Activity 7.6. Evaluating simple sequence repeat markers linked to bacterial blight resistance in cassava

Specific objectives

1. *To evaluate 486 SSR primers in a BC1 family by bulked segregant analysis (BSA).*
2. *To identify SSR markers associated with disease resistance in the field.*

Methodology

According to the results obtained in *Activity 7.5*, the selected family GM 315, a backcross between M Nga 19 and CM 9208-13, itself a progeny of M Nga 19, was evaluated, using SSR markers to search for an association between CBB resistance and segregating bulks. Parents of this family are both resistant and heterozygotic.

Bulking. The evaluation method involves comparing two pooled DNA samples of individuals from a segregating population originating from a cross. Within each pool, or bulk, the individuals are identical for the trait or interest gene but are arbitrary for all other genes. Two pools that contrast for a trait (e.g., resistant versus susceptible to a particular disease) are analyzed to identify markers that distinguish them. Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to construct the pools. Bulked segregant analysis provides a method of focusing on regions of interest or areas sparsely populated with markers (Michelmore, 1991).

The GM 315 progeny that appeared highly resistant or highly susceptible according to the severity scale for CBB (Figure 7.4A) was selected to form two contrasting bulks. Individuals from resistant bulk scored 1.0 to 2.0 on the severity scale, while susceptible ones scored between 4.0 and 5.0.

DNA extraction. DNA, concentrated at 10 ng/µL, was extracted from selected individuals forming the bulks, using the Gilbertson-Dellaporta protocol (Dellaporta et al. 1983; Table 7.10

SSR markers. M Nga 19 and bulks, comprising resistant and susceptible individuals, were evaluated, using 486 microsatellite primers, which were separated into two groups based on DNA used to design them. 153 NS primers, based on cDNA, and 345 SSRY primers, based on genomic DNA, were used.

Each PCR reaction was performed in 25-µL volumes, consisting of dATP, dCTP, dGTP, and dTTP at 0.2 mM each; 2.5 mM $MgCl_2$; 1.5 U of *Taq* polymerase, 1 µM primer; 2.5 µL 10X *Taq* polymerase buffer; and 50 ng *template* DNA. Amplification with NS and SSRY was carried out, first in a Robocycler 96 (Stratagene) thermal cycler programmed for 2 min at 95°C; 30 cycles of denaturing for 40 s at 95°C, annealing for 1.30 min at 55°C, and extension for 2 min at 72°C; and a final extension for 5 min at 72°C. Amplification continued in a second thermal cycler (MSJ-Research PTC-200) for 2 min at 95°C; 30 cycles of denaturing for 30 s at 94°C, annealing for 1 min at 55°C, and extension for 1 min at 72°C; and a final extension for 5 min at 72°C.

Table 7.10. Average and range of disease severity for cassava bacterial blight within each plot of individuals from each bulk (resistant and susceptible) in the cassava family GM 315.

Resistant bulk			Susceptible bulk		
Individual code no.	Disease severity		Individual code no.	Disease severity	
	Range	Average		Range	Average
210	1.0-1.5	1.2	45	4.0-5.0	4.6
131	1.0-2.0	1.5	51	4.0-5.0	4.3
5	1.0-2.0	1.2	114	4.0-5.0	4.4
295	1.0-1.5	1.5	357	4.0-5.0	4.4
261	1.5	1.5	36	4.0-5.0	4.4
153	1.5-2.0	1.6	246	4.0-5.0	4.4
281	1.0-1.5	1.2	79	4.5-5.0	4.9
116	1.0-2.0	1.6	223	4.0-5.0	4.4
169	1.0-2.0	1.5	200	4.5-5.0	4.9
185	1.5-2.0	1.8	345	4.5-5.0	4.7
46	1.5-2.0	1.6	265	4.0-5.0	4.5

The PCR product was electrophoresed in 6% polyacrylamide gel, where flowering time and mite resistance from another project is also presented.

Opening Bulks

Individuals from Resistant and susceptible Bulks were evaluated with candidate primers, which showed polymorphism between bulks. The marker's presence or absence in each individual can help us to confirm association between SSR marker and CBB resistance in the field.

Results

Bulked segregant analysis of cassava family GM 315 detected polymorphism between each bulk and/or between evaluated parent and bulks (Figure 7.5).

Results suggested that some primers could be chosen as candidate primers to be evaluated as SSR marker associated with CBB resistance in the field. Results from bulked segregant analysis in cassava family GM 315 are presented in the following list:

SSR primers	**Number**
Total primers evaluated	486
Polymorphic in bulks	28
Candidates as SSR markers	13

To confirm that association, 13 SRR primers were evaluated in each individual that forms part of resistance and susceptible bulks (Figure 7.6)

Primer SSRY65 showed differences between resistant and susceptible individuals and can be considered SRR marker, it can be evaluated in the whole population. Two resistant genotypes did not show resistant marker, probably because of recombination, which could cause loss of some genomic regions.

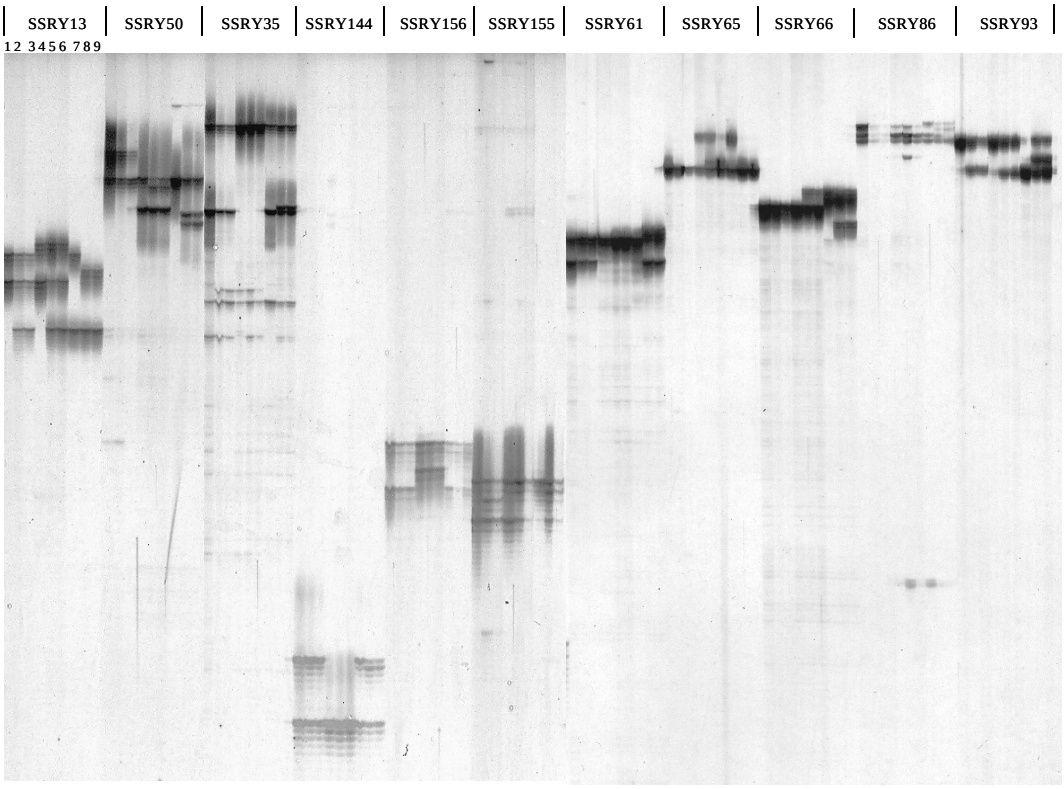

Figure 7.5. Eleven SSRY primers used to identify SSR markers by bulked segregant analysis. Nine samples were evaluated with each primer. The first three wells for each primer are from a population where flowering time is studied (lane 1 = early flowering male parent; lane 2 = early flowering bulk; lane 3 = late flowering bulk). The next three wells are from a GM 315 family where resistance to cassava bacterial blight is studied (lane 4 = resistant male parent; lane 5 = resistant bulk; lane 6 = susceptible bulk). The last three 7 = resistance male parent; lane 8 = resistant bulk; lane 9 = susceptible bulk).

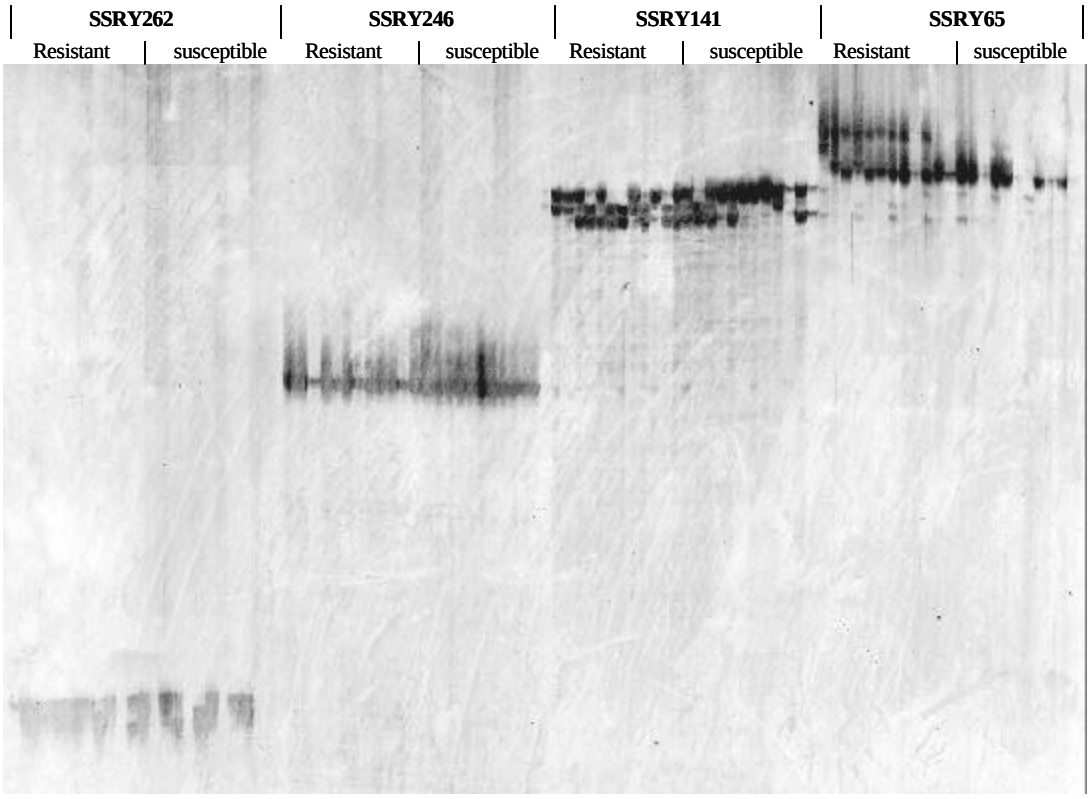

Figure 7.6. Four candidate primers evaluated in each individual that forms the bulks in cassava family GM 315. Each lane has an individual and they are separated in resistant and susceptible ones.

References

Dellaporta SL; Wood J; Hicks JR. 1983. A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19.

Michelmore, R. W., Paran, I., and Kesseli, R. V. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregant populations. Proc. Natl. Acad. Sci. USA. Vol 88: 9828-9832, November 1991. Genetics.

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