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 axonopodis* 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

Family	Cross	Individuals (No)
GM315	M Nga 19 × CM 9208-13	357
GM316	M Nga 19 × CM 9208-26	399
GM317	M Nga 19 × CM 9208-31	348
GM318	M Nga 19 × CM 9208-73	238

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). Bulked 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 (Mba 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 SRR marker associated with resistance. They were evaluated in the whole population to confirm association between SSR 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
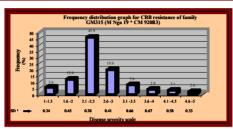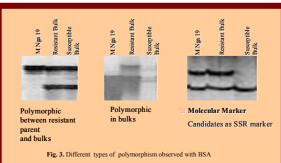
RESULTS

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

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 2.1 and 2.5, thus showing intermediate resistance, whereas 6% 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.
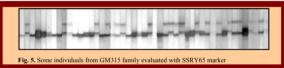

Fig. 2. Eleven SSRY primers used to identify SSR markers by bulked segregant analysis.

Polymorphic between resistant parent and bulks

Polymorphic in bulks

⇐ **Molecular Marker**
Candidates as SSR marker

Fig. 3. Different types of polymorphism observed with BSA

Table 2. 486 SSR primers were evaluated and 13 of them were Putative SSR markers

SSR Primers	Number
Total primers	48
Polymorphic between resistant parent	64
Polymorphic in susceptible	0
Candidates as SSR polymorphism between resistant bulk	2
(polymorphism between resistant bulk susceptible bulk)	8
	3

Fig. 4. Primer SSRY65 evaluated in each individual that forms the resistant and susceptible bulks in family GM 315. RP: M Nga 19. RB: Resistant Bulk. SB: Susceptible Bulk

Primer SRRY65 showed differences between resistant and susceptible individuals of each bulk (Fig. 4) and it was evaluated in the whole population (GM315) (Fig. 5)

Fig. 5. Some individuals from GM315 family evaluated with SSRY65 marker

The presence of SSRY65 marker in resistant individuals of GM315 family suggests association with CBB resistance from field evaluations. A probability of P=0.007 association was obtained for association between marker SSRY 65 and field phenotypic data.

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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