

Field Screening of Elite Cassava (*Manihot esculenta*) Mutant Lines for their Response to Mosaic and Brown Streak Viruses

ABSTRACT

Cassava Brown Streak Disease (CBSD) and Cassava Mosaic Disease (CMD) have been major limiting biotic factors to the productivity of cassava farming in Kenya. Development of resistant cassava varieties is most effective way. The study was to determine response of elite cassava lines to CBSD and CMD under field conditions on different agro-ecological zones in Kenya. Screening was done under hot-spot field conditions where five lines together with their parents were evaluated in Randomized Complete Block Design with three replications at University of Eldoret (UoE), Solai and Marakwet sites in Kenya. Severity assessment done on 1-5 rating scale where 1 very resistant and 5 most susceptible. Elite cassava lines had significant differences in their response ($p < 0.05$) to CBSD and CMD. Additionally, genotypes, site and time interval were also significant ($p < 0.05$). The three elite lines (CAS1, CAS2 and CAS3) had better tolerance to both diseases compared to their parents (KME3 and KME4) with KME4 showing visible brown rings on the root typical of CBSD. The elite lines CAS1, CAS2 and CAS3 identified to have better adaptability to both CBSD and CMD could be further exploited in breeding for new cassava varieties for Kenya and other countries in the region with similar ecology.

Keywords: Response; cassava mosaic virus; cassava brown streak

1. INTRODUCTION

Cassava Brown Streak Disease (CBSD) and Cassava Mosaic Disease (CMD) are the major diseases limiting the attainment of potential yield in cassava in Sub-Saharan Africa (Vanderschuren, Moreno, Anjanappa, Zainuddin, & Gruissem, 2012). Specifically, Cassava mosaic disease is primarily caused by the cassava mosaic virus of the family *Geminiviridae* while, cassava brown streak disease is caused by a

Cassava Brown Streak Virus, an ipomovirus of the family *Polyviridae* (Kasule, *et al.*, 2020). Global losses of CMD are estimated to reach 23 million tons of cassava (Masinde, *et al.*, 2016) resulting from more than 70% reduction in photosynthetic leaf area necessary for root growth and development. On the other hand, CBSD had been reported to steer losses in root weight to 70% (Oladokun, 2019; Shirima, *et al.*, 2020). The losses generated in Kenya by these two viral diseases have not been clearly quantified despite the increasing trends of incidences and severity in major cassava growing zones and limited access as well as availability of tolerant varieties. However, inadequate genetic sources necessary for variety improvement needed for the sustainable management of the two diseases remain a challenge not only in Kenya but also in most parts of the world. The impacts of these two diseases cannot be underestimated both in individual and dual infections especially on susceptible cassava varieties by halting growth and phenotypic appearance (Ephraim, *et al.*, 2017). Additionally, resistant varieties have been developed for CMD, but for CBSD, little success has been achieved due to its ability to evolve into more virulent strains. In Kenya, fewer varieties have been reported to express tolerance to the two viral diseases but due to increasing demand for the tolerant varieties, there is need to screen newly developed cassava to widen the genetic base for tolerance. Therefore, development of host plant resistance and utilization of elite genetic resources through mutation breeding techniques provide the most recommended and rapid strategy in conferring tolerance to CMD and CBSD (Kayondo, *et al.*, 2018). This study focused on evaluating elite mutant cassava lines alongside commercially grown parental varieties for their response to cassava brown streak and cassava mosaic viruses under the interplay of different environmental conditions.

2. MATERIAL AND METHODS

2.1 Study site characteristics

The field experiment was conducted at UoE (Uasin Gishu County), Solai (Nakuru County), and Chebara ATC-Marakwet (Elgeyo-Marakwet County) during May 2021 planting season. The UoE receives an average rainfall amount of 1055 mm annually and an average maximum and minimum daily temperatures of 25 °C and 10 °C respectively, and lies at 0.5797N, 35.3056E and 2180m above sea level (Musa, *et al.*, 2012). Solai site lies between 0.30N and 36.9E and 1677m above sea level with typical experiences maximum and minimum temperatures of 28 °C and 13 °C respectively. On the other hand, Chebara ATC-Marakwet site is located at 0.8840N and 35.5079E and receives up to 1400mm rainfall annually with temperature ranging between 14 °C and 24 °C.

2.2 Source of planting materials

The planting materials were sourced from the University of Eldoret, School of Agriculture and Biotechnology Cassava Breeding Program under the International Atomic Energy Agency – (IAEA) project The five genotypes screened were three elite mutant lines namely CAS1, CAS2 and CAS3 and

two parental lines namely *KME3* (also used as tolerant check) and *KME4* (also used as susceptible check) farmers' varieties that had been released for commercial production but with known resistance and susceptibility traits for CMD and CBSD.

2.3 Planting and experimental design

The field screening experiment was planted in Randomized Complete Block Design (RCBD) replicated three times in each site. The effective plots measured 5m by 7m with inter-plot and inter-block spacing of 1m and 1.5m respectively. The susceptible check (*KME4*) was planted one month earlier to serve as inoculum sources and spreaders to both candidates and checks that were planted one month after the guard rows. Fifteen-centimeter (15cm) stem cuttings were planted 10cm deep in ridges with an inter-plant and inter-row spacing of 1m by 1m apart, giving a total of 35 plants per plot. The plants were then subjected into natural infection by Mosaic and Brown streak viruses aided by the vectors such as white flies, spider mites and aphids that were in abundance at the experimental sites (Figure 1). Site maintenance and agronomic practices were adhered to except application of insecticides that was not done to ensure vectors were present at the screening sites.



Figure 1. The major vectors as observed during field screening of elite cassava lines in Solai, Elgeyo Marakwet and University of Eldoret experimental sites

2.4 Disease severity assessment

Severity of both CMD and CBSD were determined following a 1-5 severity rating scale adopted from Okogbenin, *et al.* (2012) and Munganyinka, *et al.* (2018) respectively. Due to the different symptoms of the diseases, description of the scale varies. For CMD, 1 = no visible symptoms; 2 = mild chlorosis on the whole leaf surface (the leaf was still healthy); 3 = average mosaic on the leaf, but the leaf begins to narrow; and the lower part of the one starts to distort, and 4 = pronounced mosaic over two-thirds of the leaflet are distorted and reduced leaf size, and 5 = the entire leaf is distorted by severe mosaic. For CBSD, 1 = no symptoms, 2 = slight chlorosis of the leaves and no symptoms on the stem, 3 = visible and progressed leaf chlorosis with wild lesions on the stem but no dieback, 4 = pronounced chlorosis and stem lesions, 5 = large lesions and dieback. Other than foliar severity, at the final phase of field screening, the severity expression on the cassava roots was quantified and recorded using a 1 to 5 scale, where: 1 = zero necrosis, 2 = less than 5% of root necrosis, 3 = 5-10% necrosis, 4 = 11 – 25% necrosis and 5 = more than 25% necrosis and severe root constriction.

2.5 Statistical data analysis

Data on severity of the two diseases (CMD and CBSD) were analyzed using Genstat statistical software version 16.0 VSN International Ltd. The mean differences between the cassava lines, experimental sites and the interactions between these two factors was tested using Tukey's test. Additionally, meta analysis (GGE Biplot) was performed on the severity data to determine the adaptability and suitability of different cassava lines to screening sites and the results were presented in terms of scatter plots, ranking and comparison biplot correlation matrices with first principal component (PC1) and second principal component (PC2).

3. RESULTS AND DISCUSSION

3.1 Response of elite cassava lines to CMD under field condition

Notably, there were significant differences in severity expression to cassava mosaic virus by the elite cassava lines and their parental lines under field conditions ($p < 0.05$; Table 1). Also, the experimental sites, time interval (days after planting - DAP) and the interactions between site and DAP, were significant ($p < 0.05$). However, the interactions between site and mutant lines, mutant lines and days after planting, as well as site, mutant lines and days after planting were not significant ($p > 0.05$; Table 1). However, both resistant and susceptible checks expressed higher disease severity slightly above 2.0 on a 1-5 rating scale at Solai and Marakwet sites in approximately one year after planting (360 DAP). Among the elite lines, only CAS3 expressed higher disease severity at Marakwet site but still within the tolerance range on the rating scale. In general, all elite lines expressed significant tolerance to cassava mosaic disease than parental lines (KME3 and KME4) at all screening sites (Table 1 and Figure 2).

Table 1: Response of elite cassava lines to CMD under varying sites, time interval and field conditions

SITE	MUTANT LINES	TIME INTERVAL (DAYS AFTER PLANTING)				MEAN	Tukey's test
		60DAP	120DAP	240DAP	360DAP		
CHEPKOILEL	CAS 2	1.00	1.00	1.00	1.33	1.08	a
	CAS 1	1.00	1.17	1.33	1.33	1.21	ab
	CAS 3	1.17	1.17	1.33	1.50	1.29	ab
	KME 3	1.17	1.50	1.33	1.50	1.38	abc
	KME 4	1.50	1.17	1.83	1.83	1.58	abc
MARAKWET	CAS 1	1.17	1.17	1.50	1.50	1.33	ab
	CAS 2	1.17	1.17	1.50	1.50	1.33	ab
	CAS 3	1.17	1.50	1.83	2.17	1.67	abc
	KME 4	1.00	1.67	2.00	2.33	1.75	abc
	KME 3	1.33	2.00	2.50	2.83	2.17	c
SOLAI	CAS 3	1.67	1.00	1.00	1.33	1.25	ab
	CAS 1	2.00	1.17	1.00	1.33	1.38	abc
	CAS 2	2.00	1.33	1.00	1.50	1.46	abc
	KME 4	1.67	2.00	1.33	2.50	1.88	abc
	KME 3	2.33	2.50	1.00	2.00	1.96	bc
MEAN		1.42	1.43	1.43	1.77	1.51	
Tukey's test		A	a	a	B		

Statistics	Site (Si)	Mutant line (ML)	DAP	Si x ML	Si x DAP	ML x DAP	Si x ML x DAP
<i>Probability</i>	0.003	<.001	0.011	0.356	<.001	0.639	0.979
<i>S.E</i>	0.074	0.0955	0.0855	0.1655	0.148	0.1911	0.331
<i>S.E.D</i>	0.1047	0.1351	0.1209	0.234	0.2093	0.2702	0.4681
<i>%CV</i>	22.1						



Figure 2. Response of elite cassava lines (CAS1 – CAS3) and Parental lines (KME3 and KME4) to mosaic virus disease under field conditions (Solai site)

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3.2 Response of elite cassava lines to CBSD under field condition

With reference to CBSD, the experimental sites, mutant lines, days after planting and the interactions between sites and mutant lines as well as mutant lines and days after planting had significant effect in terms of disease severity under field conditions ($p < 0.05$). However, the interactions between sites and DAP as well as the three-way interaction between site, mutant lines and DAP did not have significant impact on diseases severity ($p > 0.05$). Similar to CMD, the elite lines showed tolerant response to brown streak virus compared to the resistant and susceptible parental checks under the same conditions. For example, at 360 days after planting, CAS1, CAS2 and CAS3 recorded severity score below 1.5 at Chepkoilel site. In contrast, at Solai site, all elite lines recorded higher disease severities of 2.6 or below while all two checks recorded higher severity score of 3.3 and 3.7 for KME4 and KME3 respectively. However, on average, all the elite cassava lines performed better against CBSD compared to the parental checks (Table 2 and Figure 3).

Table 2. Response of elite cassava lines to CBSD under varying sites, time interval and field conditions

SITE	MUTANT LINES	TIME INTERVAL (DAYS AFTER PLANTING)				MEAN	Tukey's test
		60DAP	120DAP	240DAP	360DAP		
CHEPKOILEL	CAS 1	1.17	1.17	1.17	1.17	1.17	a
	CAS 3	1.17	1.33	1.17	1.33	1.25	a
	CAS 2	1.17	1.50	1.17	1.17	1.25	a
	KME 3	1.17	1.50	1.50	1.67	1.46	ab
	KME 4	1.00	1.67	1.67	1.67	1.50	ab
MARAKWET	CAS 1	1.00	1.33	1.50	1.67	1.38	ab
	CAS 2	1.00	1.67	1.67	1.67	1.50	ab
	KME 4	1.00	1.50	2.17	2.33	1.75	abc
	CAS 3	1.17	2.50	2.50	2.67	2.21	bcd
	KME 3	1.50	2.17	2.83	3.50	2.50	cd
SOLAI	CAS 2	1.00	1.50	1.00	2.50	1.50	ab
	CAS 1	1.33	1.33	1.00	2.50	1.54	ab
	CAS 3	1.33	1.33	1.00	2.67	1.58	ab
	KME 4	1.67	2.50	3.00	3.33	2.63	d
	KME 3	1.00	2.83	3.33	3.67	2.71	d
MEAN		1.18	1.72	1.78	2.23	1.73	
Tukey's test		A	b	B	c		

Statistics	Site (Si)	Mutant line (ML)	DAP	Si x ML	Si x DAP	ML x DAP	Si x ML x DAP
<i>Probability</i>	<.001	<.001	<.001	<.001	0.094	<.001	0.788
<i>S.E</i>	0.0773	0.0998	0.0893	0.1729	0.1546	0.1996	0.3458
<i>S.E.D</i>	0.1093	0.1412	0.1263	0.2445	0.2187	0.2823	0.489
<i>%CV</i>	24.7						



Figure 3. Response of elite cassava line (CAS1 – CAS3) and Parental lines (KME3 and KME4) to brown streak virus disease under field conditions (Solaj site)

3.3 Severity expression of CBSD elite cassava roots compared to their parents

Phenotypic expression of infection by CBSD showed that CAS2 was the most tolerant and did not indicate any sign of root discoloration compared to CAS1 and CAS3 that had slight expression of symptoms (Figure 4). In contrast, all two parents (KME3 & KME4) were susceptible to root infection just like the severity expression by the leaves with KME4 showing the highest root damage among the two (Figure 4).

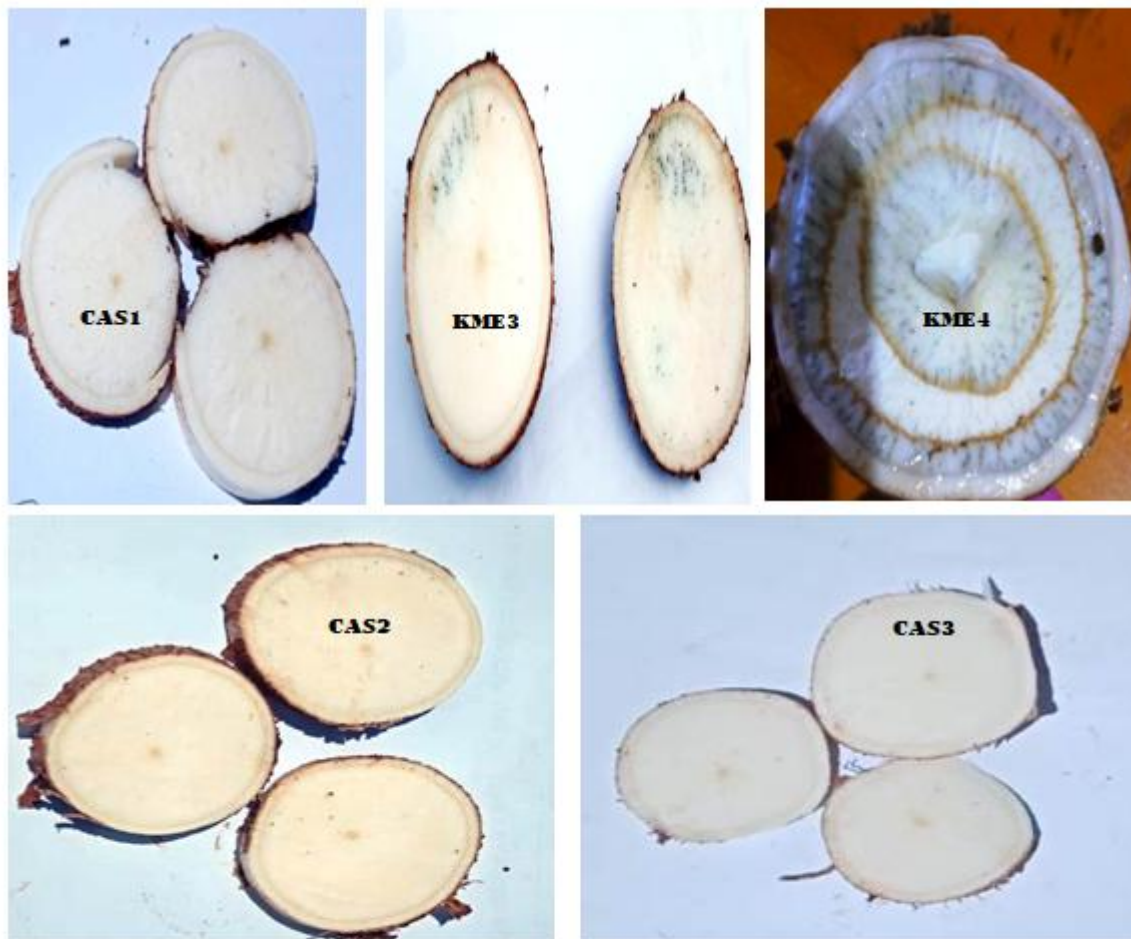


Figure 4. Expression of root infection by elite cassava line (CAS1 – CAS3) and parental lines (KME3 and KME4) to brown streak virus disease under field conditions. Elite lines were resistant compared to parental lines

3.4 Interaction effect of cassava lines and environment on severity of CMD

The genotype and genotype x environment interactions (GEI) on the response by elite cassava lines were presented in GGE biplot correlation matrices (Figure 5). The first interaction principal component sum of squares (PC1 = 82.88%) was greater than the second component (PC2 = 11.19%) contributing to a total variability of 93.71%. This corresponds to the representation of three elite cassava lines (CAS1, CAS2, CAS3), two parental lines (KM3, KM4) and three environments (Figure 5). The mega environments in scatter plot correlation matrix showed that Chepkoilel (UoE) and Marakwet sites were similar in terms of the response of cassava genotypes to CMD, but these two sites differed significantly from Solai site which formed its own mega environment (Figure 5a). Despite the differences in severity based on site variations, the ranking biplot correlation matrix for environment indicate that all the cassava genotypes responded differently from one site to the other and none showed similar response between two sites (Figure 5b). However, KM4 expressed slightly similar severity response in Chepkoilel and Solai sites while KM3 was more adapted towards Marakwet site. The remaining genotypes including CAS1, CAS2 and CAS3 responded differently to CMD across all the sites.

Similarly, the ranking biplot correlation matrix for the cassava genotypes showed that CAS1 and CAS2 had slight phenotypic variation in terms of CMD but differed from CAS3 (Figure 5c). The parental lines (KME3 and KME4) also varied in their response to this disease. However, no genotype expressed adaptation towards any environmental condition (site). The parent KME3 was identified as the most ideal genotype across all the three environmental conditions (sites) but it was also the most susceptible genotype to CMD followed by KME4 (Figure 5d). The rest of the elite cassava lines were tolerant to this disease hence appeared at the further end of the concentric ring.

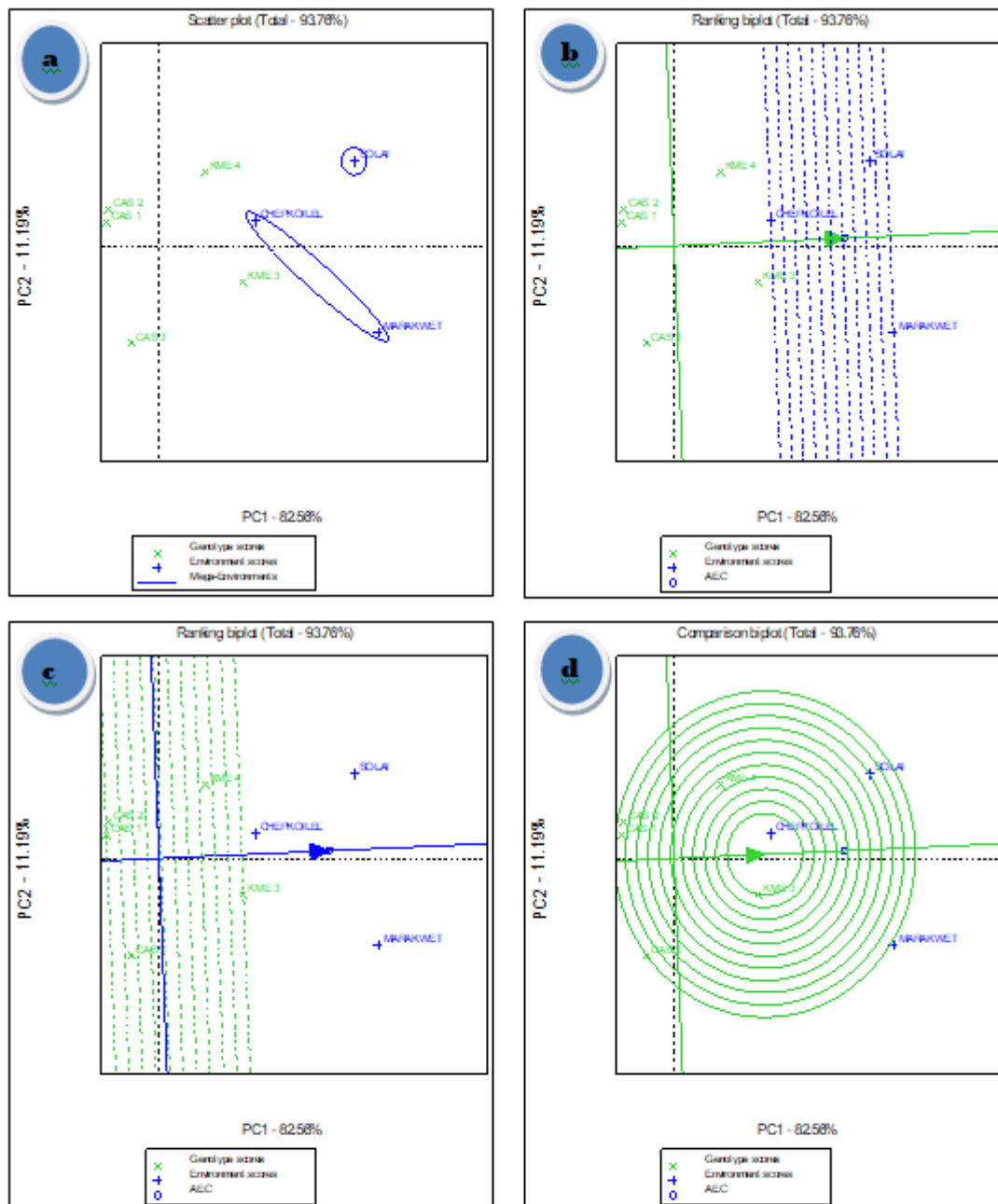


Figure 5. Biplot correlation matrices sites and elite cassava lines with respect to CMD. The figures 'a' represent scatter plot, 'b' Ranking biplot for sites, 'c' Ranking biplot for cassava lines and, 'd' Comparison biplot for both environment and cassava lines

For CBSD, the GEI showed that the first interaction principal component sum of squares (PC1 = 79.44%) was greater than the second component (PC2 = 20.31%) which indicate that the elite cassava lines differed significantly in their response to CBSD with a total variability of 99.75% considering all the factors in the GEI (Figure 6).

Unlike the CMD, the scatter plot correlation matrix for mega environments (Figure 6a) revealed that Chepkoilel, Marakwet and Solai sites did not differ in relation to how elite cassava lines responded to CBSD under field conditions. However, with respect to the ranking biplot correlation for environment (Figure 6b), it is clear that KME3 expressed similar response to CBSD across all the three agro-ecological zones, a consistency that was closely followed by KME4. The remaining elite lines responded differently from one site to the other just like the previous observation on CMD.

The ranking biplot correlation matrix for cassava genotypes (Figure 6c) revealed that all the elite cassava lines expressed almost similar response to CBSD under field condition at Chepkoilel site. However, Solai and Marakwet sites experienced dissimilar responses to CBSD by all the elite cassava lines including parental lines. Just like the response to CMD under field condition, the comparison biplot (Figure 6d) identified KME3 as the most ideal genotype across all the three environmental conditions and it was also the most susceptible genotype to CBSD followed by KME4 based on symptom expression on leaves. The remaining elite lines (CAS 1, CAS 2 and CAS 3) were tolerant to the disease and expressed low foliar severity.

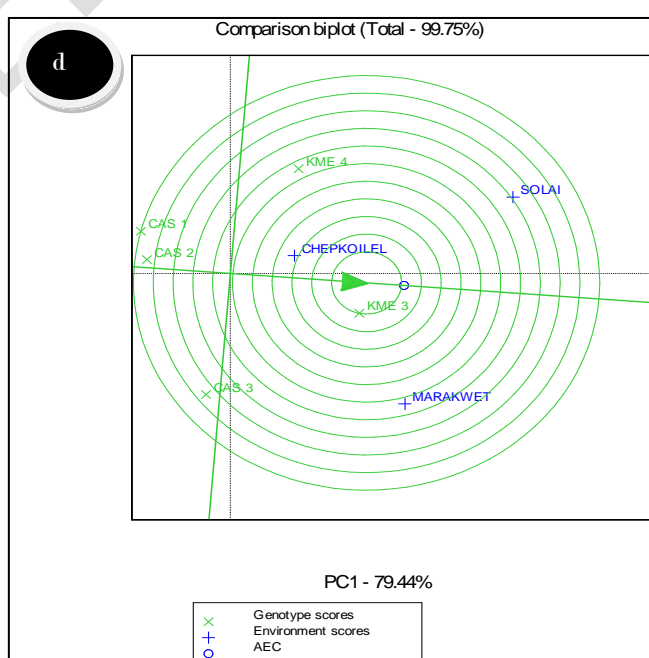
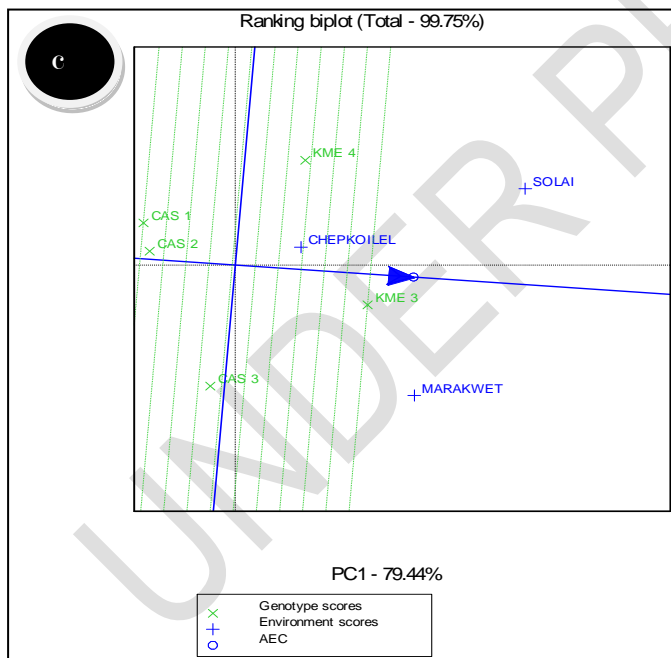
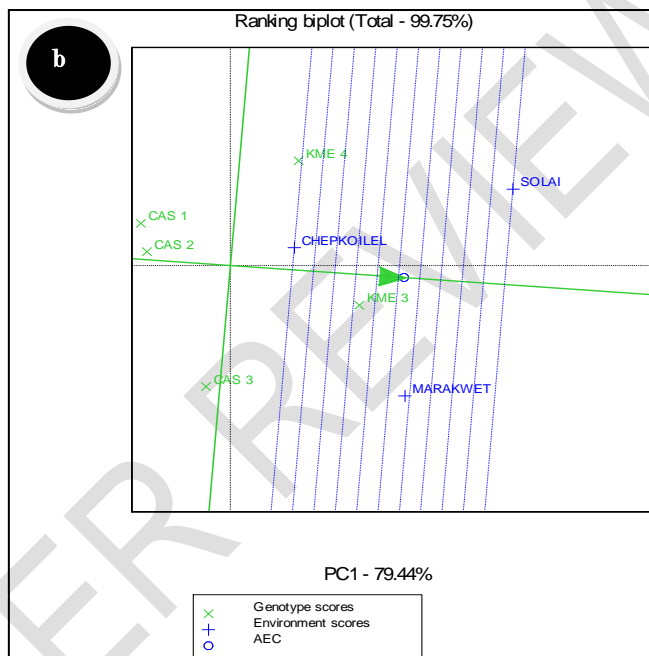
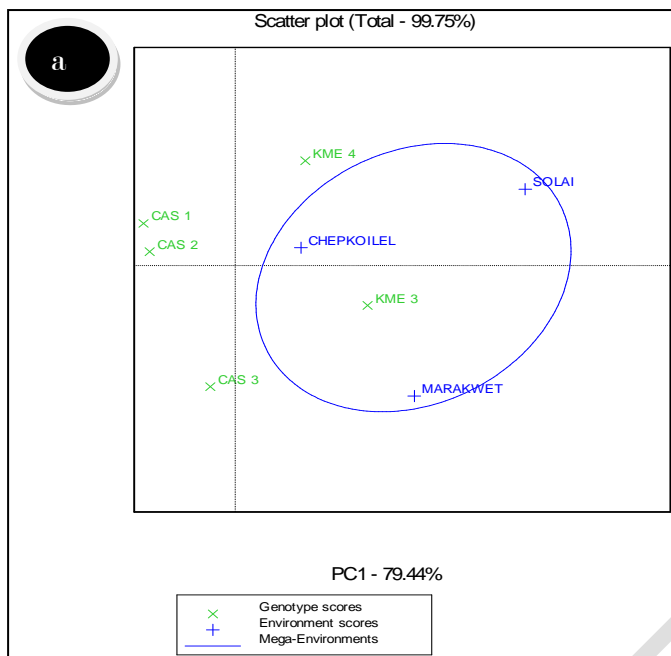


Figure 6. Biplot correlation matrices sites and elite cassava lines with respect to CBSD. The figures 'a' represent scatter plot, 'b' Ranking biplot for sites, 'c' Ranking biplot for cassava lines and, 'd' Comparison biplot for both environment and cassava lines

Discussion

Varied responses by the elite cassava lines including their parental genotypes confirms that all the screened lines against CMD and CBSD differed in their genetic constitution which played significant role in the final phenotypic observation across all sites (Wang, *et al.*, 2016) . It also suggests that if a particular line performs better than the others in a specific location, such a line could be promoted in that specific area. One that consistently performs better in all locations could be a good candidate. The significant interactions between screening sites and elite cassava lines could mean that these sites varied in terms of environmental conditions such as atmospheric temperature, atmospheric relative humidity and population densities of the potential vectors for the two viruses among others. These factors combined with host and pathogen factors could have collectively and additively influenced the varied responses from one site to the other even by the same genotypes (Chatukuta, *et al.*, 2020). Also, the relatively higher severity expressions by the parental lines (KME3 and KME4) compared to newly improved mutant elite lines indicates significant genetic improvement that confers tolerance to these two diseases under field conditions (Mukiibi, *et al.*, 2019). The inherent response by the genotypes to CMD and CBSD with respect to genotype x environmental interaction (GEI) could imply that the screened genotypes exhibit varying physiological response mechanisms when infected by viruses and possibly other pathogens. It could also mean that the final phenotypic response to diseases are not only influenced by the genetic make-up but also the environmental conditions which varied significantly for CMD and this corresponds with previous findings where a number of screened genotypes responded differently from one environment to the other (Chikoti, *et al.*, 2019).

Additionally, the similar mega environment with reference to CBSD could mean that some environmental factors responsible for severity and/or tolerance expression to CMD by the cassava genotypes are different from those needed for severity and/or tolerance to CBSD. This could be due to the influence of such specific environmental factors on the genotype-virus interactions as previously confirmed using temperature (Nakabonge, *et al.*, 2020). This could be the real situation in the study sites where temperature variation was evident (Nakabonge, *et al.*, 2020). Other than the effect of temperature variation on genotype-virus interaction, the differences in rainfall amount and intensity could have significantly influenced the number and population densities of vectors such as aphids, white flies and spider mites. This might have affected the quantities of viral inoculum and timing of inoculation in every site (Andayanie & Ermawati, 2019; Melesse & Singh, 2012).

Conclusion

Elite cassava lines namely CAS1, CAS2 and CAS3 were more tolerant to cassava mosaic and brown streak viruses compared to their parental genotypes under natural inoculation in field conditions though the tolerance levels varied from one site to the other. The three elite lines were also tolerant to root infection by brown streak virus while all parental lines were significantly affected. This study therefore recommends these three elite lines for commercial production and continuous breeding of more cassava lines with tolerance to CMD and CSBD.

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