Application of Molecular Markers to Assist Selection of Common Beans Resistant to Common Bacterial Blight (*Xanthomonas campestris pv. phaseoli*)

Kangfu Yu, Soon J. Park &Vaino Poysa
Agriculture and Agri-Food Canada, Greenhouse and Processing Crops Research Center
Harrow, Ontario, Canada, N0R 1G0

Introduction

The objectives of this study were: 1) to test whether the RAPD markers identified from XAN159 (Jung et al., 1997) can be used for indirect selection in populations derived from HR67; 2) to transform useful RAPD markers into user friendly SCAR markers; 3) to examine the distribution of SCAR markers in common bean germplasms; and 4) to compare the efficacy and economics of using molecular markers for indirect selection with conventional selection methods in the greenhouse.

Materials and methods

Plant materials: The CBB resistant material used in this study was HR67 that was derived from XAN159 from the cross of Centralia/3/HR13-621//XAN159/OAC Rico, of which the original single cross between XAN159 and OAC Rico was made in 1986. One recombinant inbred line (RI) population of 138 F_5 lines was derived from a cross between HR67 (resistant to CBB) and W1744d (susceptible to CBB). Another population of 138 F_5 lines used in this experiment was derived from another cross also using HR67 as the CBB resistant parent.

Develoment of SCAR marker: Polymorphic RAPD bands were isolated from a 1% low melting agrose gel and extracted twice by phenol and once by chloroform, followed by precipitation with ethanol. The DNA fragments were cloned into P-GEM^T vector and then transformed into high efficient DH5α *E. coli* cells as described by the manufacturer (Promega). Plasmid DNA from transformed clones was isolated by the alkaline methods and their two ends were sequenced by dideoxy sequencing with T7 DNA polymerase. PCR primers that include the random 10-mer sequence were designed and then synthesized by Gibco BRL, and their proper annealing temperature was determined by the computer program, Gene RUNNER.

Data analysis: Linkage data between molecular markers and the resistant genes were analyzed by MAPMAKER. To resolve CBB disease response into a Mendelian trait, qualitative mapping of CBB was conducted in the RI population. Briefly, lines that had DSI of less than 2 were rated as resistant, otherwise as susceptible. Meanwhile, quantitative analysis was also conducted to determine the association between molecular markers and QTLs by SAS (statistical analysis system) with a one way ANOVA model using marker genotype as the main effect on the phenotype.

Results and Discussion

Marker specificity: We selected the RAPD markers that were polymorphic and associated with genes underlying resistance to CBB identified in an RI population derived from the cross between PC50 and XAN159 (Jung et al., 1997).The markers were then used to amplify HR67 and W1744d, the parental materials used in our experiments, to verify their polymorphisms. It was not surprising that most of the RAPD markers that were polymorphic with PC50 and XAN159, were not polymorphic with HR67 and W1744d except $BC420_{900}$ and $C07_{900}$.

Linkage confirmation: Because HR67 was derived from XAN159 and is highly resistant to CBB, we expected that, at least, the major gene in XAN159 should be present in HR67 line. Therefore, cosegregation analysis of the two polymorphic markers, $BC420_{900}$ and $C07_{900}$, and plant response to the pathogen was conducted in the RI population. RAPD marker $BC420_{900}$ is tightly linked with the major loci underlying resistance to CBB, which agrees with the result of Jung et al. (1997). The single QTL explained about 62 % of the total phenotypic variation that fits the hypothesis that there is one major gene controlling CBB resistance (McElory, 1985). The linkage distance between $BC420_{900}$ and the major CBB resistance locus was estimated to be 4.6 cM by a qualitative analysis of CBB response.

SCAR development: To develop $BC420_{900}$ SCAR, the 900 bp RAPD fragments were isolated from the gel, purified and cloned into a P-GEM^T PCR cloning vector. Three distinct clones with an insert of about 900 bp were selected and sequenced. They all have identical sequences with the random BC420 10-mer sequence at both ends. Because the random10-mer sequence could generate the polymorphism, its sequences were included in the specific primers sequences. Using $BC420_{900}$ SCAR marker, usually only one reaction is required to tell the presence or absence of the band in a plant, which is not so with RAPD $BC420_{900}$. This not only improved the marker's reproducibility and accuracy significantly, but reduced the chemical cost for marker analysis.

SCAR distribution: Out of ninety-seven common bean lines/cultivars examined, only the three related resistant lines, XAN159, HR67 and HR45 [another CBB resistant line derived from XAN 159 (Park and Dhanvantari, 1994)] possessed the $BC420_{900}$ SCAR marker. This result shows that $BC420_{900}$ SCAR is probably *P. acutifolius* (PI 319443) specific.

Marker-assisted selection: To find out whether the $BC420_{900}$ SCAR marker is effective for MAS, 138 F_5 lines, derived from another cross with HR67 as the resistant parent, were screened with the marker first, and then tested for CBB resistance in the greenhouse. Based on the marker information, 28 of the 138 lines had the $BC420_{900}$ SCAR band present and were predicted to be resistant. When the results were compared with the inoculation test data, it was found that 23 of the 28 plants selected by the marker had DSI of less than 2.0, which gave an accuracy of 82% (23/28) for the 28 lines selected. In other word, only 5 of the 138 (3.6%) lines were mis-classified as resistant plants. A cost comparison was made between MAS and conventional greenhouse screening method. It was estimated that the cost of using SCAR and RAPD markers to analyze 100 bean lines would be about \$4.24 and \$4.59 per data point, respectively. This includes the costs of labor to plant seeds and water the plants for 8 days, the costs of labor for DNA extraction, PCR and electrophoresis, as well as the expendables for chemicals and the greenhouse. In contrast, conventional greenhouse screening was estimated to cost approximately \$6.99 per data point. This includes the costs of labor to prepare bacterial inoculum, inoculate the plants, take care of plants for 32 days, and rate disease symptoms, as well as the cost of greenhouse rental. Our conclusion was that MAS in this particular case costs about a third less than the greenhouse test.

References

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