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Phenotypic and molecular screening of cassava (*Manihot esculentum* Crantz) genotypes for resistance to cassava mosaic disease

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Cassava mosaic disease (CMD), caused by cassava mosaic geminivirus (CMG) is the most-important disease threatening production of cassava (*Manihot esculenta*) in Ghana. The disease is best managed through host-plant resistance. The study was conducted to assess resistance of 38 cassava genotypes to CMD, determine the associated resistance gene, and to identify the strains of CMG infecting cassava in Ghana. Both morphological and molecular markers were used to screen 38 cassava accessions against CMG infection. Morphological studies revealed one genotype (Capevars) as highly resistant whilst three others (Adehye, Nkabom and KW085) were tolerant, showing mild symptoms. PCR analyses using strain specific primers, however, detected the virus in all the three tolerant genotypes, but absent in Capevars. However, the dominant CMD resistance gene, *CMD2*, was detected in both the resistant and the tolerant genotypes. Apart from Capevars, the other 37 cassava genotypes were infected by, at least, one of the four ACMV variants of ACMV1, ACMV2, ACMV-AL and ACMV3. It is, therefore, concluded that field screening for CMD resistance, should integrate phenotypic evaluation and detection of the virus.

Key words: Cassava, African cassava mosaic virus, simple sequence repeats, resistance.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), an Euphorbiaceae (Webster, 1994), is the sixth world food crop for more than 500 million people in tropical and sub-tropical Africa, Asia and Latin America (FAO, 2008). Cassava is the number one staple food crop for majority of Ghanaians, with per capita consumption of 152.9 kg/head/year (MOFA, 2011) and has played a key role in food security in Ghana. It contributes 22% of Agricultural Gross

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Abbreviations: ACMV, African cassava mosaic virus; **CMD,** cassava mosaic disease; **EACMV,** East African cassava mosaic virus; **EACMV-Ug**, East African cassava mosaic virus-Uganda variant; **PCR,** polymerase chain reaction; **SSR,** Simple sequence repeats; **CMG,** Cassava mosaic geminivirus; **WAP,** weeks after planting.

Domestic Product (AGDP) (FAO, 2014) and is also fast becoming an important crop for industries because of its high starch content. In Ghana, cassava is grown across all agro-ecological zones and ranks first in the area under cultivation (MOFA, 2011). However, the average yield of the crop in the country, which is 13.8 Mt ha^{-1}, is far below an achievable yield of 48.7 Mt ha^{-1} (MOFA, 2011). Pests and diseases are a major contributing factor to the low yield of the crop (Akinlosotu, 1985; Thresh et al., 1994). Major pests of cassava include the cassava mealybug (*Phenacoccus manihoti*), green spider mite (*Mononychellus tanajoa*) (Akinlosotu, 1985) and whitefly (*Bemisia tabaci*) (Perrings, 2001).

Cassava mosaic disease (CMD), caused by cassava mosaic geminiviruses of the family *Geminiviridae* and genus *Begomovirus* (Fauquet and Stanley, 2003; Fauquet et al., 2005), is the most important factor limiting cassava yields in many parts of Africa (Fauquet and Fargette, 1990; Legg and Fauquet, 2004). CMD is responsible for an estimated loss of yield of over 1.5 billion US dollars a year (Thresh et al., 1994). It is undoubtedly the most important constraint to the production of cassava in Ghana (Lamptey et al., 1998). The characteristic severe distortion and stunting of leaf and entire plant associated with the disease, especially on local genotypes, indicates how serious yields could be affected (Lamptey et al., 2000). ACMV has been reported to cause 80% yield loss in susceptible cultivars in Ghana (Moses et al., 2007). Losses due to ACMV disease reported elsewhere range from 20 to 95% (Fargette et al., 1988; Hahn et al., 1989; Terry and Hahn, 1990; Otim-Nape et al., 1994; Braima et al., 2000).

The mosaic virus spread is highly linked with its whitefly (*Bemisia tabaci*) vector (Fargette et al., 1985). The virus can also be transmitted from infected planting materials. Plants grown from infected cuttings are much more seriously affected than those infested later by the whitefly vector (*Bemisia tabaci*) and plants infected at a late stage of crop growth are almost unaffected (Thresh et al., 1994).

Nine distinct cassava mosaic viruses have been characterized worldwide from CMD-affected cassava plants and seven of them are from sub-Saharan Africa (Fauquet and Stanley 2003; Alabi et al., 2011). These viruses are African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), East African cassava mosaic Cameroon virus (EACMCV) (Fondong et al., 2000), East African cassava mosaic Kenya virus (EACMKV) (Bull et al., 2006), East African cassava mosaic Malawi virus (EACMMV) (Zhou, et al., 1998), East African cassava mosaic Zanzibar virus (EACMZV) (Maruthi et al., 2004) and South African cassava mosaic (SACMV) (Berrie et al., 1998). Two other viruses, Indian cassava mosaic virus (ICMV) (Matthew and Muniyappa, 1992; Saunders et al., 2002) and Sri Lankan cassava mosaic virus (SLCMV) (Saunders et al., 2002), were reported from the Indian sub-continent.

Cassava mosaic geminivirus (CMG) strains reported so far in Ghana are ACMV (Clerk, 1974; Lamptey et al., 1998) and EACMV (Offei et al., 1999). ACMD was first observed near Accra in 1926 (Doku, 1966) and its spread was more significant in the coastal areas of the country around 1930 (Leather, 1959; Clerk, 1974). At present, ACMD is widespread and found in all the agro-ecological zones in Ghana (Lamptey et al., 1998). The EACMV was first reported in Ghana in 1999 (Offei et al., 1999). The emergence of EACMV, which has its origin from East Africa but has been documented in Central and West Africa (Fondong et al., 1998; Offei et al., 1999; Ogbe et al., 1999), raises a lot of concern to cassava growers in the sub-region including Ghana.

Effective management of the CMD-pandemic in Ghana is quite important in order to improve yields. The most effective means of controlling CMD is by the deployment of resistant varieties (Thresh et al., 1997). CMD-resistant cassava had been developed through integration of resistance traits from *Manihot glaziovii* by interspecific hybridization (Nicholas, 1947), which has become the major source dominating CMD resistance in Africa (Fargette et al., 1996). Two CMD resistance genes *CMD1* (recessive gene) and *CMD2* (major dominant gene) have so far been placed on the map and important molecular markers associated with the *CMD2* gene have been identified (Fregene et al., 2001; Akano et al., 2002). Through cassava breeding programmes, these markers are very useful and hold great promise in fast-tracking the identification of CMD-resistant germplasms (Bi et al., 2010). Knowledge of genetic diversity or an understanding of which viral strain, and strain combinations and how they are distributed, is important to such breeding programmes for resistance.

This work was, therefore, aimed at assessing the genetic diversity of ACMV currently infecting cassava in Ghana, identifying resistant cassava cultivars and determining the presence of the *CMD2* resistance gene using its associated simple sequence repeats (SSR) markers.

MATERIALS AND METHODS

Collection of cassava planting materials

Thirty-eight (38) distinct cassava genotypes were used for the study. Thirty (30) of them were obtained from the Plant Genetic Resources Research Institute (PGRRI), Bunso, Ghana and the remaining eight from the University of Cape Coast (U.C.C.) Teaching and Research Farm, Cape Coast, Ghana. Three of the materials (Capevars, Adehye, and Nkabom) have been released as cultivars for farmers.

Field experiment

Experimental site and field layout

The 38 cassava genotypes were evaluated in 2007/2008 and 2008/2009 growing seasons, on the Teaching and Research Farm,

Table 1. Disease rating and the corresponding symptom expression for cassava mosaic disease (CMD).

Rating	Symptom
1	No symptoms observed
2	Mild chlorotic pattern on entire leaflets or mild distortion at base of leaflets appearing green and healthy
3	Strong mosaic pattern on entire leaf, and narrowing cum distortion of lower one-thirds of leaflets
4	Severe mosaic distortion of two-thirds of leaflets and general reduction of leaf size
5	Severe mosaic distortion of four-fifths or more of leaflets, twisted and misshapen leaves.

U.C.C., Ghana. The location (5.1000° N, 1.2500° W) is a coastal savanna zone with a ferric luvisol soil type and is a high pressure (highly endemic) site for CMD. The soil has been described by Asamoa (1973) as Atabadze, equivalent to Ultisol in the United States Department of Agriculture, (USDA) classification. Cape Coast has a typical climate of the coastal savannah lowland characterized by an annual rainfall range of 800 to 1000 mm and mean monthly temperature of about 26.5°C.

A 380 m^2 land (38 × 10 m) was ploughed, harrowed and divided into 10-m rows with 1.0 m between rows in the 2007 and 2008 major planting seasons. A total of 38 cassava genotypes were planted in single rows in completely randomised plots. Ten 20 cm-long cuttings (bearing three to four nodes) were planted per genotype, in single rows at a spacing of 1 m within rows and 1 m between rows.

Cultural practices

The ploughed and harrowed field was lined and pegged before planting. The experiment was set out under rain-fed conditions and weeding was done manually using a hoe or cutlass when necessary.

Morphological screening of the cassava genotypes for CMD resistance

The 38 cassava genotypes were evaluated at 6, 12, 20 and 48 weeks after planting (WAP) in both 2007/8 and 2008/9 growing seasons to ascertain the resistance status of each genotype to CMD. Each plant was examined for symptom severity of the whole plant. Plants were assigned disease severity scores based on the standard 1-5 disease rating (Hahn, 1980; IITA, 1990; Ariyo et al., 2005), where 1 represents no disease symptom and 5 being the presence of the most severe symptoms, including severe chlorosis, leaf distortion and plant stunting (Table 1).

Five plants for each genotype were scored and the mean ordinal score determined. Plants with a mean CMD severity score of “1” were then classified as highly resistant (HR), those with a score of “2” were moderately resistant (MR), 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), according to Lokko et al. (2005)

Determination of population of whitefly

Since whiteflies are the vectors of CMD, their population on cassava plants were determined in order to assess their relationship with the severity of the CMD disease infection. Direct counts of adult whiteflies on the crop were made as previously described (Hill, 1968; Fargette et al., 1985; Abdullahi et al., 2003). Whitefly counting was usually done between 0600 and 0800 h when the environment was cooler and whiteflies were relatively immobile compared to later in the day as reported by Fauquet et al. (1987). Adult whitefly populations on the five topmost fully expanded leaves of the selected cassava cultivars were counted according to Otim-Nape et al. (2005) and Ariyo et al. (2005).

Whitefly count was often carried out on the five topmost fully expanded leaves. The counts were done one month after planting and were repeated at three and six months after planting. Five plants were randomly selected for each cassava genotype. On each plant, leaves were carefully turned over and the number of adult whiteflies on the abaxial leaf surfaces were counted and recorded. The mean number of whiteflies per 5 top leaves was then determined.

Screening for CMD resistance using molecular markers

Collection of cassava leaf samples

Young leaves from the 38 cassava genotypes were collected from both CMD-infected plants (symptomatic) and uninfected (non-symptomatic) plants at the experimental site.

DNA extraction and purification

Genomic DNA was extracted from the fresh samples, according to the method described by Dellaporta et al. (1983) with slight modifications. The leaf tissues were lysed using a lysis buffer, followed by extraction of DNA from the leaf tissues and DNA precipitation. DNA pellets from precipitation were washed with 700 µl of 80% ethanol, air-dried on tissue paper at room temperature (25-30°C) re-dissolved in 100 µl of 1x TE buffer and stored at -20°C until required.

PCR amplification

The ACMV strains or variants causing the mosaic symptoms in the 38 accessions were detected using the PCR method described by Zhou et al. (1997). The DNA samples of the cassava genotypes were tested for presence or absence of CMG using primers that could detect the four variants of ACMV (ACMV1, ACMV2, ACMV-AL and AVMV3). Four pairs of primer sequences designed by Zhou et al. (1997) were used (Table 2). The PCR reactions were conducted using Applied Biosystems® 2720 Thermal Cycler in 96-well plates (Life Technologies, New York, USA). The reaction mixture composed of 10 µl, which consists of AccuPower® PCR Premix (BIONEER Inc., Alameda, USA), genomic DNA, sterile distilled water (SDW) and primers. The PCR mixture contained 9 µl of PCR premix and primers and genomic DNA (10 ng μl^{-1}). The PCR programme consisted of an initial denaturation for 4 min at 94°C and then 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s depending on the annealing temperature of the primer, and

Table 2. Primers for PCR amplification and strain differentiation of cassava mosaic virus diseases.

Virus strain	Name of primer	Primer sequence (5' - 3')	Reference
ACMV1	ACMV-F1	TTC AGT TAT CAG GGC TCG TAA (F)	Zhou et al. (1997)
	ACMV-R1	GAG TG AAG TTG ACT CAT GA (R)	Zhou et al. (1997)
ACMV2	ACMV-F2	GTG AGA AAG ACA TTC TTG GC (F)	Zhou et al. (1997)
	ACMV-R2	CCT GCA ATT ATA TAG TGG CC (R)	Zhou et al. (1997)
ACMV-AL	ACMV-AL1/F	GCG GAA TCC CTA ACA TAA TC (F)	Zhou et al. (1997)
	ACMV-ARO/R	GCT CGT ATG TAT CCT CTA AGG CCT (R)	Zhou et al. (1997)
ACMV3	ACMV-1	GCTC AAC TGG AGA CAC ACT TG (F)	Zhou et al. (1997)
	ACMV-2	CCT GCA ACA TAC TTA CGC TT (R)	Zhou et al. (1997)

extension at 72°C for 1 min and final extension of 5 min at 72°C. The PCR products were separated by electrophoresis in a 1% agarose gel at 100 V for 1.5 h. The gel was stained with ethidium bromide and viewed under UV light.

Detection of *CMD2* resistance gene in ACMD-resistant cassava genotypes

Plant DNA samples that did not show presence of any of the strains of cassava mosaic virus following PCR amplification with strain specific primers were further amplified with specific SSR markers (SSRY28, NS158, NS169 and RME1) associated with the *CMD2* gene, the dominant gene, which confers resistance to ACMD. PCR amplification and gel electrophoresis were carried out as described earlier.

Data analysis

Scatter plots showing the relationship between mean whitefly population and mean CMD severity scores during 2007 and 2008 crop seasons were drawn using MICROSOFT EXCEL (Microsoft Corporation, USA). The corresponding correlation coefficients were also determined using GenStat statistical software version 12 (Payne et al., 2009).

The relationships among cassava accessions, with respect to their susceptibility to the four ACMV strains were determined based on band patterns produced in the gel. Bands of alleles were scored as 1 for presence of virus or infection, and 0 as absence of alleles, denoting no infection or healthy, for various primers-cassava accessions combinations. The band scores were then used to calculate genetic distances (Nei, 1983) between pairs of cassava accessions. Then, using the unweighted pair-group mean average (UPGMA) cluster method of Nei's genetic distance (Sneath and Sokal, 1973), a dendrogram of genetic similarity was constructed using the Power Marker software version 3.5 (Liu and Muse, 2005).

RESULTS

Cassava mosaic disease (CMD) severity

The mean CMD severity scores recorded for the cassava genotypes planted during 2007 and 2008 growing seasons showed a varying and an interesting pattern (Table 3). At 6 weeks after planting (WAP) in 2007 the mean score for all the cassava genotypes on the field was 2.8, with a range score of 1-5.

With this range of scores, five accessions had a score of 1, 12 had a score of 2, 14 had a score of 3, nine were scored 4 while three accessions registered the highest score of 5. Thus, DMA 002, ADW 004 and OFF 029, which had the highest score of 5, were the most susceptible to ACMV infection at 6 WAP.

AT 12 WAP, four genotypes had a score of 1, twelve a score of 2, sixteen a score of 3, nine a score of 4 and three had a score of 5. The mean severity score was 2.9 for 2007. In 2008 the severity scores at 12 WAP were 1, 2, 3, 4 and 5 for four, nine, seven, twenty and four accessions, respectively, with a mean score of 3.3. This indicates that the severity of infection of the cassava genotypes by the ACMV was higher in 2008 than in 2007. This indicates that the cassava genotypes were more susceptible to the ACMV infection in 2008 than in 2007.

At 20 WAP in 2007, the mean score was 2.6 and that of 2008 was 3.4 with severity scores for both years ranging between 1 and 5. At 48 WAP, which was the harvest time, ACMD severity score was recorded to assess the degree of recovery from the disease among the accessions. The mean scores reduced to 1.7 and 1.9 for 2007 and 2008, respectively.

However, in both years, 23 had severity score of 1, 12 were scored 2, five had a score of 3 while three of them had a score of 4. None of the accessions was scored the most severity score of 5.

The overall mean CMD severity responses recorded for all the 38 cassava accessions at different sampling dates and time revealed varying levels of resistance or susceptibility (Figure 1). The accessions were thus grouped into the five disease severity classes. Three genotypes were classified as highly resistant (HR) with a mean score of 1, nine as resistant (R) with a mean score of 2, 12 as susceptible (S) with a mean score of 3 and 14 as highly susceptible (HS) with mean scores of 4 and 5.

Whitefly population

At six weeks after planting (WAP), the overall mean adult whitefly population was 9.7 whiteflies plant^{-1}, with a range of 1.8 to 28.4 whiteflies plant^{-1} in 2007 (Table 4). More

Table 3. Severity of cassava mosaic disease (CMD) infections on 38 cassava accessions during 2007 and 2008 cropping seasons.

Cassava accession	2007				2008			
	WAP				WAP			
	6	12	20	48	6	12	20	48
OFF 146	3.7	4.2	3.1	2.1	5.0	4.2	3.7	3.1
AFS 136	3.0	2.7	2.2	1.9	4.7	3.7	3.1	1.4
ADW 063	4.0	3.1	3.2	1.2	5.0	4.4	2.8	1.2
DMA 002	4.7	5.0	4.0	1.0	5.0	4.1	4.1	1.3
AFS 001	4.0	3.8	2.7	2.8	4.2	4.0	5.0	4.3
AFS 027	3.1	4.1	3.0	2.2	3.1	4.4	4.3	1.0
OFF 058	4.3	2.7	2.8	2.1	4.0	3.2	4.1	3.1
DMA 066	3.1	4.1	3.1	1.3	4.1	3.0	3.3	1.0
ADW 004	4.6	5.0	4.1	4.0	5.0	4.1	4.4	1.2
AFS 131	4.4	4.3	3.2	3.2	5.0	4.0	3.6	1.1
KW 148	2.1	3.1	2.0	1.0	3.8	3.2	2.8	1.2
KW 181	3.3	3.3	3.0	2.1	4.7	4.8	4.2	1.4
ADW 051	3.1	3.1	2.1	1.0	3.3	2.4	3.1	2.4
KW 001	1.5	2.8	1.8	1.0	4.0	2.1	3.3	1.1
KW 085	1.0	1.0	1.0	1.0	1.0	1.0	2.2	1.0
OFF 029	4.6	4.8	3.5	1.8	5.0	5.0	5.0	3.2
ADW 053	2.9	3.1	1.6	1.0	3.1	2.3	4.3	1.3
OFF 086	3.1	2.6	3.0	2.0	4.7	3.4	3.1	2.2
OFF 145	2.2	3.3	4.0	3.7	4.0	4.0	5.0	4.1
KW 161	3.1	2.4	3.1	1.0	4.2	3.1	4.1	2.0
OFF 025	1.8	3.9	4.3	2.0	5.0	4.3	4.8	3.2
OFF 023	2.8	2.6	2.0	2.0	3.1	4.0	1.7	1.3
OFF 063	1.0	1.7	2.1	1.0	2.3	2.0	1.8	1.2
AFS 048	2.1	2.1	1.7	1.0	2.1	2.3	2.1	2.3
KW 070	3.8	3.0	5.0	1.0	4.3	4.6	4.8	1.0
AFS 041	2.0	2.1	2.0	2.7	1.5	2.4	2.2	2.1
OFF 093	3.0	3.0	2.8	1.0	3.2	4.3	4.2	1.0
OFF 019	2.3	2.0	2.1	1.0	3.3	2.8	2.4	2.0
AFS 126	4.1	3.7	3.9	1.0	5.0	4.1	5.0	4.4
NKABOM[a]	2.4	2.3	1.5	1.1	1.9	1.9	2.0	2.0
OFF 136	2.1	3.0	2.1	1.7	2.2	2.1	2.1	2.0
UCC 517	2.7	2.1	2.0	1.8	3.4	4.1	3.2	2.0
UCC506	2.2	2.0	1.3	1.1	1.6	3.2	4.1	1.3
B. BOTAN[a]	1.0	1.0	2.0	1.0	1.6	3.5	2.6	2.4
CAPEVARS[a]	1.0	1.0	1.1	1.0	1.0	1.0	1.0	1.0
ADEHYE	1.0	1.0	1.2	1.0	1.0	1.0	1.0	1.0
UCC 470	2.0	2.2	2.0	1.2	2.2	1.4	2.0	2.0
UCC 153	1.8	2.0	3.0	1.3	3.0	2.2	2.3	2.0
Mean	2.8	2.9	2.6	1.6	3.4	3.2	3.3	1.9
Range	1 - 5	1 - 5	1 - 5	1- 4	1 - 5	1 - 5	1 - 5	1 - 4
%CV	39.3	35.9	38.5	52.9	37.1	33.3	35.3	52.6

WAP = Weeks after planting.

than 50% of the cassava accessions had values below the overall mean value for 2007. However, in 2008 at 6 WAP, the overall mean was 93.2 whiteflies plant^{-1} with a range of 25.4 to 209.9. The mean in 2008 was almost 10 times higher than that for 2007. Capevars had the highest mean number of whiteflies plant^{-1}, being 28.4 and 209.9

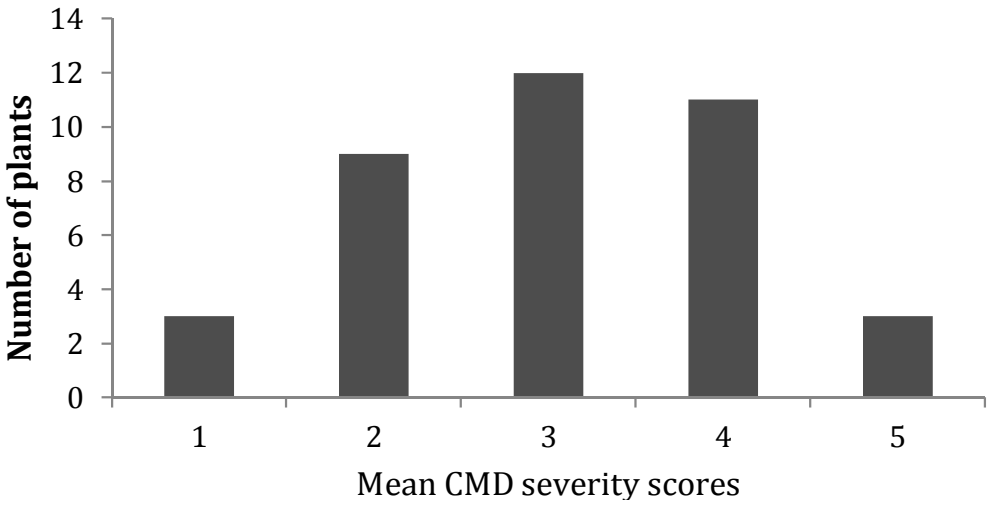

Figure 1. Distribution of 38 cassava accessions in CMD severity classes of 1 to 5. A score of 1 denotes no symptom while 5 indicates a display of severe mosaic symptoms, based on the mean CMD severity responses.

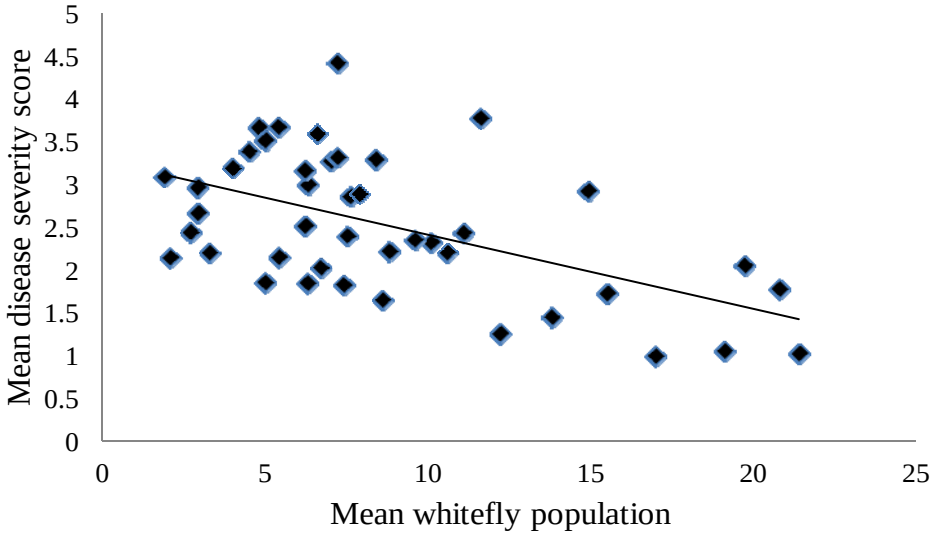

Figure 2. Relationship between mean whitefly population and mean score of cassava mosaic disease (CMD) during 2007 crop season (r = -0.543; *P* < 0.05).

for 2007 and 2008, respectively (Table 4). The lowest count was recorded on OFF 086 with a mean value of 1.8 and 25.4 for 2007 and 2008, respectively.

The whitefly population for most accessions reduced at 8WAP for both years. The mean values were 8.7 for 2007 and 33.9 for 2008. Adehye (24.4) and AFS 001 (52.7) had the highest mean counts for 2007 and 2008, respectively.

The whitefly population reduced further for most of the genotypes at 10 WAP. The mean counts ranged from 1.1 to 18.6 and 5.5 to 35.3 for 2007 and 2008, respectively (Table 4). The most infested genotypes were KW 148 for 2007 and AFS 001 for 2008. Overall, AFS 027 was the least infested by whiteflies and Capevars was the most infested in 2007. However, in 2008, genotype AFS136 was the least infested and Capevars cultivar was again the most infested. The infestation in 2008 also was clearly higher than in 2007.

Relationships between whitefly population and disease severity score

Interestingly, in both 2007 and 2008 crop seasons (Figures 2 and 3), the mean whitefly populations

Table 4. Mean number of adult whiteflies on 38 genotypes of cassava during 2007 and 2008 crop seasons.

Cassava accession	2007				2008			
	WAP				WAP			
	6	8	10	Mean	6	8	10	Mean
OFF 146	7.6	9.0	4.5	7.0	53.9	31.4	26.1	37.1
AFS 136	18.0	11.4	3.8	11.1	28.0	29.0	5.6	20.8
ADW 063	12.6	5.6	4.7	7.6	55.0	25.1	15.8	32.0
DMA 002	3.0	5.6	5.7	4.8	43.0	46.1	12.5	33.9
AFS 001	10.6	7.6	3.4	7.2	31.4	52.7	35.3	39.8
AFS 027	2.2	2.4	1.1	1.9	49.6	33.0	9.3	30.6
OFF 058	3.4	3.0	2.4	2.9	56.6	23.2	15.4	31.7
DMA 066	11.6	8.4	3.8	7.9	79.9	46.9	27.0	51.2
ADW 004	8.8	8.0	4.8	7.2	49.6	31.9	27.5	36.3
AFS 131	16.2	13.3	5.2	11.6	39.2	27.4	11.8	26.1
KW 148	25.2	15.3	18.6	19.7	98.7	38.5	9.7	48.9
KW 181	26.4	10.8	7.5	14.9	51.7	42.2	25.8	39.9
ADW 051	13.6	8.2	8.6	10.1	70.3	33.6	13.6	39.2
KW 001	28.4	17.2	16.9	20.8	90.3	44.5	25.7	53.5
KW 085	5.2	6.0	4.9	5.4	90.0	28.4	8.6	42.3
OFF 029	6.2	5.6	4.5	5.4	97.6	33.3	11.8	47.6
ADW 053	20.4	16.6	14.0	17.0	71.7	42.9	12.3	42.3
OFF 086	1.8	3.6	3.4	2.9	25.4	30.0	27.2	27.5
OFF 145	10.0	8.8	6.3	8.4	81.8	25.4	11.7	39.6
KW 161	6.5	8.6	7.5	7.5	106.8	31.7	24.4	54.3
OFF 025	5.8	8.2	4.9	6.3	47.0	27.1	12.3	28.8
OFF 023	10.3	10.8	7.8	9.6	127.5	26.5	12.4	55.4
OFF 063	14.0	13.4	14.0	13.8	89.7	45.5	5.5	46.9
AFS 048	14.2	16.6	15.6	15.5	163.8	35.9	15.0	71.6
KW 070	3.4	4.6	3.9	4.0	55.6	21.3	15.0	30.6
AFS 041	11.2	7.6	12.9	10.6	162.8	33.5	10.0	68.8
OFF 093	2.6	2.2	3.3	2.7	134.9	31.6	24.1	63.5
OFF 019	6.6	7.6	4.7	6.3	143.7	34.3	10.1	62.7
AFS 126	4.6	8.6	5.5	6.2	105.6	41.5	14.6	53.9
NKABOM[a]	8.0	8.8	9.6	8.8	165.7	32.6	11.9	70.1
OFF 136	5.8	8.2	8.2	7.4	175.9	32.7	12.1	73.5
UCC 517	3.0	1.0	2.2	2.1	128.2	35.1	9.1	57.5
UCC506	10.2	6.0	9.7	8.6	161.5	22.4	14.7	66.2
B. BOTAN[a]	11.6	16.2	8.9	12.2	162.5	26.5	17.0	68.6
CAPEVARS[a]	16.8	24.4	16.1	19.1	140.3	38.4	19.6	66.1
ADEHYE	26.6	21.6	16.0	21.4	209.9	33.9	26.7	90.2
UCC 470	7.4	1.4	6.2	5.0	93.6	43.9	27.0	54.8
UCC 153	2.2	7.0	10.8	6.7	97.9	30.1	26.2	51.4
Mean	9.7	8.7	7.6	8.7	93.2	33.9	16.4	47.8
Range	1.8 -28.4	1.0 - 24.4	1.1 - 18.6	1.9 - 21.4	25.4-209.9	21.3- 52.7	5.5 - 35.3	20.8-90.2
% CV	75.3	60.9	59.2	59.8	49.5	33.9	44.5	32.4

[a] Released varieties; WAP=weeks after planting.

significantly (P <0.05) negatively correlated with mean CMD severity scores. That is, on the average, higher populations of whitefly were found on the resistant cultivars than on the susceptible cultivars.

Detection by PCR of 4 variants of ACMV

All four ACMV-specific primer pairs (associated with the four variants of ACMV), produced allelic bands in the

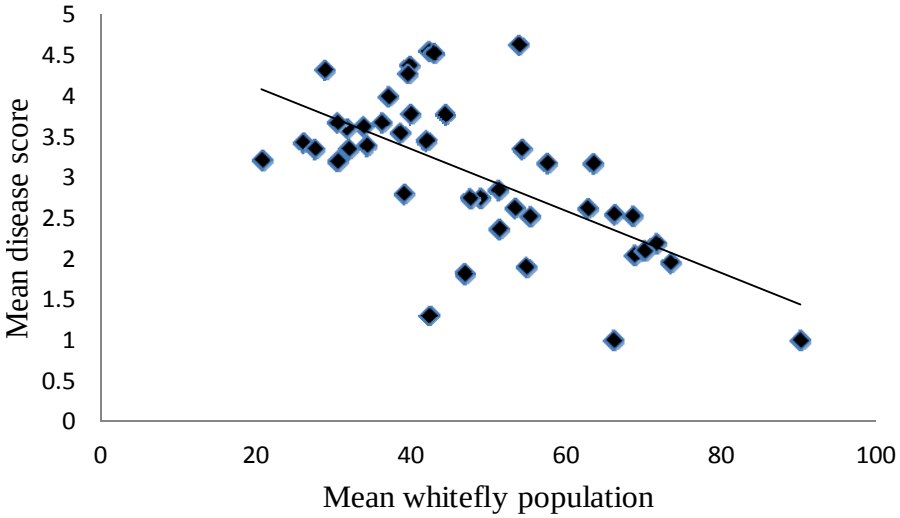

Figure 3. Relationship between mean whitefly population and mean score of cassava mosaic disease (CMD) during 2008 crop season. (r = -0.634; *P* < 0.05).

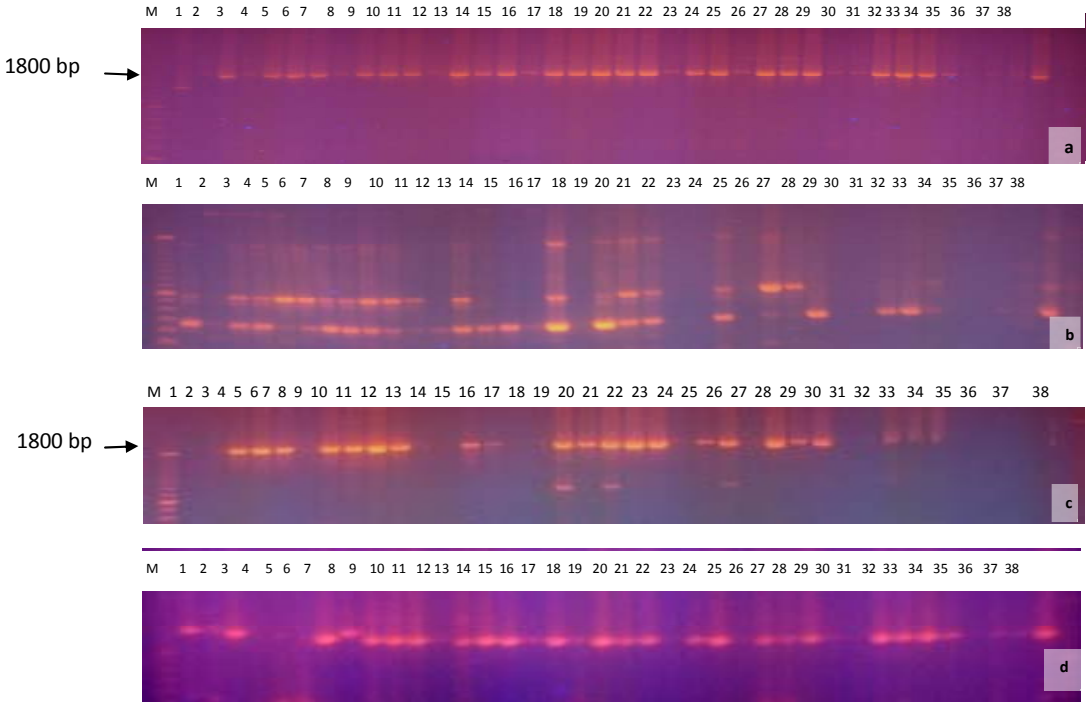

Figure 4. PCR amplification products for ACMV-specific primers: ACMV-F1/ACMV-R1 (a), ACMV-F2/ACMV-R2 (b), ACMV-AL1/F/ACMV-ARO/R(c) and ACMV-1/ACMV-2 (d) - resolved by PAGE and stained with ethidium bromide. M = 1kb+ ladder; 1-38 represent the various cassava accessions. Arrow indicates specific band for ACMV resistance.

accessions. The ACMV-specific primer pair that was most efficient in detecting the virus was ACMVF1/ACMV-R1, which detected the virus in 34 (89.5%) out of the 38 cassava accessions, whilst the primers ACMV-1/ ACMV-2, ACMV-F2/ACMV-R2, and ACMV-AL1/F/ACMV-ARO/R detected the virus in 26(68.4%), 24(63.2%) and 22(57.9%) accessions, respectively (Figure 4). With the exception of genotype Capevars, all the samples were

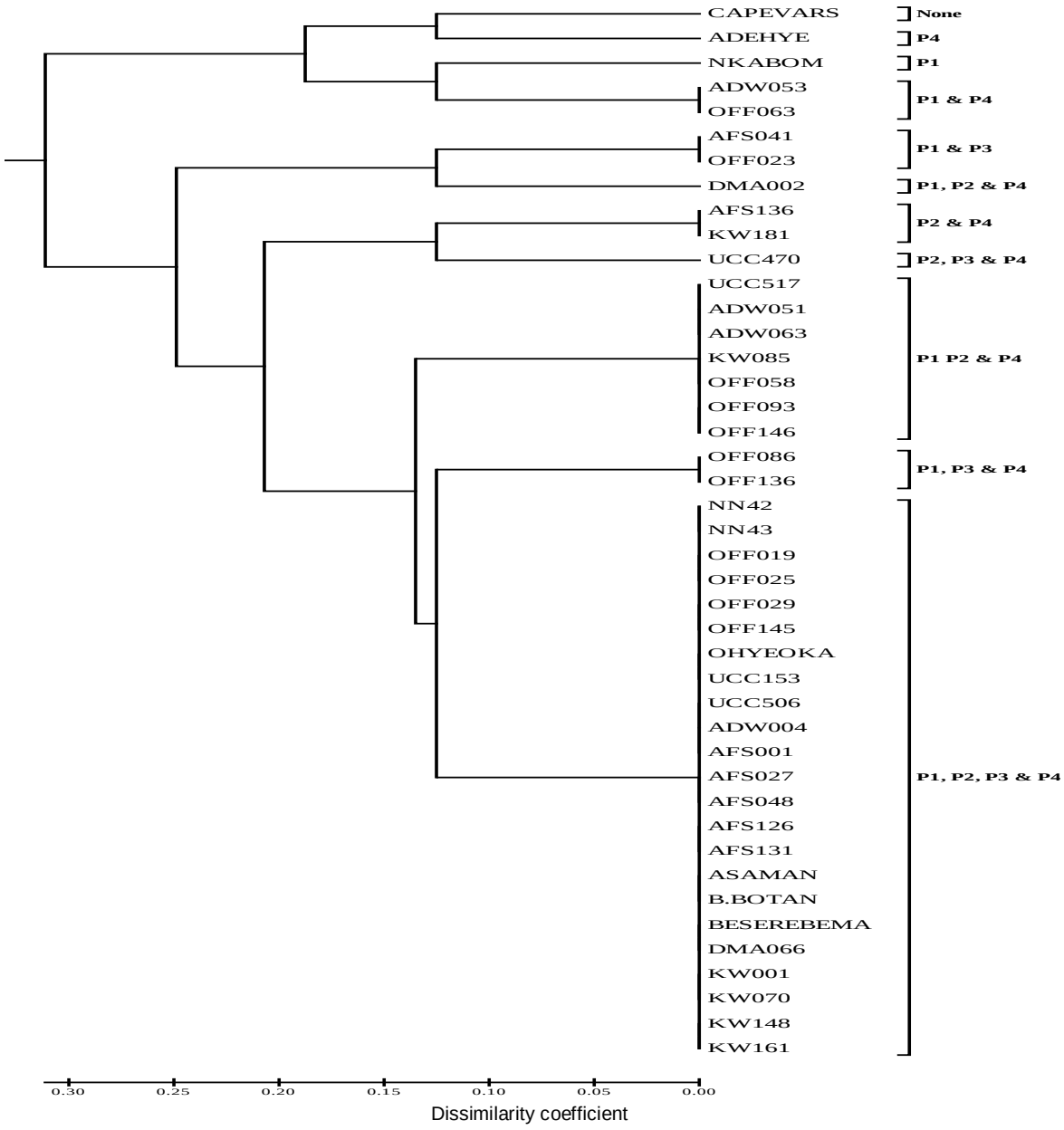

Figure 5. Genetic differences among the 38 cassava accessions based on PCR products of four ACMV primer pairs using the unweighted pair group method with arithmetic averages. P1, P2, P3 and P4 represent ACMV variants ACMV1, ACMV2, ACMV-AL, and AMCV3, respectively.

infected with one or more of the ACMV strains. The cassava genotypes were infected with two or more of the ACMV variants, with the exception of Adehye and Nkabom, which were infected with only one ACMV variant (ACMV1 and ACMV3, respectively).

The cassava genotypes were clustered into 11 groups at a similarity coefficient of 0.13 based on the PCR amplification products, indicating that the cassava genotypes were genetically diverse (Figure 5). The cluster size ranged from 1 to 23 cassava accessions. Cluster 11 had the highest number of accessions (Figure 5).

Detection of *CMD2* resistance gene

From the results obtained from PCR reactions with ACMV-specific primers and field screening for CMD resistance, four genotypes were selected for further screening with markers associated with the *CMD2* gene that confers resistance to CMD to ascertain their source of resistance. All the four accessions selected had bands of alleles of all the four markers associated with the *CMD2* gene (Figure 6). However, the bands present were more intense in two markers (NS169 and RME1), which

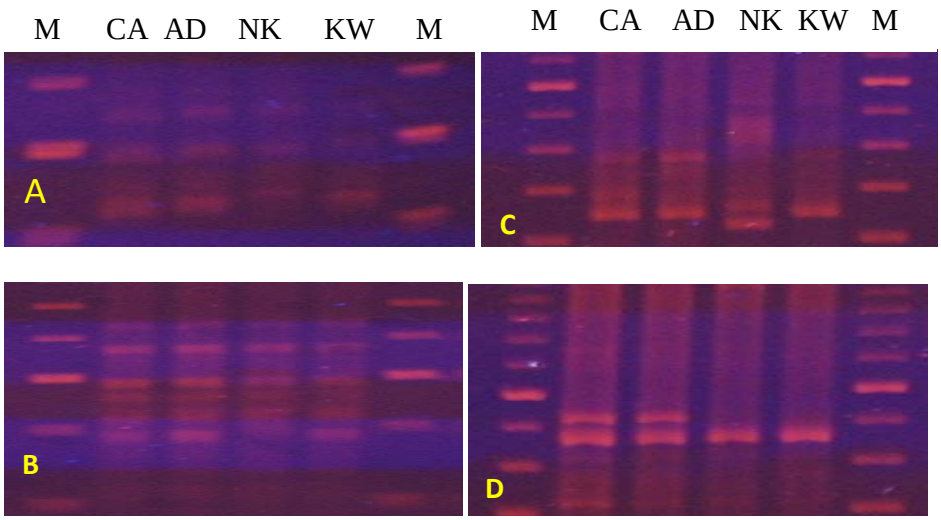

Figure 6. PCR amplification products of four markers associated with *CMD2* resistance gene (SSRY28 (A), NS158 (B), NS169 (C) and RME1 (D) resolved by PAGE stained with ethidium bromide among 4 cassava accessions - Capevars (CA), Adehye (AD), Nkabom (NK) and KW085 (KW). M is the standard marker.

are closer to the gene than the SSRY28 and NS158 markers, indicating that they were more efficient in detecting the *CMD2* gene than the latter two.

DISCUSSION

Morphological screening of the 38 cassava genotypes for CMD resistance based on the 1-5 disease rating (IITA, 1990; Ariyo et al., 2005) and classification according to Lokko et al. (2005) revealed one highly resistant genotype (Capevars) and three moderately resistant genotypes (Adehye, Nkabom and KW 085) (Table 3). However, the subsequent resistance screening using PCR with CMG strain-specific primers showed that only one genotype, Capevars, was resistant whilst the others were infected with ACMV (Figures 4 and 5). This suggests that the three genotypes (Adehye, Nkabom and KW 085) are tolerant to ACMV infection whereas Capevars was a resistant genotype. Thus, field selection of resistance should be complemented with virus detection methods such as PCR. The reason could be that the field resistance, as shown by lack of symptoms, is not necessarily an indication of resistance to virus infection as has been reported by Ogbe (2001). Therefore, the mean symptom severity scores calculated for breeding lines has a limitation, in that, the virus incidence and symptom severity are not clearly distinguished; and symptomless plants plants could be CMD-free 'escapes', or they could be extremely tolerant (Thresh and Cooter, 2005). Moreover, a low average score for a progeny or selection could mean that a few plants are infected and show severe symptoms, or that many succumb but are only slightly affected.

The ACMV-specific primer ACMVF1/ACMV-R1 was more efficient in detecting the virus in the cassava genotypes, since it detected the virus in more samples than the primers ACMV-1/ ACMV-2, ACMV-F2/ACMV-R2, and ACMV-AL1/F/ACMV-ARO/R. Whilst primer ACMVF1/ACMV-R1 detected the virus in 34 (89.5%) out of the 38 cassava accessions, the primers ACMV-1/ACMV-2, ACMV-F2/ACMV-R2, and ACMV-AL1/F/ACMV-ARO/R detected the virus in 26 (68.4%), 24 (63.2%) and 22 (57.9%) accessions respectively. In screening F_1 progeny of cassava against CMD infection, Lokko et al. (2005) also observed that the ACMV primer ACMV-F1/ACMV-R1 detected the virus in more samples than the primer ACMV-AL F/ACMV-AROR. This suggests that the ACMV1 strain detected by the primer ACMVF1/ACMV-R1 as reported by Zhou et al. (1997) is the most dominant virus among the ACMV variants detected in the study.

The detection of the resistance gene (*CMD2*) using linked SSR markers, in the four field-resistant cassava genotypes (Capevar, Adehye, KW058 and Nkabom) suggests that the *CMD2* gene is, at least, partly responsible for both CMD resistance and field tolerance. In this case Capevars can be said to be a highly resistant genotype, whereas Adehye, KW058 and Nkabom, which showed mild field symptoms are tolerant genotypes. The dominant nature of *CMD2* and its effectiveness against a wide spectrum of viral strains makes its deployment very appealing in protecting cassava against the actual or potential ravages of CMD in Africa (Boateng, 2010). Knowledge of the markers associated with this resistance gene will also facilitate the use of marker- assisted selection in a cassava breeding programmes for the development of resistant lines. It was observed in this study,

that markers RMEI and NS158 were more reliable for the detection of the CMD2 resistance gene than markers SSRY28 and NS158, as the former gave more intense bands in the gel than the latter two.

Capevars, the CMD-resistant cassava cultivar has since been released (Tetteh et al., 2005). Currently, the Government of Ghana, through the Ministry of Food and Agriculture, is multiplying the Capevars cultivar to be distributed to farmers, especially, those from the Western Region (J.P. Tetteh, pers. comm.).

The highest mean severity score for 2007 was recorded at 12 WAP. This finding agrees with Leuschner (1978) and Ogbe et al. (1996) that high incidence of CMD is achieved at 12 WAP. However, in 2008 the highest mean severity was recorded at 6 WAP. It might be due to the fact that the cuttings used were obtained from the previous crop, and these might have been already infected. This confirms the reports of Fargette et al. (1988) that plants are generally more susceptible to secondary infection.

Most (35 out of 38) of the cassava genotypes showed mixed infection with the four different ACMV variants, and this can have serious consequences for the management of CMD. It has been reported that mixed infections provide the precondition for recombination, which may contribute to the appearance of more severe viral strains (Ribeiro *et al.*, 2003). Zhou et al. (1997) has shown that EACMV-Ug, associated with the severe cassava mosaic disease in Uganda, has arisen by interspecific recombination of EACMV and ACMV**.** Mixed genotypes infections have been reported in many host-pathogen interactions (Read and Taylor, 2001; Hodgson et al., 2004; Schurch and Roy, 2004).

The whitefly, *Bemisia tabaci*, is one of the most important insect pests in world agriculture, because of its direct feeding, contamination from honeydew, and ability to transmit plant viruses (Perrings, 2001). Additional evidence of differences in whitefly infestation among a range of cassava accessions at different locations in Ghana were also found in the present study. The adult whitefly population was high at six WAP in both years. A higher number of whiteflies were found on resistant genotypes in this study, which agrees with Otim Nape et al. (2005), who recorded higher populations of *B. tabaci* on the cassava mosaic disease-resistant genotypes than in susceptible ones. Similar observations have been made by Legg et al. (2003), and are attributed to the whitefly preference for the resistant varieties of cassava. The leaves of resistant plants were broader and softer than the susceptible ones, whose leaves were mis-shapen, highly reduced and showed severe mosaic symptoms. According to Sserubombwe et al. (2001), Omongo (2003) and Ariyo et al. (2005), such leaves are usually avoided by the whitefly and this might account for the whitefly preference for the resistant plants in this study. Otim-Nape et al. (1994) has also reported the lack of any significant correlation between whitefly numbers and mosaic severity when they studied the effects of African cassava mosaic geminivirus on the main cassava varieties grown in three districts of western Uganda. On the contrary, we observed a significant negative correlation between the whitefly population and the CMD severity scores. This further supports the findings earlier made by Sserubombwe et al. (2001), Omongo (2003) and Ariyo et al. (2005).

Conclusion

Out of 38 cassava genotypes screened against CMG infection, three tolerant cassava genotypes (Adehye, KW058 and Nkabom) and a highly resistant genotype, (Capevars) were identified. Apart from Capevars, between 1 and 4 variants of ACMV (ACMV1, ACMV2, ACMV-AL, and ACMV3) were detected in the cassava genotypes including the tolerant ones. This suggests that field selection of resistance should be complemented with virus detection methods such as PCR test. Most (35 out of 38) of the cassava genotypes showed mixed infections with two or more ACMV variants, which could have serious consequences for the management of the CMD in Ghana. A higher number of whiteflies were found on resistant genotypes than the susceptible genotypes in this study, which confirms that the presence of whiteflies per se may not be an indication of possible infection with the ACMV.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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