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An RFLP marker for r_b in pea

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Summary. We have located an RFLP marker, corresponding to the locus *Vc*-5, which is linked to the r_b locus. We also show that the heterogeneity at the *Vc*-5 locus is less among $r_b r_b$ lines than among pea genotypes as a whole. The relevance of this RFLP is discussed in relation to the construction of the double recessive *rr* $r_b r_b$ genotypes.

Key words: Pea – r_b – RFLP – Vicilin gene

Introduction

The *r* and r_b loci of pea have a profound effect upon seed development. Previous investigations (Coxon and Davies 1982) have shown that one pea line, which carries the recessive allele at both of these loci, has an unusually high lipid content in mature seeds when compared to other genotypes. In order to assess the extent of variation in lipid content among *rr* $r_b r_b$ it is first necessary to construct this allelic combination in many genetic backgrounds; however, the epistatic effects of the *r* locus make it necessary to use test crosses to detect this combination, where only 1 in 16 tested plants of the required seed phenotype are expected to be of the desired genotype. To make this detection easier we have sought an RFLP marker tightly linked to the r_b locus. In this paper we show that the *Vc*-5 locus (Ellis et al. 1986) is linked to the r_b locus. A simple scheme for the construction of $r_b r_b$ lines is described that exploits RFLP markers and in which approximately half of the progeny tested by crossing are expected to be of the desired genotype.

Materials and methods

Plant material

Pisum sativum L. (pea) genotypes used in this study were John Innes accession numbers JI 15, JI 61, JI 126, JI 181, JI 281, JI 399, JI 430, JI 528, JI 601, JI 1156, JI 1194, JI 2108, JI 2109, JI 2110 and JI 2111. The lines JI 399, JI 528, JI 601, JI 1156, JI 2108, JI 2109, JI 2110 and JI 2111 carry the recessive allele at the r_b locus, while the others carry the dominant allele at the r_b locus. The lines JI 61, JI 430, and JI 1194 carry the recessive allele at the *r* locus, while the other lines carry the dominant allele at this locus.

Nucleic acid manipulation

These procedures have been described previously (Ellis et al. 1984). The plasmid pJC2-7 has also been described previously (Ellis et al. 1986) and corresponds to a vicilin genomic clone. The cosmid clone cDB107 contains several copies of the pea rDNA repeat cloned in the vector pHC79 (Hohn and Collins 1980). This cosmid clone was constructed by, and was the gift of Dr. John Gatehouse of the Botany Department of the University of Durham. DNA was extracted from young leaves of F_2 plants derived from the crosses JI 15 × JI 399 and JI 281 × JI 399. In both cases, *Eco* RI and either *Sph* I or *Hind* III digested DNAs were run out on agarose gels, Southern blotted to either nitrocellulose or nylon membranes, and then examined by hybridisation with a variety of probes. In all cases digests were tested for completeness by hybridisation with cDB107.

Linkage analysis

Non-randomness in the segregation patterns was detected using a χ^2 analysis or Fisher's exact test (Fisher 1954). When non-randomness was detected, recombination frequencies were estimated from the maximum likelihood equation of Allard (1956); iterative calculations performed by simple BASIC programmes were used to solve these equations. Recombination frequencies were converted to map units using Haldane's mapping function (Haldane 1919).

Results

In the cross JI 281 × JI 399 it was discovered, after several different probes were examined, that there appeared to be linkage between the r_b locus and the hybridisation pattern detected with the probe pJC2-7, which corresponds to the locus *Vc*-5 (Ellis et al. 1986). The parental and F_1 patterns after an *Eco* RI digest are shown in Fig. 1 a, and the segregation data are shown in Table 1. These patterns were quite difficult to score, particularly in distinguishing the F_1 type from the JI 281 pattern. For this reason the plants that were homozygous at the r_b locus were scored again. Examination of the hybridization patterns of Southern blots of various digests of parental DNAs (not shown) suggested that *Sph* I would be informative. The *Sph* I patterns for this cross are shown in Fig. 1 b. This analysis gave only one disagreement between the *Sph* I and *Eco* RI scores, with the plant being scored as a JI 281 type with *Eco* RI and a heterozygote with *Sph* I. Ignoring this single ambiguity, we calculated the linkage distance between r_b and *Vc*-5 as 8.5 ± 2.2 map units.

This linkage was then tested in another cross, JI 15 × JI 399, in which 90 F_2 individuals were tested for their digestion patterns with *Eco* RI (Fig. 2 a) and *Hin*d III (Fig. 2 b) when probed with pJC2-7. Three cases of disagreement between the scorings were found, and these have been ignored in the linkage analysis summarised in Table 2. From the results of this cross we confirmed the linkage between r_b and *Vc*-5, but the map distance is greater, 27 ± 4 map units. The reason for the difference in map distance is not known, but in the cross JI 15 × JI 399 some seed abortion was noted in most of the F_2 plants, suggesting the possibility of some meiotic abnormality that may alter recombination frequencies.

These data provided evidence of linkage between the loci *Vc*-5 and r_b. We next sampled a collection of r_br_b lines (Fig. 3) and found a restricted set of *Vc*-5 alleles (compare Fig. 4 of Ellis et al. 1986). Among the lines JI 15, JI 61, JI 126, JI 181, JI 430 and JI 1194 (all of which are R_bR_b) five different *Vc*-5 alleles can be easily identified (Ellis et al. 1986). If we include JI 281, which is also R_bR_b, this gives an identity coefficient of 0.27. In contrast in our sample of eight r_br_b lines (including JI 399 which is not shown in Fig. 3), we have identified only two classes of *Vc*-5 allele, giving an identity coefficient of 0.625. The identity coefficient (i) is related to the average heterozygosity (H) by the relationship H = (1–i). The variability of *Vc*-5 in r_br_b

Table 1. Cross JI 281 × JI 399 (repulsion)

	Vc-5		
	281/281	281/399	399/399
r_b			
r_br_b	0	4	17
r_bR_b	4	43	3
R_bR_b	14	1	1
		N = 87	
Recombination frequency		Direct	8%
		Allard	0.09 ± 0.02
Map distance		Haldane	0.09 ± 0.02

Table 2. Cross JI 15 × JI 399 (repulsion)

	Vc-5		
	15/15	15/399	399/399
r_b			
r_br_b	3	12	12
r_bR_b	13	19	5
R_bR_b	18	8	0
		N = 90	
Recombination frequency		Direct	24%
		Allard	0.27 ± 0.04
Map distance		Haldane	0.39 ± 0.06

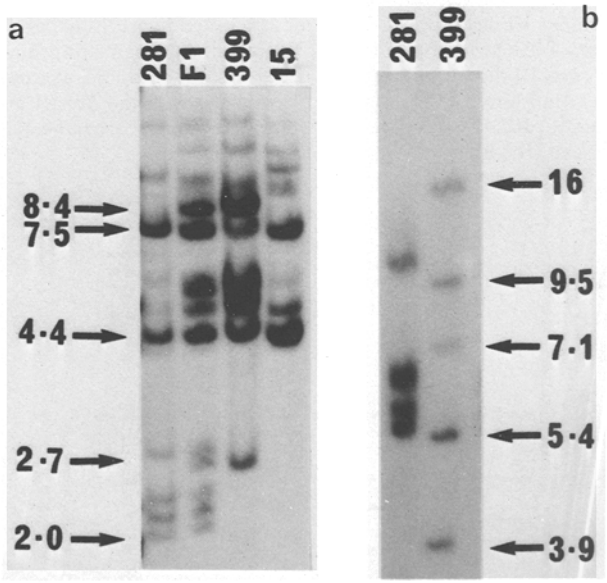

Fig. 1. **a** *Eco* RI digests of JI 15, JI 281, JI 399 and the F_1 of the cross between JI 281 and JI 399 run out on a 0.8% agarose gel, transferred to Hybond and hybridised with the *Eco* RI insert of pJC2-7. Sizes of selected hybridising bands are indicated in kbp. The filter was washed at low stringency (0.1 × SSC, 0.05% SDS at 50 °C), which accounts for the difference between the patterns for JI 15 and JI 399 in this figure and Fig. 2 a. **b** *Sph* I digests of JI 281 and JI 399 run out on a 0.8% agarose gel, transferred to Nitrocellulose and hybridised with the *Eco* RI insert of pJC2-7. Sizes of selected hybridising bands are indicated in kbp. The filter was washed at high stringency (0.1 × SSC, 0.05% SDS at 65 °C)

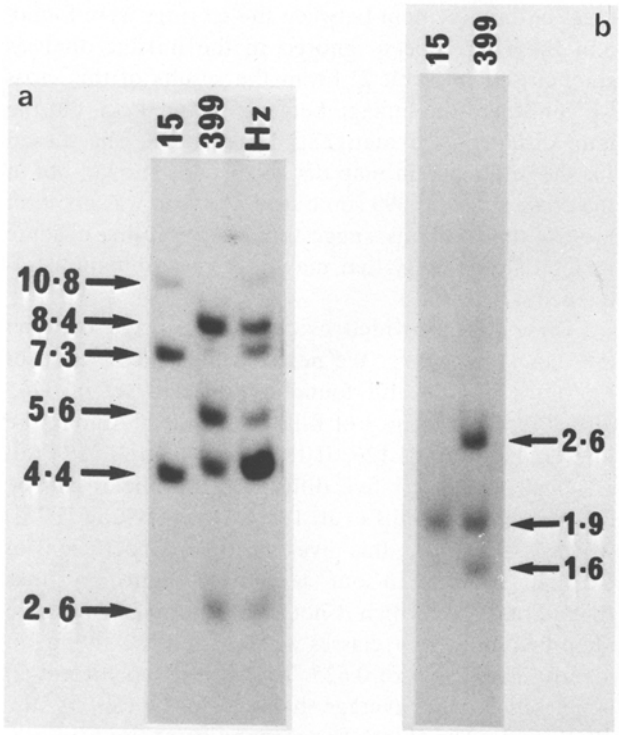

Fig. 2. **a** *Eco* RI digests of JI 15 and JI 399 and the heterozygote from the cross between JI 15 and JI 399 run out on a 0.8% agarose gel, transferred to Hybond and hybridised with the *Eco* RI insert of pJC2-7. Sizes of selected hybridising bands are indicated in kbp. The filter was washed at high stringency. **b** *Hin*d III digests of JI 15 and JI 399 run out on a 0.8% agarose gel, transferred to Hybond and hybridised with the *Eco* RI insert of pJC2-7. Sizes of selected hybridising bands are indicated in kbp. The filter was washed at high stringency

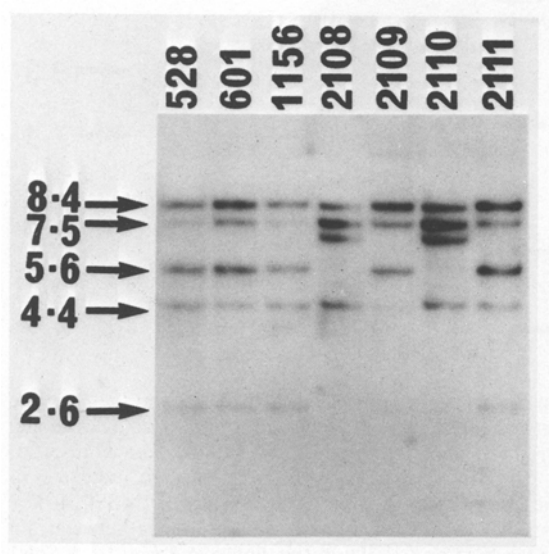

Fig. 3. *Eco* RI digests of JI 528, JI 601, JI 1156, JI 2108, JI 2109, JI 2110, and JI 2111 run out on a 0.8% agarose gel, transferred to Hybond and hybridised with the *Eco* RI insert of pJC2-7. Sizes of selected hybridising bands are indicated in kpb. The filter was washed at high stringency

lines is about three times that of the variability in r_br_b lines, which is consistent with the view that r_br_b lines are of a restricted genetic background and suggests that r_br_b lines are of a monophyletic origin.

Discussion

We have shown that the *Vc*-5 locus maps in the vicinity of the r_b locus; the present assignment of r_b to linkage group 3 (Blixt 1974) is tentative, so this information will help in the assignment of r_b to a linkage group through the analysis of RFLP linkage relationships. We have also shown that there is a linkage disequilibrium between *Vc*-5 and r_b, which is of some importance to us because it implies that different r_b lines will be similar to each other in the region around the r_b locus. Thus, it will be difficult to distinguish between the effects of the r_b locus itself and that of any closely linked marker.

Despite this difficulty and the considerable distance between the r_b and *Vc*-5 loci, we can exploit this linkage and the linkage between *r* and *Lg*-1, for which RFLPs have been described previously (Domoney et al. 1986), in the construction of *rr* r_br_b lines according to the following scheme.

In a cross between *RR* r_br_b and *rr* R_bR_b, the *rr* r_br_b progeny can be identified by assaying the alleles at *Lg*-1 and *Vc*-5. There will be some error in this assignment as a consequence of recombination. In the F_2 of this cross all the following genotypes will be wrinkled and phenotypically indistinguishable:

RR r_br_b : 2 *Rr* r_br_b : *rr* r_br_b : 2 *rr* R_br_b : *rr* R_bR_b

Since there is about 20% misassignment of *R* (or R_b) for *r* (or r_b) then the total misassignment of the putative *rr* r_br_b class will be about 50%. To attain a definitive identification of *rr* r_br_b it is necessary to undertake a test cross, which involves using another morphological marker manifest in embryonic tissues and allows for a discrimination between selfed and crossed seed.

If both the parental lines used in the programme carry the dominant allele at the *i* locus (i.e. had yellow cotyledons; Blixt 1974), then the desired genotype is *rr* r_br_b *II*.

If this is used as the pollen donor in two crosses:

rr R_bR_b *ii* × *rr* r_br_b *II*
RR r_br_b *ii* × *rr* r_br_b *II*

then the F_1 are:

rr R_br_b *Ii*
Rr r_br_b *Ii*

If the pollen donor carried at least one dominant allele at either the *r* or r_b locus, then at least half of the yellow F_1 seed in one of the crosses would be round. Any selfs will have green cotyledons and can be eliminated. Thus, it is possible to check the presumptive *rr* $r_b r_b$ lines with two test crosses, but with the need only to assay the F_1 seed borne on the maternal parent.

The implementation of this scheme would be the first exploitation of RFLP genetics in pea directed towards a practical objective, which is the creation of a variety of *rr* $r_b r_b$ lines of different genetic backgrounds, among which we may assess the extent of variation in lipid content.

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