SCAR MARKER LINKED TO THE COMMON BEAN RUST RESISTANCE GENE *Ur-11*

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Common bean (*Phaseolus vulgaris* L.) rust, caused by the fungus *Uromyces appendiculatus*, may lead to serious losses to the culture mainly in regions with mild temperatures and high humidity. The use of resistant cultivars is an alternative strategy to control the disease. In the gene pyramiding bean breeding program of BIOAGRO/UFV it was observed that the *Ur-11* gene present in cultivar Belmidak RR-3 is an important rust resistance source to be used in Brazil. This program presently uses the RAPD marker $OAE19_{890}$ linked in repulsion phase to *Ur-11* (Johnson et al., 1995) to assist the introgression of this gene into "carioca-type" cultivars. This marker was validated by Oliveira et al. (2002) in the population derived from the cross between Rudá (susceptible) and Belmidak RR-3 (resistant). In the present work the RAPD marker was converted into a SCAR in order to make its amplification more reproducible and accurate.

DNA from cultivar Rudá was amplified with RAPD primer OPAE19, and the products were fractionated in an agarose gel. The band of interest (890 bp) was excised from the gel and inserted into the pGEM-T Easy vector (Promega, Madison, WI). After sequencing the fragment, SCAR primers were designed and synthesized. The primer sequences were:

5'-CAGTCCCTGACAACATAACACC-3' (sAE19F) and

5'-CAGTCCCTAAAGTAGTTTGTCCCTA-3' (sAE19R), and the marker was designated $sAE19_{890}$. The primers were then tested in six resistant and six susceptible F_2 plants (Rudá x Belmidak RR-3). The PCR reactions (25 µL) contained 30 ng of genomic DNA, 0.2 µM of each SCAR primer, 10 mM/50mM Tris/KCl (pH 8.0), 2 mM $MgCl_2$, 0.48 mM of total dNTP, and 1 U of Taq DNA polymerase. The amplification program included a initial step of 5 min at 94 °C, 35 cycles (94 °C/15 s, 58 °C/1 min, 72 °C/1 min 30 s) and one final step at 72 °C for 7 min. Only the susceptible plants and the progenitor Rudá harbored the marker band (Figure 1).

To determine the genetic distance between the marker and the resistance gene, the reactions of 53 F_2 plants (Rudá x Belmidak RR-3) to *U. appendiculatus* pathotype 10 (Faleiro et al., 1999) were determined and they were also tested with RAPD marker $OAE19_{890}$ and SCAR $sAE19_{890}$. The plants were scored visually for the disease symptoms using a 1 to 6 scale (Stavely et al., 1983). The genetic distances were determined with the aid of MAPMAKER (Lander et al., 1987) using a LOD score of 3.0. The segregation analyses showed that $OAE19_{890}$ and $sAE19_{890}$ were located at 1.0 cM of the resistance gene *Ur-11* (Table 1). To confirm the results obtained with the F_2 population the corresponding $F_{2:3}$ families were also evaluated for resistance/susceptibility to *U. appendiculatus*. This analysis allowed us to determine the specific

genotypes of each F_2 plant. The plants harboring the marker could divided into susceptible (rr) and resistant (Rr), and the plants with no marker were resistant (RR).

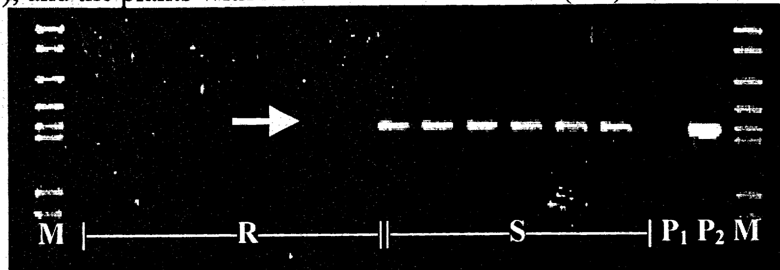

Figure 1 - Electrophoretic analyses of DNA amplification products produced with SCAR $sAE19_{890}$. Lanes are as follows: P_1, Belmidak RR-3; P_2, Rudá; R, F2 resistant plants; S, F2 susceptible plants. M refers to lambda phage DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers). The arrow indicates the SCAR marker.

Table 1. Segregation for resistance and linkage analysis between molecular markers $OAE19_{890}$ and $sAE19_{890}$, and the rust resistance gene *Ur-11* in an F_2 population derived from a cross between cultivars Rudá and Belmidak RR-3.

Locus tested	Generation	Expected ratio	Observed ratio	χ^2	Probability (%)	cM[a]
Ur-11	$F_{2:3}$	1:2:1	12RR:28Rr:13rr	0.578	74.87	
$OAE19_{890}$	F_2	3:1	13(-):40(+)	0.188	99.06	1.0
$sAE19_{890}$	F_2	3:1	13(-):40(+)	0.188	99.06	1.0

[a]Genetic distance in centiMorgan

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