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Occurrence and Distribution of Cassava Brown Streak Viruses in Western Kenya

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ABSTRACT

A survey to investigate the distribution and occurrence of cassava brown streak viruses the causal agent for cassava brown streak disease (CBSD) was conducted in major Cassava growing areas of Western Kenya in November, 2011. The highest mean disease incidence was in Busia County (11.5%) while the lowest incidence was in Homa bay County (1.5%). High mean severity score (3) was observed in farmer's fields in Busia (Mungatsi, Matayos and Mundika divisions) but all the other areas showed no foliar symptoms. CBSD incidence correlated positively with disease severity on the leaves ($r= 0.7$, $p< 0.05$) and stems ($r= 0.9$, $p<0.05$). Polymerase chain reaction (PCR) detected single infections of CBSV and UCBSV in 64% and 3.4% of the positive reactions respectively. The study revealed a first record of Uganda cassava brown streak virus (UCBSV) in the Western Kenya. Mixed infections of CBSV and UCBSV were not found in any of the samples from the surveyed fields. The widespread occurrence of CBSV in Western Kenya has implications in the management of the disease in the region

Keywords: *Disease Incidence; Disease Severity; Foliar symptoms; PCR; UCBSV.*

INTRODUCTION

Cassava, (*Manihot esculenta* Crantz) is an important crop in Western and coastal regions of Kenya, grown for both food and income generation. It is among the leading food crops of the world ranked fourth among major staples (Nassar, 2002). It is drought-tolerant and tolerates poor soils (Mabrouk A. & El-Sharkawy, 2012). However, cassava production in Kenya is constrained by lack of well adapted varieties, shortened fallow period and declining soil fertility, access to good quality planting materials, variety improvement adoption, Crop production systems, pests and diseases (CAB International, 2002). Among diseases Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD) are the most important. Cassava Brown Streak Disease is caused by two distinct virus species, the coastal endemic virus, referred to as Cassava brown streak virus (CBSV), and the highland epidemic virus, Uganda Cassava brown streak virus (UCBSV). Both species belong to the genus *Ipomovirus*, family Potyviridae (Winter, 2010) and are transmitted by the whiteflies (*Bemisia tabaci* Gennadius) (Mware, 2009).

Most disease diagnostic surveys on cassava diseases have focused on Cassava Mosaic Disease which has been identified as a major constraint to cassava production in Western Kenya. In fact severe CMD in Busia and Teso Districts of Western Province in 1995/96, prompted a major disease diagnostic survey in Western Kenya in 1997 (Legg 1999). Obiero, (2007) in a report monitoring and diagnostic survey of cassava mosaic virus disease (CMD) in Western Kenya indicated the dominance of CMD and the resurgence of CBSD in the region. Furthermore, his studies revealed that the CMD resistant varieties that were being supplied to farmers were in essence susceptible to CBSD. Similarly, there have been attempts by the Great lakes Cassava Initiative (Cassava Disease Surveillance Surveys 2009) to monitor cassava diseases through GIS mapping model.

It should be noted that effective CBSD management depends on a sound understanding of the status of the disease, and patterns of regional spread. Knowing which regions are worst affected, and which are currently threatened is vital for the effective formulation of control interventions.

As part of the efforts to mitigate the effects of this disease and guide control interventions, a disease diagnostic survey was conducted to monitor changes in disease incidence, severity and spread in Western Kenya and therefore give an update on the CBSD status in region, with a view to provide data that would be useful in development of control strategy of the disease.

MATERIALS AND METHODS

A survey for Cassava Brown Steak Disease was conducted in major cassava growing areas of Western Kenya in November, 2011. The areas surveyed were the counties of Bungoma, Busia, Siaya, Homabay, and Migori (Figure 1). Fields were selected at regular intervals along major and feeder roads. Each field was sampled for foliar symptoms, 30 plants of the dominant cultivar were examined along two diagonals. Names of other cultivars found in each sampled field were recorded.

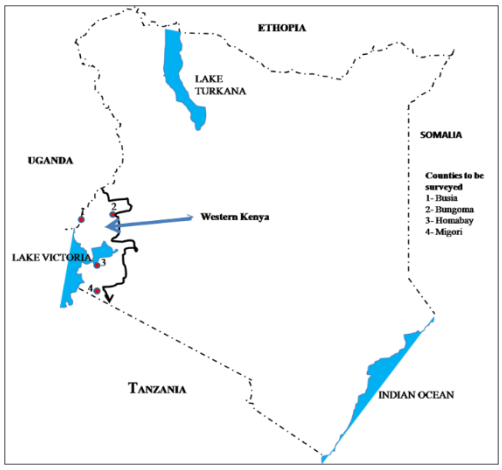

Figure 1. Map of Kenya Showing the areas sampled

Leaf and stem symptom severity were scored according to a five point scale developed by (Gondwe, 2003). In fields sampled for tuberous root symptoms, 5 plants were uprooted and the tuberous roots transversely sliced to check for root necrosis. Root symptoms were scored as proposed by (Gondwe, 2003). Disease incidence was calculated according to James, (1974) as the number of symptomatic plants expressed as a percentage of the total number of plants assessed. Using GPS (Triton 'windows CE core 5.0' X11-15302) readings of altitude, latitude and longitude of each site were recorded. Symptomatic leaf samples were collected and placed in polythene bags and kept in a cool box until use

Sample collection

A total of 131 symptomatic and asymptomatic leaf samples were collected and analyzed for viruses' detection by the:

Triple Antibody ELISA

Triple antibody sandwich ELISA was basically conducted as described by Thomas, (1986) with minor modifications using polyclonal antiserum (AS-0912) raised against particles of CBSV for coating and monoclonal antibodies that are specific for each cassava brown streak virus for detection.The reference antisera (AS-CBSV) for cassava brown streak were kindly provided by Dr. S. Winter of the German Collection of Microorganisms and cell cultures (DSMZ), Braunschweig, Germany.

Polymerase Chain Reaction

Total RNA was extracted from fifty six cassava leaf samples using the RNeasy plant min kit (Qiagen) according to manufacturer's instructions. Approximately 0.5 µg of genomic RNA was used for RT-PCR amplification. This was done in a 50- µl reaction following a one-step protocol in which cDNA synthesis was linked to PCR in the same mixture using the Superscript III/ Platinum Taq mix Invitrogen) following the manufacturer's instructions. A Qiagen one step RT-PCR mixture consisting of 1X QIAGEN OneStep RT-PCR Buffer, dNTP Mix (containing 10 mM -2.0 µl 400 µM of each dNTP of each dNTP), RNase inhibitor-5 unit, Template RNA-2µg and CBSV specific primers CBSVgenR CTCAACAGCTCTCCACGATTT and UCBSVgenF ACGTGCCTCCATCACATCBSV designed for detection of all CBSV isolates and UCBSVgenR ATTTCCAGGTTCCTTTGTCAC and UCBSVgenF AACAGACATACGTGTGCAT were used.

RESULTS AND DISCUSSION

Results

Surveyed areas

The surveyed farms were located between longitudes 0019085^{0}E (a farm in Ugenya) and 9955378^0 E (a farm in Rachuonyo) and latitudes 06272000N (a farm in Busia) and 091342^{0}S (a farm in Rachuonyo). A total of 33 farms were surveyed. In terms of altitude, Teso South was the lowest being 1181 masl (meters above sea level) while Rachuonyo 1467 masl was the highest (Table1). The mean altitude of the areas sampled was 1240.9 m. A total of 131 cassava leaf samples and 33 hardwood stem cuttings were collected from farmer's fields.

Symptoms of cassava brown streak disease in the field

A range of symptoms expressed on leaves, stems and roots of infected cassava plants were observed during the survey. However, expression of the symptoms was dependant.

on the cassava cultivar encountered. Nyakatinegi cultivar showed mild foliar symptoms although tubers had constrictions and relatively higher root disease severity. Some cultivars for instance Selele cultivar was severely affected by the disease. Cassava brown streak disease expressed as an irregular yellow blotchy feathery chlorosis which was more pronounced on lower mature leaves as in (Figure 2).

Figure 2. Symptoms of CBSD: A-Cassava stem with brown streaks, B-Cassava tuber with hard brown corky rot and C- cassava plant with mottled appearance. (*Picture taken in Matayos, Busia*)

The chlorosis on the cassava leaves was often associated with secondary and tertiary veins infections. In cassava tubers, CBSD was noted as causing a dry corky rot that was most pronounced in the periphery of the root cortex (Fig.2). This makes the tuber unpalatable and decreases its market value.

CBSD Incidence

Mild mean CBSD incidences were found in Namasanda (1.7%), Bumula (3%), Okame (2%), Ojame (3%), Okatekok (3%), Buyende (3%), Sega (3%), Rangwe (3%) and East Kamagok (1%) (Table 1). The highest CBSD incidence was recorded in Mundika (30%). Other areas where relatively high disease incidence was registered included Mungatsi (17%), Angorom (10%), Busibwabu, (15%), Matayos (25%) and Ugunja (13%) (Table 1).

CBSD incidence correlated negatively with altitude ($r= -0.5568$ $P<0.05$). Generally, there was low leaf incidence in high altitude areas and high leaf incidence in low altitude areas. Disease incidence correlated positively with disease severity on the leaves ($r= 0.5503$, $p<0.05$). Leaf Viral disease incidence was higher in samples with high disease severity. Similarly, disease incidence correlated positively with severity on the stem ($r =0.5986$, $p<0.05$). There was significant difference in viral disease incidence between the counties ($p\geq.039$, $\alpha= 0.05$).

That each county be accorded special attention in the management of CBSD.

Table 1. Summary table showing location, altitude, ELISA, PCR results, mean disease incidence and severity of sampled areas

Farm S/No	County	Sampled Areas	Number of samples	Altitude(m)	ELISA results CBSV +	PCR results CBSV	PCR results UCBSV	Visual Results	Mean incidence in %	Mean Severity		
										leaves	Stems	Roots
1	Bungoma	Bungoma South	8	1282	3	5	0	4	1.7	1	1	1
2		Bumula	10	1295	1			0	0	1	1	-
3		Bumula	6	1248	1	2	1	3	3	1	2	-
4		Bungoma South	5	1247	0	5	0	4	6	1	2	2
5		Bungoma	5	1247	0			0	8	1	2	-
6	Busia	Busia	2	1214	1	0	0	0	17	2	3	4
7		Teso South	5	1185	1			0	10	2	2	3
8		Teso South	5	1185	1			0	3	2	2	-
9		Teso South	5	1185	1			0	2	1	2	-
10		Teso South	3	1181	0	0	0	0	3	1	2	-
11		Busia	5	1161	2	4	0	4	7	2	2	-
12		Busia	5	1249	3				15	2	3	2
13		Busia	4	1242	1	4	0	1	30	2	3	-
14		Busia	3	1243	3	3	0	3	3	1	2	-
15		Busia	2	1243	0	1	0	0	25	2	4	4
16	Siaya	Sega	5	1241	4				3	1	2	-
17		Ugenya	5	1240	4	1	1	2	7	1	2	1
18		Ugunja	5	1349	0	2	0	1	6	1	2	-
19		Ugenya	2	1242	0	0	0	0	13	1	2	-
20		Ugenya	2	1247	0	0	0	0	7	1	2	1
21	Homabay	Rachuonyo	1	1381	1	1	0	1	1	1	1	-
22		Rachuonyo	1	1384	1	1	0	0	0	1	1	1
23		Rachuonyo	5	1385	0				0	1	1	1
24		Rachuonyo	5	1193	0			0	6	1	1	2
25		Rachuonyo	4	1467	0	4	0	2	0	1	1	1
26		Rachuonyo	5	1460	0				0	1	1	1
27		Homabay	5	1288	5	4	0	3	9	1	2	2
28		Homabay	5	1290	0			2	3	1	2	1
29		Homabay	2	1292	1	1	0	0	0	1	1	-
30		Homabay	3	1288	1			1	0	1	1	-
31		Migori	1	1345	0				0	1	1	1
32		Rongo	1	1346	0	0	0	0	0	1	1	1
33		Rongo	1	1347	0				0	1	1	1
		Total	**131**		**35**	**38**	**2**	**31**				
F-TEST					**S**				**S**	**S**		NS

NS-Not significant, S-Significant

CBSD Disease severity

Most divisions in Busia County (Chakol, Mungatsi, Nambale and Mundika) had a severity score of 2 except Matayos which recorded 4 on cassava stems (Table 1). Mean severity score on leaves was 1.2 while stems was 1.8. In Bungoma county (Mateka division) recorded severity score of 3. Severity score of 2 on stems was recorded in divisions of Miyanga, Chakol, Nambale, Sega, Ugunja, Ugenya, Sihai, Rangwe and Mundika.

There were no CBSD symptoms recorded on cassava leaves in divisions of Kanduyi, Mateka, Miyanga, Amukura, Sega, Ugunja, Ugenya, Sihai, Kasipul Kodongo, Kawere, Rangwe, Rodi, Maridi and Rongo though tubers were symptomatic. There was no correlation between disease severity on leaves and disease severity on stems ($r=0.1116$, $P<0.5$).

Viral disease severity on leaves and stems correlated negatively with altitude ($r=-0.8550$, $P<0.05$) and ($r=-0.2503$, $P<0.05$) respectively. There was significant difference in disease severity on leaves within the counties surveyed $p\leq0.003$, $\alpha=0.05$ and stems $p\leq0.001$, $\alpha=0.05$ respectively. The highest CBSD root severity was scored in farmers' fields in Busia County with severity score of 4 while the lowest was in Homabay County with a score of 1.

Enzyme Linked Immunosorbent Assay (ELISA) Results

From the 131 samples collected, 35 (26.7%) tested positive for cassava brown streak virus. Only 4 samples symptomatic tested negative for CBSD with TAS-ELISA. Siaya County recorded the highest percentage of samples that tested positive 42.1%, Busia county 33.3%, Homabay 23.7% while the lowest was Bungoma County with 14.7% (Table 1).

Polymerase chain reaction results

The most dominant virus strain was CBSV (64%) mostly occurring in Rachuonyo, Homabay, Rongo, Bungoma, Siaya and parts of Teso. UCBSV was only found in two samples 20 and 26 (Fig 3) which were collected from Bumula and Ugenya respectively that were symptomatic for CBSD in the field.

The highest percentage of positive samples were recorded in samples from Rachuonyo (100%), Ndenga, (80%) and Busia (70%) while the lowest number recorded in samples from Bumula, Bungoma South (10%), Ugenya (10%) and Syekumulo, (16.7%) (Table 1).

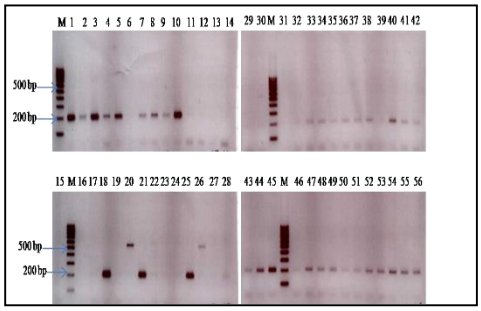

Figure 3. Gel electrophoresis of 56 PCR products amplified from CBSV and UCBSV infected samples: Lane M-100 bp ladder marker, 1-2 Rachuonyo, 3- 10 Segero Nambale, Busia, 11-13, 22-26 Ugenya, 14-16, Rongo, 17-21, 15-16 Bumula, Bungoma South, 27-31 Teso south, 32-37 Lunao, Bungoma South, 38-43 Syekumuko, Bungoma South, 44-45 Ukwala, Sihai, 46-52 Kochia, Homabay and 53-56 Matayos, Busia

None of the samples from Rongo, Ukwala (Sihai), Rachuonyo (Kodongo) and Kimaeti, tested positive for cassava brown streak virus. In total only 32.6% of the samples tested negative for CBSD viruses. Cassava brown streak disease was found in all the divisions' surveyed during the study except for Lunao, Rongo, Ndega and Ugenya. Visually, in the field only 17% of the samples collected were symptomatic for CBSD (Table 1).

RT-PCR results showed that 67% of the samples tested positive for Cassava brown streak viruses.

There were no CBSD foliar symptoms on cassava found in farms in Bumula, Mateka, Kamagok, Kasipul, Sino, Rachuonyo, Kosele, Homabay, Migori and Rongo (Table 1).

Distribution of cassava brown streak viruses

Based on survey and laboratory results a map of Western Kenya showing the distribution of the two cassava brown streak viruses was drawn (Fig 3). Cassava brown streak virus was the most dominant.

Figure 3. Map of Western Kenya showing the distribution of cassava brown streak viruses

DISCUSSION

The results of this study demonstrate a wide distribution of the disease in almost all cassava growing areas which confirms that other areas in the East African region previously unaffected by CBSD are now at risk of spread and increased prevalence of the disease. The only known causal agent for the disease before 2010 was cassava brown streak virus. However, later on Winter, (2010) confirmed the existence of two species of cassava brown streak viruses (CBSV and UCBSV).Mohammed, (2012) associated the CBSD in midaltitude areas with the new virus UCBSV. He reffered the coastal endemic virus as cassava brown streak virus (CBSV), and the highland epidemic virus as Cassava brown streak Uganda virus (UCBSV). This study has shown that the most common of the cassava brown streak viruses in Western Kenya is cassava brown streak virus (64%) while Uganda cassava brown streak virus accounted for 3.4%. A variety of factors have been implicated in the emergence of new plant viruses, including an expanded range of host and vectors, changes in climate and environment, new agricultural practices and the increasingly global movement of humans populations and plant products (Roossinck, 1997). High genetic variability has been observed in cassava brown streak virus (Mbazibwa, 2011). As a result of this there is likelihood of emergence of strains of the original species. The main sources of variation have been attributed to mutations and recombination. Recombination is known to be one of the main driving forces of evolution (Chare and Holmes, 2006; Pagan and Holmes, 2010). It has been reported in both RNA and DNA viruses that (Valli, 2007;Mangrauthia, 2008). There have been reports that recombination of 50% amongst cassava brown streak virus isolates have been observed in isolates from Uganda and north-western Tanzania (Mbazibwa, 2011). Though prevalent, cassava brown streak virus in this region may differ significantly with coastal lowland strain and could interact and recombine to produce a more virulent strain. It may be important to carry out a comprehensive survey and sequence samples that look strange to ascertain the exact strains currently available in the region.

This is a first report of UCBSV in Western Kenya, which is one of the CBSD-associated viruses responsible for the upsurge of CBSD in Uganda. For a long time since confirmation of cassava brown streak virus as the causative agent of cassava brown streak disease by Lister (1959), it had been known that the virus was endemic to the eastern and southern coastal areas. Proof of its existence and widespread nature in areas higher than 1000 m and inland areas has been a matter of concern (Alicai, 2007). The virus has been shown to be virulent just like CBSV although both have not been detected in a single cassava cultivar, a fact that this study also confirmed. There has not been any report of co-infection of CBSV and UCBSV (Abarshi, 2010). However, mixed infections with viruses are known to be common place (Mathews, 1991). For plant viruses infecting cassava, CMGs have been shown to co-infect cassava in Africa (Alabia, 2008;Omuse, 2013) and better still interactions have been reported between CMGs and CBSVs in *Nicotiana benthiamana* (Ogwok, 2010). Irungu, (2009) reported interactions among plant viruses that include cross protection, replacement, mutual suppression in some experimental hosts. Studies by Mbazibwa, (2011) have indicated that the two viruses may have evolved differently from a common ancestor. More studies have revealed that simultaneous and distinct detection of viruses in single and dual or mixed infections is crucial in breeding for single and broad spectrum resistance (Mbazibwa, 2011). Moreover, understanding of the distribution of single and mixed infection could be useful in preventing these viruses from spreading to new areas. Identification of both the two species of cassava brown streak viruses has further implications for CBSD disease management and quarantine requirements in the region and further enriches the knowledge on epidemiology of the viruses.

High mean CBSD incidences recorded in farmer's fields in Busia County in divisions of Mundika, Matayos and Mungatsi. High CBSD incidences in this county can be attributed to planting of contaminated planting material sourced from other farmers. This indicates that each county should be accorded special attention in the management of the pandemic. A disease incidence of 30% as reported in some of the areas for instance Busia, can damage root surface area decreasing the market value of cassava drastically thus requiring immediate interventions. There are some sections of the results that have not been discussed, please do discuss them.

CONCLUSION

Cassava Brown Streak Disease is widely distributed in Western Kenya with Uganda Cassava Brown Streak Virus recorded the first time in the region

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