Detection of SRR Markers Associated with Resistance to Cassava (Manihot esculenta Crantz) Bacterial Blight (CBB) in Colombia.

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INTRODUCTION

Cassava bacterial blight (CBB), caused by Xanthomonas axonopodi pv. manihotis (Xam), is a major disease of cassava (Manihot esculenta Crantz) in Africa and South America causing losses between 12 and 90%. Planting resistant varieties is the preferred method of disease control. Previous studies on the genetics and number of disease loci involved in host plant resistance to CBB revealed many QTLs that control disease resistance (Jorge et al. 2000; Jorge et al. 2001). In the present work, the evaluation of more than 400 simple sequence repeats (SSR) detected one SSR marker associated with CBB resistance in a segregating backcross family in which disease symptoms were evaluated under field conditions. The association between SSRY 65 and CBB resistance in field was obtained with a significance level $P < 0.05$.

MATERIALS AND METHODS

Characterizing F1 progeny of four backcross families. The progeny of four cassava BC1 families was characterized for their reaction to CBB under natural disease pressure in Villavicencio, Colombia.

Table 1. Four cassava backcross families evaluated

<table>
<thead>
<tr>
<th>Family</th>
<th>Cross</th>
<th>Individuals (No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM315</td>
<td>M Nga 19 × CM 9208-13</td>
<td>287</td>
</tr>
<tr>
<td>GM318</td>
<td>M Nga 19 × CM 9208-26</td>
<td>399</td>
</tr>
<tr>
<td>GM317</td>
<td>M Nga 19 × CM 9208-31</td>
<td>348</td>
</tr>
<tr>
<td>GM318</td>
<td>M Nga 19 × CM 9208-52</td>
<td>238</td>
</tr>
</tbody>
</table>

All four families have a common recurrent male parent, which is resistant to many strains of Xam (Table 1). The families’ reactions to CBB were analyzed, using a frequency distribution graph for each family, based on an average of 6 plants per plot, and a disease severity scale of 1.0 to 5.0, where 1.0 indicates plants with no symptoms and 5.0 death plants. Resistant plants scored between 1.0 and 2.0 on the scale, intermediate between 2.5 and 3.0, and susceptible more than 3.5.

Evaluating simple sequence repeat markers linked to bacterial blight resistance in cassava. One of the four BC1 families was selected based on its high standard deviation of disease response within the family for Bulk Segregant Analysis (BSA) towards the identification of SSR markers linked to CBB resistance. Bulks were constituted from 11 resistant genotypes (a score of 1.0 to 2.0) and 11 susceptible genotypes (a score of 4.0 to 5.0). Bulk Segregant Analysis (BSA) provides a method of focusing on regions of interest with molecular markers as against analyzing the entire genome (Michelmore, 1991).

M Nga 19 and contrasting bulks (11 individuals in each one) were evaluated, using 486 microsatellite primers, the SSR markers have been described elsewhere (Mbs et al 2001; CIAT 2001).

Opening of the Bulks. Individuals from resistant and susceptible bulks were evaluated with candidate primers which showed polymorphism between bulks.

Candidate primers that showed polymorphisms between resistant and susceptible individuals were considered potential SSR marker associated with resistance. They were evaluated in the whole population to confirm association between resistant marker and CBB resistance in the field.

Association between molecular marker and plant response to CBB. Association between SSR molecular marker and CBB resistance in the field was determined by a Chi-square Independence test (SAS procedure, SAS Institute, 1999-2001). An association was considered significant if the probability of the null hypothesis (no association) was less than $P<0.05$.

RESULTS

According to the frequency distribution graphs for CBB resistance, the GM 315 family (Fig. 1) showed the widest segregation for resistance/susceptibility and had the highest standard deviation (0.697) for individuals in each class: 46% of individuals of this family had a score between 1.0 and 2.5, thus showing intermediate resistance, whereas 0% scored less than 1.5 (i.e., resistant); and 2% scored more than 4.5 (i.e., susceptible).

Bulk Segregant Analysis. Bulk segregant analysis of the family GM 315 revealed polymorphism between the parents and bulks with SSR markers (Figs. 2 and 3). A summary of BSA are presented in Table 2.

Table 2. 408 SSR primers were evaluated and 13 of them were Potential SSR markers polymorphic between resistant and susceptible bulks

<table>
<thead>
<tr>
<th>SSR markers</th>
<th>Polymorphic between resistant and susceptible bulks</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM315</td>
<td>Yes</td>
</tr>
<tr>
<td>GM318</td>
<td>Yes</td>
</tr>
<tr>
<td>GM317</td>
<td>Yes</td>
</tr>
<tr>
<td>GM318</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Fig. 1. Frequency distribution graph of disease response to Xanthomonas axonopodi pv. manihotis in the BC1 family GM315. SD = standard deviation inside plot.

Fig. 2. Eleven SSRY65 primers used to identify SSR markers by bulk segregant analysis.

Fig. 3. Different types of polymorphism observed with BSA.

CONCLUSION

SSRY65 is a microsatellite marker associated with CBB resistance genes that can be used to differentiate between resistant and susceptible individuals of GM315 family to CBB in field.

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REFERENCES