Chromosomal Location of Lectin Genes Indicates They Are Not the Basis of *Rhizobium* Strain Specificity Mutations Identified in Pea (*Pisum sativum* L.)

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A lectin gene family is located on linkage group 7 in pea. The lectin genes are arranged as a cluster, with no recombination observed within the multigene family. A lectin-like cDNA clone, pEA207, and eight DNA fragments generated by random priming also were mapped in the region of the lectin genes. None of the known pea mutants altering *Rhizobium leguminosarum* strain specificity map to this region of the genome, and therefore their altered specificities appear not to be directly produced by mutations in the lectin genes.

Lectins are proteins that bind specific carbohydrates. In plants, these proteins occur in highest concentrations in seeds, but they also are present in roots and other tissues (Borrebaeck 1984; Buffard et al. 1988; Díaz et al. 1984; Vodkin and Raikhel 1986). The lectins present in roots are thought to be encoded by the same genes as those in seeds, although their sugar specificity might differ (Gatehouse and Boulter 1980). The differences in sugar-binding specificity are caused by post-translational modification of the lectin (Kaminski et al. 1987; Okamura et al. 1986). Of the numerous functions lectins have in the plant, one of the most interesting is their role in cell recognition mechanisms. They have been implicated in pollen–pistil interaction (Heslop-Harrison 1976; Knox et al. 1976), in defense response, and as receptors for the attachment of pathogens (Etzler 1986).

We have been studying the genetic basis of nodule formation in pea (*Pisum sativum* L.) and have examined several natural and induced mutants with altered specificity for strains of *Rhizobium leguminosarum* (Kneen et al. 1987; Weeden et al. 1990). Díaz et al. (1989) demonstrated that lectins function as a determinant of host-plant specificity in the *Rhizobium*-legume symbiosis. Indeed, their experiment in which *Trifolium repens* gained the ability to be nodulated by *R. leguminosarum* biovar *viciae* after its roots were transformed with the pea lectin gene suggested that lectin was a key determinant of host-plant specificity. The altered strain specificity of the natural pea mutants, *sym2* and *sym22*, as well as the induced mutants of *sym2* and *sym18*, thus may be produced by changes in the DNA sequence of the lectin gene. As lectins are known to be coded by multigene families in lentil (*Lens culinaris*) (Foriers et al. 1981), common bean (*Phaseolus vulgaris*) (Hoffman and Donaldson 1985), soybean (*Glycine max*) (Vodkin et al. 1983), and *Medicago truncatula* (Bauchrowitz et al. 1992), the distinct strain specificity mutants may represent mutations at different lectin sequences in pea. The purpose of this study was to map the lectin genes on the pea genome and compare their positions with those of the strain specificity mutants.

Materials and Methods

Mapping Lectin Genes and a Lectin-Like cDNA Clone

Twenty-seven F_2 individuals and 38 F_7 inbred lines (derived by single seed descent of F_2 individuals) from the cross of JI 1794 × Slow were used for mapping the lectin genes. JI 1794 is a *P. sativum* ssp. *humile* line obtained from the John Innes Institute, Norwich, England, and Slow is a *P. s.* ssp. *sativum* marker line developed at Geneva, New York. The two parental lines differed at 18 isozyme loci, 10 morphological loci, and 40 restriction fragment length polymorphisms. These loci were well distributed over the pea genome (Weeden and Wolko 1990; Weeden, unpublished observations). Isozyme, morphological, and lectin RFLP phenotypes were scored on F_2 or F_2:F_3 (F_2 derived F_3) plants and on the F_7 inbred lines.

For the F_2 DNA analysis, we pooled five F_3 plants. For F_7 analysis, DNA was ex-

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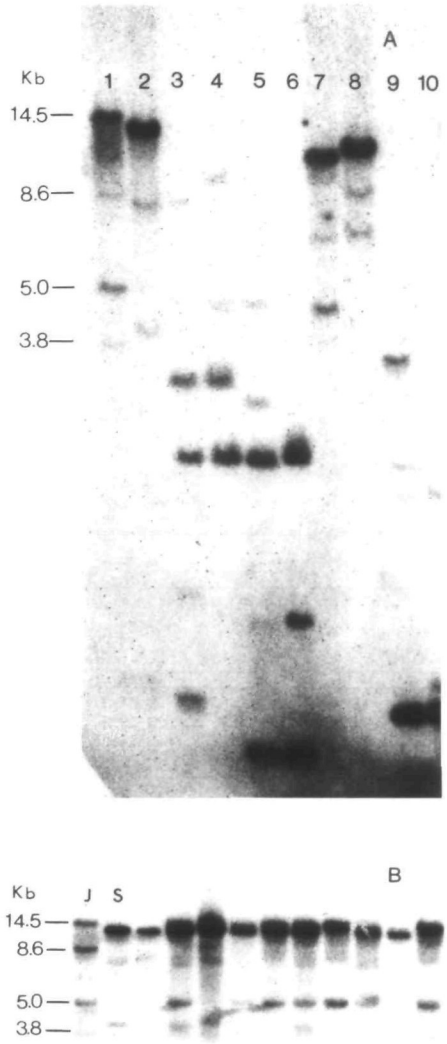

Figure 1. (**A**) Polymorphisms between the parents JI1794 (lanes 1, 3, 5, 7, and 9) and Slow (lanes 2, 4, 6, 8, and 10). Genomic DNA digested with different restriction enzymes and hybridized with labeled lectin cDNA pPS 15-50. Lanes 1 and 2 = *Eco*RI; lanes 3 and 4 = *Eco*RV; lanes 5 and 6 = *Bam*HI; lanes 7 and 8 = *Hin*dIII; lanes 9 and 10 = *Bcl*I. (**B**) *Eco*RI phenotypes observed when lectin cDNA sequence pPS 15-50 was used as a hybridization probe on blots containing parental and combined F_3 DNA (to represent the F_2 generation). The two parental and an additional heterozygous phenotype were observed in the F_2. Relative band intensities in the heterozygous phenotype varied because five to 10 F_3 plants were randomly chosen for the combined sample so that the ratio of genotypes in this sample often was skewed toward one of the parental types. J = JI1794; S = Slow.

tracted from one or more F_7 plants. We prepared DNA from young leaves according to the method of Doyle and Doyle (1987). Five restriction enzymes *Bam*HI, *Bcl*I, *Eco*RI, *Eco*RV, and *Hin*dIII were used to test restriction polymorphisms of the parental lines. *Eco*RI was chosen for the restriction digestion of the progenies because it was used in previous studies (Kaminski et al. 1986, 1987). Digested DNA was separated on 1% agarose gels. After electrophoresis, DNA was transferred to nylon membranes (GeneScreen Plus, Du Pont) under alkaline conditions (Reed and Mann 1985).

Table 1. Single locus segregation and χ^2 analysis for the lectin genes in an F_2 population from JI1794 × Slow

Probe	Ratio in progeny[a] Slow	Het.	JI1794	χ^2	*P*
pPS 15-50	6	12	9	1.00	.61
pEA207	8	12	5	0.76	.68

[a] Genotype designations: Slow = homozygous for allele in Slow parent; Het. = heterozygous; JI1794 = homozygous for allele in JI1794 parent.

The membranes were prehybridized in a bottle with 10 ml of hybridization buffer at 65°C for 5 h in a Mini Hybridization Oven (National Labnet Company, NJ). The hybridization buffer consisted of 750 mM NaCl, 175 mM citric acid, 50 mM $NaPO_4$, 2.5 mM EDTA, 5 × Denhardt's, 5% dextran sulfate, 0.6% SDS, and 0.4 mg/ml calf thymus DNA. We used a pea lectin cDNA clone containing the entire pea lectin coding region, pPS15-50 (Higgins et al. 1983), and a pea lectin-like cDNA clone, pEA207 (Dobres and Thompson 1989), as probes. The probes were labeled by random priming (Feinberg and Vogelstein 1983). We performed hybridization at 65°C overnight. Membranes were washed at 65°C in (1) 2 × SSC, 0.1% SDS, (2) 1 × SSC, 0.05% SDS, and (3) 0.5 × SSC, 0.05% SDS, for 30 min each.

Mapping with RAPD Markers

Thirty-eight F_7 recombinant inbred lines from the cross of JI1794 × Slow were used for the RAPD analysis. DNA for this analysis was extracted by the same method reported by Torres et al. (1993). For the PCR amplification, a sample with 25 μl of reaction mixture contained: 2.5 μl 10 × buffer and 0.5 units of DNA *Taq* polymerase (Promega), 100 μM dNTP, 20-25 ng genomic DNA, and 0.2-0.3 μM primer. Seventy-three 10-mers were tested. Except for the requirements of 40%-70% GC and absence of fold back symmetry, we chose the sequences of the primers arbitrarily. We performed amplification in a *COY* TempCycler (COY Corporation, Grass Lake, Michigan) programmed for 40 cycles of 1 min at 94°C, 2 min at 40°C, and 2 min at 72°C. Amplification products were resolved by electrophoresis with 2% agarose gel (1% normal molecular grade agarose plus 1% NuSieve GTG agarose, FMC). We scored segregation of markers from photographs of the ethidium bromide stained gel.

Data Analysis

LINKAGE-1 (Suiter et al. 1983) was used for linkage analysis, and MAPMAKER (Lander et al. 1987) was used for determining the sequence of markers. We calculated linkage relationships among lectin genes, cDNA pEA207, and isozyme loci from F_2 data only, although both F_2 and F_7 data were used to determine the order of these markers. The RAPDs were mapped from F_7 data only.

Results

Three to six bands were observed when endonuclease-digested DNA from either JI1794 or Slow were hybridized with the pea lectin cDNA, pPS15-50 (Figure 1A). Each of the five enzymes tested gave polymorphisms between the two parent lines. With *Eco*RI digestion, we identified four fragments, 14.5, 8.6, 5.0, and 3.8 kb, in the parent JI1794. In Slow, three *Eco*RI fragments were detected. Only three *Eco*RI patterns were observed in the F_2: the two characteristic of each parent and a third displaying a codominant or heterozygous pattern (Figure 1B). These phenotypes gave a segregation ratio fitting the 1:2:1 ratio expected for a single locus (Table 1). Thus, if the multiple bands detected in this study represent a multigene family, this family appears to be clustered. Joint segregation analysis of the lectin phenotype with the marker loci placed the lectin gene family near the end of linkage group 7, about 15 map units from *Gal*-2 (Table 2).

Probe pEA207 produced two to four bands, and these were usually polymorphic between the parental lines with each of the restriction enzymes used (Figure 2). None of the fragments detected by pEA207 matched any detected by pPS15-50. The two *Eco*RI polymorphisms revealed by pEA207 segregated as a single Mendelian locus and mapped to the same end of linkage group 7 as the lectin cluster, although pEA207 fragments mapped further from *Gal*-2 (Figure 3).

The 73 primers used in the RAPD analysis generated over 200 polymorphisms between the parents. Eight of the products, generated by seven different primers, mapped near the region where the lectin genes are located (Figure 3). Two products, one generated by primer 5'-AGTCGCTCAT and the other by 5'-CTCCAAGGCC, mapped within 10 cM of the lectin cluster. The segregation pattern produced by the latter primer is shown in Figure 4. Thus, the RAPDs not only generated a finer resolution map of linkage

Table 2. Joint F_2 segregation analysis involving lectin gene, pEA207, and associated isozyme loci

Loci	No. of F_2 plants with designated genotype[a]									χ^2	Recombination fraction
	j/j	j/h	j/s	h/j	h/h	h/s	s/j	s/h	s/s		
Skdh/Est-2	1	1	0	1	14	0	0	0	6	27.3	0.04 ± 0.03
Skdh/Aat-m	3	0	1	3	12	1	0	2	5	19.1	0.16 ± 0.06
Lectin/Aat-m	4	4	1	1	8	2	1	2	4	8.5	0.28 ± 0.07
Lectin/Amy1	5	3	1	1	8	2	0	2	5	14.9	0.20 ± 0.06
Lectin/Gal-2	6	2	1	1	9	1	0	2	5	20.7	0.15 ± 0.05
Lectin/pEA207	3	4	1	2	6	2	0	2	5	8.3	0.17 ± 0.08
pEA207/*Aat-m*	0	5	0	5	5	2	1	3	4	9.7	0.40 ± 0.09
pEA207/Gal-2	2	3	0	4	7	1	1	2	5	9.7	0.28 ± 0.08

[a] Genotype designations: j = homozygous JI1794; h = heterozygous; s = homozygous Slow.

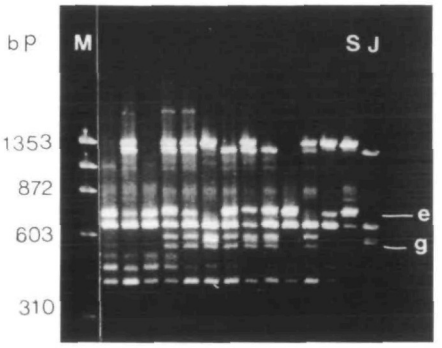

Figure 4. RAPD products generated using primer sequence 5'-CTCCAAGGCC. Seven polymorphisms segregated in the recombinant inbred lines produced from JI1794 (J) × Slow (S). Two of these, marked "e" and "g," mapped on linkage group 7 (see Figure 3). M = DNA size standard.

group 7 but also strongly confirmed the location of the lectin gene.

Discussion

Four sequences homologous to pPS15-50 have been reported in the pea genome (Kaminski et al. 1987). Our results also identify four *Eco*RI fragments in JI1794 hybridizing to the pea lectin cDNA and three fragments in Slow. The coding region of the pea lectin gene lacks an *Eco*RI site as well as introns (Higgins et al. 1983; Kaminski et al. 1987). Hence, these fragments apparently are distinct copies of the lectin sequence or pseudogenes with abbreviated or modified sequences. Our results demonstrate that these sequences are clustered on linkage group 7 near *Gal*-2.

The small number of inbred lines examined limits our ability to estimate the tightness of the lectin cluster. However, genetic linkage of the multigene family of the *Phaseolus* seed lectins has been reported (Brown et al. 1981; Hoffman and Donaldson 1985). In addition, proteins such as ribulose bisphosphate carboxylase, actin, and leghemoglobin are coded by small families of genes, and usually at least a portion of the genes within the family is tightly linked. These clusters segregate as single Mendelian units in F_2 and later generations.

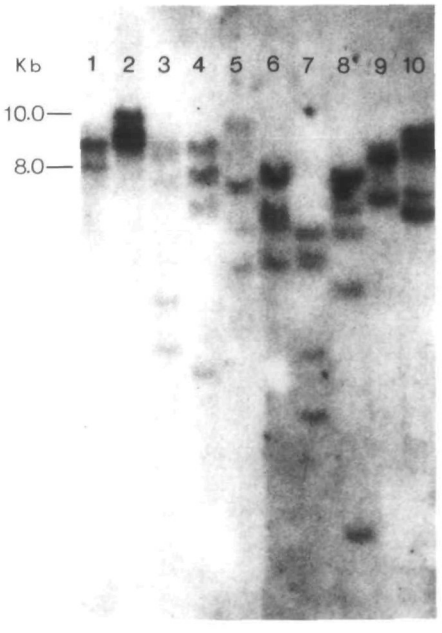

Figure 2. Polymorphisms between the parents JI1794 (lanes 1, 3, 5, 7, and 9) and "Slow" (lanes 2, 4, 6, 8, and 10) after their genomic DNA was digested with different restriction enzymes (same order as in Figure 1A) and hybridized with labeled lectin-like cDNA pEA207.

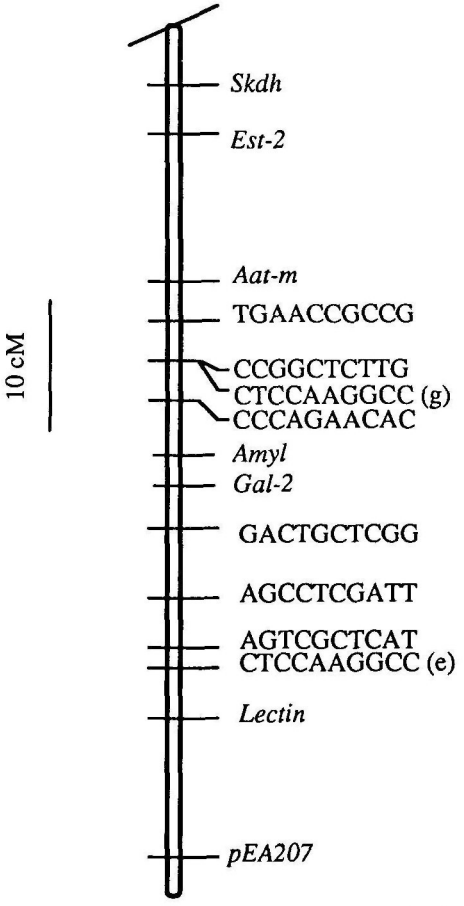

Figure 3. A portion of linkage group 7, showing the positions of the lectin genes and the lectin-like cDNA clone pEA207 relative to five isozyme loci and eight RAPD markers. *Skdh* is toward the centromere, while pEA 207 is at the distal end of the chromosome. The positions of the RAPD markers are labeled by the sequence of the oligonucleotide primer generating the polymorphism.

Our results suggest that the cDNA clone, pEA207, also may be a representative of a tightly linked multigene family. The clone was mapped to the same linkage group as the lectin genes but slightly further away from *Gal*-2. The transcript of this clone shows 37% homology to a 75-amino acid overlap of the pea major seed lectin gene encoded by pPS15-50, and Dobres and Thompson (1989) suggested that pEA207 encodes a polypeptide with lectin-like function.

The placement of the lectin genes on linkage group 7 suggests that the altered *Rhizobium* strain-specificity displayed by several *sym* mutants is not a direct consequence of a mutation in the lectin coding sequence. Mutations at *sym2* all display linkage with markers on linkage group 1 (Weeden et al. 1990; Young 1985). Preliminary data place *sym18* near *sym2* (Weeden et al. 1990), and *Sym22* is linked to markers on linkage group 2 (Temnyk, Weeden, Lu, and LaRue, unpublished observations). The only *sym* gene mapped near the lectin gene cluster is *sym*15 (Weeden et al. 1990). The phenotype of *sym*15 is short lateral roots and few or no nodules with all strains of *Rhizobium* tested. Thus the syntenic relationships between the lectin gene and *sym*15 is probably coincidental rather than functional. We conclude that none of the known *sym* genes involves a mutation of the lectin genes.

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