RAPD Marker linked to Co-15 Anthracnose Resistance Gene in Widusa

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Introduction

Random amplified polymorphic DNA (RAPD) markers have the potential to be a useful breeding tool in common bean, where monogenic disease resistance genes have been tagged. Young and Kelly (1997) found one marker in repulsion phase with Co-1 locus, while Mendoza et al. (2001) had reported one AFLP marker that is tightly linked at Co-1, in repulsion phase. The primary goal of this study was to identify random amplified polymorphic DNA (RAPD) tightly linked to Co-15 gene, present in Widusa cultivar that confers resistance to race 73.

Material and Methods

The common bean cultivar Widusa was crossed with Cornell 49-242, and 10-day-old seedlings at the primary leaf stage of the parents, F1, F2, and F2:3 families were tested for their disease reactions to the race 73 of Colletotrichum lindemuthianum. Prior inoculation with C. lindemuthianum, one foliole tissue was collected from young primary leaves, approximately six days post-emergence, from greenhouse-grown plants. The plants were spray-inoculated with a spore suspension (1.2 x 10⁶ spores/ml⁻¹) of the race. Ninety-one F2 individual plants and their respective F2:3 families, each, were used to confirm putative linkages between a RAPD marker and Co-15 in Widusa. Two contrasting bulks were formed with DNA from 6 F2 homozygous resistant and 6 F2 homozygous susceptible individual plants derived from mapping population.

DNA extraction method was conducted according to Edwards et al. (1991). An uniform concentration of 10 ng.µl⁻¹ of extracted DNA with DNA fluorometry was used to standard. Amplification reactions were performed similarly to that described by Young and Kelly (1996). The phenotypic segregation was analyzed by the Chi-square test on the F2 population, and F2:3 families from the cross Widusa x Cornell 49-242. Ten plants of each F2:3 family were inoculated, and based on the number of susceptible plants observed. The family was classified as homozygous resistant, heterozygous resistant, or homozygous susceptible and used to confirm the F2 genotype. Linkage analyses were performed using the program Mapmaker (Lander et al., 1987). The Kosambi’s function by the Linkage-1 computer program was used to determine the expression, in centimorgans (cM), of the linkage estimates between loci.

Results and Discussion

The inheritance supported an expected 3:1 ratio of resistant to susceptible individuals in the F2 population of the cross Widusa x Cornell 49-242. According to Figure 1, the RAPD marker OA181500 (generated by 5'-AGGTGACCCGT-3' decamer primer) was linked in repulsion-phase with the Co-15 gene at distance of 1.2 cM. This marker co-segregated with the resistant gene in 91 individuals in F2 population derived from the cross Widusa x Cornell 49-242, when this population was inoculated with the race 73, and one recombinant was observed. Similarly Young and Kelly (1997) found one marker in repulsion phase with Co-1 locus. Mendoza et al.
(2001) had reported one AFLP marker that is tightly linked at 2.7cM from Co-I, in repulsion phase. According to Haley et al. (1994), selection based on a repulsion-phase RAPD yields a greater proportion of homozygous resistant selections than selection based on a coupling-phase RAPD even at greater recombination frequencies between marker and resistance loci.

This marker was linked in repulsion-phase with the dominant Co-1<sup>3</sup> gene, which has proven to be effective on providing broad resistance to anthracnose. It would be useful in marker-assisted selection for the introgression of Co-1<sup>2</sup> into susceptible germplasm.

Figure 1 - Electrophoretic analysis of amplification products obtained with OA18<sub>1500</sub> RAPD marker. Lanes are as follows: 1, molecular weight marker (100bp ladder); 2, Widusa (resistant); 3, Cornell 49-242 (susceptible); 4, resistant bulk; 5, susceptible bulk; 6-11, F<sub>2</sub> plants resistant to race 73; 12-17, F<sub>2</sub> plants susceptible to race 73. The arrow indicates a DNA band of 1500 bp linked in repulsion-phase to the resistance gene.

References