

## PYRAMIDING RUST RESISTANCE GENES USING RAPD MARKERS

J. D. Kelly\*, R. Stavely\*\*, P. Miklas\*\*\*, L. Afanador\*, and S. D. Haley\*

\*Department of Crop and Soil Sciences, Michigan State University, E. Lansing, MI 48824;

\*\*USDA-ARS, Beltsville, MD; and

\*\*\*USDA-ARS, Tropical Agriculture Research Station, Maraguez, PR.

Traditional methods of gene pyramiding for disease resistance traits involve crossing, backcrossing, and test-crossing to verify the presence of the desired gene combination in the resultant progeny. Due to epistatic interaction between genes, coupled with sampling limitations and the tedium of test-crossing, not all progeny carry the desired gene combinations. The whole procedure could be made more efficient if molecular markers tightly linked to resistance genes were used to indirectly select for desired genes in the absence of the specific pathogen. Expression of the marker is not masked by any epistatic interactions which commonly occur between the resistance genes.

Using RAPD markers, our bean breeding group has successfully tagged the rust resistance *Up<sub>2</sub>* gene (Miklas et al. 1993) and the B-190 rust resistant gene block (Haley et al. 1993) using backcross populations developed by R. Stavely. These RAPD markers now offer the opportunity to more efficiently pyramid rust resistance genes into specific genotypes as a strategy to increase the durability of the same resistance genes.

To illustrate the usefulness of RAPD markers, a wide range of genotypes were surveyed for presence of *Up<sub>2</sub>* marker. Since the *Up<sub>2</sub>* gene is of Andean origin, it has not yet been incorporated into a broad array of Mesoamerican lines. The only known source of *Up<sub>2</sub>* gene in Mesoamerican germplasm are the BelMiDak rust resistance lines 1-7 (BMD) developed by Stavely, et al. (1992). In the development of the BMD lines, the common parent used was a C-20, BC<sub>4</sub> derived line 4-5753 known to possess the *Up<sub>2</sub>* gene. Line 4-5753 was derived from crosses and backcrosses between C-20 and Early Gallatin as the original source of *Up<sub>2</sub>* gene. The other common donor parent was PI 181996 identified by Stavely as possessing resistance to all known US rust races available in the Beltsville collection. Since the resistance in PI 181996 is epistatic to *Up<sub>2</sub>* gene, Stavely was prevented from selecting for *Up<sub>2</sub>* resistance in the segregating progeny during the backcrossing program. Progeny, selected with PI 181996 resistance, that possessed satisfactory seed and agronomic traits were test-crossed to a universal susceptible genotype (UI-114) to confirm either the presence or absence of the hypostatic *Up<sub>2</sub>* gene. Generally only 2 to 3 seeds of each F<sub>3</sub> progeny line were test-crossed so heterogeneous progeny risked being incorrectly catalogued as resistant or susceptible because of the sampling bias involved with the test-cross. BMD lines 1 and 2 were identified as possessing the *Up<sub>2</sub>* gene while lines 3 to 7 were reported as not carrying the *Up<sub>2</sub>* resistance gene.

The same seven BMD lines were screened by Miklas using the RAPD marker linked to the *Up<sub>2</sub>* gene and this confirmed the presence of the gene in lines 1 and 2. Unexpectedly, line 7 showed the marker, and in repeated screening of other seed samples, BMD-7 continued to show the marker suggesting that it also carried the *Up<sub>2</sub>*. These same BMD-7 plants were test-crossed to Seafarer and the resultant F<sub>2</sub> progeny sent to Stavely for rust inoculation. In screening with rust races 44, 49, and 73, Stavely confirmed that BMD-7 carried the *Up<sub>2</sub>* gene indicating that it was not detected previously because of the limiting sampling of individuals for test-crossing. The other BMD lines 3 to 6 do not carry the *Up<sub>2</sub>* gene and, as expected, do not show the marker.

The information presented here supports an important use of markers to more effectively detect hypostatic genes in segregating or heterogeneous populations in which more than one major disease resistance gene is segregating. Detection of these hypostatic genes would be impractical in a large number of lines because of the need to sample a large number of individuals for test-crossing and the time delay in verifying the reaction in an  $F_2$  population. If the  $Up_2$  marker had been available prior to the development of these BMD lines, selection using the marker for the hypostatic  $Up_2$  gene in the presence of the PI 181996 gene would have been possible. This might have resulted in development of BMD lines 3 to 6 with a broader based array of rust resistance including the  $Up_2$  gene. Marker assisted selection should be a very useful tool in the hands of the breeder interested in pyramiding resistance genes.

#### LITERATURE CITED

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