Phase	Number of progeny with designated phenotype*									Total	Joint χ^2	Prob.	RCV	SE	
			A/B	A/h	A/b	h/B	h/h	h/b	a/B	a/h	a/b						
1	<i>d-sym33</i>	R	13	47	28					2	6	15	111	8.6	<0.025	37.4	5.5
2	<i>d-sym33</i>	R	10	7	8	17	31	13		4	9	20	119	18.2	<0.005	35.5	4.1
	<i>l-sym33</i>	C	28	39	23					3	8	18	119	13.5	<0.005	31.9	5.0
3	<i>d-sym33</i>	R	16	20	8	20	66	21		12	14	18	195	16.6	<0.005	39.8	3.4
	<i>l-sym33</i>	C	45	79	22					3	21	25	195	29.7	<0.0001	27.0	3.6
	<i>af-sym33</i>	R	18	17	1	26	57	14		4	26	32	195	50.2	<0.0001	27.5	2.8
4	<i>d-sym33</i>	R	20	11	4	8	24	7		5	7	14	100	27.4	<0.0001	28.9	4.0
	<i>l-sym33</i>	C	40	60	19					8	7	20	154	24.9	<0.0001	30.9	4.3
	<i>aero-sym33</i>	R	25	10	1	10	36	8		9	16	29	144	60.6	<0.0001	24.6	3.0

*A/a first gene; B/b second gene; h, heterozygous. When both genes are dominant, the capital letter stands for the dominant allele. When the second gene is codominant, the capital A stands for the dominant allele of the first gene and capital B for an allele of the second gene in coupling with A. When both genes are codominant, the capital letter stands for an allele of the first parent.

The calculations were made using S.M. Rozov's programs PLANT and CROSS and Piet Stam's program JoinMap (10).

marker *d* (linkage group I) (Table 1). In order to confirm the position of *Sym33* on linkage group I mutant SGEFix-2 was crossed with lines NGB1515, NGB2715 and Wt10584. In the cross with line NGB1515, linkage between *Sym33* and both markers *d* and *I* was found (Table 1). In the cross with line NGB2715, linkage between *Sym33* and three markers *d*, *i* and *af* was also shown (Table 1). The cross with the line Wt10584 showed linkage between *sym33* and markers *d*, *i* and *aero*. A map of this region was constructed based on our data (Fig. 1). Previously, symbiotic mutations *nod3*, *sym2*, *sym5*, *sym10*, *sym19*, *Enod7*, *Enod40*, *Lb* (14) and *sym35* (2) have been mapped to linkage group I. *Sym33* appears to be localized near the symbiotic loci *Sym5*, *Sym19* and *Enod40*. Currently, linkage group I contains a greater number of genes involved with symbiotic associations than any other linkage group in pea. This prevalence of symbiotic loci may indicate a special role in the establishment of pea-*Rhizobium* symbiosis for this chromosome.

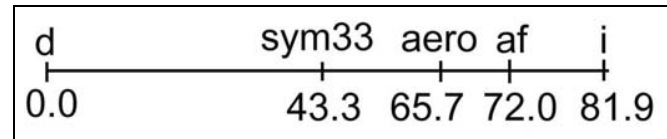


Fig. 1. The map of the *d*—*sym33*—*i* region. The distances are given in cM.

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