Response to Cassava Brown Streak Disease Infections in Local and Improved Cassava Genotypes under Field and Greenhouse Assays in Lower Eastern Kenya

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Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Cassava brown streak disease (CBSD) is caused by two cassava brown streak viruses (CBSVs) transmitted by whiteflies (Bemisia tabaci). CBSD significantly inhibits cassava production in Kenya through losses of up to 100% in farmer-preferred but susceptible varieties. As a management strategy, the present study evaluated the effect of CBSD on two local varieties (Thika-5 & Serere) and 15 improved genotypes in lower Eastern Kenya. Between October 2016 and June 2017, the genotypes were infected with CBSVs through whitefly transmission under field experiment at SEKU research farm (1.31ºS, 37.75ºE) and chip-bud grafting at KALRO-Katumani (1.35ºS, 37.14ºS) greenhouse conditions. RCBD and CRD experimental designs were respectively applied in field and greenhouse assays. CBSD symptoms were quantified through disease incidence (DIC) and severity (DSY) every 3 months for the field experiment and weekly for greenhouse assay. At harvest, storage root necrosis (SRN) was scored and non-necrotic roots weighed as marketable root yield (MRY). Molecular diagnostics was accomplished through duplex RT-PCR. Results revealed significantly (P≤0.01) higher foliar field DIC (81- 100%) and SRN (2.3 – 5.0) recorded in

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1. INTRODUCTION

Cassava (Manihot esculenta Crantz) is the most important source of calories in the tropics after rice and maize [1] and forms a major part of the diet for nearly a billion people in approximately 105 countries mostly in sub-Saharan Africa (SSA), Asia, the pacific and South America [2,3]. Cassava is now produced in 40 of the 53 countries of SSA, accounting for 61% of global production [4]. In Kenya, approximately 60, 30 and 10% cassava production occur in western, coastal and eastern regions respectively [5,6]. The crop is grown by small, poor households for subsistence and forms an important source of food security and poverty alleviation [7]. Cassava consumption in Kenya includes roasted tubers, boiled fresh roots, processed into flour for porridge, cakes, bread and dried or fried chips or crisps. Despite this potential, Kenya’s annual cassava fresh root production is estimated at 662,405 tonnes, against an annual demand of 1,662,405 tonnes, of fresh roots according to 2014 data by FAO [8]. The low production constraint cultivars [9,10]. Indeed CBSD is a serious constraint of cassava crop production for farmers and growers throughout East Africa [11].

CBSD is caused by two distinct virus species; cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) of the family Potyviridae and genus Ipomovirus [12-14]. Both CBSV and UCBSV are often referred as CBSVs. The CBSVs are transmitted by whiteflies (Bemisia tabaci) and CBSD spread through infected stakes inadvertently used for vegetative propagation [15,11,16]. CBSD-infected cassava plant exhibit yellow leaf chlorosis on secondary and tertiary veins on older leaves, brown lesions on mature stems and on severe cases shoot dieback of younger green stems [17,18]. Further, the brown necrotic lesions developed on the storage roots render the roots unpalatable and unmarketable [19,5]. All these can result in 70 – 100% yield losses especially on susceptible varieties [20,10]. In Kenya, CBSD incidences of up to 93% have been reported in western region [5], more than 95% at the coast [21] and 53% in lower eastern areas [22]. A weight loss of produced storage roots of up to 70% has been linked to CBSD in Kenya [23]. Due to its devastating impact on cassava production as reviewed above, strategies to combat or control CBSD has been proposed and imposed [24]. These include farmer sensitization, rouging infected plants, field sanitation, strict quarantine, virus-free or clean planting materials and cultivation of resistant or tolerant genotypes [19]. Breeding for CBSD resistant cassava genotypes is, however, the most sustainable or long term approach. Indeed CBSD is often more severe on susceptible than resistant genotypes [25]. However, few resistant or tolerant cassava varieties currently exist [26,11]. Further, breeding for CBSD resistant cassava is still nascent or lags in Kenya. To bridge this gap, the present study evaluated the response of cassava genotypes (previously bred for CBSD resistance) to CBSVs infections under field and greenhouse conditions in lower eastern Kenya.

2. MATERIALS AND METHODS

2.1 Cassava Genotypes

Fifteen improved cassava genotypes and two local (Thika-5 & Serere) susceptible controls...
2.2 Field Experiment

Field experiment was conducted between October 2016 and June 2017 at the South Eastern Kenya University (SEKU) research farm (1.3076° S, 37.7545° E). Experimental design was randomized complete block design with plot size of 13 m by 7 m first ploughed with inter-plow space of 1 m and a 2 m space between the blocks. Eight cuttings (each ~20 cm) from each of the selected genotypes (Table 1) were then randomly planted in each of the four plots at depths of 15 cm and spacing of 1 m by 1 m between plants [28]. Plants from two local varieties (Serere & Thika-5) that were used as susceptible controls as well “CBSVs spreaders” planted in spreader rows were obtained from fields that had 100% CBSD incidences and severities of 4.5 – 5.0 [10]. Disease incidence (DIC), calculated as the proportion of cassava plants in a plot expressing CBSD symptoms and severity (DSY), scored as degree of CBSD infection on an individual plant, were used to quantify CBSD [5,29] at 3, 6 and 9 MAP. The DSY was visually scored on a scale of 1-5 where 1- no apparent symptoms and 5-defoliation with stem lesions and pronounced dieback [30-32]. At 9 MAP, four plants per block were destructively harvested. Cross sections were made on the roots [33] to score severity of storage root necrosis (SRN) based on a 1-5 scale where 1- no apparent necrosis and 5- root necrotic and severe constriction [31,32]. Non necrotic roots (score of 1.0) were counted as marketable storage roots (MSR) and their fresh weight weighed as marketable root yield (MRY) data. The MRY was converted into tonnes per hectare (t/ha) as described by Masinde and colleagues [34].

2.3 Greenhouse Assay

Concurrent with field trials, greenhouse assay was laid in a complete randomized design at KALRO-Katumani (1º 35’S and 37º 14’E). Eighteen 4-L pots for each genotype (Table 1) were first filled with soil: manure mixture (1:1) and a single cutting (10 cm) planted per pot. The pots were then irrigated to field capacity once per day until sprouting then twice per week. Eight weeks after planting, nine potted plants from each genotype were inoculated with CBSVs from CBSD-infected local variety Serere through previously described chip bud grafting [17,35].

Table 1. List of cassava genotypes assessed for response to CBSD in the present study

| #  | Code   | Genotypes       | Parents        | Parents    | Comments                  |
|----|--------|-----------------|----------------|------------|---------------------------|
| 1  | KB-275 | Kiboko 275      | Thika-5 X Sepinde | Thika-5   | Susceptible local genotype |
| 2  | TK-279 | Thika 279       | 990183 X 990127 | 990183    | Resistant local genotype  |
| 3  | TK-272 | Thika 272       | 990127 X 990005 | 990127    | Resistant local genotype  |
| 4  | KB-281 | Kiboko 281      | 990183 X Thika-5 | 990005    | Resistant local genotype  |
| 5  | KB-277 | Kiboko 277      | Thika-5 X Sepinde | Kisimbani | Resistant genotype from Zanzibar |
| 6  | TK-280 | Thika 280       | 990183 X 990127 | Sepinde   | Resistant genotype from Zanzibar |
| 7  | KB-271 | Kiboko 271      | 990127 X 990005 |           |                           |
| 8  | TK-273 | Thika 273       | 990127 X 990005 |           |                           |
| 9  | KB-274 | Kiboko 274      | 990127 X 990005 |           |                           |
| 10 | TK-289 | Thika 289       | 990183 X Kisimbani |         |                           |
| 11 | KB-297 | Kiboko 297      | 990183 X Kisimbani |         |                           |
| 12 | KB-300 | Kiboko 300      | 990183 X Kisimbani |         |                           |
| 13 | KB-295 | Kiboko 295      | 990183 X Kisimbani |         |                           |
| 14 | TK-278 | Thika 278       | 990183 X 990127 |           |                           |
| 15 | KB-276 | Kiboko 276      | Thika-5 X Sepinde |           |                           |
| 16 | LCV1   | Serere          | Local landrace  |           |                           |
| 17 | LCV2   | Thika-5         | Local landrace  |           |                           |
The remaining nine non-grafted plants acted as controls. Two weeks after bud graft insertion, the parafilm wrapping was removed and success or failure of graft union assessed and recorded [17]. One week after grafting (WAG), development of CBSD foliar symptoms (through DIC & DSY) was visually monitored once per week [17] and the experiment terminated at 8 WAG.

2.4 Molecular Diagnostics

Cassava leaves were randomly sampled at 9 MAP (from field experiment) and at 8 WAG (from greenhouse assays) for RNA isolation, cDNA synthesis and molecular detection of CBSVs through Reverse transcriptase Polymerase chain reaction (RT-PCR). Leaves from three plants per genotype were pooled, total RNA extracted using modified CTAB-based pine tree method [36,37] and cDNA synthesised from the RNA using Biowiz’s iScript cDNA Synthesis Kit following manufacturer’s instructions. Prior to RT-PCR, concentration and integrity of RNA samples were confirmed on NanoDrop ND-1000 and 1% agarose electrophoresis. Primers to detect both CBSV and UCBSV in a sample through duplex RT-PCR were CBSVF2: 5'- GGRCCATACTYAARTGGTT-3'; CBSVR7: 5'- CCTTTTGAACCTRAAATARCC-3' and CBSVR7: 5'- CCATTTRCTTYTCCAMADCTTC-3' [38]. RT-PCR reaction and cycling conditions were adopted from Maruthi and colleagues [39].

2.5 Data Analysis

Data on DIC, DSY, SRN, MSR and MRY were subjected to analysis of variance (ANOVA) using SAS software version 9.0. Group of means were separated by Duncan’s Multiple Range Test (DMRT) (P≤0.05). Two-tailed Pearson correlation coefficient (r) was used to determine correlation between parameters. For molecular diagnostics, RT-PCR products (amplicons) were separated on 1.5% agarose gel electrophoresis.

3. RESULTS AND DISCUSSION

3.1 Genotypic Response to CBSD Infections under Field Experiment

Foliar symptoms typical of CBSD were observed as early as 3 MAP in two (Serere & Thika-5) local susceptible landraces (Fig. 1a & 1b) compared to improved genotypes that showed no symptoms under field trials (Fig. 1c). These symptoms included yellow vein banding, expressed mainly on the lower, older leaves and chlorosis which occurred along the secondary and tertiary veins, giving a feathery appearance. Similar CBSD symptoms observed from earlier related studies corroborated our results [10,17]. All these indicated presence of CBSD in the study area and thus justified screening for resistant or tolerant genotypes as a management strategy. ANOVA results showed significant (P≤0.01) genotypic variation for DIC and DSY (at 3, 6 and 9 MAP), SRN-I, SRN-S, MSR and MRY (Table 2a & 2b). Blocking effect was insignificant (P>0.05) for SRN-I, SRN-S and MSR but significant (P≤0.01) for MRY (Table 2b). Such significant genotypic variation in morphological and yield parameters in response to CBSD infections is a vital criterion for selection of virus resistant or tolerant genotypes with high yields.

As previously reported [40], the response to CBSD in the present study was also genotype-dependent. It was stated [10] that understanding the cultivar response to CBSD is important if appropriate control measures based on breeding are to be implemented to restrict the spread of the virus. Specifically, all the fifteen (15) improved genotypes were foliarily asymptomatic (0% DIC and mean DSY of 1.0) compared to the two local varieties (Serere and Thika-5) that showed DIC of 65, 87.5 and 100% and 55, 81.3 and 87.5% at 3, 6 and 9 MAP respectively (Fig. 2a & 2b). Similarly mean DSY of 4.25, 4.75 and 5.0 for Serere and 3.5, 3.75 and 3.75 for Thika-5 were recorded during the periods (Fig. 2a & 2b). Upon dissection, storage roots for all improved genotypes were healthy or non-necrotic (Fig. 3a & b) while severe and highly severe root necrosis (SRN) were observed on local varieties Thika-5 (Fig. 3c) and Serere ((Fig. 3d). This unexpectedly translated to 0% SRN-I and mean SRN-S of 1.0 in all improved genotypes compared to 80% SRN-I and SRN-S of 5.0 in Serere as well as 45% SRN-I and SRN-S of 2.25 in Thika-5 (Table 3).

The asymptomatic (both shoot & root) response to CBSD infection exhibited by improved genotypes compared to high shoot and root incidence and severity observed in local varieties Serere and Thika-5 could be attributed to two factors. First, the improved genotypes had been bred using one CBSV resistant parent (Table 1; M Yussuf, KALRO-Thika, Kenya, Unpublished results), therefore a likelihood that resistance mechanism was conferred. Secondly, natural transmission of CBSVs through *Bemisia tabaci* could have been insignificant or ineffective in inducing CBSD symptoms in improved genotypes. Thus, resistance mechanism in these improved genotypes is not MACP mediated. Further, the genotypes displayed high yields (Table 3).

The expected low frequency of transmission in improved genotypes compared to the local varieties (45% and 55% SRN) could have also contributed to low severity of infection. These findings indicate that resistance in improved genotypes is likely to be virus specific. Therefore, resistant genotypes should be identified and bred into local varieties to expand the genetic diversity and create virus-specific resistant landraces. The improved genotypes should also be field tested in the areas that are highly prevalent with CBSD.
genotypes. This is probably due to low population of _Bemisia tabaci_ that could not transmit high titres of CBSVs to induce CBSD symptoms. Indeed [22] recorded 53% CBSD prevalence with an average of 0-3 whiteflies per cassava plant in AEZ-LM5 where the present study was carried out. The 0% DIC in improved genotypes could thus be linked to the low _Bemisia tabaci_ population. This concurs with previous findings that significantly and positively correlated whitefly population with CBSD incidences [15]. Recently, 53 – 30% reduction in CBSVs transmission was observed when number of whiteflies per plant was reduced from 100 to 20 [41]. Whitefly population, which could have been linked with ineffective CBSD transmission, was however not scored in the present study.

The high CBSD symptoms recorded in variety Serere and Thika-5 were expected as cuttings used to establish the two local varieties for the field trial were derived from infected stocks. As part of any experimental design, such susceptible controls or infected materials should be included as “CBSVs spreaders” [10]. The non-necrotic storage roots of improved genotypes compared to high severity of root necrosis on both susceptible varieties were consistent with previous findings which indicated that root necrosis often develop after foliar symptoms and that it is more severe in the most sensitive cultivars where the planting material has been derived from infected stock [32]. This observation was further corroborated by the significant (Ps0.01) positive correlations observed between foliar DSY with SRN (r = 0.951; Table 4). Similarly, [30] reported a strong association between CBSD foliar and root symptom as frequent genotypes that showed foliar symptoms and root necrosis.

Fig. 1. CBSD symptoms observed in cassava plants during field trials: 1a = Thika-5; 1b = Serere and 1c = Improved genotypes

Table 2a. Analysis of variance (ANOVA) for incidence and Severity under field trials

| SoV    | df | 3 MAP |           | 6 MAP |           | 9 MAP |           |
|--------|----|-------|-----------|-------|-----------|-------|-----------|
|        |    | MS (G) | F value | Pr>F  | MS (G) | F value | Pr>F  | MS (G) | F value | Pr>F  |
| DIC    | 16 | 3652.64 | 105.58  | <.01  | 3881.39 | 308.23  | <.01  | 1594.32 | 33.86   | <.01  |
| DSY    | 16 | 3.702  | 31.32    | <.01  | 4.77    | 63.92   | <.01  | 5.2     | 91.98   | <.01  |

_MAP = months after planting; SoV = source of variation; df = degree of freedom; MS = mean squares; G = genotypes; DIC = disease incidence; DSY = disease severity_

Table 2b. ANOVA for storage root necrosis and root yield under field trials

| SoV        | MS for B (df = 3) | F value (B) | Pr>F (B) | MS for G (df = 16) | F value (G) | Pr>F (G) |
|------------|-------------------|-------------|----------|--------------------|-------------|----------|
| SRN-I      | 68.63             | 1.94        | 0.135    | 1876.47            | 53.17       | <.01     |
| SRN-S      | 0.015             | 0.32        | 0.811    | 4.09               | 89          | <.01     |
| MSR        | 11.06             | 0.79        | 0.502    | 66.49              | 4.79        | <.01     |
| MRY        | 236.76            | 35.98       | <.01     | 74.08              | 11.26       | <.01     |

_MAP = months after planting; SoV = source of variation; df = degree of freedom; MS = mean squares; B = blocking; G = genotypes; SRN-I = storage root necrosis Incidence; SRN-S = storage root necrosis severity; MSR = marketable storage roots; MRY = marketable root yield_
Fig. 2a. Mean percent disease incidence (DIC) under field experiment
MAP = Months after planting, CBSD-I = CBSD Incidence; KB = Kiboko; TK = Thika

Fig. 2b. Mean disease severity (DSY) under field experiment
MAP = Months after planting, CBSD-S = CBSD Severity; KB = Kiboko; TK = Thika

Fig. 3. Storage root necrosis (SRN) from field-harvested root tubers
Tubers exhibiting CBSD-associated root necrosis: Fig. 3a & b = non-necrotic tubers from improved genotypes; Fig. 3c = severe necrosis on variety Thika-5; Fig. 3d = high severity of necrosis of variety Serere
The genotypic differences for yield data included significantly lower MSR (mean of 2.0 per plant) counted in both susceptible controls (Serere & Thika-5) compared to 7 – 15 MSR per plant counted in all (except KB-274) improved genotypes (Table 3). Similar trends were observed for MRY where 5.81 – 9.21 t/ha MRY produced by 11 of the improved genotypes were significantly (P≤0.01) higher than 2.16 and 1.99 t/ha MRY respectively harvested from Serere and Thika-5 (Table 3). Among improved genotypes, TK-273, TK-289, TK-280 and TK-272, produced the highest (8.31 – 9.21) MRY (Table 3). Thus, these improved genotypes were not only tolerant or CBSD-free but were also high yielding compared to local varieties. This is often a highly sought-after performance in a genotype. A resistant or tolerant plant should exhibit no or only mild disease symptoms with little or no loss in growth or vigor and yield [42, 43]. Development of high yielding and CBSD resistant cassava genotypes is pre-requisite towards sustainable CBSD management.

The significant (P≤0.01) negative correlation observed between DSY with MRY (r = -0.697), DIC with MRY (r = -0.710) as well as SNR with MRY (r = -0.679) (Table 4) could perhaps be linked with significantly (P≤0.05) lower yield (MSR & MRY) produced by the two susceptible varieties (Thika-5 & Serere) compared to significantly (P≤0.05) higher yield (MSR & MRY) harvested from most of the improved genotypes (Table 3). DIC, DSY and SRN also negatively correlated with MSR (Table 4). Similar inverse correlations that validated already known deleterious CBSD effect on cassava production has been reported [44]. Indeed below ground symptoms associated with CBSD such as reduction in root size or numbers, formation of radial constrictions and corky brown necrotic lesions, often render the tubers unfit for human consumption or valueless at the market [30, 19, 32].

3.2 Foliar CBSD Symptoms under Greenhouse Assays

Successful graft unions were observed 1½ weeks after grafting (WAG) through sprouted buds on the grafted scions and rootstock (Fig. 4) with subsequent development of foliar CBSD symptoms first recorded at 2 WAG in local controls Serere and Thika-5 that exhibited ~47 and 7% DIC respectively (Fig. 5a). By 8 WAG, variety Serere had 90% DIC and mean DSY of 3.7 while Thika-5 had 81% DIC and mean DSY of 2.3 (Fig. 5a & 5b). CBSD symptoms were first observed at 6 WAG in improved genotypes. Additionally, only four (KB-281, TK-280, KB-277 & KB-276) out of the 15 improved genotypes developed symptoms (DIC of 20 - 35% & DSY of 1.7 – 2.2) by 8 WAG (Fig. 5a & 5b). The remaining 11 improved genotypes (KB-300, KB-297, KB-295, TK-289, TK-279, TK-278, KB-275, KB-274, TK-273, TK-272 & KB-271) did not show
CBSD symptoms at the end of 8 WAG (Fig. 5a & 5b). A number of implications were deduced from above results. First, through sprouting of the buds and subsequent pronounced CBSD symptom development, the chip-bud grafting technique effectively transmitted CBSVs. Previous successful transmission of CBSVs through chip bud grafting resulted in 70 – 100% CBSD incidence [17,45].

Secondly, the period taken for symptoms to develop or show and severity in symptom expression was genotype-dependent. For instance, symptoms developed as early as 2 WAG in local varieties Serere and Thika compared to delayed symptoms observed at 6 WAG in some improved genotypes. Similar variations in time taken for symptoms to develop upon grafting have also been reported. Such includes 1 - 2 WAG [17], 4 WAG [45,46] and 6 – 10 WAG [12]. The delayed CBSD symptom development by the four improved genotypes compared to sensitive varieties that rapidly expressed CBSD, can be described as a resistance mechanism where the genotypes tolerate or systemically harbor the virus without symptoms which merely delay symptom expression after inoculation followed by mild symptoms [47]. This perhaps was also corroborated by 'severe' symptoms (81 & 90% DIC) as indicated by varieties Serere and Thika-5 compared to 'mild' symptoms (20 - 35% DIC) observed in four (KB-281, TK-280, KB-277 & KB-276) improved genotypes. Moderately resistant genotypes delays symptom expression and exhibits mild or low DIC [48].

Table 4. Pearson correlation coefficient for field data

|            | DIC (9 MAP) | DSY (9 MAP) | SRN-I | SRN-S | MSR  | MRY  |
|------------|-------------|-------------|-------|-------|------|------|
| DIC (9 MAP)| 1           | .992**      | .976  | .906  | -677 | -710 |
| DSY (9 MAP)| .992**      | 1           | .995  | .951  | -666 | -697 |
| SRN-I      | .976**      | .995**      | 1     | .976  | -651 | -679 |
| SRN-S      | .906**      | .951**      | .976  | 1     | -593 | -616 |
| MSR        | -.677**     | -.666**     | -.651 | -.593 | 1    | .994 |
| MRY        | -.710**     | -.679**     | -.679 | -.616 | .994 | 1    |

**correlation is significant at P ≤ 0.01 level; *correlation is significant at P ≤ 0.05 level; MAP = months after planting; DIC = disease incidence; DSY = disease severity; SRN-I = storage root necrosis incidence; SRN-S = storage root necrosis severity; MSR = marketable storage roots; MRY = marketable root yield

![Fig. 4. CBSD symptoms on plants inoculated with CBSVs through chip bud grafting](Image)
Thirdly, unlike field trials where all improved genotypes showed no CBSD symptoms, the chip bud grafting technique produced a 20 – 35% DIC in four (KB-281, TK-280, KB-277 & KB-276) improved genotypes. This perhaps elucidated variation in efficiency of CBSVs transmission between the two methods. Indeed transmission or inoculation of viruses in cassava through grafting has been reported to be more efficient both in the field and greenhouse conditions compared to vector-borne (whitefly) transmission [45,17,49]. Thus transmission of CBSVs through whiteflies in the current field experiment was considered insignificant.

3.3 Molecular Diagnostics

RT-PCR detected CBSV (345 bp) in two susceptible controls (Thika-5 & Serere) and one improved genotype (KB-276) while the remaining fourteen improved genotypes were all CBSV-free under field trials (Fig. 6a). Under chip-bud grafting (at 8 WAG), the two susceptible controls (Thika-5 & Serere) and five improved genotypes (KB-276, KB-281, TK-280, KB-277 & KB-271) were positive for CBSV (345 bp) while the remaining eleven improved genotypes (KB-300, KB-297, KB-295, TK-289, TK-279, TK-278, KB-272, KB-275, KB-274 & KB-273) were negative for CBSV (Fig. 6b). In either experiments (greenhouse and field), no UCBSV (441 bp) were detected (Fig. 6a & 6b).

Molecular detection of CBSV in five improved genotypes further provides evidence on effectiveness of transmission of CBSV through chip-bud grafting. Although visually asymptomatic under field trial (Fig. 2a & 2b), improved genotype KB-276 was positive for CBSV (Fig. 6a). Similarly genotype KB-271 showed no CBSD symptoms upon grafting (Fig. 5a & 5b) but presence of CBSV was detected through RT-PCR (Fig. 6b).

The two genotypes exhibited CBSV latency, a situation where some infected plants may harbor the virus but remain visually symptomless or where some varieties express symptoms in roots rather than on leaves [23,9]. It is, therefore, prerequisite to carry out molecular diagnostics in addition to visual monitoring of CBSD symptoms in cassava. Although the duplex RT-PCR was to detect both CBSV and UCBSV in a sample, only CBSV was detected from both field and greenhouse leaf samples. This could be associated with differential interaction between CBSV and UCBSV. CBSV is a more aggressive and virulent CBSD viral pathogen inducing more rapid and severe CBSD symptoms compared to UCBSV that produces milder foliar symptoms [50,51]. Additionally, tolerant cassava varieties have been infected with only CBSV, but free of UCBSV, suggesting their resistance UCBSV [52]. Indeed CBSV isolates have been reported

![Fig. 5a. Disease incidence (DIC) of genotypes inoculated with CBSVs through Chip-bud grafting](image-url)

*Wag = weeks after grafting; DIC = Disease or CBSD incidence*
Fig. 5b. Disease severity (DSY) of genotypes inoculated with CBSVs through Chip-bud grafting

$WAG = \text{weeks after grafting}; DSY = \text{disease or CBSD severity}$

to be more detectable, having higher more severe symptoms compared to UCBSV infection rates by graft inoculation and inducing [53].

Fig. 6a. RT-PCR for detection of CBSVs from field leaf samples

$M = 1 \text{ kb marker}; 1 = \text{KB}-300; 2 = \text{KB}-297; 3 = \text{Thika-5}; 4 = \text{Serere}; 5 = \text{KB}-295; 6 = \text{TK-285}; 7 = \text{TK-279}; 8 = \text{KB}-276; 9 = \text{TK-278}; 10 = \text{KB}-281; 11 = \text{TK-280}; 12 = \text{KB}-277; 13 = \text{KB}-272; 14 = \text{KB}-271; 15 = \text{KB}-275; 16 = \text{KB}-274; 17 = \text{KB}-273; W = \text{water (sterile)}$
4. CONCLUSION

Conclusively, of the 17 genotypes screened, the present study indicated that, two local varieties (Thika-5 & Serere) and five improved genotypes (KB-276, KB-281, TK-280, KB-277 and KB-271) exhibited CBSD symptoms while ten improved genotypes (KB-300, KB-297, KB-295, TK-289, TK-279, TK-278, TK-272, KB-275, KB-274 and TK-273) were asymptomatic. The ten genotypes were not only CBSD-free, but also produced significantly higher marketable root yield compared to susceptible local varieties. As suggested by [30], such genotypes can potentially be considered as parental breeding stocks for CBSD resistance breeding. The genotypes should however be subjected to further molecular characterisation to identify underlying tolerance or resistance mechanisms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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