Resistance levels of cassava landraces to CMD, CBSD and vector whiteflies in Malawi

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Abstract

Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) have been two major threats to cassava production in eastern and southern Africa. This study was designed to identify CMD- and CBSD-resistant cassava landraces and farmer-preferred varieties in Malawi for effective disease control. Thirty cassava landraces were collected from 17 districts across Malawi and evaluated for disease resistance a field experiment at the Chitala Research Station in a randomized complete block design (RCBD). Cultivars Mbundumali and Pwani were used as susceptible and resistant controls, respectively. Data collected on foliar and root disease symptoms indicated that CMD was more prominent than CBSD during the study. Cultivars Pwani, Mkumba, R23-Sangwala, Sagonja, R31-Kamphunobi, R33-Chimphuno, R76-Kamphuno, MZ126, and R63-2020 showed relative dual resistance to both CMD and CBSD with the highest incidence of 25 and 18%, respectively. Some cultivars showed resistance to one disease and not the other. The lowest cumulative number of whiteflies (0.68 per plant) was observed on Pwani while the highest was seen on R42-Mwenemisuku with 6.33 per plant. However, various cultivars supported varied numbers of whiteflies and nymphs. Assessing relative virus quantities of the prevailing viruses, *East African cassava mosaic virus* (EACMV) and *Cassava brown streak virus* (CBSV) indicated that Pwani, Mkumba and Sagonja supported lowest amount of EACMV and CBSV. Our results collectively identified the presence of dual-resistant cassava which can be further exploited for managing both CMD and CBSD in Malawi.

Introduction

Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) have posed a significant challenge to cassava production in Malawi. It is possible for farmers to lose the entire yield or harvest much less due to infection of the two diseases (R. J. Hillocks et al., 2008); (Mcsween et al., 2006; Bisimwa et al., 2015). Cassava yield losses due to CMD depend on the incidence and severity of disease symptoms (Tembo et al., 2017). CBSD causes losses of yield in quality and quantity leading to loss of monetary opportunities to farmers (R. J. Hillocks & Maruthi, 2015).

Over the years, suggestions have been put forward on the best practices worthy of adopting to control CMD and CBSD of which the major ones include phytosanitation, use of resistant genotypes and management of the whitefly vector (Thresh, 2004). While important and helpful, most of the disease control proposals may not be as cost effective as the use of resistant varieties. This is the case because practices such as phytosanitation usually require legislative interventions leading to enactment of laws to regulate the use and movement of the planting materials. In countries where majority of people involved in cassava production, like Malawi, are subsistence smallholder farmers, this may be burdensome and to some extent ineffective (Bhatti et al., 2021). The recent cassava breeding research studies on CMD and CBSD resistance in cassava have been carried out to complement research that has been done internationally since the 1930s (Storey & Nichols, 1938; Nichols, 1947). Despite all these efforts there has not been a total breakthrough because most of the studies concentrated on controlling a single disease i.e., either CMD or CBSD. It is imperative that cassava resistance breeding involve both diseases so that dual resistance to

both CMD and CBSD can be achieved. In reality, farmers have different preferences when it comes to crop variety selection of which disease resistance is just one of the many factors for consideration.

In Malawi, diagnostics of the two diseases was previously implemented by dominantly observing foliar and root symptoms without looking at the virus quantities (Legg & Hillocks, 2003; Mbewe et al., 2015). Concisely, it was based on visually observed parameters.

Malawi has a number of landraces found throughout the country of which some might be good sources of resistance to CMD and CBSD, and vector whiteflies coupled with other preferred agronomic traits. This study was designed to screen and identify CMD and CBSD disease resistant landraces.

The specific objectives of this study were to:

(1) determine the severity and incidence of CMD among cassava landraces in Malawi.

(2) determine the severity, incidence and percentage root necrosis of CBSD among cassava landraces in Malawi.

(3) quantify the two most prevailing viruses, EACMV and CBSV in cassava landraces in Malawi.

(4) determine the performance of landraces based on a combination of variables that determine resistance to CMD and CBSD.

Materials And Methods

Collection of landraces and experimental design

Cassava landraces were collected in Malawi from 17 districts of Chitipa, Karonga, Rumphi, Mzimba, Nkhatabay, Kasungu, Mchinji, Nkhotakota, Salima, Lilongwe, Ntcheu, Balaka, Mangochi, Blantyre, Zomba, Chikwawa and Nsanje in September, 2018. Leaf samples were collected from the plants where cuttings had been taken from for laboratory analysis to check if the plants were free from EACMV and CBSV. The plants were then raised in a nursery at Bvumbwe Agricultural Research Station in Thyolo, a low pressure area for CMD and CBSD, where planting materials were then collected for field trials at Chitala Agricultural Research Station.

The cassava landraces were planted at Chitala Agricultural Research Station in the lakeshore district of Salima from February, 2019 to February, 2020. Chitala is one of the sites with high CMD and CBSD inoculum pressure and many cassava disease resistance research activities take place there. The experiment depended on natural field inoculation of diseases from surrounding plants.

Cassava cuttings were planted on ridges containing 5 plants per ridge with 2 border plants on each end for each treatment. The cuttings were planted at 1m between planting stations and 1m between ridges. The experiment was laid out in a randomized complete block design (RCBD) with 3 replicates. Mbundumali and

Pwani were used as susceptible and resistant controls to both CMD and CBSD, respectively. The experiment was kept weed-free.

Data collection

Foliar CMD and CBSD symptoms, whitefly adult and nymph counts, and collection of leaf samples

Leaf samples and data on CMD and CBSD symptom severity, whitefly adult and nymph abundance was collected at 3, 6, 9 and 12 months after planting (MAP). CMD and CBSD incidence and severity were determined by observing foliar symptoms. Disease severity was based on a 1–5 scale as shown in Table 1. Foliar incidence was calculated as a percentage of symptomatic plants out of a total of 15 plants assessed as shown in the formula below (Fargette et al., 1985):

$$\%Foliarincidence = \frac{Numberofsymptomaticplants}{Totalnumberofsampledplants} X100$$

Table 1
Severity scale for CMD foliar and CBSD foliar and root symptoms

Severity scale	Description		
	Foliar CMD	Foliar CBSD	Root CBSD
1	No symptoms	No symptoms	No necrosis
2	Mild chlorosis and distortions at the base of the leaves with remaining parts of the leaves or leaflets appearing green and healthy	Slight chlorosis on leaves or stems	Trace of necrosis
3	Mosaic patterns on most leaves, narrowing and distortion of lower one-third of the leaflets	Chlorotic spots that are easily observable on leaves or stems. Small lesions are observed on stems	Clearly defined areas of necrosis but necrotic areas can be easily removed
4	Severe mosaic distortion of the thirds of most leaves and general reduction of leaf size and stunting of shoots	Severe chlorotic spots on leaves and severe necrotic lesions enlarged into streaks on stems	Most of root necrotic but may still be possible to remove necrotic areas for home consumption
5	Very severe mosaic symptoms on all leaves, twisting, distortion, misshapen and severe leaf reduction of most leaves accompanied by severe stunting of plants	Very severe chlorotic/ necrotic blotches and leaf wilt. Severe necrotic lesions, streaks, withering and die-back on stems	Most or all roots necrotic and unsuitable for human consumption

The presence of CBSD symptoms on stems was observed as a binary variable where 1 was recorded if a plant had symptoms on the stem and 0 if not. The number of adult whiteflies and whitefly nymphs were counted on the ventral side of the top most five fully-expanded apical leaves of the tallest shoot of each

plant (Fargette et al., 1985). Counting was done by gently turning the leaf on the underside in order not to disturb the adult whiteflies (Fargette et al., 1985).

Three fresh leaf samples from the top, middle and bottom of each plant were collected from 5 plants of each plot. A composite sample for each plot was taken by combining leaves from the 5 plants and immediately preserved in liquid nitrogen and there were a total of three composite samples for each treatment. The samples were then taken to Chitedze Molecular Biology Laboratory where they were stored at -80°C.

Cassava root necrosis by CBSD

At 12 MAP, the cassava plants were uprooted and the roots were detached and counted. All the harvested roots per plant were weighed and pooled together. Fifteen roots were randomly selected for each cultivar before being cut longitudinally to check the presence of CBSD root symptoms. Root CBSD scores were based on the standard 5 point scoring scale (R. Hillocks et al., 2016) as shown in Table 1.

Necrotic regions were removed from the randomly selected roots and weighed on a scale. Percentages necrosis was then calculated as follows:

$$\%necrosisinroots = \frac{Totalweightofroots - (weightofnon - necroticpartoftheroots)}{Totalweightoftheroots} X100$$

Determining virus accumulation using Real-Time qPCR

Laboratory analysis of samples was done at Chitedze molecular biology laboratory in Lilongwe, Malawi. The collected leaf samples were pulverized in a geno/grinder® (Spex SamplePrep, 2010). Total nucleic acid was extracted using modified Cetyl trimethyl ammonium bromide (CTAB) method (Maruthi et al., 2002). The extracted nucleic acid was quantified using DU730 Life Science UV/Vis spectrophotometer (Beckman Coulter). EACMV and CBSV were quantified separately using StepOne real time PCR (Applied Biosystems) using TaqMan qPCR protocol. Virus quantification was done on two viruses only (EACMV and CBSV) because we could not get a working positive control for the other viruses.

DNA was used as template in a duplex qPCR reaction with PP2A as an endogenous control (Moreno et al., 2011) for quantification of EACMV. Specific primers and probes (Alabi et al., 2008; Moreno et al., 2011) and (Otti et al., 2016), were used in this study are shown in Table 2. A total reaction volume of 20 µl was made which contained 10 µl of 2X Express qPCR supermix (Invitrogen, Thermo Fisher Scientific, UK), 2 µl of 100 ng DNA template, 500 nM of EACMV-UG primers, 300 nM of PP2A primers and 100 nM of both probes. A total of 375 nM ROX [25 µM solution of 5- carboxy-X-rhodamine in 10 mM Tris-HCl (pH 8.6), 0.1 mM EDTA, and 0.01% Tween®-20] was used as a passive reference. The qPCR was carried out for 2 minutes at 50 ^{0}C and another 2 minutes at 94 ^{0}C which was followed by 40 PCR cycles at 94 ^{0}C for 15 seconds, 54 ^{0}C for 20 seconds and 60 ^{0}C for 30 seconds.

Two step qPCR was used for the detection of CBSV with the first step being the synthesis of cDNA using Improm II™ Reverse transcription kit (Promega, Southampton, UK) following manufacturer's instructions. A volume of 5 µl Mastermix I and 15 µl of mastermix II were prepared for reverse transcription. Mastermix I and II were later mixed in PCR tubes to form a total reaction volume of 20 µl. The mixture was incubated for 5 min at 25 ^{0}C, 60 min at 40 ^{0}C and 15 min at 70 ^{0}C. The cDNA underwent a 10X dilution before being used for qPCR.

Table 2
Primer and probe sequences used for qPCR analysis of EACMV-UG, CBSV and PP2A

Target	Primer/Probe	Sequence (5 – 3)	Reference
EACMV	CMB Rep/F	CRTCAATGACGTTGTACCA	Alabi et al. (2008)
	Neweac-alt/R	CATGGAGACCGATCAGTATTGTTC	Otti et al. (2016)
	Probe	FAM-TCTTKGGAGACAGATCCAGGTGTCCACAT-IABkFQ	Otti et al. (2016)
CBSV	CBSV F3	GGARCCRATGTAYAAATTTGC	Abarshi *et al.* (2012)
	CBSV R4	GCWGCTTTTATYACAAAMGC	Otti et al. (2016)
	Probe	JOE-TTCCAGCCA/ZEN/AGCAATWYTGATGTATCAGAATAGTGTGA-IABkFQ	Otti et al. (2016)
PP2A	PP2A F	TGCAAGGCTCACACTTTCATC	Moreno et al. (2011)
	PP2A R	CTGAGCGTAAAGCAGGGAAG	Moreno et al. (2011)
	Probe	JOE-CTTTCTGTT/ZEN/GCCCCCACCATGC-IABkFQ	Otti et al. (2016)

A total reaction volume of 20 µl was consisted of 10 µl of 2X Express qPCR supermix (Invitrogen, Thermo Fisher Scientific, UK), 2 µl of cDNA template, 700 nM of CBSV primers and 400 nM of the probe. A total of 375 nM ROX [25 µM solution of 5- carboxy-X-rhodamine in 10 mM Tris-HCl (pH 8.6), 0.1 mM EDTA, and 0.01% Tween®-20] was used as a passive reference. The reaction was carried out following thermal cycling conditions described above in quantification of EACMV.

CBSV was quantified in uniplex qPCR reaction with cDNA as a template and PP2A as an endogenous control (Moreno et al., 2011) as explained above. Each sample was duplicated to form two technical replicates on each qPCR plate when quantifying EACMV and CBSV. Each run included a virus-infected sample as positive control, a virus-free sample as negative control and sterile nuclease free water as a no template control.

Threshold cycle values for the target virus and reference gene obtained from qPCR were used to calculate relative virus quantities. Specifically, Delta delta CT method was used to calculate relative quantities where relative virus quantity was equal to $2^{-\Delta\Delta Cq}$ (Livak & Schmittgen, 2001) which were then log transformed to the base two for statistical analysis.

Statistical data analysis

Time series data on disease severity, incidence, adult whitefly and whitefly nymph and the presence of CBSD on stems and EACMV and CBSV virus quantities was analysed using mixed effect model with MAP as repeated measurements using nlme package (Pinheiro & Bates, 1998). Analysis of variance (ANOVA) was used to analyse overall data of EACMV and CBSV relative quantities for the four time points using MASS package (Venables & Ripley, 2002). The overall adult whiteflies and whitefly nymph, percentage necrosis and CBSD root incidence data was analysed using generalized linear model (glm) negative binomial. Analysis of Deviance (ANODEV) using glm quasibinomial was used to analyse overall data for CMD severity, CBSD severity, presence of CBSD symptoms on stems, percentage necrosis and CBSD root incidence. Spearman rho was used to compute correlations among CMD and CBSD incidence, foliar symptom severity, total number of whiteflies, the presence of CBSD on stems and relative virus quantities. Coefficients of at least 0.8 were considered as strong correlations, those between 0.6 and 0.7 moderate and those less than or equal to 0.5 weak (Bolboacă & Jäntschi, 2006). Coefficients of + 1 or -1 were considered perfect correlations while 0 represented no relationship. Wilcoxon signed rank test was used to determine the differences in expression between foliar symptoms of CMD and CBSD. These statistical analyses were done using R version 3.6.1 (R Core Team, 2019).

The genotypes were then clustered for their performance to separate CMD or CBSD and a combination of CMD and CBSD using hierarchical agglomerative clustering (HAC) through dendrograms using Ward's method (Ward, 1963) in R version 3.6.1 (R Core Team, 2019) using pvclust package (Suzuki & Shimodaira, 2006). Dendrograms for CMD and CBSD resistance were compared by constructing a tanglegram using dendextend package (Galili, 2015). For CMD, the following diseases variables depicting resistance or susceptibility to the disease were used: CMD severity, log 2 of EACMV relative quantity and CMD incidence. The diseases variables depicting resistance or susceptibility to the CBSD used were CBSD severity, log 2 of CBSV relative quantity, CBSD incidence, the presence of CBSD on stems, CBSD root incidence, CBSD root severity and percentage CBSD root necrosis. To cluster cultivars based on dual performance to CMD and CBSD, a combination of variables for CMD and CBSD resistance was used.

Results

Foliar CMD incidence and severity

Plants infected with CMD showed typical foliar symptoms while those not infected showed no symptoms (Fig. 1). CMD severity was significantly different among cultivars ($F_{32} = 25.0$, $p < 0.0001$) and at different months after planting ($F_3 = 124.0$, $p < 0.0001$). There was a significant interaction of cultivar and time after planting on symptom severity ($F_{96} = 4.4$, $p < 0.0001$). CMD incidence was significantly different among

cultivars (F_{32} = 31.6, p < 0.0001) while time did not have significant effect on CMD incidence (F_3 = 0.1, p = 0.95). There was no interaction between cultivar and time on CMD incidence (F_{90} = 0.8, p = 0.92). Cultivar MZ 126, R23-Sangwala, Sagonja, Pwani and Mkumba had minimum CMD severity (< 1.5) and incidence (< 30%) at all four time points. The highest CMD incidence was observed on R42-Mwenemisuku, R44-Mwenemisuku, R41-Dyongo and R56-Mwenemisuku with 93, 87, 73 and 73%. The Mean CMD severity was highest on R42-Mwenemisuku with 3.5 (Chi-square = 69.24, df = 32, p < 0.0001).

Foliar (leaf) CBSD incidence, severity and presence on stems

Plants infected with CBSD showed typical foliar symptoms while those not infected showed no symptoms (Fig. 2). CBSD severity was significantly different (F_{32} = 19.9, p < 0.0001) among cultivars. There were significant differences in CBSD severity among time points (F_3 = 23.78, p < 0.0001). Time after planting and cultivar had a significant interaction on CBSD severity (F_{96} = 6.6, p < 0.0001). CBSD incidence significantly varied among cultivars (F_{32} = 18.6, p < 0.0001) and time after planting (F_3 = 5.7, p < 0.00001).

The interaction between cultivar and time after planting on CBSD incidence was significant (F_{90} = 1.2, p < 0.00001). Cultivars MZ126, Sagonja, Sauti and Pwani had the lowest CBSD severity of 1 at all the four time points. The minimum CBSD incidence of 0% at all 4 time points was observed on MZ126, Sagonja, Sauti and Pwani. The presence of CBSD symptoms on stems was significantly different among cultivars (F_{32} = 8.3, p < 0.00001) and time after planting (F_3 = 21.6, p < 0.00001). There was also a significant interaction between cultivar and time after planting (F_{96} = 2.7, p < 0.00001). CBSD symptoms were absent on MZ126, R31-Kadamphuno, R47-Chitembwele, R57-Chitembwele Sweet, Sagonja, Sauti, Mkumba and Pwani.

Highest mean CBSD severity was observed on R73-Matakolembwende (2.8) (Chi-square = 53.4, df = 32, p < 0.0001). Cultivars MZ126, Sagonja, Sauti and Pwani had the lowest mean CBSD severity (1). Other cultivars, Mkumba, R57-ChitembweleSweet, R42-Mwenemisuku, R56-Mwenemisuku, R53-Nachisalanzo, R23-Mtutumusi and R31-Kamphunobi had relatively low mean symptom severity between 1.0-1.2. R55-Kasekeleman had the highest CBSD symptoms of 0.6 on stems (Chi-square = 59.1, df = 32, p < 0.00001). It was seconded by R73-Matakolembwende and R70-Nyautonga with 0.5 each. No CBSD symptoms were observed on stems of MZ126, R31-Kamphunobi, R47-Chitembwele, R57-ChitembweleSweet, Sagonja, Sauti and Pwani.

Whitefly adult and nymph abundance

Whitefly adults and nymphs were observed on all cultivars. The mean number of adult whiteflies varied significantly among the cultivars (Chi-square = 124.4, df = 32, p < 0.0001). There were significant differences in the number of whitefly nymphs (Chi-square = 99.9, df = 32, p < 0.0001) for all 4 time points. The lowest number of whitefly adults was observed on Pwani with 0.68 whiteflies per plant (Table 3). The lowest number of whitefly nymphs was observed on Mkumba with 0.92 nymphs per plant (Table 3).

Table 3
Whitefly adult and nymph count on different cultivars at Chitala agricultural research station, Malawi 2019-20

Cultivar	Mean whitefly adult count	Mean whitefly nymph count
R42-Mwenemisuku	6.33	3.67
R41-Dyongo	5.22	4.88
R48-Mpama	5.12	3.72
R38-Mtutumusi	4.58	4.32
R63-2020	4.33	1.27
R51-Chitembwelemtuwa	4.32	2.83
R61-Nyamukhunga	4.17	2.05
R47-Chitembwele	4.13	3.03
R58-Buladifulu	4.07	1.62
Kachamba	3.97	1.73
R44-Mwenemisuku ACC 05	3.83	1.32
R55-Kasekelemani	3.32	5.42
R66-Chitembwele	3.13	2.82
Sagonja	3.10	2.22
R57-ChitembweleSweet	2.88	1.83
R70-Nyautonga	2.83	3.22
R31-Kamphunobi	2.72	3.23
R76-Kadamphuno	2.70	2.52
R56-Mwenemisuku	2.62	1.67
Sauti	2.37	5.17
R32-Buchiya ACC 30	2.30	3.48
MZ 123	2.28	3.35
R46-Gomanimtuwa	2.18	1.43
R53-Nachisalanzo	2.17	3.27
R74-Lwinga	2.07	2.58
R33-Chimphuno	2.02	2.70
R23-Sangwala	2.00	1.08

Cultivar	Mean whitefly adult count	Mean whitefly nymph count
R73-Matakolembwende	1.82	1.25
Mbundumali	1.77	3.13
R36-Gomani	1.55	2.50
MZ 126	1.33	4.37
Mkumba	1.18	0.92
Pwani	0.68	2.45
P value	**< 0.001**	**0.001**

EACMV and CBSV accumulation

EACMV relative quantities were significantly different among cultivars and time (F_{32} = 54.3, p < 0.0001). Time and cultivar had a significant interaction on EACMV (F_{96} = 6.2, p < 0.00001). EACMV was not detected in Pwani at all the four time points. EACMV was not detected in Mkumba, Sagonja and Sauti until 12 MAP. EACMV was detected from 3 MAP in many cultivars (Fig. 3). R31-Kamphunobi had the highest EACMV quantities of all cultivars at 3 and 6 MAP (Fig. 3). At 9 and 12 MAP, EACMV quantities were highest in R44-Mwenemisuku and R42-Mwenemisuku, respectively. There were significant differences in mean EACMV relative quantities among the cultivars (Chi-square = 730.4, df = 31, p < 0.0001). There was no EACMV detected in Pwani. However, for the cultivars in which the virus was detected, Mkumba and Sagonja had the lowest virus quantity with 0.001 and 0.002.

CBSV relative quantities were significantly different among cultivars and time (F_{32} = 38.5, p < 0.0001). Time and cultivar had a significant interaction on CBSV relative quantities (F_{96} = 4.9, p < 0.0001). CBSV quantities were highest of all cultivars in R73-Matakolembwende at 3 and 6 MAP (Fig. 4). At 9 and 12 MAP, CBSV quantities were highest in R55-Kasekelemani and MZ123, respectively (Fig. 4). CBSV titre was not detected in Pwani, and it was lowest in Mkumba and Sagonja with 0.001 and 0.02, respectively. The mean CBSV quantity for all 4 time points was highest in R55-Kasekelemani with 1207.47, followed by R73-Matakolembwende with 1101.52.

Correlation analysis of CMD, CBSD and whitefly parameters

Spearman rank correlation analysis was carried out to determine the correlation among various parameters related to CMD. There was high correlation between CMD incidence and severity (r = 0.85, p < 0.00001) (Table 4). EACMV relative quantity was significantly positively correlated with CMD incidence (r = 0.39, p < 0.00001) and severity (r = 0.32, p < 0.00001) (Table 4).

Table 4
Correlation between CMD incidence, symptom severity, number of adult whiteflies and EACMV relative quantity

	Severity	Number of adult whiteflies	Relative virus quantity
Incidence (%)	0.85***	0.16**	0.39***
Severity		0.17**	0.32***
Number of adult whiteflies			0.01 ns
***p < 0.001; **p < 0.01; *p < 0.05; ns = not significant			

Spearman rank correlation analysis was carried out to determine the correlation among various parameters related to CBSD. There were positive correlations among CBSD severity, the presence of CBSD on stems, CBSD symptom severity, incidence and Relative virus quantities (Table 5).

Table 5
Correlation between CBSD incidence, symptom severity, number of adult whiteflies, CBSV relative quantity and presence of CBSD on stems.

	Severity	Number of adult whiteflies	Relative virus quantities	Presence on stems
Incidence (%)	0.74***	0.07 ns	0.42***	0.54***
Severity		0.02 ns	0.33***	0.79***
Number of adult whiteflies			-0.08 ns	-0.09 ns
Relative virus quantities				0.29***
***p < 0.001; **p < 0.01; *p < 0.05; ns = not significant				

CBSD root severity, incidence and percentage necrosis

There were significant differences among cultivars in root CBSD severity (Chi-square = 21.2, df = 32, p < 0.0001). R73-Matakolembwende had the highest root severity of 3.0 and least severity of 1.0 was observed in MZ126, MZ123, R32-Buchiya, R38-Mtutumusi, R41-Dyongo, R44-Mwenemisuku, R47-Chitembwele, R53-Nachisalanzo, R61-Nyamunkhunga, R74-Lwinga, Sauti, R66-Chitembweremtuwa, Pwani and Mkumba (Fig. 5). There was significant difference among cultivars in CBSD root incidence (Chi-square = 1245.4, df = 32, p < 0.00001). CBSD incidence (0.0%) was not observed on MZ126, MZ123, R32-Buchiya, R38, Mtutumusi, R41-Dyongo, R44-Mwenemisuku, R47-Chitembwele, R53-Nachisalanzo, R61-Nyamunkhunga, R74-Lwinga, R66-Chitembweremtuwa, Sauti, Pwani and Mkumba (Fig. 6) while the highest incidence of 33.3% was observed on R73-Matakolembwende followed by Sagonja (Fig. 6). Percentage necrosis in roots

varied among cultivars with R73-Matakolembwende having the highest of 24.2% loss (Chi-square = 767.5, df = 32, p < 0.00001) (Fig. 7). There was no necrosis in 17 (52%) of the cultivars (Fig. 7). The percentage necrosis of the rest of the other cultivars with CBSD root incidence did not exceed 20%.

Correlation analysis of CBSD root incidence, CBSD root symptom severity and CBSV relative quantities.

There were positive correlations among CBSD root incidence, severity of root necrosis and percentage necrosis (Table 6). There was, however, no correlation between CBSD relative quantities in leaves and the other parameters of CBSD.

Table 6
Correlation analysis of CBSD root incidence, root severity, percentage necrosis and CBSV relative quantity from leaf samples

	Severity	Necrosis (%)	Relative virus quantity
Incidence (%)	0.96****	0.89****	0.07 ns
Severity		0.89****	0.09 ns
Necrosis (%)			0.02 ns

Wilcoxon signed rank test of CBSD and CMD incidences and severities showed significant differences among these parameters of CBSD and CMD (Table 7). CMD incidence was statistically significantly higher than CBSD incidence (p < 0.001). Similarly, CMD severity was higher than CBSD severity (p < 0.001) (Table 7).

Table 7
Wilcoxon signed rank test for corresponding parameters of CBSD and CMD

Parameter	Mean CBSD	Mean CMD	P value
Incidence	20.1	36.1	0.001***
Severity	1.5	1.8	0.001***

Cluster analysis to determine the resistance or susceptibility of cassava genotypes based on a combination of disease response parameters.

Cassava cultivars were grouped into different clusters based on their performance to CMD and CBSD. Pwani (resistant control) was in the same cluster with six genotypes MZ126, Sagonja, Sauti, Mkumba, R47-Chitembwele and R23-Sangwala, in response to CMD. The susceptible control (Mbundumali) was in the same cluster with R31-Kamphunobi and R56-Buladifulu (Fig. 8).

The resistant control (Pwani) was clustered together with three other genotypes in response to CBSD (Mkumba, MZ126 and Sauti) (Fig. 9). However, the closest genotype was Mkumba. R73-Matakolembwende which performed poorly in many disease measurement parameters was in the cluster with R55-Kasekelemani, R48-Mpama, R58-Buladifulu and R70-Nyautonga. The susceptible control (Mbundumali) was in a cluster with three other genotypes.

Pwani (resistant control) was in the same cluster with eight other genotypes (Mkumba, MZ126, R23-Sangwala, Sagonja, R31-Kamphunobi, R76-Kadamphuno, R33-Chimphuno and R63-2020) in response to both CMD and CBSD (Fig. 10). Mbundumali (susceptible control) was clustered together with three other genotypes.

Discussion

Crops that differ in genetic makeup perform differently toward certain attributes such as pest and disease resistance (Kover & Schaal, 2002). The current study has confirmed observations from several other studies in which cultivars performed differently toward CMD, CBSD and whitefly resistance (Kaweesi et al., 2014; Navangi et al., 2020; Shirima et al., 2020). This is not unusual considering that Malawi has high diversity of cassava (Benesi, 2005). Resistance to vector whitefly is measured by observing the number of adult whiteflies and nymphs. Resistance to CMD and CBSD in Malawi has for years largely been determined by observation of symptom severity and incidence. Additionally, the presence of CBSD on stems, root CBSD severity, root CBSD incidence and yield loss have been crucial elements for consideration when identifying resistant genotypes. Although time was another source of variation, it nonetheless, was insignificant on CMD incidence which computationally led to lack of interaction with the cultivar. This might be as a result of constant numbers of infected plants whose severity changed over time. It was, however, in contrast with observations on CBSD where all disease parameters under investigation significantly varied over time.

Adult whiteflies and nymph numbers vary from cultivar to cultivar as observed in this study. This is because whitefly populations differ due to differences in the genetic makeup of the host plants. Both adult and nymph numbers were highest at the initial point of data collection (at 3 MAP in May). This was a result of a combination of age of plants and rainfall which affect whitefly abundance (Kalyebi et al., 2018); (Shirima et al., 2019). In the month of May, the rainfall had stopped which provided a conducive environment for whitefly development. (Shirima et al., 2019) reported increased abundance of whiteflies during the short rainfall season (between October and December) which indicates that the abundance of whiteflies can be affected by rainfall duration. A cultivar, Mkumba, had the lowest number of both adult whiteflies and nymphs indicating some level of resistance in this cultivar.

This study has confirmed the existence of the monotonic relationships among various CMD and CBSD parameters. CMD and CBSD severity and incidence had strong positive correlations. These parameters were also positively correlated with the respective relative virus quantities though weakly. This entails that in a plant with increasing quantities of the virus, one is likely to observe an increase in symptom severity and incidence. Therefore, resistance of cassava cultivars to CMD and CBSD was correctly defined using

these parameters. The results in this study are partially consistent with (Maruthi et al., 2017) who reported a positive correlation between the number of whiteflies and CBSD incidence at 18 weeks after planting but no correlation at 22 weeks after planting. In field experiments, however, it would not be easy to always get consistent results on the relationship between foliar disease symptoms and number of adult whiteflies due to the cosmopolitan nature of the insect. This study has shown that the number of adult whiteflies do not determine the quantity of viruses in cassava host plants, and no correlation between the number of whiteflies and relative quantities of EACMV and CBSV. Furthermore, this shows that a plant that accommodates a large number of whiteflies at a particular time might not necessarily mean it will have rapid multiplication of the viruses or show more severe symptoms. Symptom severity has been reported to be positively correlated with viral load by some studies (Moreno et al., 2011; Kuria et al., 2017). (Luckew et al., 2022) reported significant differences in whitefly numbers among *Cucurbita pepo* genotypes. However, the viral loads of *Cucurbit leaf crump virus* (CuLCrV) and *Cucurbit yellow stunting disorder virus* (CYSDV) in the genotypes were not significantly different despite the varying numbers of whiteflies. Plants showing increased presence of CBSD stem symptoms are likely to have increased symptom severity and incidence. This is explained by the positive correlations between the presence of CBSD symptoms. Different cassava genotypes express CBSD root symptoms with varying degrees. In this study root CBSD severity and incidence significantly varied among cultivars. However, there was generally low severity, incidence and percentage necrosis. This might be because the study was conducted in one season so the buildup of the disease was slow. Additionally, low severity, incidence and necrosis could mean that the varieties in Malawi are generally resistant to CBSD. EACMV and CBSV served as examples since plants of genotypes inoculated with an isolate would behave the same way if all of them are inoculated with a different isolate (Kuria et al., 2017).

Resistance to CMD and CBSD can best be described by analyzing a collection of responses of cassava plants rather than looking at only one dependent variable. Just like in this study, for years now, research on cassava virus disease resistance has been done on the actual disease parameters and the vector whiteflies. Although it has been observed that genotypes with high virus quantities exhibit high disease severity, there are other disease resistance parameters that are put into consideration in determining the resistance or susceptibility of the genotypes. These parameters include incidence and percentage necrosis depending on the disease and the nature of symptoms exhibited. There is also great interest in resistance to whiteflies in addition to the actual diseases as we would also want to limit the whitefly populations which if not checked lead to epidemics (Legg et al., 2011). Getting a genotype which does not support high whitefly populations is crucial. Considering that there are a number of disease response parameters that determine resistance of plants, cluster analysis would be an important step to incorporate many observations and take into account their effect. In this study dual resistance was determined by observing the clustering of genotypes based on a combination of CMD and CBSD parameters separately and collectively. Eleven genotypes which were in the same cluster with the resistant genotype (Pwani) were regarded as having dual resistance. The study has also shown through a comparison of dendrograms that although there might be dual resistance, there are many cultivars with resistance to a single disease.

Generally, foliar symptoms for CMD were higher than the corresponding parameters of CBSD. This can testify to the prominence of CMD in cassava fields as reported previously. Many cassava cultivars in this study are susceptible to CMD than CBSD which can be regarded as exhibition of more resistance to CBSD than CMD. Many cultivars that showed some resistance in this study were resistant to one disease and susceptible to the other. Generally, Pwani, Mkumba, R23-Sangwala, Sagonja, R31-Kamphunobi, R33-Chimphuno, R76-Kamphuno, MZ126, and R63-2020 exhibited dual resistance to both CMD and CBSD than the rest of the cultivars by looking at multiple parameters. It would be important to test for the presence of virus resistance genes (e.g., CMD1 and CMD2) in these cultivars showing resistance to the diseases. Pwani and Mkumba which performed well against both CMD and CBSD were among the genotypes that were accessed by the Department of Agricultural Research Services in Malawi through the germplasm exchange programme under the 5CP project (Tumwegamire et al., 2018). These genotypes have, thus, been maintained as separate varieties in Malawi. However, of recent, they have been discovered through genotyping to be the same genotype (Perez-Fons et al., 2020). It is therefore, not surprising that the cluster analysis grouped them together.

Conclusions

Combining foliar and root symptoms, percentage root necrosis and virus quantities is an accurate approach in determining disease resistance in cassava. Cultivars that showed dual resistance to CMD and CBSD should be multiplied and promoted through the national seed system for improved cassava yields and food security. Some cultivars were resistant to one disease and not the other, as such they need to be adopted for cultivation in areas where the diseases are less prevalent. The cultivars can also be incorporated into resistance breeding programs to develop progenies with dual resistance and improved agronomic traits. Finally, this study has shown that CMD was more prominent among cultivars under investigation than CBSD based on foliar symptoms expressed by plants. This might help explain why nationally CMD is likely to be encountered more than CBSD.

Declarations

Author Contributions: All authors contributed to the design and conceptualization of the study. Material preparation and data collection were done by Hastings T. Musopole and Andrew Mtonga. Data analysis was done by Hastings T. Musopole. The first draft of the manuscript was prepared by Hastings T. Musopole. Review and editing of the manuscript was done by M.N. Maruthi and Andrew Mtonga.

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Figures

Figure 1

Cassava cultivars showing distinct resistance to CMD A. R44-Mwenemisuku with severe CMD symptoms B. Mkumba without CMD symptoms

Figure 2

Cassava cultivars showing distinct resistance to CBSD at ChitalaA. R58-Buladifulu with CBSD symptoms B. Sagonja without CBSD symptoms.

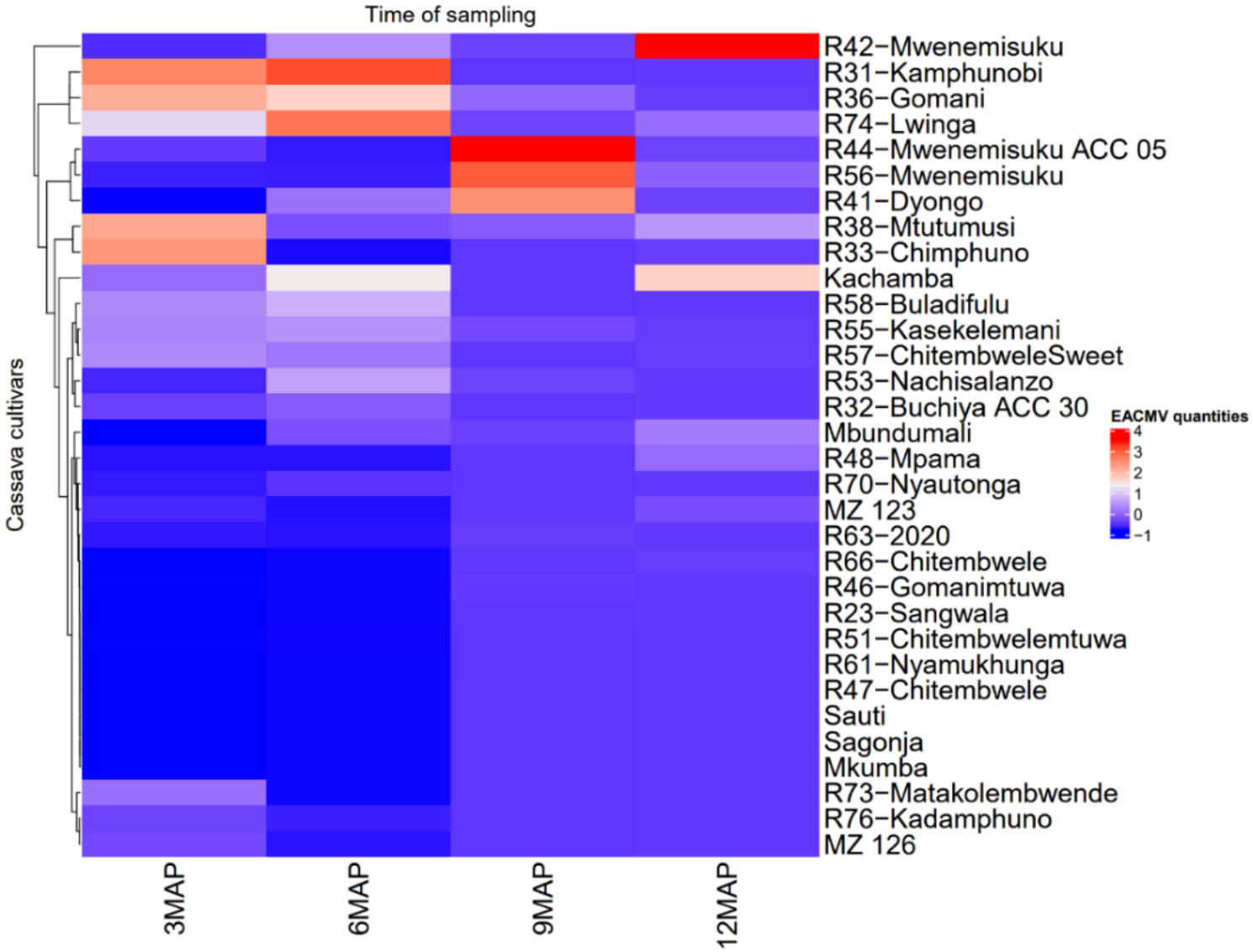

Figure 3

Quantities of EACMV in different cassava cultivars at 3, 6, 9 and 12 MAP relative to EACMV quantity in Mbundumali at 3MAP. Mbundumali was chosen as EACMV was detected in it since 3 MAP. At 3 MAP the relative quantity of Mbundumali was 1

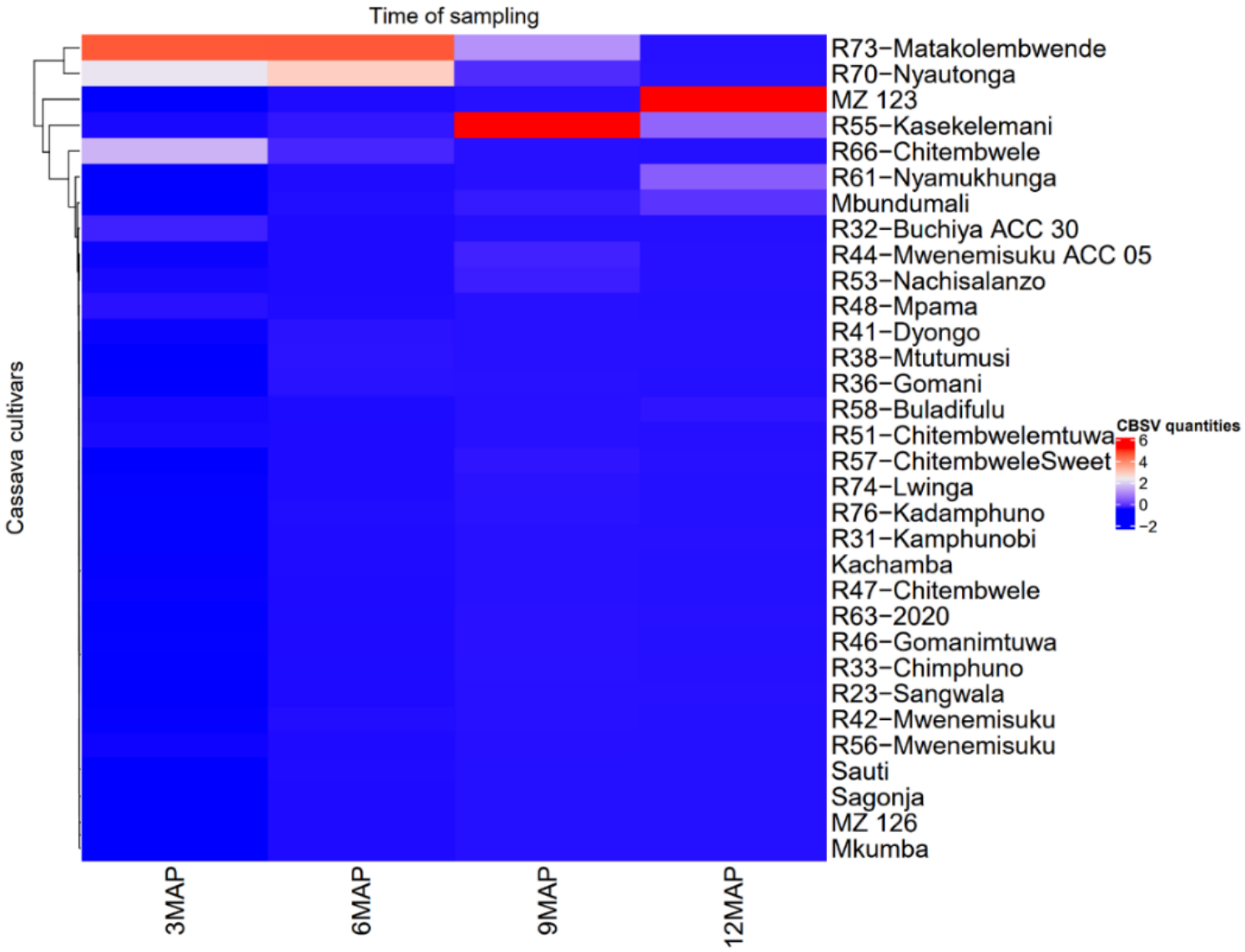

Figure 4

Quantities of CBSV in different cassava cultivars at 3, 6, 9 and 12 MAP relative to CBSV quantity in cv. Mbundumali at 3 MAP. Mbundumali was chosen as EACMV was detected in it since 3 MAP. At 3 MAP the relative quantity of Mbundumali was 1

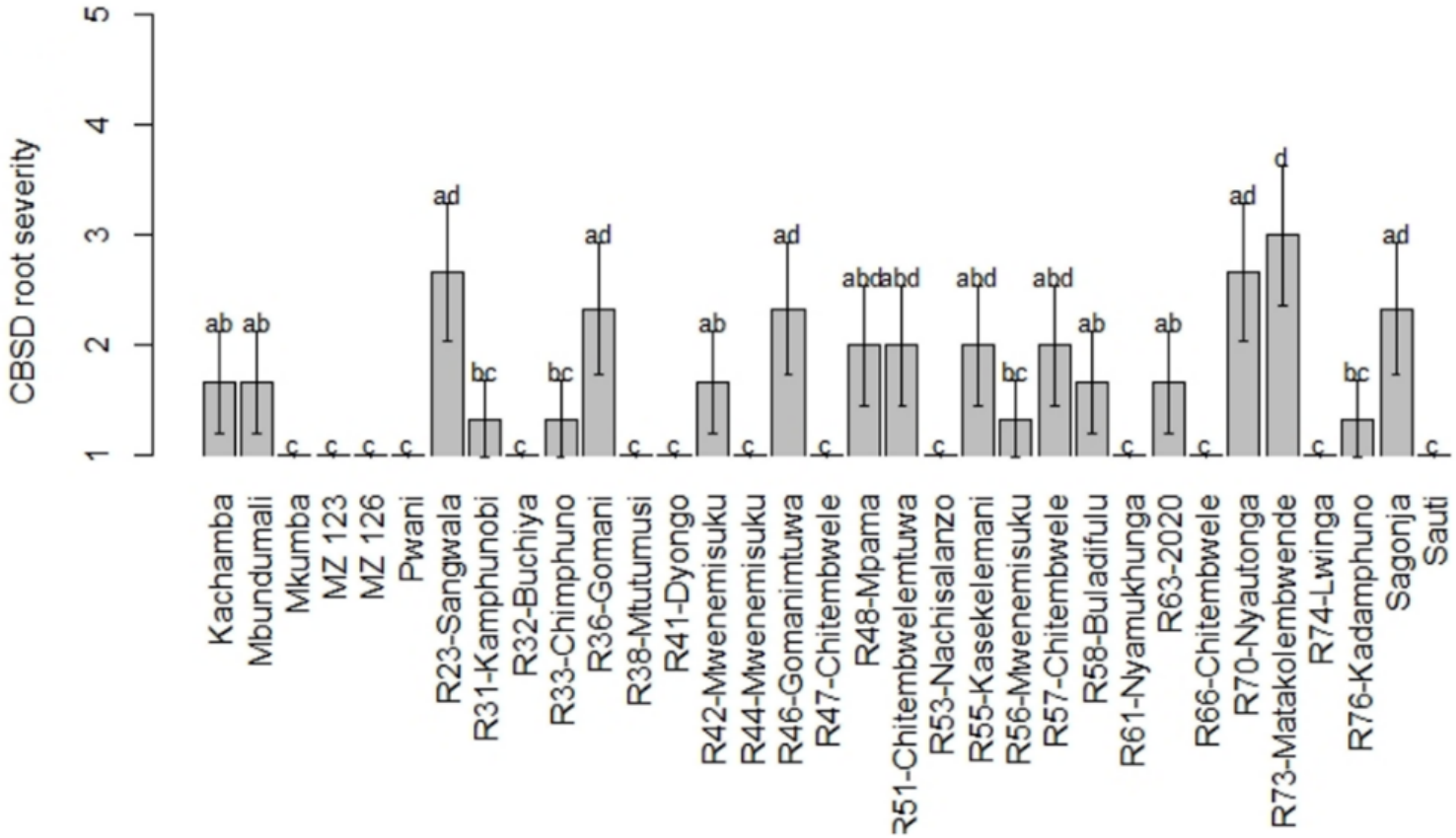

Figure 5

Root CBSD severity on different cassava varieties at 12 MAP at the Chitala agricultural research station, Malawi 2019-20. Bars with the same letters were not significantly different. The error bar represents standard errors of the mean

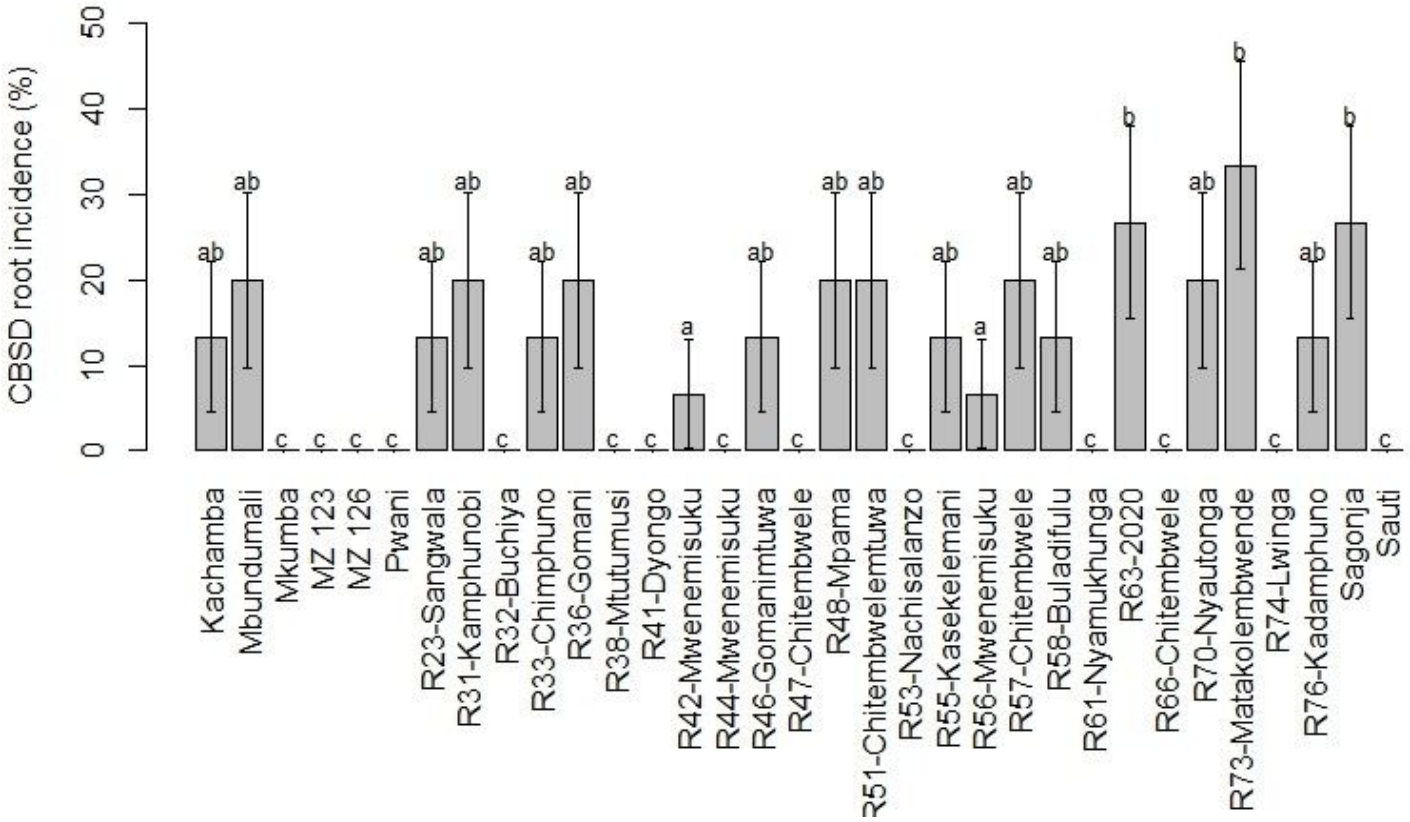

Figure 6

Mean CBSD root incidence on different cassava varieties at 12 MAP at the Chitala agricultural research station, Malawi 2019-20. Cultivars with the same letters were not significantly different. The error bar represents standard errors

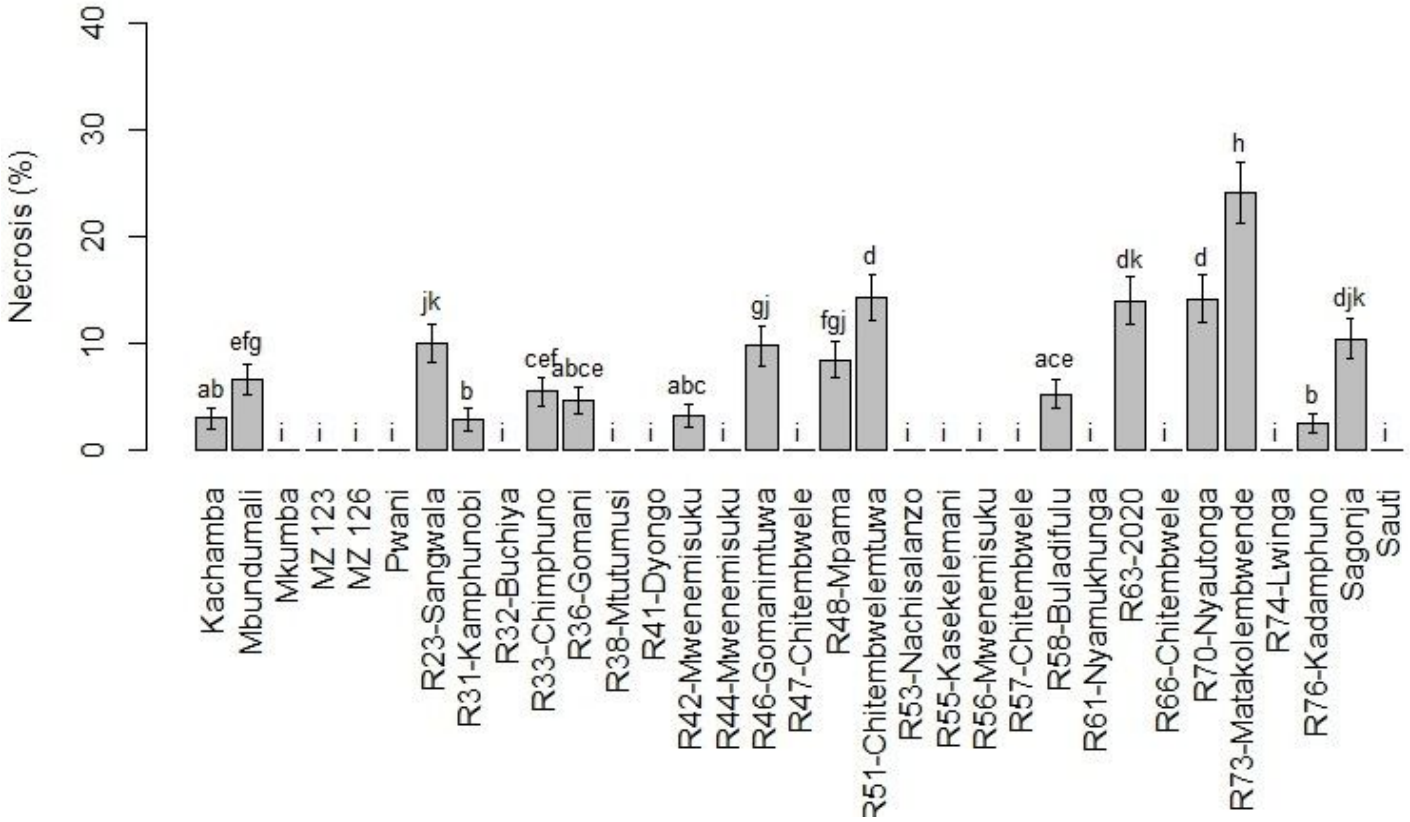

Figure 7

Mean percentage necrosis in roots of different cassava varieties due to CBSD at 12 MAP at the Chitala agricultural research station, Malawi 2019-20. Percentage necrosis was calculated by weight. Cultivars with the same letters were not significantly different. The error bar represents standard errors

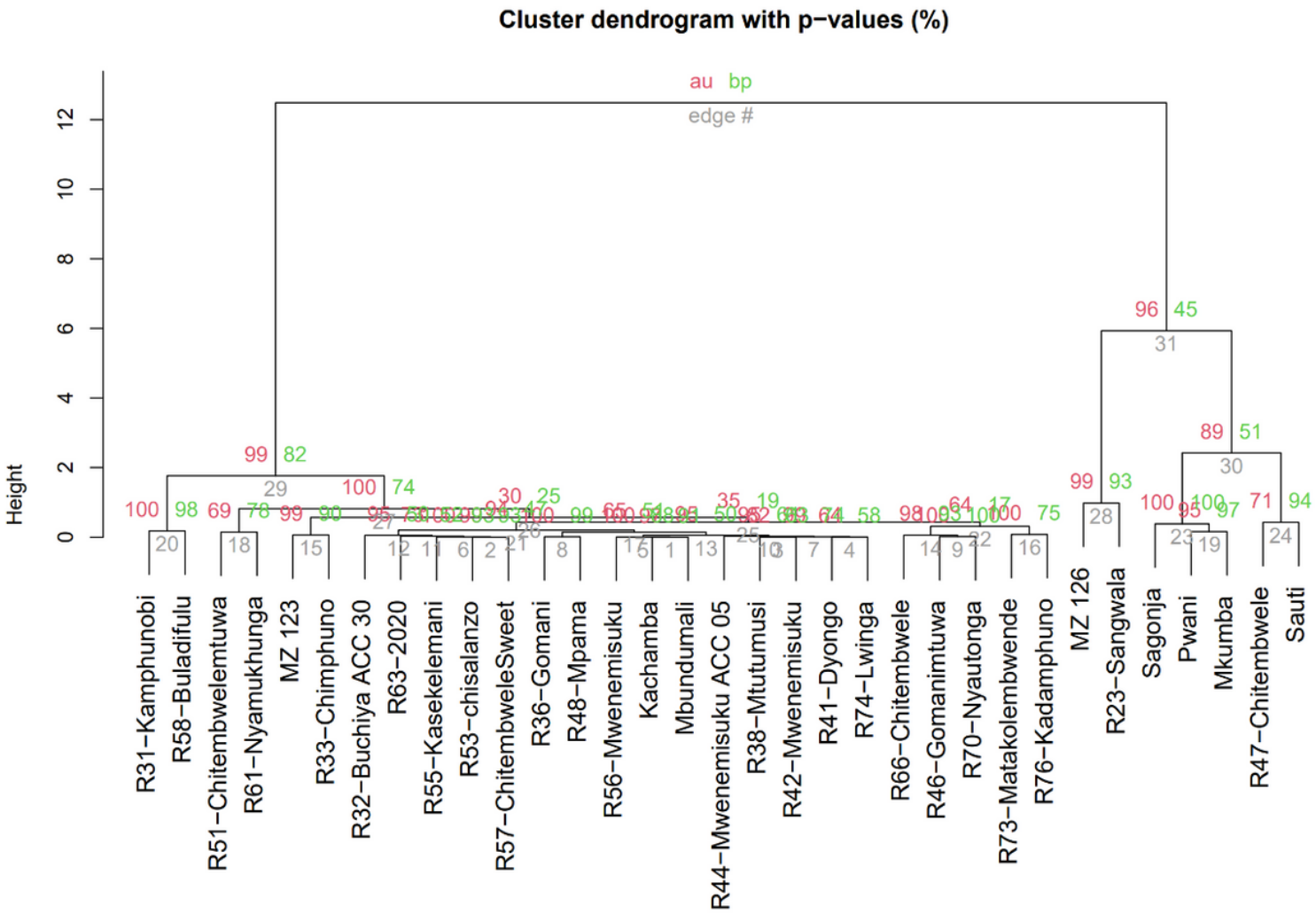

Figure 8

Genotypes clustered based on resistance to CMD and whitefly infestation at the Chitala agricultural research station, Malawi 2019-20. The dependent variables used for clustering were CMD severity, log2 EACMV relative quantity and CMD incidence calculated as the averages of data between 3 to 12 MAP. Au= approximate unbiased probability and bp= bootstrap probability. Edge # indicates the branch numbers at which clusters merge starting from the most similar clusters. Height axis indicates the dissimilarity between clusters

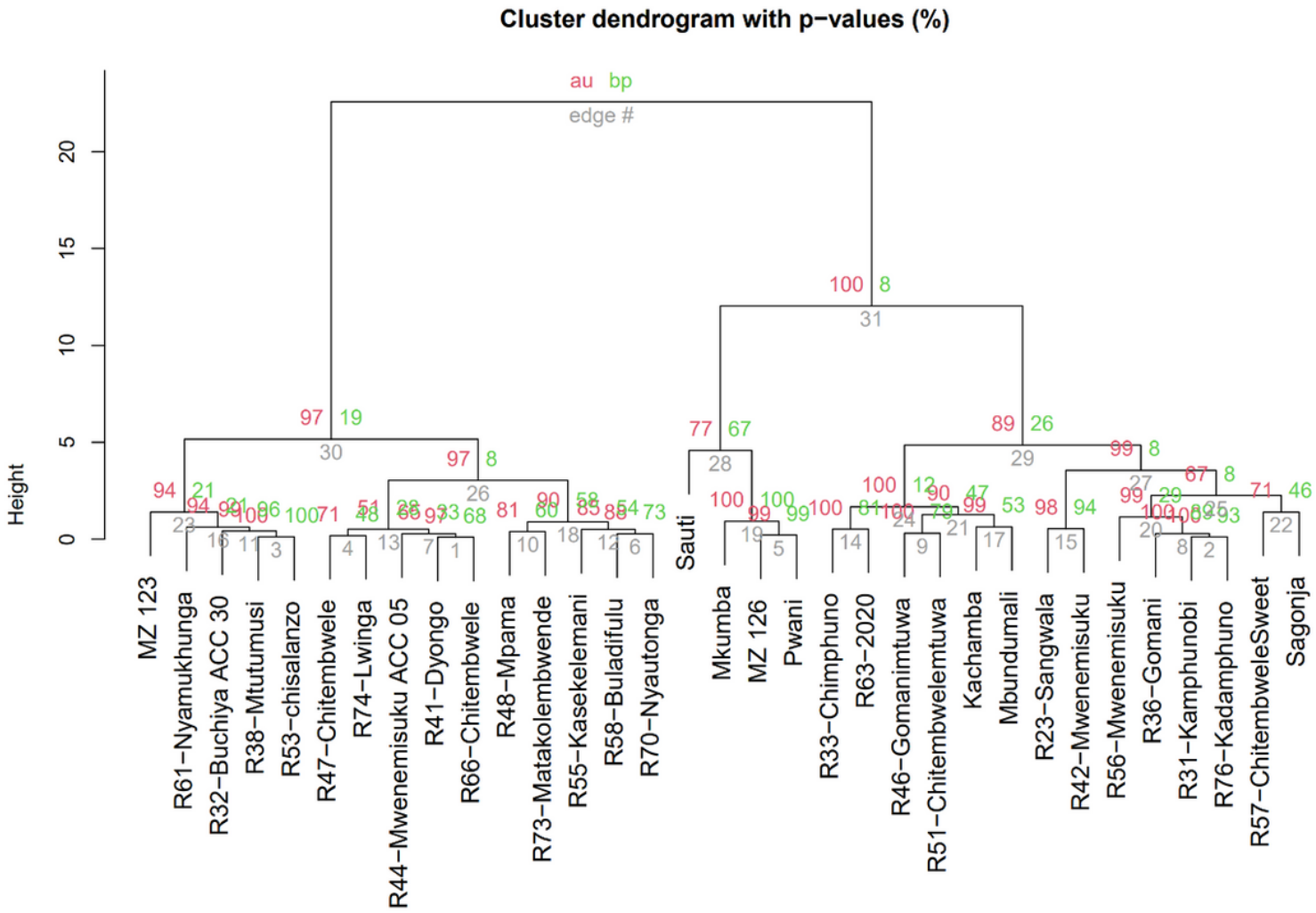

Figure 9

Genotypes clustered based on resistance to CBSD and whitefly infestation at the Chitala agricultural research station, Malawi 2019-20. The dependent variables used for clustering were CBSD severity, log2 CBSV relative quantity, CBSD incidence, root incidence, root severity and percentage necrosis. Au= approximate unbiased probability and bp= bootstrap probability. Edge # indicates the branch numbers at which clusters merge starting from the most similar clusters. Height axis indicates the dissimilarity between clusters

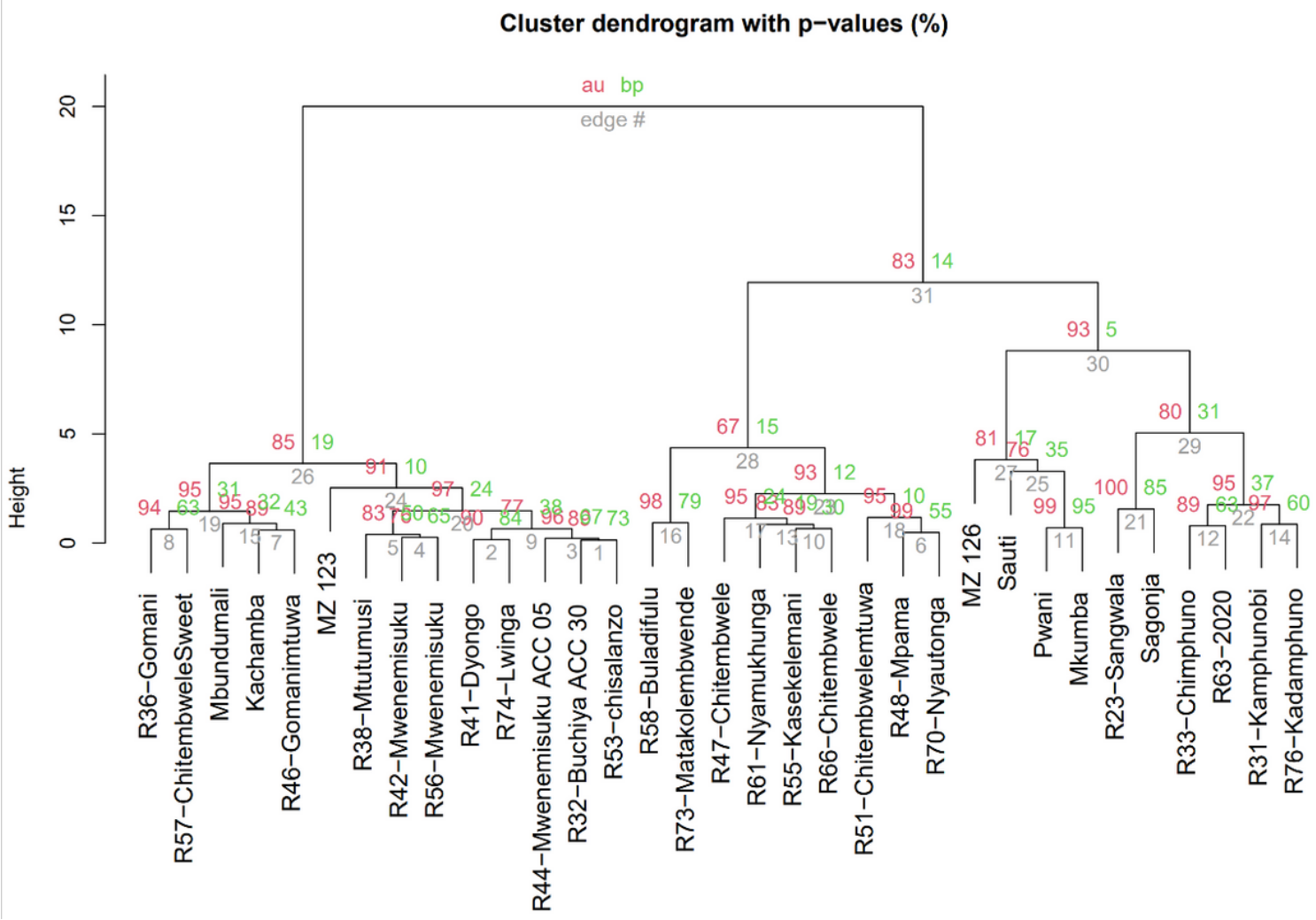

Figure 10

Genotypes clustered based on resistance to both CMD and CBSD, and whitefly infestation. at the Chitala agricultural research station, Malawi 2019-20. The dependent variables used for clustering were CMD and CBSD severity, log2 EACMV and CBSV relative quantity, CMD and CBSD incidence, CBSD root incidence, CBSD root severity and percentage necrosis. Au= approximate unbiased probability and bp= bootstrap probability. Edge # indicates the branch numbers at which clusters merge starting from the most similar clusters. Height axis indicates the dissimilarity between clusters