

Development of an ASAP marker for resistance to bean yellow mosaic virus in *Pisum sativum*.

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Pea mosaic, caused by bean yellow mosaic virus (BYMV), is a disease of economic importance in many pea growing areas of the world. Resistance to this virus is conferred by a single recessive gene, *mo* (6), located on linkage group II about 5 cM from *K* (3). The relatively close linkage between *K* and *Mo* has permitted the use of the mutant *k* as a marker for *mo* in a segregating progeny. However, several disadvantages are associated with it as a marker. The mutant phenotype is fully recessive, allowing only two progeny classes to be distinguished, and the phenotype is expressed in the flower, delaying selection for resistance until the plants bloom. More recently an isozyme marker (*Pgm-p*), mapping between *k* and *mo*, has been identified (5). However, isozyme analysis is cumbersome for large-scale screening, whereas PCR-based DNA markers offer many advantages in marker-assisted selection programs. Here we describe two DNA fragments linked to *Mo*, the conversion of one of these fragments into an allele-specific marker, and determine the linkage intensity between this latter marker and *Mo*.

Four F₂ families from the cross 'Ranger' (susceptible) x 'Bonneville' (resistant) were used for linkage analysis. In addition, a set of 19 F₅ plants derived by single seed descent from the same cross and 50 F₈ recombinant inbred lines from the cross JI1794 (susceptible) x Slow (resistant) were included to confirm the linkage. DNA was extracted by the method of Torres et al. (4). Approximately 300 different 10-base oligonucleotide primers were screened using a polymerase chain reaction (PCR) procedure described previously (7). Two of the primers, BC302 (sequence 5'-CGGCCACGT) and BC355 (sequence 5'-GTATGGGGCT), gave fragments closely linked to *Mo*. The 1300-bp fragment from BC302 (BC302₁₃₀₀) was linked in coupling with the susceptible allele, while another 1300-bp fragment generated by BC355 (BC355₁₃₀₀) was linked in coupling with the resistant allele. Both of these fragments were cut from the gel, reamplified, and ligated into the vector pCRII using the TA Cloning® Kit (Invitrogen Corp., San Diego). The clone containing BC302₁₃₀₀ was sequenced by the dideoxy chain termination method using M13 sequences as primers. Allele-specific associated primers (ASAPs) were designed with the sequences: 5'-CGGCCACGTCTACTAGTGA and 5'-CGGCCACGTCTGGGGTATC. This pair of primers produced a single band in susceptible plants but no conspicuous product in resistant plants. The BC355 fragment was also sequenced, but in this case the longer primers that we had synthesized generated the marker in both resistant and susceptible individuals.

Analysis of 100 F₂ individuals gave only three recombinants between BC302₁₃₀₀ and *Mo* (Table 1). Heterogeneity tests among the F₂ families indicated the data were homogenous and could be combined, generating an overall recombination frequency of 3%. Similar analysis of the 50 RILs gave 3 recombinants. Using the equation $r = R/(2-2R)$, where R is fraction of lines displaying a recombinant phenotype, for calculation of linkage intensity (1), we obtained a linkage value of 3.2%, very similar to that obtained for the F₂ population (Table 1). In the F₅

plants, no recombinants were found between BC302₁₃₀₀ and *Mo*, but two recombinants were identified between BC355₁₃₀₀ and the virus resistance gene. From these results we conclude that the latter marker is further from *Mo* than BC302₁₃₀₀ although we do not know whether the two markers are on the same side of or flank the gene.

Table 1. Joint segregation analysis of *mo* and BC302₁₃₀₀ in F₂ and F₈ populations.

Family	Phenotype designation ¹				Total	Joint seg. Chi-sq.	Recombinant fraction ± SE
	S/+	S/-	R/+	R/-			
F ₂ families							
A185-46	48	1	0	26	75	70.7	1 + 11
A185-36	5	0	0	2	7	7.0	<10
A185-39	4	1	0	4	9	5.8	8 ± 33
A185-38	3	1	0	5	9	5.6	8 + 33
Total F ₂	60	3	0	37	100	88.1	3 ± 10
F ₈ recombinant inbred lines							
87-18,19	23	1	2	24	50	38.8	3 ± 5

¹ R = resistant, S = susceptible, + = present, - = absent.

The use of DNA markers simplifies the process of mapping major genes and provides the opportunity for 'tagging' disease resistance genes with genetic markers that are suitable for marker-assisted selection in plant breeding programs. Marker-assisted selection using ASAPs appears to offer a very efficient and cost-effective approach for screening large numbers of samples (2). Our dominant ASAP marker is linked in coupling with the susceptible allele, *Mo*. Having the dominant alleles in coupling permits selection for the resistant phenotype (absence of marker) but does not permit unambiguous identification of heterozygous individuals. The BC355₁₃₀₀ marker is at least 10 cM from *mo* and thus is probably not an improvement over the isozyme marker, being both further away and requiring electrophoresis to separate the various products produced by amplification with the short primers.

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