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Screening Selected Cassava Cultivars for Resistance against Cassava Viruses and Cassava Green Mites under Advanced Yield Trials in Kenya

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Abstract: *Cassava (Manihot esculenta Crantz) is one of the most cultivated tuberous crops as a sustainable source of food security and family income among the poor in the developing world. Despite this economic significance, cassava's tuber yield is significantly reduced by viral diseases and pests among them cassava mosaic disease (CMD), cassava brown streak disease (CBSD) and cassava green mites (CGM). CMD and CBSD are respectively caused by cassava mosaic begomoviruses (CMBs) and cassava brown streak viruses (CBSVs) which often result in 100% yield losses in susceptible cultivars. Through a field-based randomized complete block designed experiment, the present study screened fifteen cassava genotypes sourced from different breeding programs and local landraces in Kenya for resistance against CMD, CBSD and CGM. Genotypic differences for either diseases incidence (INC) or severity (SVY) and marketable root yield (MRY) was significant (P≤0.05. Both disease and pest incidences were generally low (0-15%) indicating potential suppression. Genotypes were grouped into disease tolerant (DT) and disease susceptible (DS) classes with significantly higher MRY of 23.8 t/ha bulked by a DT genotype TME-419 and least MRY of 2.1 t/ha recorded in a DS cultivar Thika2. The negative correlation observed between MRY and both disease incidence and severity indicated the inhibitory role of CMD and CBSD on cassava production. Molecular diagnostics two CMB species, African cassava mosaic virus (ACMV) and East Africa cassava mosaic virus (EACMV) and CBSVs in some tolerant and all susceptible genotypes. Four (990005, TC4, TC14 & TME419) high yielding and DT cassava genotypes identified in the current study could potentially be used as parents in future breeding programs for introgression of tolerance traits in farmer preferred but susceptible local landraces.*

Keywords: *Cassava mosaic disease, cassava brown streak disease, cassava green mites, tolerance, yield.*

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I. Introduction

Cassava (*Manihot esculenta* Crantz) ranks as third largest source of carbohydrates for food consumption for over 800 million people worldwide [34, 49]. The crop is also a source of raw materials in industries including production of biofuels [22]. Africa accounts for over half of the world's cassava production [50]. Furthermore, the importance of cassava production to the world economy is shown by reports that an estimated 8.5M tons is exported annually [15]. It is an important food crop in the marginal and drought prone regions of Kenya [50], as it can survive harsh environmental conditions such as poor soils and drought [55, 62]. Despite the significance of cassava for food security and economies of various developing countries, the crop is affected by a myriad of biotic, abiotic, management and socio-economic constraints [13, 63]. The major biotic constraints especially in developing countries includes viral diseases such as cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) and insect pests among them cassava green mites (CGM) and whiteflies [13].

Both CMD and CBSD are transmitted by whiteflies (*Bemisia tabaci*) [12] and propagated through infected cuttings during vegetative planting [40]. CMD is caused by cassava mosaic begomoviruses (CMBs) and contains a single stranded (ss) DNA genome. CMB has nine species with East African cassava mosaic virus (EACMV), Africa cassava mosaic virus (ACMV) and East African cassva mosaic- Uganda (EACM-Ug) being the most widespread in East Africa [30]. CMD symptoms include stunted growth, distorted and twisted leaves with mosaic and mottling which result to production of very few or no tubers at all [2]. CMD can cause up to 95% yield losses [61] amounting to an annual yield loss in Africa of approximately US$1.9-2.7 billion [29]. CBSD is caused by cassava brown streak viruses (CBSVs) that contain a positive-sense ss RNA genome [64]. Two species, cassava brown streak virus (CBSV) and Uganda brown streak virus (UCBSV) are known to cause

the disease which has rapidly spread throughout East and Central Africa threatening cassava production [29]. CBSD symptoms include chlorosis along leaf veins and brown necrotic streaks on stems. Roots of infected plants may have a scaly skin surface and show brown corky necrosis on the starchy tissues and this may be accompanied by constrictions [19, 36]. This renders the storage roots inedible and unmarketable [47]. Cassava green mites (CGM) (*Mononychellus tanajoa) have* been shown to cause numerous cassava production losses across the world [9, 65]. The pest causes serious leaf damage on cassava leading to low root yield in the dry savanna regions of Africa [43]. They feed on the lower side of leaves by removing the cell contents causing blotchy whitish-yellow spots which may result in reduced leaf size of loss of the leaves altogether [11]. CGM can cause about 50% reduction in leaf weight and up to 80% tuber yield loss in susceptible varieties [18, 53, 59]. In seasonally dry areas of Kenya, CGM reduced storage root dry matter by 29 percent [60].

Of all cassava diseases and pests, CMD, CBSD and CGM have received most attention due to their devastating effect to crop lands and are known to cause over $1 billion worth of losses every year [20, 29, 43, 62]. Losses occur due to damage to above-ground vegetative material on the crops resulting in leaf chlorosis, root necrosis and reduced root sizes [17, 32, 64]. Several efforts have been made to develop strategies to reduce yield losses caused by the biotic stresses through use of "clean" cuttings, breeding for resistance among others. However, it is also essential to identify germplasm that in addition to being resistant or tolerant to diseases and pests can produce high yield of marketable roots even in severely infested regions. An advanced yield trial was therefore carried out in Kiboko, Eastern Kenya, to identify resistant or tolerant, high-yielding cassava varieties and determine the yield losses resulting from CMD, CBSD and CGM. This is essential for future breeding programs or enhanced cassava production across the Kenya.

II. Material and Methods

Plant Materials and Trial Site

Plant materials (Table 1) screened in the current advanced yield trial included cassava genotypes sourced from previous breeding programs as well as local Kenyan landraces. These had been selected based on their yield and response to CBSVs, CMBs and CGM. The cultivars had been bred for resistance to CBSD and CMD and had shown resistance/tolerance in a previous study in Thika [66]. The advanced yield trial was carried out at KALRO-Kiboko research station (37° 43"E; 2° 12"S & 975 m asl) located in semi-arid region of lower Eastern Kenya [37]. The site was chosen because of its high pressure zone for CMBs, CBSVs, CGM and white flies[44]. The nine month trial period was between January and September 2016.

Table 1: Cassava cultivars used in the current field trial, their sources and status

Cultivar	Source	Type / Comment
Thika2	KALRO-Kandara	Improved for CMD & CBSD resistance
Thika6	KALRO-Kandara	Improved for CMD & CBSD resistance
92/00061	Cuba	Improved for CMD & CBSD resistance
TME419	Cuba	Improved for CMD & CBSD resistance
TC2	Cuba	Improved for CMD & CBSD resistance
Thika5	KALRO-Kandara	Improved for CMD & CBSD resistance
TC4	Cuba	Improved for CMD & CBSD resistance
TC14	Cuba	Improved for CMD & CBSD resistance
TC17	Cuba	Improved for CMD & CBSD resistance
TC19	Cuba	Improved for CMD & CBSD resistance
TC20	Cuba	Improved for CMD & CBSD resistance
Wakahiu3	Thika	Local landrace
Kileleshwa	Kileleshwa	Local landrace - Susceptible control
Wakahiu4	Thika	Local landrace
990005	Cuba	Improved for CMD & CBSD resistance

Source: Breeding cassava resistant to virus diseases and pests at KALRO (Yussuf, 2015).

Experimental Design and Data Collection

Randomized complete block design was employed with 9m by 3m plots arranged in three blocks. Each plot had 5 columns by 10 rows. The 15 varieties (Table 1) were replicated three times to make 45 plots. Among these, susceptible local cultivar Kileleshwa was used as a virus spreader or positive control. By excluding insecticidal sprays, natural virus infections through whiteflies were permitted across all blocks. Incidences and severity of CMD, CBSD and CGM was recorded at 3 and 6 months after planting (MAP). Data was collected from each plot, 10 plants per column from 3 inner columns with the outer ones left out to avoid the effect of data biasness. Incidence and severity were scored using the visible symptoms on the leaves. Symptoms of CBSD were confirmed following appearance of chlorosis on the leaf veins and subsequent yellowing of the leaves and the roots had necrotic patches. CMD was confirmed following appearance of misshapen leaves with a mosaic shaped coloration on the leaves and the plants had lesser roots and those present were smaller compared to healthier roots. Sightings of the mites and presence of prick marks on the leaves confirmed presence of CGM. Disease and mite incidence was calculated as the number of plants affected by the viruses or CGM and expressed as a percentage of the total plants in the plot. Disease and mite severity was assessed by how widespread the attack was, using the standard 1-5 disease rating (1-0% (no disease), 2-25%, 3-50%, 4-75% and 5-100% (most severe) [7, 48, 56].

Nine MAP, root tuber data (number of palatable storage roots and their weights) was collected from four randomly picked plants of each cultivar in each plot. A transverse dissection was then made on the roots and presence of root necrosis was then investigated to determine whether the roots were marketable according to severity scores [25]. Marketable roots were then weighed to determine marketable root weight of yield (MRY). Estimation of MRY per hectare of land was then extrapolated from the weight and size of each plot using the formula described by Masinde et al [33]. Effect of CGM on the cultivars were extrapolated or correlated with tuber data since the mites cause leaf loss which directly affects tuber yield [9].

Molecular detection of CMBs and CBSVs

Using a herbarium, leaf samples were randomly collected from cassava plants at 6 MAP. DNA and RNA were extracted for detection of CMBs and CBSVs respectively. Polymerase chain reaction (PCR) was applied for detection of CMBs and Reverse transcriptase PCR (RT-PCR) for detection of CBSVs [26]. For PCR, genomic DNA was extracted as described in Osena et al [51], while for RT-PCR, total RNA was first isolated using the modified pine tree method [41]. The cDNA was then synthesized from the extracted RNA using Bio-Rad's iScript cDNA Synthesis Kit. In the PCR process, JSP002/JSP003 primers were used for the amplification of EACMV and CMBCP/F and ACMVCP/R for ACMV (Table 2); these primers are commonly used in CMB diagnostics for detection of ACMV and EACMVs [3, 16] because they do not discriminate the EACMV species [6].

Prior to diagnosis, concentration and integrity of each DNA and RNA sample was confirmed on NanoDrop ND-1000 and 1% agarose electrophoresis respectively [26]. The primers for the diagnosis of CMBs (ACMV & EACMV) and CBSVs (CBSV and UCBSV) were respectively sourced from Maruthi et al [32] and Abarshi et al [1].

Table 2: Details of primers used in virus diagnosis

Target Virus	Primer name	Primer sequence (5'-3')	Expected PCR product	Reference
A) Uniplex PCR				
• CBSVs:	CBSVF2	GGRCCATACATYAARTGGTT	283bp	Abarshi et al., 2012 [1]
	CBSVR7	CCCTTTGCAAARCTRAAATARC		Abarshi et al., 2012 [1]
• ACMV	CMBCP/F	GKCGAAGCGACCAGGAGAT	650bp	Alabi et al., 2008 [2]
	ACMVCP/R	CCCTGYCTCCTGATGATTATA		Alabi et al., 2008 [2]
• EACMV	JSP001	ATGTCGAAGCGACCAGGAGAT	770 bp	Fondong et al., 2000 [16]
	JSP003	CCTTTATTAATTTGTCACTGC		
B) Duplex PCR				
• CBSV+ UCBSV	CBSVF2	GGRCCATACATYAARTGGTT	345bp	Abarshi et al., 2012 [1]
	CBSVR7	CCCTTTGCAAARCTRAAATARC		Abarshi et al., 2012 [1]
	CBSVR8	CCATTRTCTYTCCAMADCTTC	441bp	Abarshi et al., 2012 [1]

III. Data Analysis

Data on both disease and CGM incidences (INC), severities (SVY) and marketable root yield (MRY) were subjected to analysis of variance (ANOVA) at 95% confidence interval to compare means across cultivars. A Turkey's honest significance difference (HSD) test was carried out to assign mean separations and therefore indicate whether there were any significant differences. Disease development over time was monitored using area under disease progress curve (AUDPC) and area under severity index progress curve (AUSiPC) according to Shaner and Finney [58]. Data used for this section had been collected at 3 and 6 months after planting. The curves calculated using the formulae below:

Formula 1: disease incidence

$$\text{AUDPC} = \sum_{i=1}^{n}\left[\left(x_i + x_{i+1}\right)/2\right] * t\,,$$

Where; xi is disease incidence at the time i, n is the number of data taken and t is the number of days between the registration of xi and xi+1.

Formula 2: disease severity

$$\text{AUSiPC} = \sum_{1}^{n-1}\left(\frac{SS_1 + SS_2}{2}\right) x\left(t_2 - t_1\right)$$

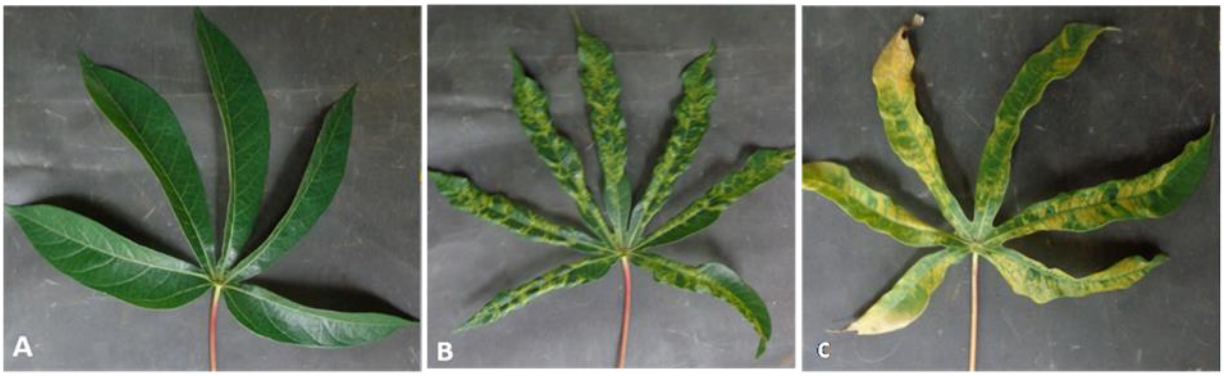
Where; SS1 is the disease severity score at time t1 and SS2 is the disease severity score at time t2.

To show relationship between yield and disease and CGM parameters under this study, means of yield per hectare for each cultivar were correlated with INCs and SVYs using the linear regression model in Graphpad Prism at 95% confidence interval. PCR and RT-PCR products were analyzed or separated in 1.5% agarose gel electrophoresis.

IV. Results

Foliar disease symptoms typical of CBSD (Fig. 1) and CMD (Fig. 2) were visually scored or observed in some genotypes or cultivars and used to calculate disease incidence and severity. Below ground symptoms such as brown necrotic spots and rots on roots (Fig. 3b & c) and small-sized tubers (Fig. 3d) respectively linked with CBSD and CMD were also observed. Asymptomatic plants (foliar & roots) for both diseases were also recorded (Fig. 1a, 2a, 3a & 3d). Incidence of CGM was identified by sighting of the mites on the lower side of the leaf and prick marks on its surface.

Figure 1: Cassava brown streak disease foliar symptoms observed in selected genotypes under field trial



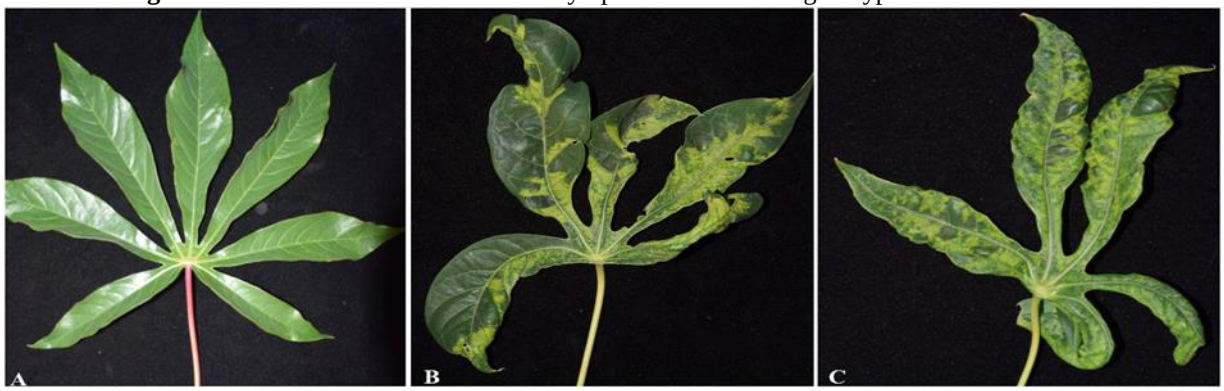
(A) Symptomless genotype-TC20 ***(B) symptomatic genotype-TC17*** ***(C) symptomatic cultivar-Thika2***

Analysis of variance revealed significant differences for disease incidences and severities i.e. at P≤0.001 for CMD, at P≤0.03 for CBSD and marketable root numbers as well as marketable root yield (P≤0.04) across the genotypes while non-significant variance (P>0.05) was analyzed for CGM (Table 3). Incidences and severities were recorded as means of three replicates for each cultivar. INC and SVY of CGM in the cultivars were generally higher than in CBSD and CMD. It should also be noted that CMD INC and SVY were generally higher than for CBSD (Fig. 4). A Turkey's honest significance difference (HSD) test carried out on the means of INC and SVY for all the cultivars revealed significant differences in the means. However, means of CGM INC and SVY across the cultivars were not significantly different (Table 4).

Upon quantification, most genotypes exhibited a consistent trend of increasing disease incidences between 3 and 6 MAP, while some cultivars recorded lower readings at 6 MAP than at 3 MAP. For example

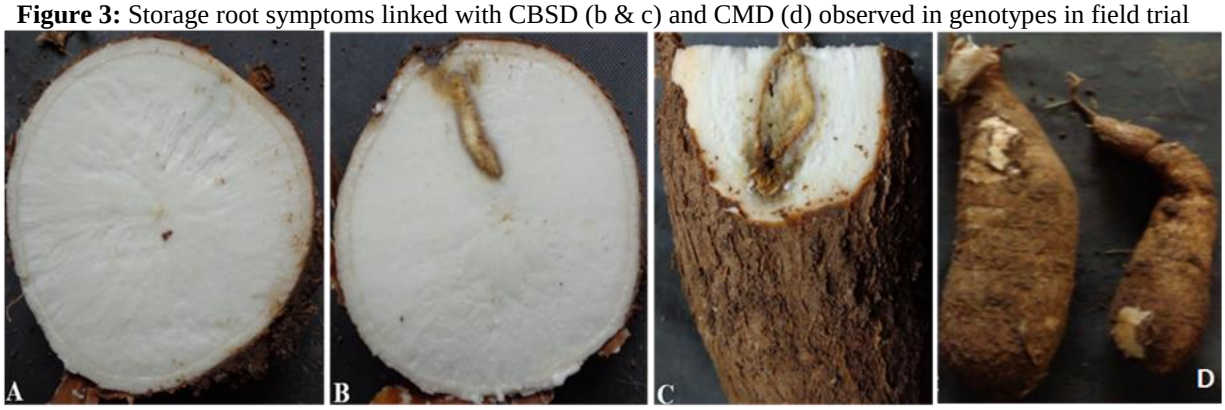
cultivars TC17 and Wakahiu4 recorded lower CBSD incidences at 6 than at 3 MAP with similar trends observed in cultivars Kileleshwa, TC14, TC17 and TC19 for CMD.

Figure 2: Cassava mosaic disease foliar symptoms observed in genotypes under field trial



(A) Symptomless genotype-TC4 *(B) symptomatic landrace-Wakahiu3* *(C) symptomatic landrace-Kileleshwa*

Figure 3: Storage root symptoms linked with CBSD (b & c) and CMD (d) observed in genotypes in field trial



(A) non-necrotic *(B) brown necrotic spots* *C) rotting flesh & scaly skin* *(D) reduced sized roots*

To carter for these disparities, disease progression and severity over time was monitored. This study determined the area under disease progression and area under severity index curves for CMD and CBSD after 6 months. With regard to CMD, cultivar TC17 recorded the highest AUDPC of 5894.56 while the lowest AUDPC of 68.18 was recorded in cultivar TC14 (Fig. 4a). Four cultivars (990005, 92/00061, TC2 & TC4) did not record any AUDPC for CMD (Fig. 4a). With regard to CBSD, TC17 also recorded the highest AUDPC of 5595.68 compared to the least 426.47 AUDPC observed in 990005 (Fig. 4b). No CBSD-AUDPC was recorded in five (92/00061, TC14, TC20, TC4 & TME-419) other cultivars (Fig. 4b). Analysis of AUSiPC after the 6 month trial period revealed significant differences ($P \leq 0.05$) among the cultivars for both diseases. For instance the highest CMD AUSiPC value of 292 and least value of 105 were respectively calculated in cultivars TC19 and TC14 (Fig. 4c) while for CBSD, AUSiPC of 210 and 112.5 were respectively observed in cultivars TC17 and TC2 (Fig. 4d).

Table 3: ANOVA for incidences and severity of CMD, CBSD, CGM, MRN and MRY

Source of variation	df	SS	MS	F-value	P-value	R^2%
CMD Incidence	14	2041.6	113.74	7.59	0.000	0.7799
CMD Severity	14	76.00	3.667	4.46	0.000	0.6754
CBSD Incidence	14	998.6	47.37	4.24	0.000	0.6642
CBSD Severity	14	35.11	1.365	2.56	0.015	0.5443
CGM Incidence	14	2046.0	45.05	0.95	0.517	0.3082
CGM Severity	14	50.80	1.343	1.26	0.289	0.3701
Marketable Root Yield (MRY)	14	5019	180.10	2.16	0.037	0.5024
Marketable root numbers (MRN)	14	35262	1346.5	2.46	0.019	0.5346

df = degree of freedom; SS = sum of squares; MS = mean squares; R^2 = Coefficient of determination

Table 4: Average incidence and severity for CMD, CBSD and CGM in cassava genotypes at 6 MAP

Genotypes	CBSD-I	CBSD-S	CMD-I	CMD-S	CGM-I	CGM-S
990005	1±1.00^{b}	1.33±0.33^{ab}	0±0.00^{c}	1±0.00^{bc}	5±2.00^{a}	3±1.00^{a}
92/00061	0±0.00^{b}	1±0.00^{b}	0±0.00^{c}	1±0.00^{bc}	1.33±2.31^{a}	1.33±0.58^{a}
Kileleshwa	5±5.00^{ab}	2.66±1.53^{a}	16.33±4.04^{a}	3.3±0.58^{abc}	10.33±9.07^{a}	2.33±1.16^{a}
TC14	0±0.00^{b}	1±0.00^{b}	0±0.00^{c}	1±0.00^{bc}	5±8.66^{a}	1.66±1.16^{a}
TC17	14.67±7.58^{a}	2.33±0.58^{ab}	13.33±2.31^{ab}	3.3±0.58^{ab}	12.67±11.02^{a}	2.33±1.16^{a}
TC19	3±2.00^{b}	2±1.16^{ab}	6.33±6.03^{bc}	4.00±2.31^{a}	4.00±5.29^{a}	1.33±1.16^{a}
TC2	3±2.89^{b}	1.33±1.00^{ab}	0±0.00^{c}	1.00±0.58^{bc}	11.67±10.69^{a}	1.66±1.53^{a}
TC20	0±0.00^{b}	1±0.00^{b}	1.67±2.08^{c}	1.33±0.58^{abc}	9.67±8.39^{a}	2±1.00^{a}
TC4	0±0.00^{b}	1±0.58^{b}	0±0.00^{c}	1.00±0.58^{ab}	0±0.00^{a}	1.00±0.58^{a}
Thika2	7.33±5.13^{ab}	2.33±0.58^{ab}	12.33±11.24^{a}	3.66±2.08^{abc}	11±9.64^{a}	2±1.00^{a}
Thika5	4±2.00^{b}	2±0.00^{ab}	10.33±2.89^{ab}	2±0.00^{abc}	10±1.00^{a}	2.33±0.58^{a}
Thika6	3.67±3.21^{b}	1.66±0.58^{ab}	12±3.46^{ab}	2.66±0.58^{abc}	8±6.56^{a}	2±0.00^{a}
TME419	0±0.00^{b}	1±0.00^{b}	0.33±0.577^{c}	1.33±0.58^{abc}	3.33±3.51^{a}	1.33±0.58^{a}
Wakahiu3	2.33±2.08^{b}	1.66±0.58^{ab}	6±3.00^{bc}	2±0.00^{abc}	6.33±4.93^{a}	2±0.00^{a}
Wakahiu4	6±5.20^{ab}	2.33±1.16^{ab}	12.67±2.52^{ab}	3.66±0.58^{a}	6.67±5.77^{a}	3±2.00^{a}

Data is a mean of three replicates recorded 6 MAP. Means that do not share a letter in a column are significantly different (P≤0.05). I = incidence; S = severity; ± = standard deviation; a b c letter codes = significance at P≤0.05

To determine how the genotypes ranked with regard to resistance to CBSD, CMD and CGM collectively, a heat map was used for classification based on mean severity counts of the cultivars at 6 MAP. The heat map clustered the cultivars into two distinct categories "A" and "B" (Fig. 6). In this study, the cultivars that clustered in category A had a collective average severity of ≤ 2.33 for CBSD, CMD and CGM and were termed tolerant. Category B consisted of cultivars that had a collective average severity of > 2.33 for CBSD, CMD and CGM and these were termed the susceptible cultivars (Fig. 5). In category A, TC4, TME419, TC14 and 92/00061 had the lowest severity to CBSD, CMD and CGM while in category B Thika2, TC19 and TC17 had the highest severities (Fig. 5).

Marketable root yield under CBSV,CMD and CGM infestation

Marketable storage roots were non-necrotic (Fig. 3a) while non marketable storage roots included those affected by CBSD that appeared malformed and constricted with corky brown spots with rots upon dissection with a knife (Fig. 3b & 3c). Roots affected by CMBs appeared smaller in size compared to unaffected tubers (Fig. 3d). Cultivar TME419 bulked significantly higher (P≤0.05) average marketable root numbers per plant (~68) followed by Wakahiu4 (~59), Wakahiu3 (~46) and 990005 and TC14 both at ~40 (Fig. 6a). Significantly (P≤0.05) lower average marketable root numbers was recorded in the rest of the cultivars with no roots harvested from TC17 and TC20 with ~8 tubers (Fig. 6a). The roots were then weighed in kilograms and extrapolated into tons/ha. Genotype TME19 had significantly (P≤0.04) higher average root weight at 23.8 t/ha, followed by TC4 at 21 t/ha and 990005 at 20.9 t/ha (Fig. 6b). Kileleshwa and Thika2 had the least marketable root weights with no roots weighed in TC17 (Fig. 6a).

To gain insight into the relationship between disease incidence, severity and CGM effect on cassava production, a correlation analysis was carried out. Generally, incidence and severity of CGM and marketable root number did not show a significant difference (P>0.05) with the other variables. However, marketable root weight showed a significant difference (P≤0.05) with the rest of the variables. Marketable root weight showed an inverse correlation with the disease and mite incidences and severity. Marketable root weight had a significant positive correlation with marketable root number. There was an inverse correlation too between marketable root number and the incidences and severities except for CGM severity (Table 4).

Molecular detection of CBSVs and CMBs

Gel electrophoresis revealed the presence of both EACMV (770 bp) and ACMV (650 bp) that causes CMD (Fig. 7a & b). Out of the 15 genotypes, 6 varieties tested positive for EACMV and 6 were positive for ACMV (Fig. 7; Table 5). Of these, Wakahiu3, Kileleshwa, TC17 and Thika2 exhibited mixed ACMV and EACMV infections (Fig. 7a & 7b). Uniplex RT-PCR detected CBSVs (283 bp) in 8 of the 15 samples (Fig. 8a; Table 5). Seven of these genotypes also tested positive for CBSV (345bp) and UCBSV (441bp) except Thika2 which was negative under duplex RT-PCR (Fig. 8b; Table 5). Bands on the gel at 441bp representing UCBSV were very faint (Fig. 8b). Wakahiu3, Kileleshwa and TC17 tested positive for all the viruses, Wakahiu4 and

TC19 had a combination of EACMV and CBSV and Thika6 and Thika5 had a combination of ACMV and CBSV (Table 5). None of the asymptomatic plants tested positive for CMBs and CBSVs

Figure 4: Monitoring CMD and CBSD progression in cassava based on AUDPC and AUSiPC

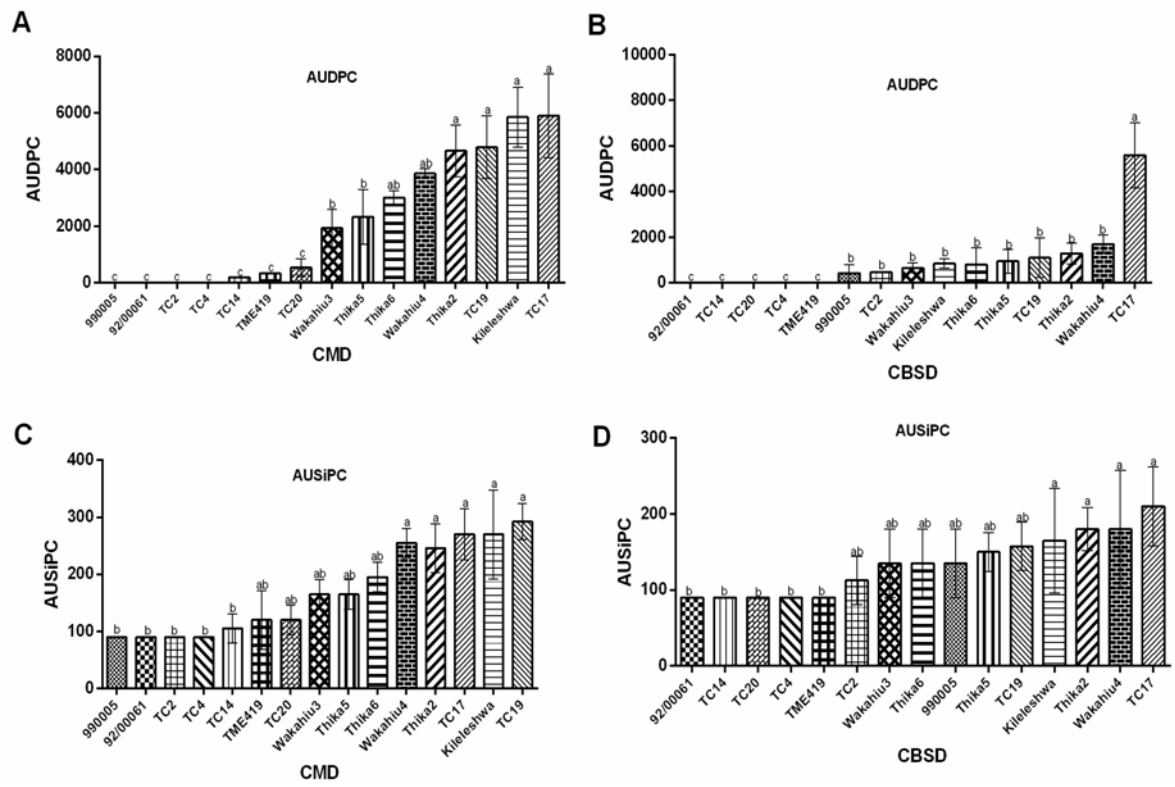


Fig. 4A = CMD-AUDPC; Fig. 4C = CMD- AUSiPC; Fig. 4B = CBSD-AUDPC; Fig. 4D = CBSD- AUSiPC. The AUDPC and AUSiPC used average data sets from disease incidence and severity respectively between the third and sixth month after planting. Data is a mean and standard errors shown by vertical bars. Letters above each bar show significance between means

V. DISCUSSION

Cassava response to CMD and CBSD infections

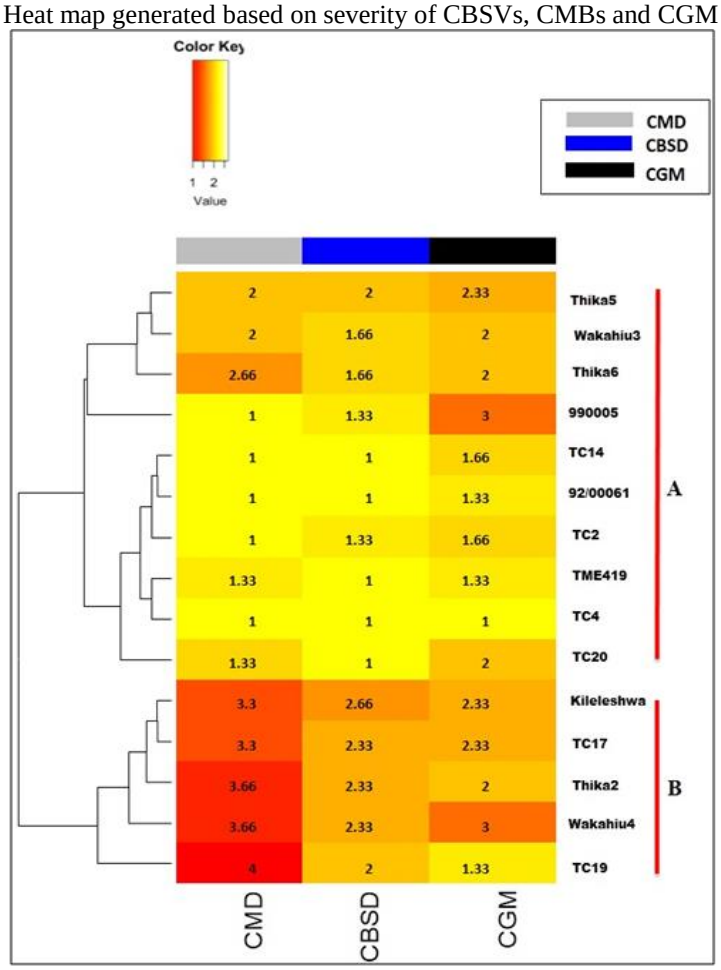
The presence of foliar and root symptoms associated with CMD and CBSD among some of the 15 varieties in this study, confirmed the presence of both viruses at the trial site (KALRO-Kiboko) and successful natural infections. Recently, Koima et al [26] reported incidences and severity of both diseases in different agro-ecological zones (AEZ) of lower Eastern Kenya including Kiboko. Although the cultivars (excluding the local varieties Kileleshwa, Wakahiu3 and Wakahiu4) had previously been bred for and had shown resistance to CBSD and CMD [66], only one genotype, TC4 was asymptomatic to both diseases and CGM with the remaining cultivars exhibiting varying levels of incidences and severities. This phenomenon of cultivars deemed resistant in other agro-ecological zones expressing symptoms in other zones has been noted in some studies [31, 46]. It has been attributed to the fact that, while resistance or susceptibility of a variety depends on its innate resistance, its expression of disease symptoms is pegged on the climatic conditions and disease pressure in the AEZ [46].

The significant differences in incidences and severities of CBSD and CMD was an indication that the cultivars had different resistance levels to the viruses; some were tolerant while others susceptible. This difference in disease sensitivity among different cultivars was also recorded in previous studies [19, 33]. Incidence and severity of CMD were generally high across all cultivars compared to CBSD, perhaps implying a more successful suppression of CBSD compared to CMD. It is noted that the cultivars had been bred for resistance against both diseases [66]. Further, this could also be linked to relatively higher CMD incidence (20-100%) compared to lesser CBSD (10-80%) recently surveyed by Koima et al [26] in this AEZ. Earlier research [24, 33] reported relatively similar findings.

CMD showed increasing intensities in both incidence and severity between 3 MAP and 6 MAP. Cultivar Thika2 and Kileleshwa recorded the highest CMD incidences at 6 MAP. The highest average disease severity index of 3.66 was recorded in the most CMD-susceptible cultivars such as Thika2 and Wakahiu4. Cultivars TC14, TC17, TC19 and Kileleshwa, however, recorded lower severity readings at 6 MAP than at 3

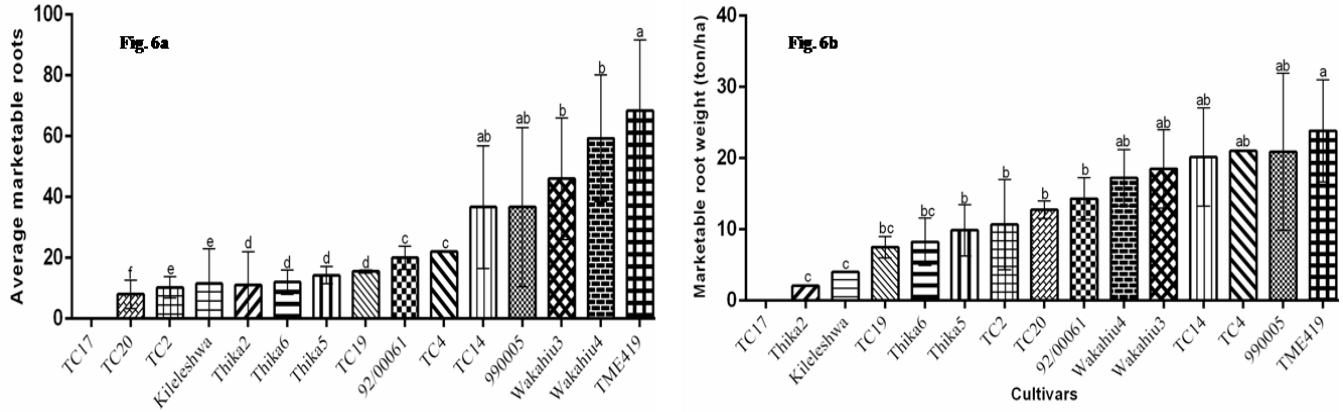
MAP. This was attributed to shedding of multiple leaves due to prolonged drought period at the trial site between June-October [45], an adaptation to combat effects of drought. Similar indices of CMD severity have been previously reported in various cassava cultivars [42] albeit under different environmental conditions and host genotypes, stages of crop growth and virus strains.

Figure 5: Heat map generated based on severity of CBSVs, CMBs and CGM at 6 MAP



Class A = tolerant genotypes; Class B = susceptible genotypes. The heat map was generated using a hierarchical clustering of an R script in R software.

Figure 6: Average marketable root number (A) and marketable root weight in ton/ha (B) harvested at 9 MAP



Data is a mean of 3 replicates with respective standard errors (vertical bars). Letters above each bar represent significances between the means. Means with the same letter are not significantly different (p≤0.05).

Table 4: Correlation between incidence and severity of CBSD, CMD, CGM, MRN and MRY

	CMD-I	CMD-S	CBSD-I	CBSD-S	CGM-I	CGM-S	MRN	MRY
CMD-I	1							
CMD-S	0.907*	1						
CBSD-I	0.767*	0.708*	1					
CBSD-S	0.898*	0.835*	0.775*	1				
CGM-I	0.388**	0.284**	0.502**	0.482**	1			
CGM-S	0.405**	0.307**	0.424**	0.582*	0.288**	1		
MRN	-0.213**	-0.149**	-0.321**	-0.138**	-0.535*	0.219**	1	
MRY	-0.762*	-0.687*	-0.743*	-0.698*	-0.694*	-0.114**	0.735*	1

**Significance at P≤0.05; **Significance at P>0.05; I=incidence; S= Severity; MRN= Marketable Root Number; MRW= Marketable Root weight of Yield.*

Figure 7: PCR detection of EACMV and ACMV from leaves of cassava genotypes under field trials

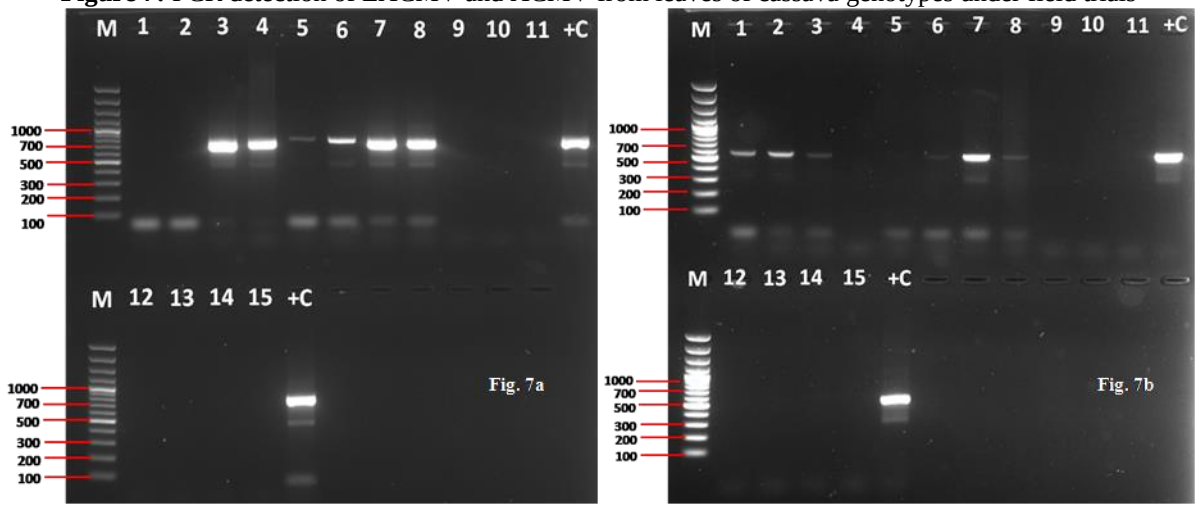


Fig. 7a = EACMV (770 bp) **Fig. 7b = ACMV (650 bp)**

M = 1.0 Kb molecular weight marker; Samples: 1 = Thika; 2 = Thika5; 3 = Wakahiu3; 4 = Wakahiu4; 5 = TC19; 6 = Kileleshwa; 7 = TC17; 8 = Thika2; 9 = TC2; 10= 92/00061; 11 = TC20; 12 = TC4; 13 = 990005; 14 = TME-419; 15 = TC14; +C = positive control

Cultivar-dependent (P≤0.05) significant differences for CBSD incidences were observed after 3 and 6 months of growth. Furthermore, appearance of CBSD symptoms on the rest of the cultivars after this period indicated a variation in their susceptibility. Cultivar TC17 showed CBSD symptoms much earlier than the rest. This, coupled with a high severity index confirmed its susceptibility while other cultivars including TME419, TC14 and 92/00061 did not show incidences of CBSD even after 6 months of growth under the current field conditions and were therefore considered tolerant to CBSD. Kileleshwa, which at 3 MAP had a severity of 1 recorded an average severity of 2.7 at 6 MAP, the highest in the group confirming its susceptibility to CBSD. TC17 and Wakahiu4 exhibited lower incidences of CBSD at 6 months than at 3months. This was also attributed to loss of leaves due to an extended period of drought [45].

These differential susceptibility to CBSD exhibited by cassava cultivars under the current study conditions follows trends previously reported by other studies. For instance, Pariyo et al [56] demonstrated how different cultivars respond to CBSD in agro-ecological zones of Uganda. Similarly, Mohammed et al [38] examined the diversity of brown streak viruses in various cultivars in East Africa. This initial screening for symptoms, while not confirmatory, is vital for projecting potential effects the virus would have on root production and therefore yield. It would be expected that cultivars that show high susceptibility to CBSD would subsequently have more necrotic root tubers and therefore result in less yields [57].

Figure 8: RT-PCR detection of CBSV and UCBSV from leaves of cassava genotypes under field trials

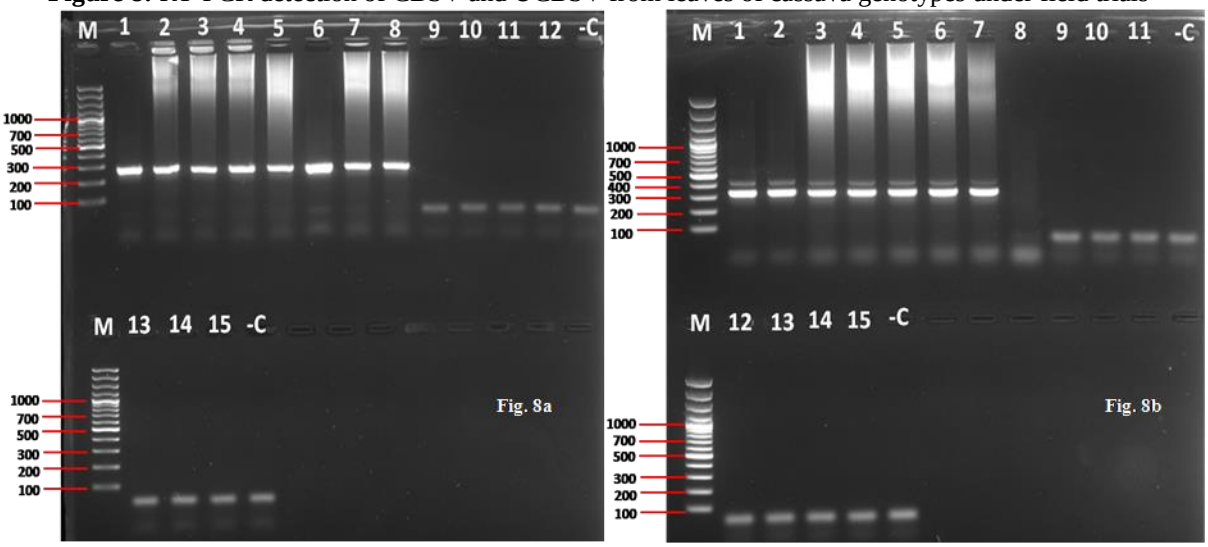


Fig. 8a = CBSVs (283bp) **Fig. 8b = CBSV (345 bp) & UCBSV (441 bp)**

M = 1.0 Kb molecular weight marker; Samples: 1 = Thika; 2 = Thika5; 3 = Wakahiu3; 4 = Wakahiu4; 5 = TC19; 6 = Kileleshwa; 7 = TC17; 8 = Thika2; 9 = TC2; 10= 92/00061; 11 = TC20; 12 = TC4; 13 = 990005; 14 = TME-419; 15 = TC14; -C = negative control

Table 5: Summary for molecular diagnostics for EACMV, ACMV, CBSV and UCBSV

#	Genotype	EACMV	ACMV	CBSVs	CBSV & UCBSV
1	Thika 6	-	+	+	+
2	Thika 5	-	+	+	+
3	Wakahiu 3	+	+	+	+
4	Wakahiu 4	+	-	+	+
5	TC19	+	-	+	+
6	Kileleshwa	+	+	+	+
7	TC17	+	+	+	+
8	Thika 2	+	+	+	-
9	TC2	-	-	-	-
10	92/00061	-	-	-	-
11	TC20	-	-	-	-
12	TC4	-	-	-	-
13	990005	-	-	-	-
14	TME 419	-	-	-	-
15	TC14	-	-	-	-

+ = virus present; - = virus absent; EACMV = 770 bp; ACMV = 650 bp; CBSVs = 283 bp; CBSV = 345 bp; UCBSV = 441 bp

Tracking CMD and CBSD progression over time.

Viral assays in cassava have employed the use of AUDPC and AUSiPC to monitor disease progression over time and therefore factor in any other environmental parameters that may lead to different levels of susceptibility like the prolonged drought experienced when carrying out the current study [23, 58]. The disease progression over time revealed high AUDPC and AUSiPC values at 6 MAP. Significant differences among the cultivars across the 2 curves further indicated that these cultivars respond differently to continued exposure to CMD and CBSD. There was a higher disease progression in cultivar TC17 than any other cultivar indicating its susceptibility over time. Similar studies have demonstrated that severely infected cultivars generally express higher AUDPC and AUSiPC values than those less affected and this can therefore be used as a basis for selecting germplasm that are resistant to diseases [8, 42].

In their study, Paraschivu et al [54] found that the varieties that recorded higher AUDPC values exhibited severe disease symptoms and concluded that the formulae is an efficient instrument to measure disease development over time.

Cassava response to CGM infestation

CGM was observed at the late stages of plant growth. For instance, incidence at 3 MAP indicated few to no CGM but by 6 MAP all but TC4 had CGM incidence following a pattern from previous studies that indicated occurrence of these pests from the fourth month after plant establishment [43]. The non-significant variance ($P>0.05$) of CGM incidences and severities among the cultivars indicated that there were no significant differences in the readings. The low or no CGM incidence recorded at 3 MAP was attributed to the rains experienced during data collection (Feb. – May, 2016) compared to the high CGM incidence at 6 MAP that was associated with the dry spell in June 2016. Previous research has shown that CGM thrives during the dry season and when it rains; their population drastically reduces due to high mite mortality [9, 10]. Generally, high numbers of CGM in Kiboko has been attributed to the area being a lowland tropic, a favorable environment for the mites, while wet cool midland areas like Embu were shown to be less favorable for CGM population growth [10, 21, 43]. CGM feed on young foliar leaves causing them to misshapen and become smaller in size, heavy infestation result in loss of leaves altogether and this damage to the leaves greatly reduces photosynthesis and consequently reduces tuber yield and in some cases causing death of the plant [11]. This was corroborated by the inverse correlations between CGM and yield in the present study. Indeed cassava genotypes that recorded high incidence and severity of green mite infestation, example TC17, consequently, had lower tuber yield.

Effect of CMD, CBSD and CGM on marketable root yield

Summarily, the cultivars in this study were categorized as tolerant and susceptible based on their level of response to CGM, CBSD and CMD and their average marketable root weight. The tolerant varieties recorded a collective average severity of ≤ 2.33 for CBSD, CMD and CGM (Fig.6), and had significantly higher marketable root weight compared to the susceptible varieties. These included TC4, TC14 and TME419. Of these, TME-419 had the highest average marketable root weight of 23.8 t/ha. The susceptible category comprised of varieties that had a collective average severity of >2.33. These varieties also had significantly lower average marketable root weight than varieties in the tolerant category. These included TC19, Kileleshwa, Thika2 and TC17 which did not have any marketable root weight. Thika2 had 2.1 tons per hectare of marketable root weight and was the most infected of all the varieties. Notably, Wakahiu4, despite having high severity indices for CBSD, CMB and CGM, recorded a high marketable root weight of 17.2 ton/ha which can be a quality that can be used to improve other low-yielding, but resistant cultivars through introgression of the genes controlling the good quality. Cooper and Jones [14] described tolerant plant as those that, on infection, exhibit mild disease symptoms while at the same time being able to produce good yield. Susceptible plants, on the other hand, are plants that express marked symptoms and the yield is greatly affected by the disease [26].

Incidences and severity of CMD, CBSD and CGM showed a negative correlation with marketable root number and marketable root yield and this confirmed the negative impact of both diseases and CGM to cassava yield. Cultivars with high disease and mite severity consequently recorded low yields. Marketable root number had a positive correlation with marketable root weight an indication that the higher the number of roots the higher the yield. This should be put into consideration when selecting a suitable variety. Related studies have also used estimation of yields to screen for cassava cultivars that are resistant or tolerant to cassava viral diseases [2, 25, 33]. All these studies share a common conclusion where inverse relationships are reported when yield is correlated to disease occurrence and severity. Cultivars that give high yields even under heavy infection may be desirable for introduction into heavily-infested / infected areas.

Molecular Diagnostics

In addition to the morphological leaf and root symptoms, molecular diagnostics using PCR and RT-PCR further confirmed the presence of CMBs and CBSVs in some tolerant and all susceptible cultivars in the present study. The variants of CMBs and CBSVs that were identified were EACMV & ACMV and CBSV & UCBSV respectively. The tolerant cultivars in this study could potentially be recommended for multiplication or as parents for further breeding trials. The cultivars can also be used for further molecular analysis on the genes or molecular pathways responsible for tolerance in these varieties. For CBSD, it was necessary to carry out molecular diagnostics since both CBSV and UCBSV exhibit similar morphological symptoms [4, 5]. Identification of specific virus species is essential for proper design of management strategies. In the RT-PCR gels, CBSV showed brighter and more visible bands at 345 bp compared to the faint bands for UCBSV at 441 bp. This revealed probably that there was minimal viral titre of the UCBSV concluding that most of the leaf symptoms and yield losses were attributed to CBSV. Studies have also shown that CBSV isolates are more

readily detectable in diagnostics and have higher rates of infection compared to UCBSV [4, 5]. In addition to that, CBSV has been found to cause more severe CBSD symptoms than UCBSV as was observed [4, 5, 26].

An experiment carried out by Mohammed et al [39] where they inoculated CBSD tolerant or resistant plants with CBSV and UCBSV, the plants infected with UCBSV exhibited lesser symptoms and the most disease free plantlets compared to plants infected with CBSV. This difference has been attributed to the superior genetic makeup of CBSV compared to UBSV. For instance, CBSV has more non-synonymous than synonymous substitution at individual amino acid sites which has conferred it with more ability to evade cassava immune system compared to UCBSV [4, 5]. Furthermore, CBSV has 66 advantageous sites in the CP and HAM1-like sites [35] of its genome while UCBSV has none, therefore increasing its ability to combat the cassava plant's immune system [5]. This knowledge will be useful to breeders as they continue to develop cassava varieties that are resistant to CBSD.

In screening for the viruses, precedence should be given to CBSV even in areas with higher incidence of UCBSV [4, 5]. The eight cultivars that showed presence of CBSV showing their susceptibility to the virus and this were in line with their low marketable root numbers. Several studies have been carried out documenting the yield loss caused by CBSVs due to the brown streak in the tubers and constricted tubers [19, 29]. The symptoms were also present in the tubers harvested. The results in this study did not show any presence of viruses in asymptomatic leaf samples, this differed from Abarshi et al [1] where CBSV or UCBSV was detected in both symptomatic and asymptomatic leaves. The molecular detection of the viral isolates from asymptomatic leaves was thought to be because the plants were infected but were yet to develop symptoms or the leaves were wrongly labelled as asymptomatic owing to the difficult to identify CBSD symptoms [36].

For CMD, PCR results revealed evidence of mixed infections of ACMV and EACMV similar to other studies in literature [6, 26, 61]. The mixed infections usually result in severe symptoms [16, 67] which consequently result in low marketable root yield [52]. This was evident in Thika2, TC17 and Kileleshwa which showed evidence of mixed ACMV and EACMV and had very low marketable root numbers. The mixed infections could also result in the advent of new species or variants [61]. Thika5 and Thika6 had a single infection of ACMV but both mixed and single infections by CMBs were attributed to yield losses [52]. ACMV and EACMV were however not detected in some genotypes such as 990005 and TME419 that had recorded significantly lower incidence or severities of CMD. This could have been as a result of random selection of leaf samples for diagnostics where uninfected leaves might have been sampled, or the virus (ACMV and EACMV) titres might have been too low for detection via PCR in the two genotypes. All the 8 cultivars that tested positive for one or both variants of CBSVs also tested positive for at least one of the two CMBs therefore the yield loss was attributed to both the viruses. In total, both CMBs and CBSVs could not be detected in the remaining 50% of the other cultivars despite the study being carried out in a high virus pressure zone. This could be attributed to the fact that the varieties used in this study had been previously bred for resistance against both viruses and indeed they were the best performing.

VI. Conclusion and recommendation

The present study successfully identified cassava varieties or genotypes that showed tolerance to CMD, CBSD and CGM and had high marketable root yield as well. The cultivars that were earlier bred for resistance to CMD and CBSD showed that they developed a better tolerance to the latter. There was a negative correlation between yield and disease and mite infection which revealed the negative impact these had on the marketable root yield of susceptible and tolerant varieties. While it was difficult to quantify the effect of individual stresses (CMD, CBSD and CGM) to cassava yield loss owing to the fact that some plants were affected by both viruses and the mites at the same time, it was nevertheless accurate to relate the loss to their combined action. The significant inverse relationships between yield and CMD/CBSD/CGM incidences and severity further validate this observation. The tolerant varieties in the study will be potentially recommended for further breeding trials. Further molecular analysis is recommended to identify molecular pathways and genes responsible for tolerance in these varieties.

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