SSR analysis of F_1 intra specific crosses of cassava for the identification of new sources of CMD resistance in African germplasm

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INTRODUCTION

In Africa, the most important disease of cassava is the cassava mosaic disease (CMD) which is a major constraint to stable root production. CMD is one of the most globally damaging plant virus diseases with an annual economic loss of USD 1.9-2.7 billion (Legg and Fauquet, 2004). Host-plant resistance is the most effective control strategy to tackle the disease. CIAT in collaboration with African NARs has been using a single gene dominant gene (Akano et al., 2002) in breeding for resistance. However, the use of single gene is highly vulnerable to resistance breakdown. Due to the dynamic evolution of the virus, with the emergence of new recombinants, there is need to identify new sources of resistance. CMD resistance has been identified in some landraces and elite collection of African germplasm but information are currently lacking to indicate if they represent new sources of resistance. High CMD resistance in cassava genotypes is often indistinguishable phenotypically for the different resistance genes making it unavoidably important to employ molecular tools in breeding for the disease. Molecular marker technology can rapidly differentiate and identify new sources genes for use in breeding for resistance against a broad spectrum of the virus. In this study simple sequence repeat (SSR) markers were used to determine new additional sources of resistance to CMD in the elite germplasm of National Root Crops Research Institute (NRCRI) in Nigeria. We report here results of SSR analysis and the discovery of new sources of CMD resistance and its significance for CMD resistance breeding in cassava for Africa.

MATERIALS AND METHODS

Plant materials and Field experiment

The plants used in this study were developed from crosses between CMD resistant and susceptible parents. (Table 1). The families (Table 2) were planted in two seasons (2006 and 2007) for CMD evaluation at NRCRI, Umudike, Nigeria. The seedling nursery of the F1 progenies was evaluated in 2006. In 2007, 6 to10 cuttings (depending on vigor) of each genotype were evaluated in a randomized complete block design of three replicates. The F1 progenies were scored for CMD at peak disease pressure with symptom severity rated on a scale of 1 (highly resistant) to 5 (highly susceptible).

Table 1. .List of parental genotypes used for survey of polymorphism in markers linked to CMD2

Serial #	Genotype	Response to CMD
1	TMS2205	Resistant
2	NR8212	Susceptible
3	NR8083	Susceptible
4	TMS0505	Resistant
5	TMS30555	Susceptible

Table 2. Familes evaluated for cassava mosaic disease

Cross code	Female	Male	No. of plants
COB4	TMS2205	TMS30555	106
COB5	TMS2205	NR8212	116
COB6	TMS2205	NR8083	52
COB7	TMS0505	TMS30555	186

SSR screening

Genomic DNA of the Four F1 families and their parents was extracted as described by Dellarporta et al. (1983). The parents of the F1 families were screened with four flanking markers - two each of simple sequence repeats (SSR) and sequence cleaved amplified regions (SCARS) linked to *CMD2* dominant gene conferring resistance to CMD (Fig. 1). Amplification by polymerase chain reaction (PCR), polyacrylamide gel electrophoresis, and silver staining were done as described by Mba et al. (2000) for SSR markers. PCR products of the SCAR markers were run on 1% agarose gels at 60 V for 2h and stained with ethidium bromide. Markers were scored based on the presence or absence of the alleles associated with *CMD2* gene. Families showing no association with the *CMD2* gene were selected and used to identify new sources of resistance to CMD via bulked segregant analysis (BSA) following the scheme shown in Fig 2. A total of 530 SSR markers were used for BSA.

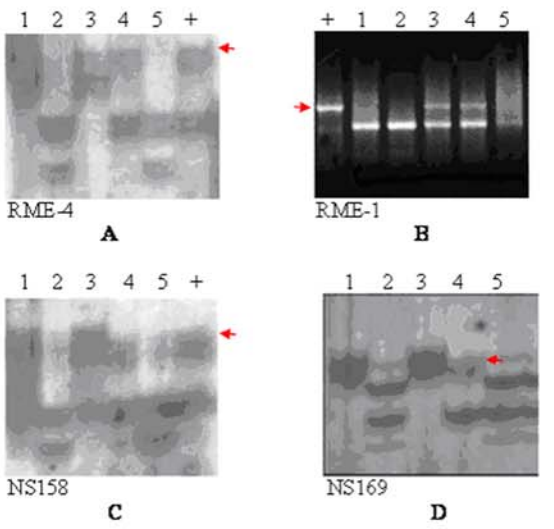

Fig 1. Polymorphism survey in parents for *CMD2* gene

Data analysis

Phenotypic data for CMD in 2006 and 2007 were correlated using Pearson correlation test in Microsoft Excel. Phenotypic and genotypic data were correlated for each polymorphic marker. The selected candidate markers from correlation analysis were subjected to t-test analysis to identify markers significantly associated to new sources of CMD resistance using Genstat discovery edition2. Mapmaker linkage analysis software version 2.0 (Lander et al., 1987) was used to calculate the genetic distance between the genes and the markers significantly associated to new sources of CMD resistance in each family.

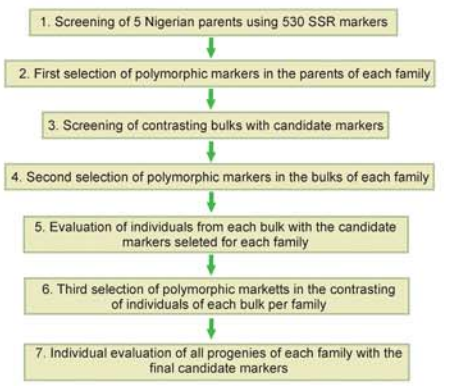

Fig. 2. BSA scheme. The composition of the resistant bulk for each of the F_1 families was 24 individuals. For the susceptible bulk, 12 individuals were used for three families (COB4, COB5 and COB6).

RESULTS AND DISCUSSION

The proportion of resistant genotypes in each family was 56.6% in family COB4, 60.3% in family COB5, 65.4% in family COB6 and 73.1% in family COB7. CMD mean severity scores increased slightly from 2006 to 2007 in COB5 (2.0 to 2.22), in COB6 (2.18 to 2.42) in COB6 (2.08 to 2.11). The slight increase in the severity index in 2007 over 2006 was due to the increased severity index of susceptible genotypes due to primary and secondary infection that occurs with increased time on the field. Family COB4 with a slight decrease in mean severity score (1.85 to 1.82) from 2006 to 2007 had the best resistance among the families. Correlation of CMD response between 2006 and 2007 was highly significant (r = 0.69 (*P* =0.001) to r = 0.72 (*P* = 0.001). Parental survey of polymorphism of *CMD2* gene flanking markers are presented in Fig 2. BSA analysis revealed the absence of *CMD2* gene resistance in families COB4, COB5 and COB6. These families were therefore used to identify new sources of CMD resistance. Of the total 530 SSR markers screened, polymorphic markers detected were 150 in COB4, 154 in COB5 and 169 in COB6. Screening of contrasting bulks with polymorphic markers identified five preliminary candidate markers. With t-test analysis, only two (NS 198 and NS 199) of the candidate markers were significantly associated to new sources of CMD resistance (Table 5). NS198 was more significantly associated to CMD resistance at *P* = 0.015 while the second marker (NS119) was significant at *P* = 0.049 (Table 3). The histogram, box plot, and residual plots for NS198 are shown in Figs 3, 4, and 5, respectively. The CMD gene linked to NS198 is designated *CMD3* while that linked to NS119 is designated *CMD4*. The alleles for these markers in both families were from the male parent TMS2205. Genetic distances as computed with Mapmaker shows that genetic distance between NS198 and CMD3 is 47. 5 cM while the interval between NS119 and CMD4 is 91.4 cM. The marker alleles associated with both genes were derived from TMS97/2205 (Fig. 6) which was a common parent in both families. Variety TMS97/2205 which was developed from a cross between TMS30572 and TME3 at the International Institute of Tropical Agriculture (IITA), is a highly resistant variety with near immunity to CMD (Egesi et al. 2006). The use of molecular markers in disease resistance in breeding program is rapidly permitting faster access to new genes thus allowing more direct identification of the resistance genes than is possible through phenotypic selection. Based on the results obtained from this study, TMS2205 is being utilized in CMD resistance breeding to pyramid the new genes identified in this genotypes with *CMD2* gene.

Table 3. T-test analysis candidate markers for significant association to CMD resistance

Family	Markers	Mean (CMD scores)		T-test (P level)
		Band presence	Band absence	
COB4	NS119	2.096	2.745	0.049*
	SSRY319	2.277	2.519	0.474 (ns)
COB5	EST-SSRY88	2.157	2.305	0.614 (ns)
	NS915	2.322	2.180	0.645 (ns)
COB6	NS198	1.591	2.611	0.015**

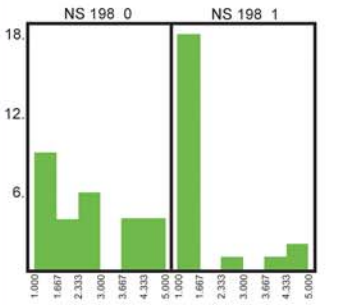

Fig.3 Histogram for cassava mosaic disease for marker NS198

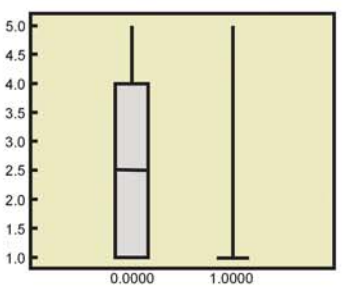

Fig. 4 Box plot of phenotypic and genotypic data for cassava mosaic disease for marker NS198

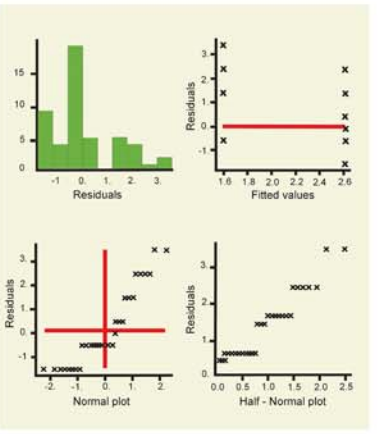

Fig 5. Residual plots of phenotypic data of cassava mosaic disease for NS198

Fig 6. Variety TMS97/2205

PERSPECTIVES

- The discovery of new genes for CMD resistance should minimize the high vulnerability associated with the routine use of the CMD2 gene alone. This should facilitate the development of super resistant cassava varieties in maintaining the food security status of this crop in Africa.
- We are presently exploring more markers around the regions of CMD resistance to identify closely linked markers to the new genes for efficient application in molecular breeding via marker assisted selection.

REFERENCES

Akano AO, Dixon AGO, Mba C, Barera E, Fregene M, 2002. Genetic mapping of a dominant gene conferring resistance to cassava mosiac disease. Theor Appl. Genet. 105:521-535.

Dellarporta SL, Wood J, Hicks JR. 1983. A plant DNA minipreparation:version II. Plant Mol Biol Rep1:19-21

Egesi CN, Ogbe FO, Akoroda M, Ilona P, Dixon A. 2006. Resistance profile of improved cassava germplasm to cassava mosaic disease in Nigeria. Euphytica 155:215-224

Lander ES, Green P, Abrahainmson J., Barlow A, Daly MJ, Lincoln SE. 1987 MAPMAKER : an interactive computer pakage for constructing primary genetic linkage maps of experimental and natural populations. Genomis 1:174-181.

Mba REC, Stephenson P, Edwards K, Melzer S, Mkumbira J, Gullberg U, Apel K, Gale M, Tohme J, Fregene MA (2001) Simple sequence repeat (SSR) markers survey of the cassava (Manihot esculenta Crantz) genome: towards an SSR-based molecular genetic map of cassava. Theor Appl Genet 101:21–31

Legg JP and Fauquet CM. 2004. Cassava mosaic geminiviruses in Africa. Plant Molecular Biology 56:585-599.

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