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GENETIC ANALYSIS OF POD DEHISCENCE IN PEA (*PISUM SATIVUM* L.)

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Abstract: The inheritance of the dehiscent pod character was investigated in two recombinant inbred populations using a simplified correlation analysis. The approach identified three regions on the pea genome that affect the expression of pod dehiscence. The region on linkage group III corresponded to the expected position of *Dpo*, a gene known to influence pod dehiscence. A locus on linkage group V appeared to have a slightly smaller effect on expression of the phenotype. The third region was observed only in one cross, had a greater effect than *Dpo*, and was postulated to be yellow pod allele at the *Gp* locus.

Key words: *Pisum*, Domestication, Dehiscent Pod, QTL Analysis

INTRODUCTION

The cultivated pea, *Pisum sativum* ssp. *sativum* originated from wild forms of this same species approximately 10,000 years ago. According to cpDNA and 45S RNA analysis, a taxon very closely related to domesticated pea is *P. sativum* ssp. *elatius* var. *pumilo* [1,2]. Accession JI 1794 is a representative of this taxon and crosses easily with domesticated pea, although it differs significantly in appearance from the pea of commerce and possesses a number of primitive characters, including the tendency for the pods to dehisce when mature. Dehiscent pods are useful in wild pea as a seed dispersal mechanism. However, such a trait complicates the harvesting of the seeds, and selection of a pea with indehiscent pods probably was one of the first modifications made during the domestication of the crop.

The genetic basis of the difference between dehiscent and indehiscent pod phenotypes has been partially established. A recessive allele at the locus, *Dpo*,

has been assigned responsibility for the loss of pod dehiscence [3]. However, the inheritance of the dehiscent pod trait appears to be more complex than that expected for a simple monogenic character. Segregation ratios in the F_2 often diverge significantly from the expected 3:1 ratio (unpublished data), possibly due to the action of a second gene. We undertook this study to investigate if other loci besides *Dpo* play an important role in the expression of the dehiscent pod phenotype.

MATERIALS AND METHODS

Plant material

Three well-characterized pea lines (JI 1794, 'Slow' and MN313) were used as parents. 'Slow' is a multiple-marker line derived primarily from domesticated germplasm. It possesses indehiscent pods, but it differs from most commercial lines in possessing yellow pods (gene *gp*), colored flowers (gene *A*), reduced wax on stems and certain other plant parts (gene *wb*), caterpillar-pea (seeds sticking together in pod, gene *s*) and brown-violet testa (gene *oh*). MN313 is a typical cultivated pea released from the pea breeding program at the University of Minnesota. It lacks anthocyanins and displays indehiscent pods.

Two populations of recombinant inbred lines (RILs) were generated from crosses between JI 1794 and each of the domesticated lines. The cross JI 1794 x 'Slow' generated 53 F_{12} RILs that were derived from individual F_2 plants by single seed descent. This population will be referred to as the JxS population. Genetic characterization of the lines was performed on the F_8 and later generations. Various segregating markers (morphological, protein and DNA) were scored, and by the F_{12} over 800 markers had been arranged into seven linkage groups [4]. The second RIL population, consisting of 47 F_7 lines, was generated from the cross MN313 x JI 1794 (designated the MxJ population). As before, these lines were generated by single seed descent. Markers were scored on the F_5 and later generations, the greatest proportion of the markers being scored on the F_7 plants.

One plant of each line from both populations was grown in the glasshouse during the fall of 2000, and seed collected from these plants were used as the primary generation for analysis. Three seeds from each line were planted the following January in 15 cm diameter pots filled with an artificial soil mix and the plants grown in a glasshouse at Bozeman, Montana under 15 hr daylight.

Evaluation of the dehiscent pod character

Pods were removed from the plant just as they became dry (to prevent seed loss by pods dehiscing on the plant). The pods were stored in a 5 cm x 10 cm envelope at approximately 25°C and low (20 to 40%) relative humidity for at least 10 days. Inspection of the pods in each envelope after this time permitted the separation of the lines into five categories: (1) lines in which all pods spontaneously dehisced (often before or as harvested from plant) with the

pericarp often displaying tight coiling, (2) lines in which many pods spontaneously dehisced in the envelope with some twisting of pericarp, (3) lines in which most pods split open but little twisting was observed, (4) lines in which the pods were mostly indehiscent, but some pods split along the suture, and (5) lines in which the pods were completely indehiscent. These results were compared with less comprehensive observations on the dehiscent pod character in previous generations (lines were generally scored as displaying dehiscent pods or lacking dehiscent pods).

Marker and joint segregation analysis

The segregation pattern for each DNA or isozyme marker in both populations was determined by electrophoresis on starch or polyacrylamide gels (for isozymes) or agarose gels (for DNA markers). The conditions used have been described previously [5,6]. The linkage map for the JXS RIL population has been presented previously [4]. Additional STS markers have been added to this map [7]. All of the anchor markers and over 99% of the RAPD markers mapped onto the seven linkage groups that were assembled from the segregation data. There were no major gaps (>5 cM) between markers, and the relative length of the linkage groups corresponded approximately to the relative lengths of the respective chromosomes. Thus, the marker coverage of the genome in the JXS population appeared to be excellent.

For initial genetic analysis of pod dehiscence only those lines displaying a parental phenotype were subjected to joint segregation analysis with all the segregating markers using the EXCEL macro 'QUIKMAP' (N. Weeden and J. Barnard, unpublished). The markers/regions displaying a linkage value of less than 20 cM with the segregation pattern of those lines parental phenotypes were combined into multilocus genotypes and the distribution of all lines across the various genotypes was examined for the partitioning of phenotypes.

RESULTS

The linkage map developed for the MxJ RILs is shown in Fig. 1. Forty-one anchor markers and over 250 total markers were located on the map. Relatively few clusters of markers were observed, and only a few gaps of >10 cM and none >15 cM were present between markers. All the segregating anchor markers and over 90% of the DNA markers could be placed on this map. When the MxJ map was compared to the JxS map [4], the former appears to be missing only a portion of the upper section (satellite arm) of linkage group IV. Small changes in linkage intensity between anchor markers can be recognized, but the order of the markers is the same as on the JxS map except for a slight change in the position of *hsfA* (linkage group III), which mapped nearly coincident with *M* on the JxS map.

More than half of the 47 MxJ RILs could be placed in one of the two parental categories (categories 1 and 5) with regard to dehiscent pod phenotype (Table 1).

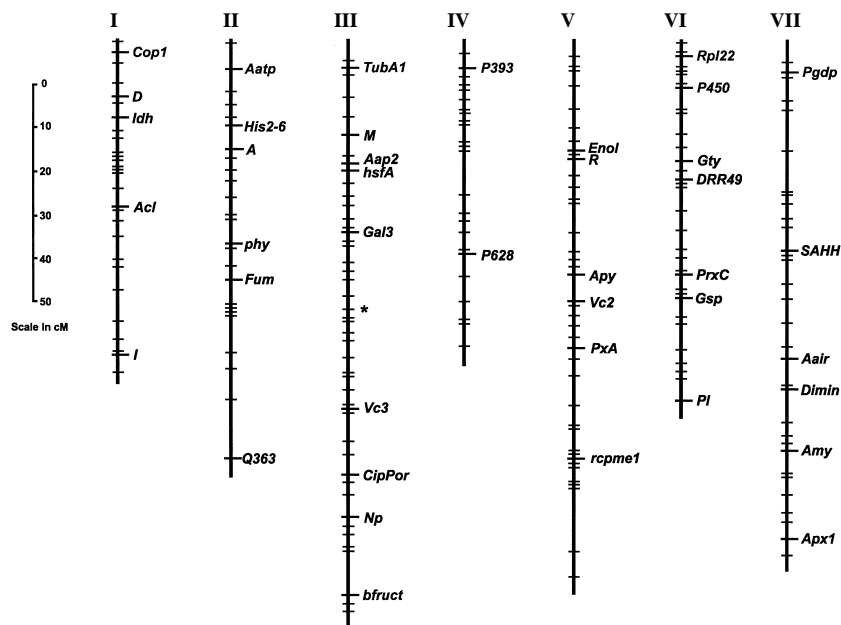


Fig. 1. Linkage map for the MxJ population. The anchor markers are labeled in bold print. The small cross bars indicate the positions of other markers (RAPDs and SSRs) segregating in the population. The position of the RAPD marker B432e on linkage group III is indicated by an asterisk. This marker identifies the approximate position of the *Dpo* locus.

Tab. 1. Joint segregation analysis of the dehiscent pod trait with alleles at the OB7g and OM14 RAPD loci in the MN313 x JI 1794 population.

Dehiscent pod	Number of lines with given OB7g, OM14 genotype ¹				Total
	J, J	J, M	M, J	M, M	
Phenotype (score)					
All dehiscent (1)	4	2	2	0	8
Mostly dehiscent (2)	3	3	2	0	8
Pods splitting (3)	1	2	3	1	7
Some pods splitting (4)	0	1	4	3	8
Indehiscent pods (5)	<u>0</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>15</u>
Average score for genotype	1.6	3.0	3.5	4.5	46

¹Genotype designation J = JI 1794 allele, M = MN313 allele. All genotypes were homozygous.

The remaining 23 lines displayed phenotypes intermediate between the parents. This distribution is not what is expected for control by a single locus, which

should have given a 1:1 ratio of dehiscent and non-dehiscent. Instead only eight lines (about 17% of the population) displayed the dehiscent phenotype of JI 1794. These results indicate that two or three major genes control loss of pod dehiscence. Joint segregation analysis identified two regions of the MxJ linkage map that displayed a significant correlation with that of the dehiscent pod character. One of these regions is in the middle of linkage group (LG) III, the same region that *Dpo* is believed to be located [3]. The segregation pattern of two RAPD markers on LG III, OB7g and OB11b, displayed the strongest correlation with that of dehiscent pod. These RAPD markers gave identical segregation patterns and were located about 3 cM from B432e, a marker also mapped in the JxS population. Only OB7g will be referred to in the presentation of the data. The other region mapped to LG V near *R* (Mendel's round/wrinkled seed locus). The closest marker to this 'QTL' was the RAPD OM14. Joint segregation analysis of the dehiscent pod phenotype with the two markers (OB7g and OM14) is presented in Table 1. Marker data were missing for one of the RILs so that only 46 lines could be analyzed, but the data display a clear additive trend. When both markers were from the same parent (e.g. either J, J or M, M in Table 1) the range in phenotype only slightly exceeded the range observed in the original parent, and none of these plants had the phenotype of the alternate parent. When the genotype was mixed (e.g. either J, M or M, J for OB7 and OM14 genotypes, respectively, in Table 1) the observed phenotypes included the entire range, although the J, M genotype had a slightly lower average (favored the dehiscent phenotype) than did the M, J genotype. Thus, it appeared that the locus on LG III (presumably *Dpo*) had a slightly stronger effect.

Tab. 2. Joint segregation analysis of the dehiscent pod trait with the two-locus combination B341f, *Gp* in the JI 1794 x Slow population.

Dehiscent pod Phenotype	Number of lines with given B341f, <i>Gp</i> genotype ¹				
	J, J	J, S	S, J	S, S	Total
All dehiscent	8	2	2	0	12
Mostly dehiscent	5	0	1	0	6
Pods splitting	2	0	0	0	2
Some pods splitting	8	0	4	0	12
Indehiscent pods	0	7	6	8	<u>21</u>
Average score for genotype	2.4	4.1	3.8	5.0	53

¹Genotype designation J = JI 1794 allele, S = Slow allele. All genotypes were homozygous.

Analysis of the segregation of the dehiscent pod character in the JxS population also revealed two regions of the genome having major influence. The two regions were again on LG III and LG V; however, the region on LG V was not near marker OM14 but rather approximately 50 cM away, very near *Gp*. The marker on LG III displaying the segregation pattern most closely correlated with that of the dehiscent pod phenotype was B341f, located about 10 cM from B432e. Joint segregation analysis of the dehiscent pod phenotype with the two markers (B341f and *Gp*) is presented in Table 2. In this population, the region on LG V had the stronger influence, with 15 of the 17 yellow-podded lines (*gp*) exhibiting only indehiscent pods. Both green-podded (*Gp*) genotypes had a greater tendency to express pod dehiscence, those possessing the JI 1794 allele on LG III being more likely to exhibit dehiscent pods (average of 2.4 for the dehiscent pod character). We did not observe an effect of the region around *R* on the dehiscent pod phenotype.

DISCUSSION

Our analysis of the genetic basis of dehiscent pods confirmed a major role for the *Dpo* locus. The effect of *Dpo* polymorphism could be observed in both populations. The relative position of *Dpo* to a marker mapped in both populations (B432e) differed by about 5 cM. However because basically a different set of RAPD primers were used to generate each of the two maps and each mapping population was relatively small, the 5 cM difference lies within the precision of the analysis. The broad range of phenotypes observed for the mixed genotypes (J, M or M, J) probably reflect the difficulty in scoring the intermediate phenotype. Possibly the influence of the environment became more pronounced, or segregation at other loci became more important. It is of interest that in lentil (*Lens culinaris*) the dehiscent pod character also is controlled by a dominant gene on the homologous linkage group to that carrying *Dpo* in pea [8]. Hence, the same gene may have been eliminated during the domestication of both crops.

The identification of a second locus influencing the dehiscent pod trait in the MxJ population suggests that more than one gene might have been selected during domestication to produce the indehiscent pod character now expressed in commercial pea lines. This second locus, to which we will tentatively assign the symbol *Dpo2*, has not been described before, nor are any loci controlling morphological variation pod structure known from that region. The locus *R* is sufficiently close (<5 cM) to be a possible candidate gene, but it is difficult to envision how the round/wrinkled polymorphism could significantly affect pod dehiscence. This second locus may have been incorporated into the domesticated germplasm much later. A genetic analysis of pod dehiscence in early pea cultivars might reveal when *dpo2* was incorporated into this germplasm.

We were not able to confirm the action of *Dpo2* in the JxS population, perhaps because of the major effect of the *Gp* region. We propose that *gp* is the gene

directly producing the indehiscent pod effect identified with that region of linkage group V in the JxS population. The influence of the *Gp* region was observed only in the population in which *gp* was segregating, and the effect was stronger than that of *dpo*. Indeed, the joint segregation of *gp* and indehiscent pods suggests that yellow pod is almost completely epistatic to *Dpo*. In addition, lines with yellow pods tend to have thinner pod walls than lines with green pods (N. Weeden, personal observation), suggesting a possible mechanism for the role of *gp* in that the thinner pericarp may exert less force on the sutures. However, if *Gp* is the locus responsible, it does not reflect a selection made early in the domestication of the crop.

The relatively simple approach we used, similar to single-factor ANOVA, clearly identified the expected effect of *Dpo* on linkage group III, as well as a second gene in each population. No other regions of the linkage map correlated with the segregation of dehiscent pod expression. The availability of a comprehensive linkage map for each population provided strong evidence that we identified all the major genes affecting pod dehiscence. Our success indicates that this approach can be applied in other situations in which the number of recombinant inbred lines is small and the number of loci influencing the phenotype is also limited.

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