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APPROACHES TO DIAGNOSIS AND DETECTION OF CASSAVA BROWN STREAK VIRUS (*POTIVIRIDAE*: *IPOMOVIRUS*) IN FIELD-GROWN CASSAVA CROP

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ABSTRACT

Cassava brown streak disease (CBSD) has been a problem in the East African coastal cassava growing areas for more than 70 years. The disease is caused by successful infection with *Cassava Brown Streak Virus* (CBSV) (Family, *Potyviridae*: Genus, *Ipomovirus*). Diagnosis of CBSD has for long been primarily leaf symptoms-based. This is unreliable due to the irregular pattern and variability of the disease phenotype in roots and leaves. The suitable method to undertake reliable field diagnostic survey and derive acceptable analysis of the disease situation has never been standardized. Zigzag and diagonal approaches for disease assessment have been used successfully on other diseases infecting cassava such as Cassava mosaic disease but neither of them has ever been tested and proven suitable for CBSD assessment. In addition, the suitable sample for successful molecular detection of the causal virus has never been optimised. The number of samples to be collected from large plant stands which would be a true representation of the population has never been determined. The effect of sample bulking on possible detection or non detection of infection particularly when un-infected samples are combined with infected ones is not known. In this study, the comparative efficiencies of diagonal and zigzag approaches to CBSD field diagnosis were tested through surveys conducted in 20 randomly selected farmers' fields in major cassava growing areas of the Coastal and Lake Zones in Tanzania. Using molecular diagnostic techniques, the plant parts which are suitable for *Cassava brown streak virus* (CBSV) detection were determined. Sample bulking was tested for rationalized laboratory detection of CBSV over large cassava stands. The study revealed that CBSD incidences and severities obtained using either diagonal or zigzag approach did not differ significantly. Suitable parts for CBSV detection were identified to be flowers, fruits, apical buds, young tender leaves, newly-opened leaves, youngest symptomatic leaves, the tender top-green portion of the stem and non-necrotic storage root tissues. CBSV was not detected in seeds. In bulked leaf samples, CBSV was detected from ratios of 1:1 up to 1:19 of CBSV-infected to CBSV-free tissues in cultivar Albert. It was concluded that either zigzag or diagonal can be used for CBSD field diagnosis. A choice of the suitable sample is of absolute necessity, and bulking of many samples for collective CBSV detection over a large crop stand is effective.

Key words: Cassava, Disease, Diagnosis, Detection, Sampling

INTRODUCTION

Cassava brown streak disease (CBSD), a disease known in the East African coast since 1936 [1], has been one of the most damaging viral diseases of cassava (*Manihot esculenta* Crantz). More than 70 % of tuberous yield loss per plant has been attributed to CBSD [2]. The disease is caused by *Cassava brown streak virus* (CBSV) (family, *Potyviridae*: genus, *Ipomovirus*), [3, 4]. Cassava brown streak disease has been reported in various countries including Kenya, Malawi, Mozambique, Tanzania and Uganda [4, 5, 6]. Other countries suspected to be affected are Burundi, Democratic Republic of Congo, Rwanda and Zambia [7].

Most published reports on CBSD have been based solely on observable CBSD foliar and root symptoms established during surveys [8, 9]. However, the standard approaches to field diagnosis of CBSD are not yet established. The diagonal method of field traversing has been commonly used to assess the field incidences and severities of CBSD in farmers' fields [10, 11]. This method has also been used in field assessment of cassava mosaic virus disease [12]. On the other hand, the zigzag method of field survey has been used in field assessment of several crop diseases and in some cases, preferentially recommended to other methods [13]. The suitability of the zigzag method was indicated by Turner *et al.* [14], in assessment of the incidence and severity of eyespot disease (*Tapesia* spp.) and sharp eyespot (*Rhizoctonia cerealis*) in winter wheat. Thackray *et al*. [15] preferentially used the method to assess the spread of cucumber mosaic virus in narrow-leaf lupins (*Lupinus angustifolius*). Whether diagonal or zigzag methods of field traversing may lead to similar or significantly different CBSD field indices has never been explored.

Although commonly used, the symptom-based diagnosis of CBSD may not be solely reliable [6, 16]. Recent observations [17] indicated that some plants may exhibit CBSD-like foliar symptoms without being CBSV-infected. In some cases, symptomless plants may be CBSV-infected. Furthermore, variability of CBSD phenotypes in different cultivars and under variable abiotic conditions, and in different organs of cassava such as roots and leaves, may lead to unreliable diagnosis. Consequently, the requirement for additional molecular detection techniques for CBSV becomes important. Molecular techniques for CBSV-detection have been reported [4, 5, 18]. However, the efficiencies of these techniques have never been tested on bulked samples for fast screening of large volume of samples. In addition, the possible detection of virus from infected tissue depends on the use of the right sample [19]. The plant parts that are suitable for optimal CBSV detection in infected cassava have never been delineated. The studies reported here aimed at identifying the best approach for field diagnosis of CBSD, identifying the most suitable plant parts for CBSV detection and standardizing the ratios for sample bulking for rationalized laboratory detection of CBSV over large cassava stands.

METHODOLOGY

Approaches to field assessment in CBSD diagnosis

Field surveys were conducted in 20 randomly selected farmers' fields in major cassava growing zones of Tanzania. These included 10 fields in each of the Coastal and Lake Zones. Diagonal method and Zigzag method [13] were used to traverse across fields and assess foliar and stem incidences and severity of CBSD based on visual symptoms. In each field, 30 plants were assessed. Both diagonal and zigzag methods were tested in parallel in the 20 fields to compare if the two approaches produce different results. In smaller fields with plants less than 30 in one diagonal, a second course of diagonal was tracked to assume an 'X' sect of sampling, likewise for the zigzag a 'Z'. A total of 600 plants were assessed. The survey data were analysed using GenStat 4.24DE statistical package [20], (Lawes Agricultural Trust, UK) for Spearman ranked correlation between the two methods.

Determination of suitable plant samples for CBSV detection

Distribution of CBSV in different parts of cassava plant was determined in four infected cassava cultivars, namely Albert, Cheupe, Kibaha and Nachinyaya. Test samples included seeds, fruit, flower, apical leaf buds, tender young leaves, immediately fully open leaves, mature symptomatic leaves and old-near senescent leaves. The stem samples included peelings from young green tender portion, the middle section of the stem and mature section. Root tissue samples included the total necrotic tissue, thin layer of soft tissue of the necrotic margin, cortical tissue outside the necrotic margin of the test root and the non-necrotic roots from diseased plant (Figure 1).

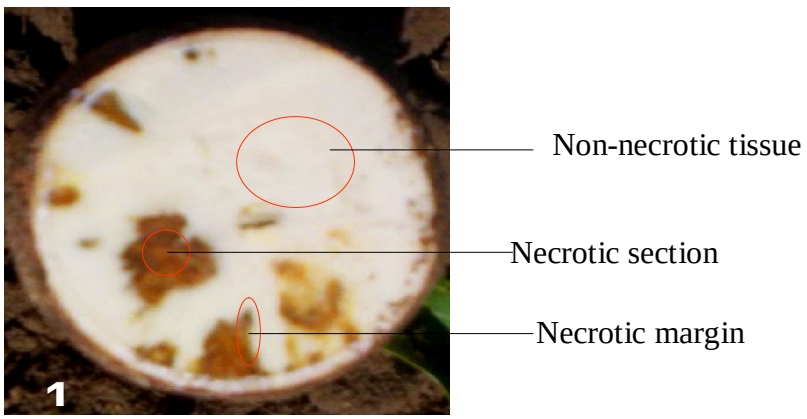

Figure 1: Cross section of CBSD-affected cassava root showing sources of tissues used for CBSV detection. Red circles indicate areas of excision of the tissue.

Five plants were sampled in each of the four test cultivars. Considering the 14 different tissues types sampled, the number of tested samples was 14 tissue types x 5 plants x 4 cultivars totalling to 280 different samples. One CBSV-free leaf sample for each cultivar was included as a negative control during testing. The characteristic

symptoms of CBSD on each of the collected plant/tissue samples were recorded. CBSD incidence was assessed as the percentage proportion of symptomatic leaves or stem. The severity of CBSD was assessed according to the method of Hillocks and Thresh, [8] with minor modifications (Table 1). The presence of an infective virus, CBSV was detected by the reverse transcriptase polymerase chain reaction (RT-PCR) using the (coat protein) CP gene specific primers [4].

Sample bulking

Leaf samples were collected from a third fully open top leaf of the CBSV-infected field grown cassava plants and the potted CBSV-free cassava cv. Albert, pre-tested for CBSV and maintained in the screen-house and bulked at different ratios. The leaf samples bulking ratios (BR) of infected to CBSV-free tissues included 5:5, 5:10, 2:8, 2:18, 1:9 and 1:19. This aimed at testing if the virus may be detected from the mixture of infected and non-infected leaves. Samples were processed as strips and discs. Thus, the BR for each sample was tested in duplicate.

RNA Isolation from Plant Tissues

Total RNA was extracted from 0.1g of fresh leaf, stem or root tissues in 4 M guanidium thiocyanate (Sigma, 59980) buffer mixed with 2-mercaptoethanol (Sigma, M3148) at a ratio of 1:125 (2-mercaptoethanol to guanidium) using a sterile mortar and pestle. Standardization for the amount of extractable RNA from each organ was established by ensuring that equal weight of starting tissues for RNA isolation was maintained, that is 0.1g for each sample. Five hundred micro litres (µl) of the lysate was transferred to a 1.5 ml eppendorf tube, 500 µl of 2.0 M sodium acetate added and the tube contents were thoroughly mixed. Four hundred microliters of 24:1 (chloroform ($CHCl_3$) to isoamyl alcohol (C_5H_2O) was added to the tube, the contents mixed and incubated on ice for 10 minutes. The chilled contents were centrifuged at 13000 rpm for 15 minutes and 450 µl of supernatant transferred to new tubes. Five hundred microliters of ice-cold isopropanol was added to precipitate the RNA and the tubes were incubated at –20 ^{0}C for 10 minutes. The chilled contents were centrifuged at 13000 rpm for 10 minutes and the supernatant discarded. The RNA pellet was air-dried after washing with 500 µl of 75 % ethanol and centrifuging at 13000 rpm for 2 minutes. Finally, 35 µl of RNase free water was added to dissolve the RNA pellet for analysis in RT-PCR.

Nucleic Acids Amplification by RT-PCR

Triplicate RT-PCR was performed in a one-step reaction with superscript ^{TM} III RT/Platinum® *Taq* Mix System (Invitrogen Life Technologies) using a GeneAmp PCR system 9700 (Applied Biosystems, UK). The primers, CBSV 10F and 11R [4] designed to amplify a 231 bp segment of the CP gene were used. Each 50 µl reaction mixture comprised 25 µl of 2X reaction mix [buffer with 0.4 mM of dNTP, 3.2 mM of $MgSO_4$ and stabilizers], 1 µl of RNA, 0.4 µl of each of the forward and reverse primers, 1 µl of superscript RT-Taq (Invitrogen 12574-026) and 22.2 µl of sterile water. Utmost care was taken to maintain the same volume of RNA in the reaction mixture. The PCR conditions were as follows: Initial cDNA synthesis and denaturation at 55°C for 0.5 min and 94°C for 1 min, denaturation at 94°C for 1 min,

annealing at 52°C for 1 min, extension at 72°C for 1 min (in 35 cycles) followed by final extension at 72°C for 10 min.

Analysis of RT-PCR product

RT-PCR products were electrophoretically separated in 1.2 % agarose gels in 0.5X Tris Acetate EDTA (TAE) buffer, for 1 hour at 92 volts. Amplicons were visualised by staining with ethidium bromide (0.01 µl/ml) under ultraviolet (UV) light and recorded using an image analyser (Syngene). A 1kb plus DNA marker (Invitrogen, cat.10787-018) was used on the agarose gels.

RESULTS

Approaches to field assessment on CBSD diagnosis

The Pearson's linear correlation analyses of the survey data suggested that there was no significant difference ($r > 0.9$) between the diagonal and zigzag methods of field traversing in diagnosing the foliar and stem incidences and severity of CBSD (Table 2). Highest correlation value ($r = 0.998$) was obtained on foliar incidences of CBSD by the two methods. The correlation trend was similarly reflected on regression analysis as shown (Figures 2a and 2b).

Detection of CBSV in different plant tissues

Triplicate RT-PCR tests done on different plant organs indicated variation in amounts of PCR product despite being extracted from tissues of equal weight of 0.1g (Figure 3). Since there was no absolute quantification of the actual amount of extracted RNA, this observation was only qualitative based on the intensity of amplification bands. CBSV was detected in almost all live tissues tested except in seeds and peelings from the middle and mature sections of the stem. The virus (CBSV) was not detected from necrotic tissues of the roots. Brighter bands were apparent in samples from non-necrotic root tissues, young tender leaves and the youngest symptomatic leaves of the CBSV-infected plants.

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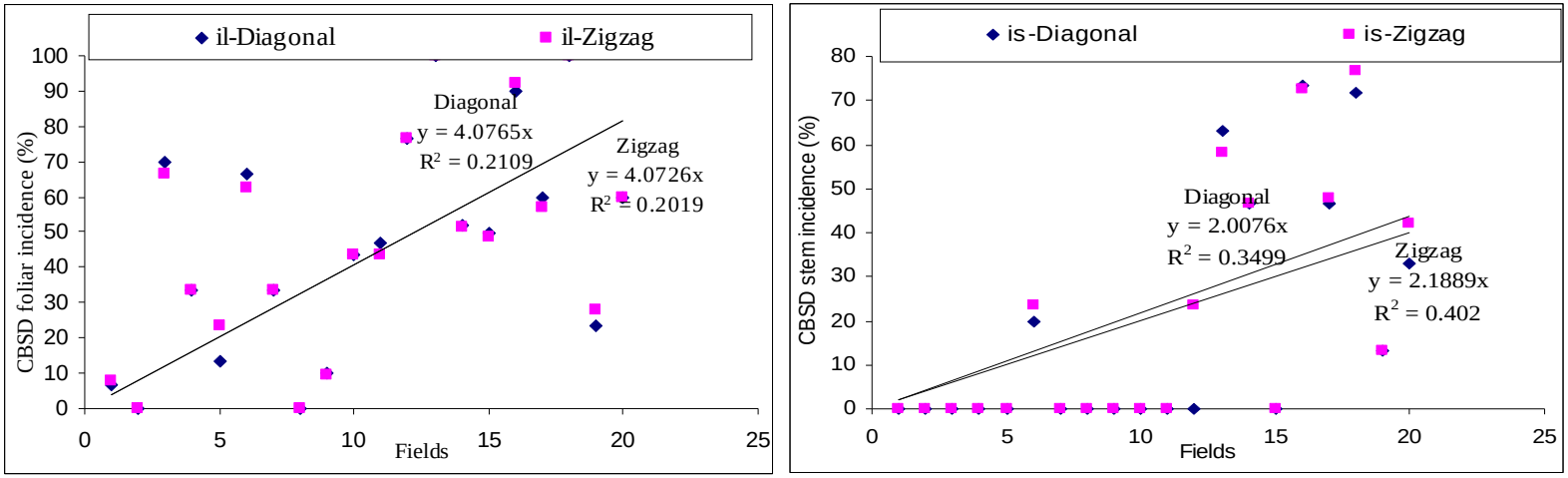

Figure 2a: Relationship between Diagonal and Zigzag method for measuring CBSD foliar (il) and stem (is) incidences

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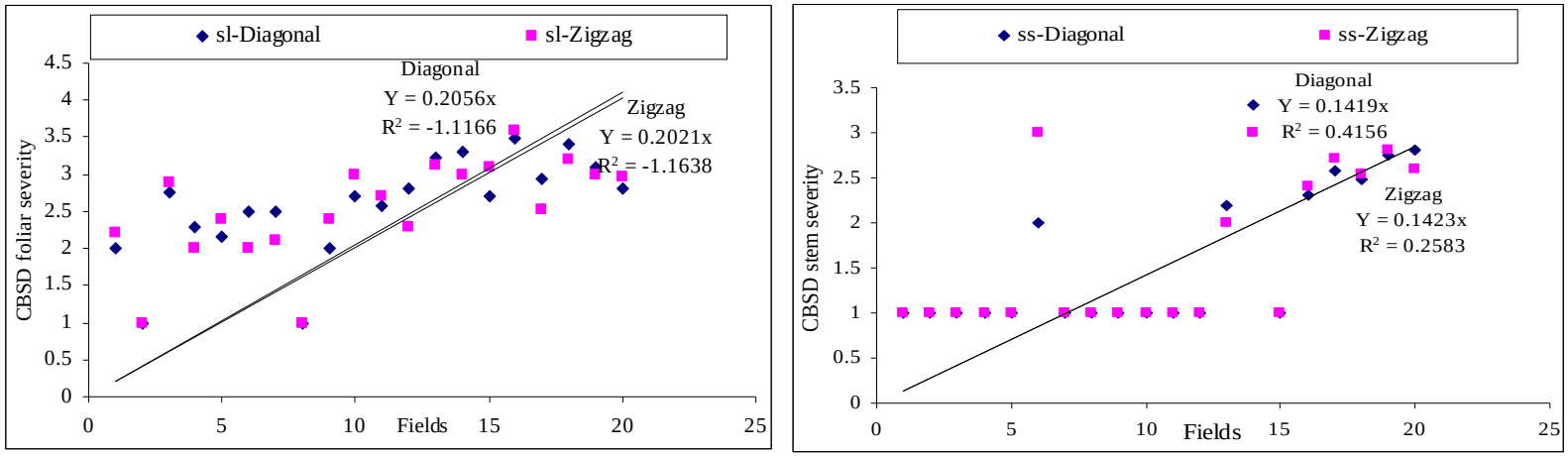

Figure 2b: Relationship between Diagonal and Zigzag method used in measuring CBSD foliar (sl) and stem (ss) severity

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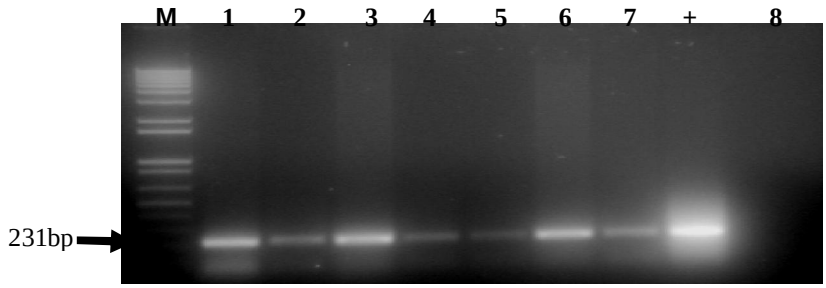

Figure 3: Agarose gel electrophoresis of RT-PCR amplified products (231bp CBSV coat protein gene fragment) using CBSV-specific primer pair CBSV 10F and CBSV 11R. RNA isolated from different parts of CBSD affected plants cv. Albert: M; 1kb plus DNA marker, 1; non-necrotic root tissue from diseased plant, 2; peelings from tender stem with necrotic lesion, 3; young tender leaves, 4; flowers, 5; fruits, 6; youngest symptomatic leaves, 7; mature leaves with CBSD clear symptoms, +; positive control and 8; seed from CBSV-infected plant.

Given the fact that equal weights of tissue were used for RNA extraction and equal volume of RNA used for RT-PCR, the brighter bands suggested the suitability of tissues from these organs for CBSV detection. It should be noted, however, that this judgment is only qualitative, based on band brightness and does not depict the actual quantity of extractable RNA from the studied tissues. Further studies may be required to establish the comparative quantity of extractable RNA from each sample type.

Triplicate assessment of the proportionate distribution of extractable and detectable virus in plant tissue in three other cultivars, Cheupe, Kibaha and Nachinyaya, gave results similar to those of Albert (Table 3). CBSV was detected in flowers, fruits, apical buds, young tender leaves, newly open leaves, youngest symptomatic leaves, tender-top green stem portions and non-necrotic storage root tissues. The virus was also detected from non-necrotic storage root tissues obtained from cv. Nachinyaya, which only exhibited foliar symptoms.

Sample bulking

Test results for CBSV by RT-PCR on bulked samples revealed that the virus could be detected from 1:1 to 1:19 of the CBSV-infected to CBSV-free cassava leaf tissues in cv. Albert (Figure 4). Based on the intensity of amplification bands, the disc and strip techniques of samples preparation prior to CBSV detection did not differ significantly. The two techniques yielded similar results.

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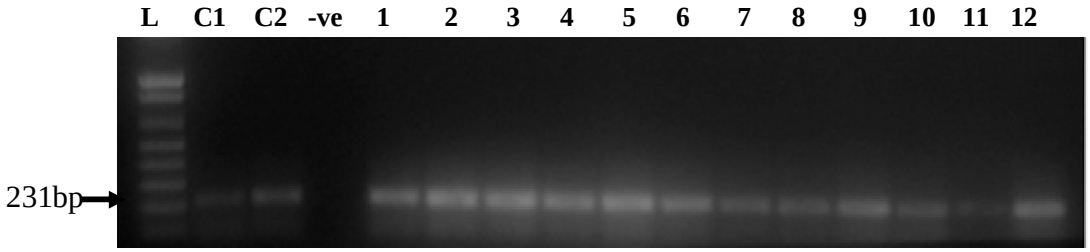

Figure 4: Agarose gel electrophoresis of RT-PCR amplified products (231 bp CBSV coat-protein gene fragment) using CBSV-specific primer pair CBSV 10F and CBSV 11R. RNA isolated from bulked cassava leaf samples cv. Albert processed as discs and strips at varied dilution ratios (DR) of CBSV-free to CBSV-infected tissues. L; 1kb plus DNA marker, C1; positive control disc, C2; positive control strips, -ve; negative control, 1; discs (DR 5:5), 2; strips (DR 5:5), 3; discs (DR 5:10), 4; strips (DR 5: 10), 5; discs (DR 2:8), 6; strips (DR 2:8), 7; discs (DR 2:18), 8; strips (DR 2:18), 9; discs (DR 1: 9), 10 strips (1: 9), 11; discs (1: 19), 12 strips (DR 1: 19).

DISCUSSION

Comparison of methods for field traversing

The field traversing methods used for the CBSD surveys did not differ significantly. This suggests that the same information would be obtained when either diagonal or zigzag method was used for CBSD diagnosis. Although the diagonal method has been previously preferred by some workers in CBSD survey [10, 11], the findings in the current study suggest that the zigzag method may also be used to produce similar results. The fact that there were high correlation values ($r > 0.9$) between diagonal and zigzag methods on CBSD incidences and severities obtained, suggests that CBSD affected plants were somewhat uniformly distributed across the assessed fields.

Suitable samples for CBSV detection by RT-PCR

Cassava brown streak virus (CBSV) was detected from non-necrotic cortical root tissue, peelings from tender stem, young tender leaves, flowers, fruits, youngest symptomatic leaves and mature leaves with clear symptoms. This suggests that most portions of infected cassava plants could be used to sample for CBSV. Based on PCR amplification intensity, however, it was apparent that non-necrotic cortical root tissue, young tender leaves and youngest symptomatic leaves offer the best sample for the RNA isolation technique used. These observations could be explained by the classical studies on virus distribution in plants inoculated with *Tobacco mosaic virus* (TMV), which was found to move from the point of inoculation to the root system first, successfully infecting the youngest leaves and thereafter, the older leaves [21]. Thus, cassava roots and tender actively-growing leaves are assumed to contain a higher virus titre than the older leaves.

In addition, the presence of limited amounts of phenolic compounds in young tissue [22] could also explain these observations. The detection of CBSV in root tissue provides a basis for separating root necrosis due to CBSD and that caused by invasive facultative soil-inhabiting fungi species [10, 23]. .

Cassava brown streak virus was not detected in seeds, mature stem peelings or necrotic root tissue. This observation suggested an uneven distribution of viruses in infected plants. Similar observation was reported in *Tobacco vein mottling virus* (TYMV), in which the virus concentration in the stem, root system, and the midrib and leaf petiole of expanded leaves was one one-tenth to one-twentieth of that found in the leaf lamina [24]. In a similar study on the distribution of *Tobacco rattle virus* (TRV) in different parts of tulips cv. Apeldoorn, highest concentration of the virus was mainly in the basal stem and basal leaf parts compared to the rest of the plants [25].

The absence of CBSV in seeds obtained from diseased plants was consistent with findings of Maruthi *et al.* [18]. The detection of CBSV in flowers and fruits of CBSV-infected plants but not in seeds suggests the possible existence of mechanisms that excludes the virus from the embryo as reported by Carroll [26] in *Barley stripe mosaic virus* (BSMV). This suggests the certainty of regenerating CBSV-free cassava plants from seed. Failure to detect CBSV in peelings from mature infected stems suggests that the cell wall lignification and high phenolic compounds, compared to young tender stems (where CBSV was detected), could have negatively impacted on RNA extractions. Stem tissues of most plants are known to contain lignified compounds and cellulose [22, 27, 28]. Viruses are not likely to target cells in lignified non-dividing tissue. Virus extraction from this lignified tissue may be difficult, causing unavailability or limited amount of detectable viral RNA. Other workers have reported difficulties in extraction of nucleic acid from woody species such as grapevines (*Vitis vinifera* L) [27]. Based on these results, it was established that stem peelings may not make a good sample for CBSV detection.

Cassava brown streak virus was neither detected from the necrotic tissue nor the necrotic margin of the root cortex. This could be explained by the obligate nature of viruses [23]. The necrotic portions of plant tissues are usually comprised of dead plant cells, which may not support the survival of viruses [29]. This observation further confirms the observations in other studies [17] in which CBSV could not be transmitted through infected root debris because the virus does not survive in dead tissues.

CBSV detection in bulked samples

The virus could be detected in a mixture of CBSV-infected and CBSV-free samples bulked at different ratios to utmost 1:19 (infected to CBSV-free), suggesting that many samples could be bulked for CBSV detection. This makes it easy to assess large fields from which collection of a single sample would not truly represent the whole field. It also reduces the number of samples that would be required to be tested particularly on general

testing for the presence of CBSV. Similar sample bulking techniques have been used in diagnostic studies of other important viruses such as *Citrus tristeza virus* [30, 31].

CONCLUSION

The current study demonstrated that the diagonal and zigzag methods of traversing the field during CBSD diagnostic survey do not yield significantly different disease data. CBSV was demonstrated for the first time to be unevenly distributed through the plant system. *Cassava brown streak virus* was detected in flowers, fruits, apical buds, young tender leaves, newly-opened leaves, youngest symptomatic leaves, and the tender top green portion of the stem and non-necrotic storage root tissues. The most suitable tissue samples for CBSV-detection were young tender leaves, youngest symptomatic leaves and the non-necrotic storage root tissues. The virus may be detected in bulked samples at a wide range of dilution ratios from 1:1 to 1:19.

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Table 1: Description of visual diagnostic scale for CBSD as used during field survey

Disease parameter	Plant part assessed	symptomatic /damaged	Score scale	Description
CBSD severity	Foliar	0 %	1	None of the leaves has chlorosis characteristic for CBSD
		1 - 5 %	2	Slight chlorotic spots characteristic of CBSD seen on leaves
		5 - 12 %	3	CBSD chlorotic spots/blotches easily observable on leaves
		12 - 30 %	4	Appreciable CBSD chlorotic spots/blotches seen on leaves
		30 - 100%	5	Very severe chlorotic/necrotic blotches and leaf wilt
	Stem	0 %	1	No necrotic spot or lesion is seen on stem
		1 - 5 %	2	Slight chlorotic spots on tender portion of the stem
		5 - 12 %	3	Necrotic spots are numerous, coalesced to small lesions
		12 - 30 %	4	Severe necrotic lesions enlarged into streaks
		30 - 100%	5	Severe necrotic lesions, streaks, withering and die-back
	Root	0 %	1	None of the roots has necrosis characteristic for CBSD
		1 - 5 %	2	Small portion of roots bears necrotic spots
		5 - 12 %	3	Appreciable proportion of the roots is obviously necrotic
		12 - 30 %	4	Roots mostly necrotic, not suitable for consumption
		30 - 100%	5	Roots are almost/totally necrotic, started

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Table 2: Partial linear correlation values between the diagonal and zigzag methods for CBSD incidences and severities based on diagnostic foliar and stem symptoms

Test parameters	IL-Random	IL-Zigzag	SL-Random	SL-Zigzag	IS-Random	IS-Zigzag	SS-Random	SS-Zigzag
IL-Random	1.000							
IL-Zigzag	0.998	1.000						
SL-Random	0.747	0.741	1.000					
SL-Zigzag	0.697	0.701	0.813	1.000				
IS-Random	0.723	0.732	0.408	0.603	1.000			
IS-Zigzag	0.766	0.778	0.442	0.578	0.979	1.000		
SS-Random	0.347	0.360	0.435	0.498	0.677	0.673	1.000	
SS-Zigzag	0.433	0.442	0.448	0.440	0.719	0.722	0.943	1.000

Key to abbreviations used in table 2: IL; CBSD foliar incidence, SL; CBSD foliar severity score, IS; CBSD stem incidence, SS; CBSD stem severity score

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Table 3: CBSV detection in different plant tissues of CBSD-affected cassava plants

*Plant parts tested	Tested cultivars and band strength			
	Albert	Cheupe	Kibaha	Nachinyaya
Flowers	+	+	+/-	+
Fruits (symptomless)	+	+	+	+
Seeds	-	-	-	-
Apical buds	++	++	++	++
Young tender leaves (symptomless)	++	++	++	++
Newly open leaves (symptomless)	++	++	++	+
Youngest symptomatic leaves	++	+	++	++
Mature leaves (clear symptoms)	+/-	+/-	+/-	+/-
Senescent leaves	-	-	-	+/-
Tender, top green part of the stem	+	+	+	+/-
Mid stem peelings	-	-	-	-
Bottom woody peelings	-	-	-	-
Necrotic root tissue	-	-	-	-
Necrotic margin tissues	-	-	-	-
Non-necrotic cortical root tissue	++	++	++	++

* Different plant parts tested for the presence of CBSV. Sources were four CBSD-affected cultivars obtained from field plants at SRI. Annotations to RT-PCR amplification band strength are; -; negative (no amplification band), +; normal & clear amplification band, ++; very clear amplification band, +/-; amplification band present but not clear

REFERENCES

1. **Storey HH** Virus Diseases of East African Plants VI. A progress report on studies of the disease of cassava. *E. A Agric. J.*, 1936; **12**: 34-39.

2. **Hillocks RJ, Raya MD, Mtunda K and H Kiozia** Effects of cassava brown streak virus disease on yield and quality of cassava in Tanzania. *J. Phytopath.* 2001; **149**: 389-394.

3. **Adams MJ, Antoniw JF and CM Fauquet** Molecular criteria for genus and species discrimination within the family *Potyviridae*. Arch. Virol. 2005; **150**: 459-479.

4. **Monger WA, Seal S, Isaac AM and GD Foster** Molecular Characterization of cassava brown streak virus coat protein. Plant Pathology 2001; **50**: 527-534.

5. **Alicai T, Omongo CA, Maruthi MN, Hillocks RJ, Baguma Y, Kawuki R, Bua A, Otim-Nape GW and J Colvin** Re-emergence of Cassava Brown Streak Disease in Uganda. Plant Disease 2007; **91**: 24-29.

6. **Jennings DL** Observations on virus diseases of cassava in resistant and susceptible varieties to brown streak disease. *Emp. J. Exp. Agric.* 1960; **28:** 261-269.

7. **EARRNET.** Statement on the Consequences of New Spread of Cassava Brown Streak Virus Disease for Movement of Cassava Germplasm in the East and Central African Region. *Int. Inst. Trop. Agric.* 2pp; 2005. www.iita.org. Retrieved 7th August, 2008.

8. **Hillocks RJ and MJ Thresh** Cassava Mosaic and Cassava Brown Streak Virus Diseases in Africa: a comparative guide to symptoms and aetiologies. Natural Resources Institute, UK. 10pp, 1998.

9. **Hillocks RJ and DL Jennings** Cassava Brown Streak Disease: a review of present knowledge and research needs. *Int. J. Pest Man.* 2003; **49**: 225-234.

10. **Hillocks RJ** Cassava virus disease and their control with special reference to southern Tanzania. Integrated Pest Management Reviews 1997; **2**: 125-138.

11. **Legg JP and MD Raya** Survey of cassava virus diseases in Tanzania. International *J. Pest Man.*, 1998; **44:** 17-23.

12. **Fargette D, Fauquet C, Grenier E and JM Thresh** The spread of African Cassava Mosaic virus into and within cassava fields. *J. Phytopath.* 1990; **130**: 289-302.

13. **Anonymous** Field Corn Scouting Procedure. Cornell University Dairy and Field Crops IPM, 1994. http://www.nysipm.cornell.edu/fieldcrops/scouting_info/fcorn/procedures/field.pdf . Retrieved 5th March, 2009.

14. **Turner AS, Nicholson P, Edwards SG, Bateman GL, Morgan LW, Todd AD, Parry DW, Marshall J and M Nuttall** Evaluation of diagnostic and quantitative PCR for the identification and severity assessment of eyespot and sharp eyespot in winter wheat. Plant Pathology 2001; **50**: 463-469.

15. **Thackray DJ, Jones RAC, Bwye AM, and BA Coutts** Further studies on the effects of insecticides on aphid vector numbers and spread of cucumber mosaic virus in narrow-leafed lupins (*Lupinus angustifolius*). *Crop Protection* 2000; **19:** 121-139.

16. **Jennings DL** Further studies in breeding cassava for virus resistance. *E. A. Agric. J.* 1957; **22**: 213-219.

17. **Rwegasira GM** Aspects of Epidemiology of Cassava Brown Streak Virus Disease in Tanzania. PhD Thesis. University of the Witwatersrand, South Africa. 2009; 220pp.

18. **Maruthi MN, Hillocks RJ, Mtunda, K, Raya MD, Muhanna M, Kiozia H, Rekha AR, Colvin J and JM Thresh** Transmission of *Cassava brown streak virus* by *Bemisia tabaci* (Gennadius) *J. Phytopath*, 2005; **153**: 307-312.

19. **Lodhi MA, Ye GN, Weeden NF and B Reisch** A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Pla. Mol. Biol.Rep.*, 1994; **12**: 6-13.

20. **GenStat 4.24DE** The GenStat Discovery 2nd Edition. Lawes Agricultural Trust. Rothamstead Experimental Station. 2005. http://discovery.genstat.co.uk.

21. **Samuel G** The movement of *Tobacco mosaic virus* within the plant. Annals of Applied Biology 1934; **21**: 90-111.

22. **Lerner HR** Plant Responses to Environmantal Stresses: From Phytohormones to Genome Reorganization. CRS Press. 730pp, 1999.

23. **Matthews REF** Plant Virology. Third Edition. Academic Press Inc.,UK. 835pp; 1991

24. **Nichols RFW** The brown streak disease of cassava: distribution, climatic effect and diagnostic symptoms. *E. A. Agric. J.*. 1950; **15**: 154-160.

25. **Van der Vlugt CIM, Linthorst HJM, Asjes CJ, Van Schadewijk AR and JF Bol** *Eur. J. Plan Path* 1988; **94**: 149-160.

26. **Carroll TW** Seed transmissibility of two strains of *Barley stripe mosaic virus*. *Virol.*, 1972; **48**: 323-336.

27. **Newbury HJ and JV Possingham** Factors Affecting Extraction of Intact Ribonucleic Acid from Plant Tissues Containing Interfering Phenolic Compounds. *Pla. Physiol.*, 1977; **60**: 543-547.

28. **Sharma KK, Lavanya M and V Anjaia** A Method for Isolation of Peanut Genomic DNA Suitable for Analytical Applications. Plant molecular Biology Reporter 2000; **18**: 393a-393h.

29. **Agrios GN** Plant Pathology. Fourth Edition, Academic Press. New York. 1997; 561pp.

30. **Herron CM and HH Sabah** Incidence of *Citrus tristeza virus* and *Toxoptera citricida* in Belize. *J. Trop. Agric*. 1997; **74**: 1-5.

31. **Hughes G and TR Gottwald** Survey methods for assessment of Citrus tristeza virus incidence. Phytopathology 1998; **88**: 715-723.