African Journal of Biotechnology Vol. 4 (9), pp.873-881, September 2005
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Full Length Research Paper

Molecular markers associated with a new source of resistance to the cassava mosaic disease

Y. Lokko[1]*, E.Y Danquah[2], S.K Offei[2], A.G.O Dixon[1] and M.A Gedil[3]

[1]International Institute of Tropical Agriculture, Ibadan, Nigeria. [2]Dept. of Crop Science, University of Ghana, Accra, Ghana. [3]Georgetown University Medical Centre, Washington, DC USA.

Accepted 24July, 2005

The predominant source of resistance to the cassava mosaic disease (CMD) is known to be polygenic requiring evaluation in multiple environments to characterise resistant genotypes, which makes the detection of genes for resistance using segregation analysis inefficient. Recently, some landraces have been identified which exhibit high levels of resistance to CMD. In this study, molecular markers associated with resistance to CMD in a resistant landrace were identified, using F1 progenies derived from a cross between the CMD resistant landrace TME7 and the susceptible line TMS30555, as a first step in marker assisted breeding for CMD resistance. Bulk segregant analysis (BSA) on the parents, resistant and susceptible DNA pools, using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers revealed that an SSR marker, SSRY28-180, donated by the resistant parent was linked with resistance to CMD. Marker-trait association detected by regression analysis showed that the marker, accounted for 57.41% of total phenotypic variation for resistance. The analysis further showed that another SSR marker, SSRY106-207 and an AFLP marker, E-ACC/M-CTC-225, accounted for 35.59% and 22.5% of the total phenotypic variation for resistance, respectively. The implication of the results in breeding for resistance to CMD is discussed.

Key words: Cassava mosaic virus, genetic linkage map, quantitative trait loci.

INTRODUCTION

The cassava mosaic virus disease (CMD), which is caused by any one or a combination of the white-fly-transmitted cassava mosaic begomoviruses, is an important constraint to cassava production in Africa (Geddes, 1990; Zhou et al., 1997). It is spread mainly through the use of infected planting material. Estimated total crop yield losses due to CMD on the continent amounts to about US $440 million per annum (Thresh et al., 1997). The most severe effects of the disease is associated with Ugandan variant of the East African cassava mosaic virus (EACMV-UgV) and in cases of mixed infection of the African cassava mosaic virus (ACMV) and the East African cassava mosaic virus (EACMV) (Ogbe et al., 2003). The most effective means of controlling CMD is by the deployment of resistant varieties (Thresh et al., 1997).

Resistance to CMD was first obtained from a cross between cassava and its relative Manihot glaziovii Muller von Argau (Nicholas, 1947). After three backcrosses into cassava to obtain suitable storage roots, the clone 58308 was selected, and for decades this clone and its derivatives have been extensively used as the main source of resistance in breeding for resistance to the disease. This has resulted in the selection of several improved cultivated cassava genotypes of the Tropical Manihot Selection (TMS) series, with resistance to CMD (Hahn et al., 1989). Some African landraces, the Tropical

*Corresponding Author's E-mail: Y.Lokko@cgiar.org.

Abbreviations: ACMV, African Cassava Mosaic Virus;AFLP, amplified fragment length polymorphism; BAC, bacterial artificial chromosome; BSA, bulk segregant analysis; CMD, cassava mosaic disease; CP, cross between two heterogeneously heterozygous and homozygous diploid parents; EACMV, East African cassava mosaic virus; EACMV-UgV, East African Cassava Mosaic Virus-Ugandan variant; EST, expressed sequence sites; HR, highly resistant; HS, highly susceptible; K, Kruskal Wallis rank-sum test; LOD, logarithm of the odds (to base 10); MAS, marker assisted selection; MDSS, mean disease severity scores; MLS, mean leaf severity; MQM mapping, composite interval mapping method; MST, mean shoot tip severity; MWP, mean whole plant severity; PCR, polymerase chain reaction; QTL, quantitative trait loci; R, resistant; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; S, susceptible; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; TME, tropical *Manihot Esculenta*; TMS, tropical Manihot Selection; WAP, weeks after planting.

Manihot Esculenta series (TME), collected in West Africa have also been identified to be resistant to CMD (Mignouna and Dixon, 1997) and could serve as alternative sources of resistance to increase the genetic base of resistance to the disease.

Resistance in clone 58308 has been described as recessive and polygenic (Hahn et al., 1980). A recent study on the inheritance of resistance to CMD in some of the African landraces has revealed polygenic and recessive inheritance with susceptible accessions also contributing to resistance (Lokko et al., 1998). Hahn et al. (1980) inferred from the polygenic mode of inheritance that resistance to the disease must be attributed to the combined action of a number of loci, which are linked on a chromosome, or a set of chromosomes in a genome. Population improvement and recurrent selection were, therefore recommended in breeding for resistance to the disease.

Quantitative genetic analysis has also shown allelic differences and complementarity of genes for resistance to CMD in the African germplasm (Lokko et al., 2003). Recently, a major gene responsible for resistance in a Nigerian landrace (TME3 "2nd Agric") was reported (Akano et al., 2002) suggesting that there could be different mechanisms of resistance to CMD in cassava genotypes. An understanding of these resistant mechanisms will be useful in breeding efforts particularly in marker assisted selection (MAS) programmes. The detection of multiple genes for virus resistance using segregation analysis alone is not efficient because of the confounding effect of the environments and its interaction with the genotype (McMullen and Louie, 1989). The expression of CMD in different cassava genotypes is known to be dependent on the environment, and there is a strong relationship between the range of symptoms produced and genotype x environment interaction (GXE) (Fargette et al., 1994). Hence molecular marker analyses are suitable for such studies. Marker systems such as Isozymes, RFLP's, RAPD's, SSR's and EST's have been used to develop a cassava framework map consisting of two geographic divergent parents (Fregene et al., 1997).

The two strategies frequently used to identify molecular markers associated with traits of interest include genetic linkage mapping and bulk segregant analysis (BSA) (Giovannoni et al., 1991; Michelmore et al., 1991; Tanksley et al., 1989). Akano et al. (2002) recently used BSA of land races to identify a SSR marker linked to another CMD resistance gene, designated CMD2. To date two CMD resistance genes CMD1 and CMD2 have been placed on the map (Akano et al., 2002; Fregene et al., 2001). The detection of more molecular markers associated with resistance to CMD in cassava genotypes will enhance breeding programmes. The objectives of this study were to determine molecular markers associated with resistance to CMD in the African landraces.

MATERIAL AND METHODS

Genetic material

Segregating F1 progenies from a cross between a breeder's accession TMS30555 and landrace TME7 (Oko-Yawo) were used in this study. TMS30555, which exhibits moderate susceptibility to CMD, is from the earlier breeding selections, and derived from a cross between 58308 and a Nigerian landrace Oyarugba dudu, while TME7, a landrace from the Niger State, Nigeria is resistant to CMD.

Resistance screening

The F1 progenies were evaluated in two growing seasons, 1998 and 1999 in Ibadan, Oyo state Nigeria which is in the forest-savannah transition zone with a ferric luvisol soil type and is a high pressure site for the disease, to ascertain the CMD resistance status of each progeny. In the 1998 growing season, the progenies were evaluated at 6, 12, 20 and 50 weeks after planting (WAP), while during the 1999 season they were evaluated at 6 and 12 WAP. Each plant was examined for symptom severity of the whole plant. Plants were assigned disease severity scores based on the standard five point scoring scale for CMD. Where there were no obvious symptoms plants were assigned a score of "1". Plants with mild chlorotic patterns or mild leaf distortion at the base were scored "2", while those with strong mosaic on the entire leaf, distortions of leaves were scored "3". Severe mosaic distortion, reductions of leave lamina affecting about 2/3 of the leaves were scored as "4" and plants with the most severe mosaic symptoms, with severe distortion of leaves, stunting of entire plant and about 4/5th of leaves affected were assigned a score of "5' (IITA, 1990). In the 1999 season, the first 10 leaves of each plant were individually scored in addition to the shoot tip and whole plant scores.

Using the GLM procedure in SAS, least square means of mean shoot tip severity (MST), mean whole plant severity (MWP) and mean leaf severity (MLS) for each F1 plant were computed and a mean CMD response score determined as an average of the three disease responses. Plants with a mean CMD scores of "1" were then classified as highly resistant (HR), those with a score of "2" were resistant (R), those with a score of "3" were classified as susceptible (S) and those with scores of "4" and "5" were classified as highly susceptible (HS).

In March 2000, cuttings of the progenies and parents were planted in nursery beds and watered twice a week to produce young leaves for DNA extraction. After three weeks the shoot tips were excised to enhance symptom severity from any existing primary infection in the cutting, for a final confirmation of resistance or susceptibility to CMD.

DNA extraction and PCR-based diagnostics of CMGs

Young leaves (100 mg) were excised from each F1 plant for DNA extraction using the DNA extraction kit, DNeasyTM (Qiagen, Germany) following the manufacturer's instruction.

The virus strain(s) causing the symptoms in the population was examined using the PCR method described by Zhou et al. (1997). DNA samples of the mapping population were tested with primers designed to identify ACMV using primers ACMV-F1+ACMV-R1 and ACMV-AL1/F+ACMV-AR0/R, EACMV using primers UV-AL1/F1+EACMV-CP/R and EACMV UV-AL1/F1+ EACMV UV-AL1/R1 (Zhou et al., 1997) and the Uganda variant (UgV) using primers UV-AL1/F1+ACMV-CP/R3 (Harrison et al., 1997). All Oligonucleotides were synthesised by Integrated DNA technologies (IDT, Coralville, USA).

Bulk segregant analysis (BSA) and genotyping mapping population

The bulks were constructed by selecting ten highly resistant plants to make up the resistant bulk and ten plants from the highly susceptible classes to make up the susceptible bulk. The parents and bulked segregant DNA were screened with 186 simple sequence repeat (SSR) markers and by amplified fragment length polymorphism (AFLP). The SSR primers have previously been reported (Chavarriaga-Aguirre et al., 1998; Mba et al., 2001). The AFLP analysis was performed with 64 EcoR1/Mse1 primers combinations obtained from Gibco Life Technologies Inc (Gibco BRL, Paisley, UK), AFLP analysis system I primer starter kit, and 24 Pst1/Taq1 primer combinations. Primers that were polymorphic between the parents and the two bulks were used to screen the members of each bulk then the entire mapping population to determine the relationship between resistance to CMD and markers associated with resistance.

SSR analysis: The two DNA pools or bulk segregants were constructed by combining 10 µl (10 ng/µl) of DNA from each of the 10 highly resistant and the 10 highly susceptible selected samples. Each 25 µl reaction contained 2.5 µl DNA (10 ng/ul), 2.5 µl each of the forward and reverse primers (2.0 µM each), 1 µl dNTPs (5 mM stock), 2.5 µl 10X PCR buffer, 1 or 1.5 µl MgCl2 (25 mM) and 0.75 units of Taq polymerase (Roche). The touchdown PCR profile involved an initial denaturation for 5 min at 94oC. The first cycle then had a denaturation at 94°C for 30 s, annealing at 65oC for 1 min, and extension at 72°C for 1 min. This was followed by 10 cycles, which were similar to the first cycle, but the annealing temperature was lowered by 0.7oC for each cycle. Thereafter, the annealing temperature was maintained at 55°C for 1 minute, for 25 cycles. The final extension was 72°C for 7 min.

AFLP analysis: AFLP analysis using the EcoR1/Mse1 restriction enzymes was performed following the protocol of Life Technologies Inc, and for and the Pst1/Taq1 analysis, digestion with the restriction enzyme Taq1 (Phamarcia) preceded digestion with Pst1 (Roche), using the method described by Wong et al. (1999). After digestion, adapter ligation and preamplification, equal aliquots (10 µl) of preamplified DNA samples constituting a bulk group were bulked together prior to selective amplification. Adapters and primers for the Pst1/Taq1 analysis were synthesised by Integrated DNA Technology (IDT, Coralville, USA) according to sequences published by Barrett and Kidwell (1998) for Pst1 and Wong et al. (1999) for Taq1.

Electrophoresis and detection: About 3.5 µl of SSR or AFLP product was electrophoresed on 6% polyacrylamide sequencing gel (19:1 Acrylamide:Bis acrylamide, 8 M urea) at 50oC for 2 h in 1X TBE buffer (0.09 M Tris-borate and 0.002 M EDTA). DNA was visualised by silver staining using the Promega's silver staining kit.

Linkage analysis

A total of 15 SSR primers, which showed differences between the parents and bulks, were used to genotype the mapping population. These were primers SSRY6, SSRY7, SSRY28, SSRY40, SSRY49, SSRY51, SSRY91, SSRY95, SSRY102, SSRY106, SSRY110, SSRY113, SSRY135, SSRY170 and SSRY179 (Mba et al., 2001). EcoR1/Mse1 +3 AFLP primers, with the +3 extension to the EcoR1 primer (E-ACC/M-CTC, E-AAC/M-CAT, E-ACA/M-CAC, E-AAG/M-M-CTC, E-ACA/M-CAT, E-AAG/M-CTG, E-ACT/M-CAG, E-ACC/M-CAT, and E-ACC/M-CTC) and Pst1/Taq1 +3 AFLP primers, with the +3 extension to the Pst1 primer (P-ACC/T-CTA, P-ACT/T-CTT, and P-ACC/T-CAG) were also used to genotype the mapping population.

Polymorphic markers were tested for 1:1 and 3:1 segregation ratios using chi-square test. Markers with chi square values less than 6.63 (chi value at 1% probability) were then used in linkage mapping analysis using the computer software package JoinMap version 3.0 (Van Ooijen and Voorrips, 2001). Markers assignment to linkage groups and calculation of pairwise recombination frequencies and corresponding LOD scores for all pairs of markers that belong to a certain linkage group were based minimum LOD score of 3.0 (Stam, 1993). Centimorgan units were calculated using the Kosambi mapping function (Kosambi, 1944).

QTL analysis

Simple phenotypic correlation analysis was performed on the markers and the CMD responses. The association between molecular markers and CMD resistance was then determined by simple linear regression of mean CMD response score and all the markers using the SAS computer package (SAS, 1999), and also with the non-parametric mapping procedure (Kruskal Wallis rank-sum test) of the software package MapQTL versions 3.0 (Van Ooijen et al, 2002). The mean CMD response scores of the F1 individuals were used as the phenotypic trait scores and significant associations were searched on the linkage groups. Associations between markers and the CMD response traits were declared significant at a significance threshold of $p = 0.005$ (Lander and Botstein, 1986). This stringent threshold was adopted to avoid type 1 error (Dudley, 1993). The proportion of the phenotypic variance explained by the marker was determined by the R2 value.

Map QTL's permutation test was then carried out on all the mapped markers to determine appropriate threshold value for declaring a significant QTL effect with interval mapping. The LOD values at $P = 0.05$ and $P = 0.01$ were taken as the estimated critical values at which to declare the presence of a QTL. Markers close to the detected QTL were selected as cofactors and used in MQM mapping analysis to search for other segregating QTL's.

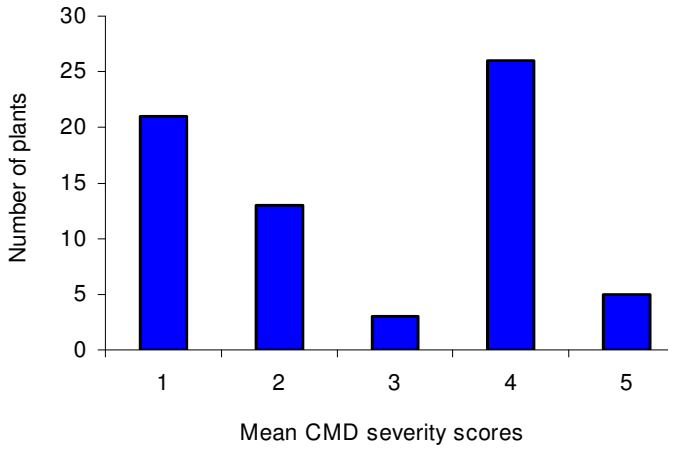

Figure 1. Distribution of F_1 progeny in CMD severity classes of 1 to 5 scale, where 1= no symptom and 5=severe mosaic symptoms, based on F_1 progeny mean CMD severity responses.

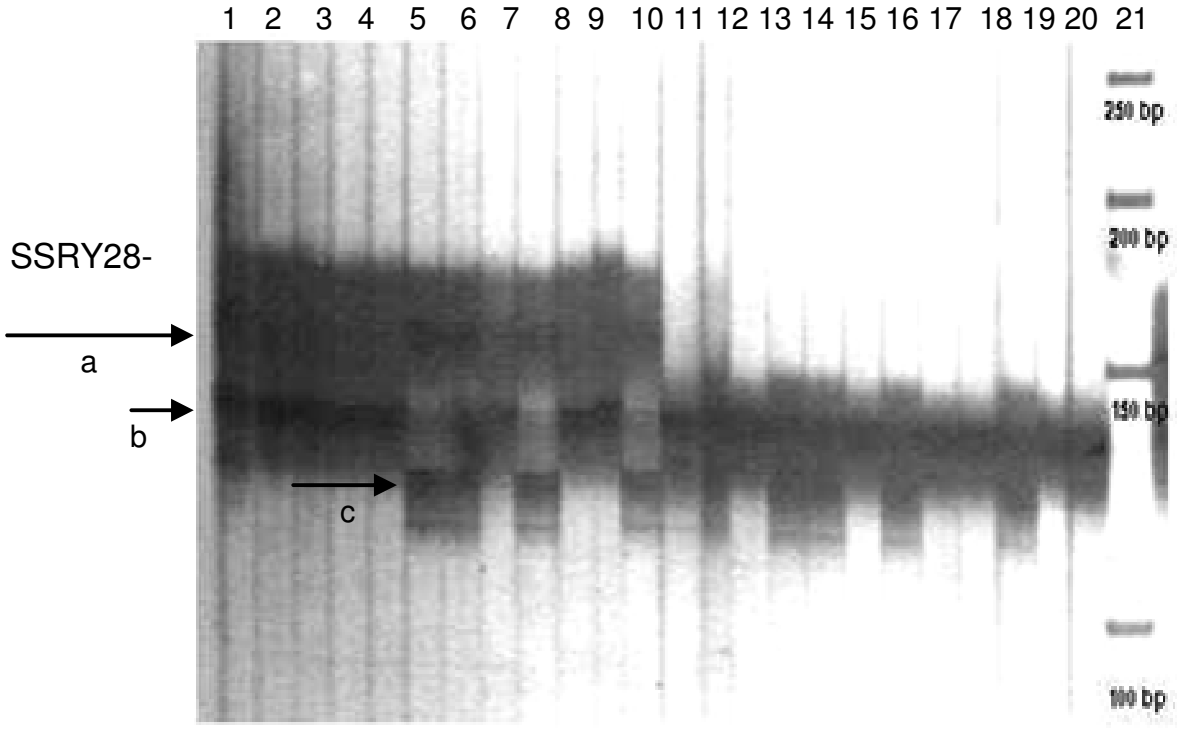

Figure 2. Amplification products of SSRY28, on parents, bulks, and bulk members, showing three alleles "a", "b" (SSRY28-180) and c. Lane 1, resistant parent TME7; lane 2, resistant bulk; lanes 3 to 12, ten HR progeny; lane 13, susceptible parent TMS30555; lane 14, susceptible bulk, lane 15 to 24 ten HS progeny; lane 25, 50bp marker (Promega).

RESULTS

Resistance screening

The population was characterised for its reaction to CMD in different environments based on three phenotypic symptomson each genotype and the virus strain causing the symptoms identified. The mean CMD response scores, based on MST, MWP and MLS of individual F1 progeny overall score dates and years, revealed varying levels of resistance and susceptibility. The progenies associated into the five disease severity classes (Figure 1). Thirty-one genotypes were classified as highly resistant (HR), five as resistant (R), eight as susceptible (S) and twenty-five as highly susceptible (HS).

The PCR diagnostics test detected only ACMV as the causal agent responsible for the characteristic symptoms in the population. The ACMV specific primers ACMV-F1/ACMV-R1 and ACMV-ALF/ACMV-ARO/R detected ACMV in 61 and 37 DNA samples, respectively. ACMV-F1/ACMV-R1 detected the virus in 29 resistant samples (24 HR and 5 R samples) while ACMV-ALF/ACMV-ARO/R detected the virus in nine resistant samples (8 HR and 1 R) including one, which was not detected by ACMV-F1/ACMV-R1. ACMV-F1/ACMV-R1 also detected the virus in 32 susceptible samples (24 HS and 8 S) and ACMV-ALF/ACMV-ARO/R detected the virus in 28 susceptible samples (21 HS and 7 S).

BSA and linkage analysis

SSR primer SSRY28-180, which had three alleles (a, b, and c) in the two parents, consistently distinguished between the two DNA bulks and the members of each as SSRY28-180-180, was present in the resistant landrace TME7 but absent in the susceptible improved line I30555. The band was also present in all the members of the resistant bulk, but absent in the members of the susceptible group (Figure 2).

The CP segregating marker types for a population resulting from a cross between diploid heterozygous parents and the linkage phase is unknown, were assigned to the data generated for JoinMap analysis (Van Ooijen and Voorrips 2001). The 17 primers used to genotype the population yield three segregating marker types <aaxab>, <abxaa> and <abxac>. In all cases for this scoring nomenclature, the two characters to the left of the "x" represent alleles from the first parent in the cross (in this case the female parent TMS30555) and the two characters to the right of the "x" represent alleles from the second parent in the cross (in this case the male parent TME7).

Table 1. Association between markers E-ACC/M-CTC-225, SSRY28-180 and SSRY106-270 with CMD resistance in mapping population based on linear regression and nonparametric mapping analysis.

Markers	Map Position (cM)	CMD Response	Regression Analysis		Kruskal-Wallis Test	
			R^2 (%)	p-value	K	p-value
E-ACC/M-CTC-225	0	MST	15.79	0.0004	11.3	0.001
		MWP	22.01	0.0001	16.7	0.0001
		MLS	27.13	0.0001	20.26	0.0001
		Mean	22.5	0.0001	15.2	0.0001
SSRY28-180	40.2	MST	53.76	<0.0001	37.56	0.0005
		MWP	58.48	<0.0001	35.58	0.0001
		MLS	50.43	<0.0001	32.49	0.0001
		Mean	57.41	<0.0001	35.04	0.0001
SSRY106-270	55.8	MST	27.82	<0.0001	24.75	0.0005
		MWP	33.59	<0.0001	19.96	0.0005
		MLS	29.87	<0.0001	22.51	0.0001
		Mean	32.24	<0.0001	21.44	0.0001

MST, mean shoot tips; MWP, mean whole plant; MLS, mean leaf severity; Mean, mean CMD response; R^2, proportion of the phenotypic variation; andK, Kruskal Wallis rank-sum test.

Thirty-six <aaxab> and 41 <abxaa> markers were scored from the nine EcoR1/Mse1+3 primers and three Pst1/Taq1+3 primers analysis of the F1 mapping population, while five <aaxab>, three <abxaa> and five <abxac> markers were scored for the SSR analysis giving a total of 90 markers. The chi square analysis revealed that 34 (37.78%) <aaxab>markers, 37 (41.11%) <abxaa> markers and five (5.55%) <abxac> markers were significant for the1:1 segregation ratio, while 10 bulk group. The polymorphic marker (allele “a”) designated (11.11%) <abxaa> markers and 4 (4.44%) <aaxab> markers were significant for 3:1 segregation ratio. Forty-five out of the 90 markers mapped in 15 linkage groups with a total length of 812.32 cM of the genome.

QTL analysis

Markers associated with resistance to CMD based on single-marker analysis using regression and the non-parametric method of QTL mapping are presented in Table 1. Both analyses yielded three significant ($p<0.0005$) marker associated effects due to SSRY28-180, SSRY106-270 and E-ACC/M-CTC-225 based on the three mean disease severity responses. All three markers were donated by the resistant parent TME7 and mapped on the linkage group IV. The R2 values, which indicated the proportion of the phenotypic variation explained by each of these markers was, 58.48% for SSRY28-180, 33.59% for SSRY106-270 and 22.01% for E-ACC/M-CTC-225 based on the mean CMD response. The Kruskal-Wallis test further revealed that these markers explained 38.58, 19.96 and 16.70% respectively of the total variation based on the mean CMD response.

A more rigorous QTL analysis was then carried out using the interval mapping option of the MAPQTL software. Using the estimated genome-wide empirical LOD score thresholds of 19.0 for $P<0.01$, and 18.1 $P<0.05$, three QTL regions were detected on the linkage group IV based on MWP, MLS and the mean CMD response each revealed two QTL regions and MST revealed one QTL region (Figure 3). The first, QTL associated with MWP was at map position 0 cM and associated with the AFLP marker E-ACC/M-CTC-225, and explained 93% of the variation. The second QTL spread across the linkage group from to map position 10 to 35 cM, between E-ACC/M-CTC-225 and SSRY28-180. This QTL was also detected by the other CMD responses, starting from map position 15 to 30 cM based on the MST and from map position 5 to 35 cM for MLS and the mean CMD responses. The third QTL region was at map position 45.2 cM between SSRY28-180 and SSRY106-270 (Table 2). Further analysis with MQM, which uses the three markers E-ACC/M-CTC-225, SSRY28-180 and SSRY106-270 as cofactors in the analysis did not reveal any more QTLs.

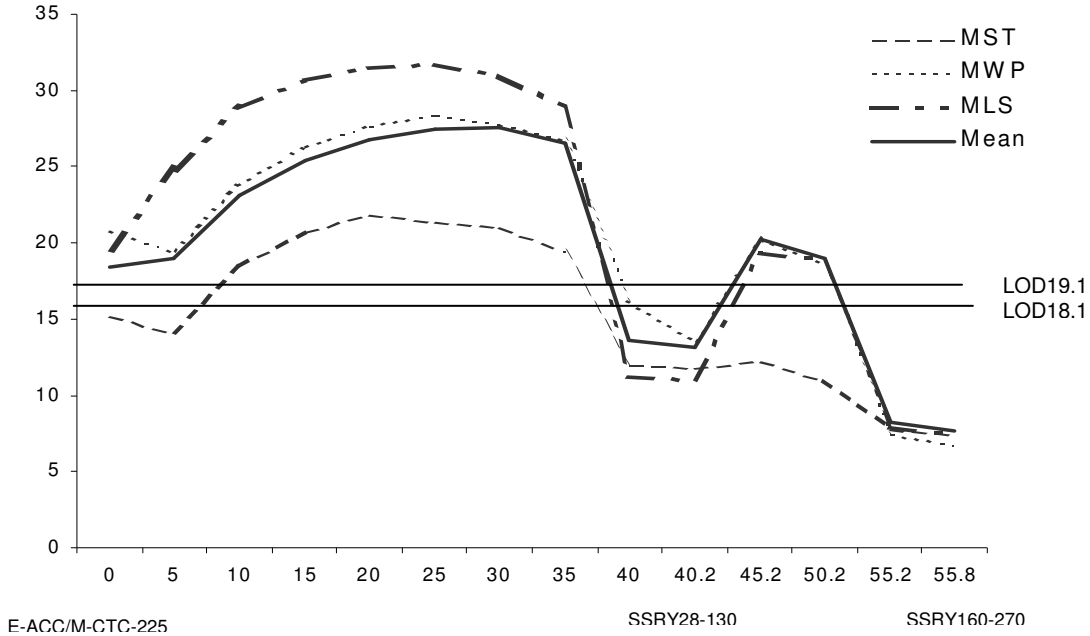

Figure 3. Likelihood profiles for QTL associated with resistance to CMD at the significant threshold p<0.01 LOD 19.1 and p<0.05 LOD 18.1 on linkage group IV based on MST (Mean shoot severity), MWP (Mean whole plant severity), MLS (Mean leaf severity) and the mean (Mean CMD responses).

Table 2. Interval Mapping Analysis of markers on linkage group IV associated with resistance to CMD based on CMD responses.

Marker	Map Position (cM)	MST		MWP		MLS		Mean	
		LOD	% var.	LOD	% var.	LOD	% var.	LOD	% var
E-ACC/M-CTC-225	0	15.26	89.5	20.81	92.9	19.51	92	18.36	91.3
	5	14.02	91.8	19.48	92.3	24.84	96.5	19.01	96.3
	10	18.47	92	23.83	95.9	28.81	96.5	23.05	94.4
	15	20.68	92	26.28	95.9	30.63	96.5	25.38	93.8
	20	21.89	92	27.68	95.9	31.48	96.5	26.73	93.6
	25	21.4	91.1	28.39	95.8	31.63	96.5	27.43	93.3
	30	21.09	90.4	27.8	93	30.96	96.3	27.51	93
	35	19.43	89.2	26.76	92.7	28.91	96.3	26.5	92.7
	40	11.96	56.2	16.19	92.1	11.22	60.6	13.57	70.1
SSRY28-180	40.2	11.82	54.6	13.49	59.6	11	54.7	13.12	58.8
	45.2	12.27	63.4	20.23	92.2	19.3	92.3	20.22	91.3
	50.2	10.94	61.8	18.7	92.1	18.91	92.3	18.96	91.1
	55.2	7.8	43.2	7.38	44.3	7.88	44.7	8.24	46.8
SSRY106-270	55.8	7.39	39.3	6.71	36.6	7.38	39.3	7.63	40.3

LOD, LOD scores values at $P = 0.01$
% var, proportion of the phenotypic variation
MST, Mean shoot tips
MWP, Mean whole plant
MLS, Mean leaf severity
MEAN, Mean CMD response

DISCUSSION

Although mixed infections of ACMV and EACMV have been reported in West Africa (Ogbe et al., 1999; Offei et al., 1999), the results of this study showed that the only CMG strain responsible for the disease symptom in the mapping population was ACMV. The ACMV primer ACMV-F1/ACMV-R1 was more efficient in detecting the virus in the samples in that it detected the virus in more samples than the primer ACMV-AL F/ACMV-AROR.

The presence of the virus in some of the resistant samples suggests that field resistance observed as symptoms, was not necessarily an indication of resistance to virus infection. The A genome of gemini-viruses of which the cassava mosaic viruses belong, encode a protein required for their replication and must recruit the remaining DNA replication mechanism from the host plant, while the B genome is responsible for spread and symptom production (Estessami et al., 1991; Fontes et al., 1992). Since DNA replication is part of the natural growth and development, it is possible that the virus is able to replicate and probably even spread in the resistant plant but the subsequent disease symptoms are inhibited. Using field evaluation, axial bud inoculation and PCR, Ogbe (2001) concluded that field resistance as shown by lack of symptoms was not necessarily an indication of resistance to virus infection, but could be partly due to lack of virus multiplication, which suggests that field selection of resistance should be complemented with PCR or inoculation test.

While this result is of direct relevance to CMD control and management in that use of such symptomless genotypes in controlling the spread of the disease could actually be a reservoir of the virus. This finding in our study is an indication of different CMD resistance mechanism within the population.

In BSA, where DNA samples are pooled from individuals which are similar for a particular trait, it is expected that the low frequency allele(s) in the pooled DNA sample will not be amplified (Liu 1999; Michelmore et al., 1991), therefore increasing the chance of identifying markers linked to the trait of interest. This study identified SSRY28-180 to be linked with resistance to CMD using BSA. The SSR marker SSR28-180, which explained about 58% of the total phenotypic variation, has also been identified in another cassava population involving the CMD resistant landrace, TME3 (known in Western Nigeria as 2nd Agric). In that study, the marker accounted for 68% of the total phenotypic variation (Akano et al., 2002), and was mapped on linkage group R of the male cassava framework map developed by Fregene et al. (1997) with a recombination distance of 8 cM from a dominant cassava resistance gene CMD2. The results of our current study imply that the resistant gene CMD2 from TME3 is related to a gene or genes for resistance in TME7.

QTL analysis further established associations between resistance to CMD and three markers, SSRY28-180 SSRY106-270 and E-ACC/M-CTC-225. The three markers, donated by the resistant parent TME7, were linked with CMD resistance in coupling phase. Correlation coefficients for SSRY28-180 and E-ACC/M-CTC-225 with CMD responses were significant and negative while correlation between SSRY106-270 and the CMD responses were significant and positive. This suggests that the CMD resistance genes associated with E-ACC/M-CTC-225 and SRY28-180 are different from genes associated with SSRY106-270. The interval mapping analysis further revealed the large QTL region between SSRY28-180 and E-ACC/M-CTC-225 covering about 50% of the length of the linkage group and 3% of the length of genome covered, which explained most of the phenotypic variation. These markers could therefore be useful in marker assisted selection for CMD resistance. There is however a need to saturate this and other CMD segregating mapping populations with more DNA markers, to obtain markers that is more tightly linked to resistance to CMD.

Currently, further development of SSR markers involving untranslated regions of cassava ESTs for SSR repeats are underway. Another project involves fine mapping the region of the cassava framework map at CIAT, followed by contig mapping with bacterial artificial chromosome (BAC) libraries (Fregene et al., 2001). Since the markers, which had the highest association with CMD resistance were developed from microsatellite loci with CT repeats, SSRY28 ((CT) 26 (AT) 3 AC(AT) 2), SSRY106 ((CT) 24) (Mba et al., 2001), exploring more markers based on such repeats could facilitate efforts at fine mapping and isolating CMD resistance genes. Alternatively, high throughput markers such as from expressed sequence sites (EST) and single nucleotide polymorphism (SNP), the most abundant type of DNA polymorphism, may be employed in mapping CMD resistance genes. Due to the high frequency of SNPs in the genome, there is high possibility of them being closely associated with resistance (Lohmann et al., 2000).

Recently, several cassava EST projects have been initiated using different cassava accessions including CMD resistant accessions TMS30572 and TME3 (Anderson et al., 2004; Lopez et al., 2005). These resources when available would facilitate mapping genes associated with traits of interest in cassava including CMD resistance by including gene markers on existing maps.

The results of this study have other implications in cassava molecular genetics and breeding. To date, three CMD segregating mapping populations including the current population have been developed and markers associated with CMD identified (Akano et al., 2002; Lokko et al., 2004). QTLs associated with the polygenic and recessive source of resistance to CMD in the TMS 30572 × TME 117 population based on the mean

disease severity scores (MDSS) of the progeny in two growing seasons in Nigeria have also been reported (Lokko et al., 2004). Another CMD resistance gene from TMS30572 CMD1 has also been identified (Fregene et al., 2001). With the availability and enhancement of the computer package JOINMAP a consensus map based on these and the cassava TMS 30572 × CM 2177-2 (Fregene et al., 1997) could be to developed which would facilitate planning experiments, to constructing a genome database, comparing QTL identities in different genetic backgrounds, and comparative mapping with other species.

In conclusion, this study has identified three DNA markers associated with resistance to CMD. While the efficiency of these markers in MAS for CMD resistance still needs to be investigated, their identification forms a basis for further studies to develop more markers with high association to CMD in that the inclusion of additional markers based on sequenced data such as ESTs and RFLPs converted to PCR-based markers on the map, would provide better coverage of the genome and increase their usefulness in gene tagging and MAS of resistance to CMD and other traits of agronomic importance.

ACKNOWLEDGEMENT

This work was supported by the Rockefeller Foundation grant RF 97007 #28 and 2000 FS 137 and managed by the International Institute of Tropical Agriculture (IITA), Ibadan Nigeria under its Graduate Research Fellowship programme.

REFERENCES

Anderson JV, Delsen M, Fregene MA, Jorge V, Mba C, Lopez C, Restrepo S, Soto M, Piegu B, Verdier V, Cooke R, Tohme J, Horvath DP (2004). An EST resource for cassava and other species of Euphorbiaceae. Plant Mol. Biol. 00:1–13.

Akano AO, Dixon AGO, Mba C, Barrera E, Fregene M (2002). Genetic mapping of a dominant gene conferring resistance to the cassava mosaic disease (CMD). Theor. Appl. Genet. 105: 521–525.

Barrette BA, Kidwell KK (1998). AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci. 38:1261-1271.

Chavarriaga-Aguirre P. Maya MM, Bonierbale MW, Kresovich S, Fregene MA, Tohme J, Kochert G (1998). Microsatellites in cassava (Manihot esculenta Crantz): discovery, inheritance and variability. Theor. Appl. Genet. 97: 493–501.

Dudley JW (1993). Molecular markers in plant improvement: Manipulation of genes affecting quantitative trait. Crop Sci. 33: 660-668.

Estessami P, Saunder K, Watt J, Stanley J (1991). Mutational analysis of complementary sense genes of ACMV DNA-A. J. Gen. Virol. 72: 1005-1012.

Fargette D, Jeger M, Fauquet C, Fishpool LDC (1994). Analysis of temporal disease progress of Afr. cassava mosaic virus. Phytopathology 84: 91-98.

Fontes EPB, Luckow VA, Hanley-Bowdoin L (1992) A geminivirus replication protein is a sequence specific DNA binding protein. Plant Cell 4: 597-608.

Fregene M, Okogbenin E, Mba C, Angel F, Suarez MC, Guitierez J, Chavarriaga P, Roca W, Bonierbale M, Tohme J (2001). Genome mapping in cassava improvement:: Challenges, achievements and opportunities. Euphytica 120: 159-165.

Fregene M, Angel F, Gomez R, Roduriguez F, Chavarriaga P, Bonierbale M, Roca W, Tohme J (1997). A molecular genetic map of cassava (Manihot esculenta Crantz). Theor. Appl. Genet 95: 431-441.

Geddes AM (1990). The relative importance of crop pests in sub-Saharan Africa. Bulletin 36; Chatham Natural Resource Institute (NRI). pp.???

Giovannoni JJ, Wing RA, Ganal MW, Tanksley SD (1991). Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res. 19(23): 6553-6558.

Hahn SK, John C, Isoba G, Ikoun T (1989). Resistance breeding in root and tuber crops at the International Institute for Tropical Agriculture (IITA), Ibadan Nigeria. Crop Protection 8: 147-168.

Hahn SK, Terry ER, Leuschner K (1980). Breeding cassava for resistance to cassava mosaic virus disease. Euphytica 29: 673-683.

Harrison BD, Zhou X, Otim-Nape GW, Liu Y, Robinson DJ (1997). Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. Ann. Appl. Biol. 131:437-448.

IITA (1990). Cassava in tropical Africa. A reference manual. Chayce Publication Services, U.K (ed) p.196

Kosambi DD (1944). The estimation of map distances from recombination values. Ann. Eugen. 12:172-175.

Lander ES, Botstein D (1986). Mapping complex genetic traits in humans: New method using a complex RFLP linkage map Cold Spring Harbor Symposia on Quantitative Biology 51: 49-62.

Liu BH (1997). Statistical Genomics: Linkage mapping and QTL analysis. CRC Press LLC, Boca Raton, Florida, USA, p. 611.

Lohmann S, Lehmann L, Tabiti K (2000). Fast and flexible single nucleotide polymorphism (SNP) detection with the LightCycler System. Biochemica 4: 23-28.

Lokko Y, Dixon AGO, Offei SK (1998). Combining Ability of resistance to the cassava mosaic virus disease. In Akorada MO, Ngeve JM (Eds). Root Crops in the 21st Century. Proceedings of the 7th Triennial conference of the International Society for Tuber and Root Crops, Africa Branch, 11 –17 Oct. 1998 Cotonou, Benin. IITA Ibadan, Nigeria. pp. 438-442.

Lokko Y, Gedil M, Dixon A (2004). QTLs associated with resistance to the cassava mosaic disease: new directions for a diverse planet. In Proceedings of the 4th International Crop Science Congress, 26 September-1 October 2004, Brisbane, Australia. http://www.cropscience.org.au/icsc2004/poster/3/7/4/1154_lokko.htm

Lokko Y, Dixon AGO, Offei SK, Danquah E (2003). Complementarity of genes for resistance to the cassava mosaic disease. Proceeding 5th African Crop Science Conference s 21-26 Oct. 2001, Lagos, Nigeria. IITA Ibadan, Nigeria Vol 5: 29-33

Lopez C, Piegu B, Cooke R, Delsen M Tohme J, Verdier V (2005). Using cDNA and genomic sequences as tools to develop SNP strategies in cassava (*Manihot esculenta* Crantz). Theor. Appl. Genet. 110: 425-431.

Mba REC, Stephenson P, Edwards K, Melzer S, Nkumbira J, Gullberg U, Apel K, Gale M, Tohme J, Fregene M (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. 102:21–31.

McMullen MD, Louie R (1989). Linkage of molecular markers to a gene controlling the symptom response in maize to maize dwarf mosaic virus. Mol. Plant-Mic. Int. 2(6): 309-314.

Michelmore RW. Paran I, Kesseli RV (1991). Identification of markers linked to disease-resistant genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl Acad. Sci. 88: 9828-9832.

Mignouna HD, Dixon AGO (1997). Genetic relationships among cassava clones with varying levels of resistance to the African mosaic disease using RAPD markers. Afr. J. Root and Tuber Crops 2 (1and2): 28-32.

Nichols RFW (1947). Breeding cassava for virus resistance. East Afr.

Agric. J. 15: 154-160.
Offei SK, Owuna-Kwakye M, Thottappilly G (1999). First report of East African cassava mosaic begomovirus in Ghana. Plant Dis. 83(9): 877.
Ogbe FO (2001). Survey of cassava begmoviruses in Nigeria and the response of resistant cassava genotypes to African cassava mosaic begmovirus infection. PhD Thesis University of Ibadan, Nigeria. p. 197.
Ogbe FO, Atiri I, Robinson D, Dixon AGO, Winter S, Quin FM, Thottappilly G (1999). First report of East African cassava mosaic begomovirus in Nigeria. Plant Dis. 83(4): 398.
Ogbe FO, Thottappilly G, Dixon AGO, Atiri GI, Mignouna HD (2003). Variants of East African cassava mosaic virus and its distribution in double infections with African cassava mosaic virus in Nigeria. Plant Dis. 87: 229-232.
SAS Institute (1999). SAS companion for Microsoft windows environment, version 6, 1st ed. SAS Institute, Cary, North Carolina, USA. p. 1028.
Stam P (1993). Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. 3: 739-744.
Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989). RFLP mapping in plant breeding: New tools for an old science. Bio/Technology 7: 257 264.
Thresh JM, Otim-Nape GW, Legg JP, Fargette D (1997). African cassava mosaic virus disease: The magnitude of the problem. Afr. J. Root and Tuber Crops 2: 13-18.
Van Ooijen, JW, Voorrips RE (2001).JoinMap® 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands.
Van Ooijen JW, Boer MP, Jansen RC, Maliepaard C (2002). MapQTL® 4.0, Software for the calculation QTL positions on genetic linkage maps. Plant Research International, Wageningen, the Netherlands. p.45
Wong HL, Yeoh HH, Lim SW (1999). Customisation of AFLP analysis for cassava varietal identification. Phytochemistry 50: 919-924
Zhou X, Liu Y, Calvert L, Munoz C, Otim-Nape GW, Robinson DJ, Harrison BD (1997). Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by inter-specific recombination. J. Gen. Virol. 78: 2101-2111.