Molecular Markers Linked to the Ur-7 Gene
Conferring Specific Resistance to Rust in Common Bean

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Bean rust, caused by Uromyces appendiculatus, is a major disease of common bean (Phaseolus vulgaris L.). The most economic and environmentally sustainable method for controlling bean rust is the use of host plant resistance (Mmbaga et al., 1996). Bulked segregant analysis (Michelmore et al., 1991) is an efficient method to rapidly identify molecular markers linked to a specific gene using bulked DNA from F2 plants. This technique has been used to tag Ur-5 and Ur-4 genes, Middle American (MA) and Andean (A) gene pools, respectively, for rust resistance in common bean using RAPD markers (Haley et al., 1993; Miklas et al., 1993).

Pyramiding monogenic resistance genes into a single bean cultivar is a strategy recommended to obtain durable rust resistance (Mmbaga et al., 1996). Molecular markers linked to major genes for rust resistance are useful to pyramid these genes into a single cultivar, especially if epistasis is present (Kelly, 1995). Tagging the Ur-7 gene from the MA gene pool for specific resistance to rust race 59 with RAPD markers has not been reported. Our objective was to identify RAPD markers linked to the Ur-7 gene using bulked segregant analysis in an F2 segregating population from the common bean cross GN1140 (resistant to race 59) x GN Nebr. #1 (susceptible to race 59).

Materials and Methods

Plant material and inoculation. The parents and 89 F2 plants from the cross GN1140 x GN Nebr. #1 were grown in 1997 and 78 F3 families (12 to 16 plants per F3) from randomly selected F2 plants were grown in 1998 in the greenhouse. All plants were inoculated by a spore suspension of race 59 sprayed on the abaxial leaf surfaces of the unifoliate leaves. Rust reaction was recorded on all plants as resistant (hypersensitive) or susceptible (uredinia >300 μm in diameter) at 14 days after inoculation.

RAPD. Total genomic DNA was extracted from lyophilized leaf tissue of the parents and 89 F2 plants. Polymerase chain reactions (PCR) were performed in an air thermalcycler (model 1605; the Idaho Technology, Idaho Falls, ID) in thin-walled glass capillary tubes. A total of 280 primers were obtained from Operon Technologies. Two different bulked DNAs were prepared from eight homozygous rust resistant and eight homozygous rust susceptible plants. The 280 primers were used to simultaneously screen between resistant and susceptible bulked DNAs and between the two parental lines. Eighteen primers generated marker polymorphisms between the resistant and susceptible bulked DNAs, and they were tested subsequently on the F2 population. The name of each RAPD marker is derived from an ‘O’ prefix for Operon primers, the letters identifying the Operon kit, Operon primer number, and the approximate length (bp) of the marker.

Statistical analysis. Chi-square was used to test goodness-of-fit of observed to expected ratios in the F2 and F3 generations. The F2 plant segregating for each marker identified by presence and absence of RAPD amplification was tested for goodness-of-fit to a 3:1 ratio. The segregation analysis of the markers and the locus for specific rust resistance was performed using MAPMAKER Macintosh version 2.0. Map distances (cM) between markers and gene were calculated using
recombination fractions and Kosambi mapping function.

**Results and Discussion**

A good fit to a 3:1 ratio ($P=0.95$) of number of resistant to susceptible F2 plants to rust race 59 was observed. It was hypothesized that a single dominant gene controlled specific resistance to the rust race. This hypothesis was confirmed in the F3 based on a satisfactory fit to a 1:2:1 ratio ($P=0.42$) of number of families non-segregating for resistance, segregating for resistance and susceptibility and non-segregating for susceptibility.

Bulked segregant analysis was utilized to identify RAPD markers linked to the *Ur-7* gene for specific resistance to rust race 59. Eighteen RAPD markers were polymorphic for the resistant and susceptible bulked DNAs. Among the 18 markers, ten were linked to the *Ur-7* gene. However, the remaining eight markers were proved false positives, suggesting that the markers were not linked to the gene. A good fit to a 3:1 ratio ($P=0.50-0.95$) for band presence to band absence for each of ten markers was observed in 89 F2 plants.

Six RAPD markers that displayed an amplified DNA fragment in the resistant bulked DNA were detected in a coupling phase linkage with the *Ur-7* gene. Three coupling-phase markers OAA11.500, OAD12.550 and OAF17.900 showed no recombination with the *Ur-7* gene. Two markers OAB16.850 and OAD9.550 were tightly linked to the gene at a distance of 2.2 cM. Marker OAI12.1000 was also closely linked to the gene at a distance of 2.4 cM. Four RAPD markers that displayed an amplified DNA fragment in the susceptible bulked DNA were identified in a repulsion phase linkage with the *Ur-7* gene. Repulsion-phase marker OAB18.650 was the most closely linked to the *Ur-7* gene among the four markers at a distance of 8.2 cM. Three markers OAH20.1100, OAI10.1250 and OAI19.1500 were also linked to the gene at 14.0 cM to 16.8 cM. This is the first report on coupling- and repulsion-phase RAPD markers linked to the *Ur-7* gene in common bean.

Kelly (1995) reported that pyramiding three major genes resulted in resistance to 63 of the 65 bean rust races reported in the USA. Recombining resistance genes from both MA and A gene pools should provide more durable resistance to rust. The coupling- and repulsion-phase RAPD markers linked to the *Ur-7* gene for specific rust resistance of MA origin detected here, along with other independent rust resistance genes from other germplasm, could be utilized to pyramid multiple genes into a bean cultivar for more durable rust resistance.

**Literature Cited**


