ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 15 No. 4
September 2015
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

EFFICIENCY OF NON-VECTOR METHODS OF *CASSAVA BROWN STREAK VIRUS* TRANSMISSION TO SUSCEPTIBLE CASSAVA PLANTS

Rwegasira GM*[1] and MER Chrissie[2]

Gration Rwegasira

*Corresponding Author's e-mail: grwegasira@yahoo.co.uk

[1]Department of Crop Science and Production, Sokoine University of Agriculture, P. O. Box 3005 Chuo Kikuu, Morogoro, Tanzania

[2]School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, Private Bag X 3 Wits 2050, South Africa

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 15 No. 4
September 2015
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

ABSTRACT

Several etiological and epidemiological studies have been undertaken to determine the disease causal agent and the mechanism of spread of Cassava brown streak disease (CBSD). Until recently, two distinct potyviruses have been reported to cause the disease. These are *Cassava brown streak virus* (originated in Tanzania but most widely spread) and *Ugandan Cassava brown streak virus* (reported in Uganda and a few areas in Tanzania). Limited knowledge on the transmission mechanisms of the virus curtailed the designing of practical CBSD management techniques. Transmission by the whitefly vector, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), and dissemination of virus-infected cuttings are the reported mechanisms through which *Cassava brown streak virus* (CBSV) is mostly spread. However, the occurrence and subsequent spread of the disease in originally un-infected stock and in absence of *B. tabaci* is not uncommon. Thus, the need to explore further, other transmission mechanisms and their efficiency was paramount. In the current study, CBSV was successfully transmitted through a series of non-vector techniques. Subsequent detection and confirmation of CBSV infections were done by RT-PCR using coat protein gene-specific CBSV primers. In replicated screen-house experiments, transmission of CBSV was achieved through cutting tools (22 %) using susceptible cassava cv. Albert as test plants. Up to 54 % transmission efficiency was achieved through sap inoculation of CBSV from infected cassava to CBSV-free cv. Mreteta. Grafting CBSV-free susceptible scions onto CBSV-infected rootstocks was the most efficient CBSV transmission technique with up to 100 % of scions infected within 4-weeks. The infected plants developed characteristic foliar vein chlorosis and blotches on the previously symptomless CBSV-free scions. The virus was not transmitted from infected root debris to cassava seedlings or virus-free cuttings. The findings suggest that the non-vector methods, such as sap transmission, cutting tools and leaf harvesting, could contribute significantly to CBSV spread in field and non-field conditions, such as in propagation nurseries or cassava leaf handling for food. Moreover, grafting was justified to be an effective technique to quickly test for susceptibility or resistance of any newly bred cultivar for CBSD resistance.

Key words: Cassava, Cassava brown streak, Disease, grafting, potyviruses, non-vector transmission, RT-PCR

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 15 No. 4 September 2015 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

INTRODUCTION

Cassava brown streak disease (CBSD) is the second most important virus disease after Cassava mosaic disease (CMD), infecting cassava (*Manihot esculeta* Crantz) in Africa [1]. The disease is caused by two distinct viruses, *Cassava brown streak virus* [2, 3] and *Ugandan Cassava brown streak virus* [4, 5] (family, *Potyviridae*: genus, *Ipomovirus*). The disease was first reported from the East African coast in 1936 [6]. Disease symptoms of CBSD were documented by Nichols [7] and updated later by Hillocks and Jennings [8]. Since CBSD does not occur in Latin America, from which cassava is believed to originate [9] the virus is believed to have come from an unknown host plant in East Africa. No naturally-occurring hosts of CBSV have been recorded to date, other than cassava. The economic loss attributed to the disease in susceptible local cultivars in Tanzania is *ca*. 70 % [10].

Since the first report [6] CBSD was known to be most prevalent in coastal East Africa below 500 meters above sea level (masl) [7]. It was rarely observed above 1000 masl [11, 12, 13, 14]. Recent observations indicated the wide spread occurrence of CBSD in all cassava growing areas in Tanzania regardless of altitude [15]. In addition, a new outbreak was reported from parts of Uganda all above 1000 masl [16]. The cause for such recent wide-spread detection of the disease is not known.

Transmission of CBSV from one plant to another is reported to occur through grafting CBSV-free with infected cuttings [6, 7, 17, 18] and subsequent dissemination by infected cuttings. However, natural spread of CBSD to originally uninfected cassava has been reported [19, 20]. Suspected transmission of CBSV by the whitefly vector *B. tabaci* [18] was confirmed in greenhouse and field experiments [21], although at rate *ca* 2 %. Previous attempts to transmit the virus by the aphid, *Myzus persicae* Sulz (Hemiptera: *Aphididae*) and other aphid species failed [22].

Since vector transmission was demonstrated to be inefficient under controlled greenhouse and field conditions, non-vector mechanisms could contribute more widely to the spread of the virus. Success with artificial sap transmission of CBSV was reported [17], although the infection rate was not established. Moreover, the reported graft transmission of the virus [6, 18] did not examine the infectivity and efficiency of the technique. The time taken for symptoms to appear when the infected and non-infected parts of the plants are used interchangeably as scion and rootstock was not determined. Other CBSV sap-based transmission methods including cutting tools, leaf harvesting and CBSD-affected root debris (crop residues) on cassava have never been explored. Moreover, the contribution of seed-based transmission of CBSV onto new cassava plants remains unknown.

The current study aimed at identifying other non-vector mechanisms of CBSV transmission and comparing their effectiveness and efficiency. The specific objectives of the study were: (i) to determine the possible role of sap transmission by cutting tools and leaf harvesting in virus infection; (ii) to determine the efficiency of sap and graft-based transmission; (iii) to explore the comparative effect of using infected and non-infected scions and rootstocks in grafting; (iv) to establish if CBSV-infected root debris

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 15 No. 4 September 2015 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

might cause new infection, and (iv) to determine if CBSV is transmitted through seeds of infected plants.

METHODOLOGY

Series of transmission experiments were conducted in the screenhouse. CBSV-free plants for experiments were generated either by meristem tissue culture or obtained as in-vitro cultures from the International Institute of Tropical Agriculture (IITA). All tissues were tested in triplicate by reverse transcriptase polymerase chain reaction (RT-PCR) using CBSV-CP gene specific primers (CBSV10 and CBSV11) [3]. The CBSV transmission experiments included grafting, seeds, CBSV-infected cassava root debris, or mechanical transmission by sap, leaf harvesting or cutting tools (Figure 1). All experiments were replicated (three to six times) and controls were included.

Generation of CBSV-free cassava plants

Cuttings from symptomless plants cv. Albert, Mreteta, Namikonga and Supa were collected from experimental plots at Naliendele Research Institute, Mtwara and pre-germinated at a nursery unit at the Sugarcane Research Institute (SRI), Kibaha in Tanzania. Plant tops were harvested and used for meristem tissue culture at Mikocheni Agricultural Research Institute (MARI) in Dar es Salaam. Triplicate RT-PCR tests using coat protein gene specific primers for CBSV [3] were done on the tissue culture plants (molecular indexing). Only plants proven to be virus-free were used for experimentation.

Figure 1: Non-vector transmission of CBSV:
1A: Sap inoculation of CBSV with buffers A and B. (Buffer A contained 0.05 M monobasic potassium phosphate, 50 mM glycine, 1 % bentonite and 1 % celite in distilled water at pH 9.0. Buffer B contained 0.1M monobasic potassium phosphate and 1 % celite in distilled water at pH 7.5). **1B:** Graft inoculation with CBSV-infected and CBSV-free scion and rootstocks cv. Albert. **1C:** Seedlings from CBSV-infected mother plants. **1D:** CBSV infected root debris as used in 3.3.5. **1E:** Harvested leaves with contaminated hands for CBSV transmission. **1F:** Plants stems chopped down using a knife (cutting tools) initially cut through an infected stem.

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 15 No. 4 September 2015 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Sap inoculation experiments

Sap transmission experiments (Figure 1A) were conducted in the screenhouse at MARI from March to December 2007. Two formulations of inoculation buffer (annotated as SA and SB) were prepared and used to sap-inoculate CBSV-free tissue culture-generated cv. Mreteta plants. Buffer SA comprised 0.05 M potassium phosphate monobasic (KH_2PO4), 50 mM glycine ($C_2H_5NO_2$), 1 % bentonite and 1 % celite (SiO_2), dissolved in double-distilled water and adjusted to pH 9.0. Buffer SB comprised 0.1M solution of monobasic potassium phosphate (KH_2PO4) mixed with 1 % celite (SiO_2) in double-distilled water and adjusted to pH 7.5. The control leaf samples were collected pre-inoculation from each test plant.

The youngest symptomatic leaves collected from CBSV-infected plants cv. Albert were ground in pre-chilled mortar and pestle with each of the inoculation buffer at 1:1 ratio and inoculated carefully onto the second top (fully open) leaf of each test plant. The mortar and pestles were disinfected and treated with diethyl pyrocarbonate (DEPC) water prior to use. Inoculation assays were done in triplicate with each treatment comprising eight plants. Young top leaf samples were collected for virus detection by RT-PCR after 2, 3 and 5 days and thereafter weekly up to 24 weeks. Collected leaf samples were stored at -80^0C prior to CBSV testing.

Graft inoculation

Graft transmission experiments (Figure 1B) were conducted in a screenhouse at MARI from June to December, 2007. Two grafting procedures were made in which the CBSV-infected and CBSV-free seedlings were used as either scion or rootstock. A modified cleft/wedge grafting technique was used as described previously [23, 24]. The virus-indexed pre-sprouted plants of the cv. Albert developed by tissue culture and pre-germinated infected seedlings from diseased plants of the same variety were used. The former were developed in the laboratory and plantlets acclimatized in the screenhouse, while the latter were obtained from the field and pre-sprouted in pots in the screenhouse. Grafting was done when the stem girth at 15 cm from the soil level was at least 10 mm. The graft portions were tightly taped with parafilm to promote union and prevent desiccation. The grafted plants were protected from excessive evaporation by a plastic bag which was removed after 4 days. Grafted plants were maintained in the screenhouse and monitored for symptom expression and samples were collected regularly for post-inoculation detection of CBSV. In the infected rootstock-healthy scion treatment, the samples were collected from the top leaf lobe after 2, 3 and 5 days and thereafter weekly up to 24 weeks and stored at -80^0C for CBSV detection by RT-PCR. In the infected scion-healthy rootstock treatment, leaf lobes below the graft union were collected at similar intervals.

Seeds transmission of CBSV

The seed transmission experiment was conducted at SRI, Kibaha, in Tanzania from August 2006 to October 2007. Seeds were collected from CBSV-infected plants of six different local cultivars; Albert, Limbanga Red, Limbanga White, Mreteta, Sharia and Supa in farmers' fields in Mtwara Region. Leaf samples were collected from each of the source plants for confirmation of the presence of CBSV by RT-PCR using CP gene-specific primers at MARI. The seeds were surface sterilised with 75 % ethanol, 10 %

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 15 No. 4 September 2015 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

sodium hypochlorite and distilled water then planted in heat-sterilised soil. Two experiments were conducted, each for six months. Seeds of each cultivar were planted in six pots (six replications) and thinning of surplus seedlings done a week after germination (Figure 1C). Five seedlings of each cultivar were maintained in each of the six pots giving a total of 180 plants (6 varieties x 6 pots x 5 seedlings). Leaf samples for CBSV detection were collected starting at two weeks (at three fully open leaves) after germination, and thereafter monthly, for six months. Samples from each pot were bulked into one sample for RT-PCR.

Effects of CBSV-infected root debris on seeds and CBSV-free cuttings

Two different experiments of similar set up were conducted from July 2007 to January 2008 at SRI, Kibaha to assess whether infected cassava debris might be a source of new infection of CBSV. In the first set of experiments, the roots from infected plants cv. Cheupe were dug up, chopped into smaller pieces and mixed with sterile soil at ratios (debris: soil) of 0:4, 1:3 and 2:2 (v/v). These ratios were determined in a preliminary experiment in which none of the planted seeds germinated at a ratio of 3:1 (debris: soil). Water was applied to the soil-plant debris mixture in 10 litre volume pots to field capacity and six surface-sterilised seeds cv. Albert were planted per pot. Each treatment was replicated thrice to give a total of 54 test plants (3 ratios x 6 seeds x 3 replications). Leaf samples were collected monthly for CBSV detection. The duration of the experiment was four months.

A similar experiment was set up in the same screenhouse at SRI, Kibaha which involved soaking CBSV-free cassava cuttings cv. Albert in distilled water-root debris mixture at varied volumes ratios. Distilled water and chopped root debris from CBSV-infected plants cv. Cheupe were mixed at ratios 0:4, 1:3, 2:2 and 3:1 (v/v of debris by water). The soaked cuttings were retrieved from the debris-water mixture after 6-12 hours and planted in potted sterile soil (Figure 1D), three cuttings per pot. Ten litre capacity plastic pots were used in this experiment.

Effect of leaf-harvesting

An experiment to test the possibility of CBSV spread through leaf harvesting, which is carried out by people eating cassava leaves as a vegetable, was conducted in the screenhouse (Figure 1E) at SRI, Kibaha from September 2007 to March 2008. Fifteen CBSV-free plants cv. Albert were grown in pots alongside five CBSV-infected cuttings of the same variety. At five months of age, CBSV transmission by leaf harvesting was attempted. A leaf was harvested from an infected plant and then the same hand was used to harvest leaves from three CBSV-free plants. Leaf harvesting was done for all fifteen initially CBSV-free plants on a monthly basis for four months. Leaf samples were collected monthly for CBSV detection by RT-PCR. CBSD symptoms expression was also monitored and recorded each month.

Effect of cutting tools

A pot experiment to assess cutting tools (knives) as a possible means of transmission of CBSV was conducted in the screenhouse at SRI-Kibaha from January 2007 to March 2008. Ten CBSV-infected cuttings cv. Albert were established individually in pots and thirty CBSV-free cuttings (ten for each of the cvs. Albert, Mreteta and Supa) were also

planted in pots. After six months a knife used to cut through an infected stem of cv. Albert was used immediately afterwards to cut through CBSV-free stands of cvs. Albert, Mreteta and Supa. (The CBSV-free plants were previously developed through tissue culture and maintained in the screenhouse at SRI, Kibaha). A single cut through an infected stem was followed by a cut on each of three different CBSV-free stems (Figure 1F). Three treatments were made per variety in a three replicate experiment to give a total of 27 inoculated plants (3 plants x 3 varieties x 3 replications). One plant of each cultivar was maintained as a control that is was not inoculated through cutting. The experimental plants were 30 in total, (27 cut-inoculated and 3 controls). Leaf samples were collected monthly starting at three months after sprouting (post-inoculative cuts) for CBSV detection. Sample collection for detection of the virus continued for six months.

Isolation of RNA and Amplification by RT-PCR

Total RNA was extracted from 0.1g fresh leaf tissue 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 followed by the triplicate RT-PCR performed in a one-step reaction using superscript ™ III RT/Platinum® *Taq* Mix System (Invitrogen Life Technologies) in a GeneAmp PCR system 9700 (Applied Biosystems, UK) as per Rwegasira [25].

RESULTS

Data obtained from this study suggest that CBSV may be transmitted through a number of non-vector mechanisms. These include mechanical transmission (by sap inoculation, leaf harvesting and cutting tools) and by grafting. The virus was not transmitted through seeds germinated from CBSV-infected plants or by cassava root debris from CBSV-infected plants.

Sap transmission of CBSV

A period of 1–4 months was required before the virus could be detected in the sap-inoculated plants. A total of 54% (13/24) of plants inoculated with infected leaves ground in buffer SB tested positive for CBSV compared to 27% (7/24) which tested positive when inoculated with the leaves crushed in buffer SA. An average of 27 days after inoculation (DAI) was required before detection of CBSV in plant tissues, when either sap in buffer SA or SB was used for inoculation. The proportion of plants that developed CBSD symptoms was 33 % with buffer SB and only 16 % with buffer SA. Although initially more plants *ca* 8 % were positive with buffer SA compared to *ca* 4 % with buffer SB, 4-weeks after inoculation (Figure 2), more infections were increasingly recorded over time with buffer SB than buffer SA. The suitability of buffer SB was similarly observed in the rate of symptoms expression. The decline in percentage of symptomatic plants ten weeks after inoculation with buffer SA was caused by reduced number of older symptomatic leaves due to senescence.

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 15 No. 4
September 2015
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

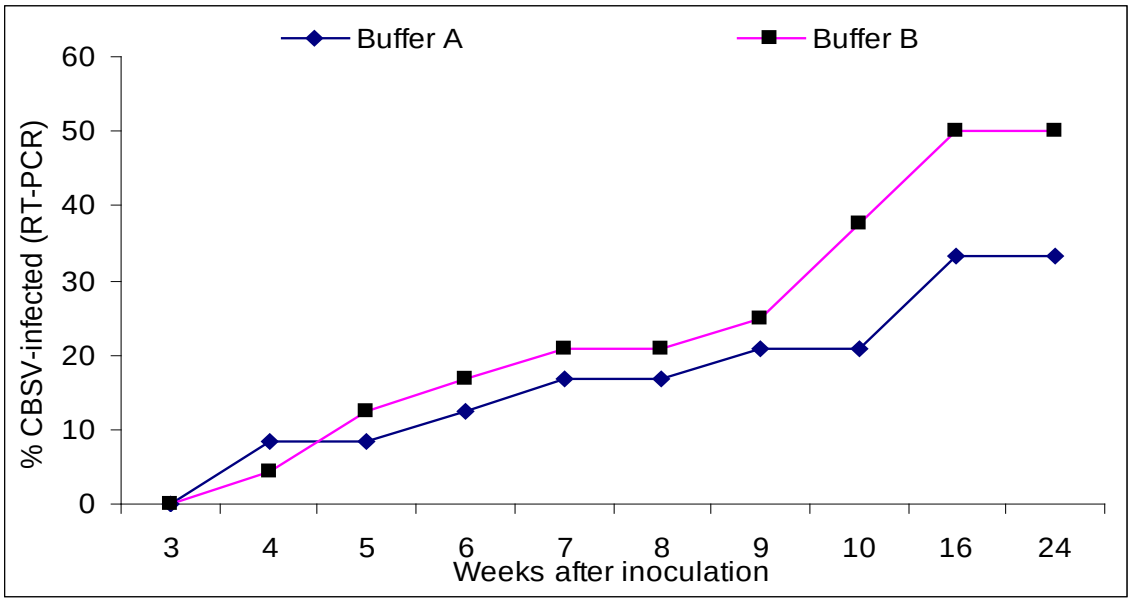

Figure 2: Relationship between inoculation buffers SA and SB and subsequent detection of CBSV in leaf tissues of inoculated plants (cv. Mreteta). Buffer SA comprised 0.05 M potassium phosphate monobasic (KH_2PO4), 50 mM glycine ($C_2H_5NO_2$), 1 % bentonite and 1 % celite (SiO_2), dissolved in double-distilled water and adjusted to pH 9.0. Buffer SB comprised 0.1M solution of monobasic potassium phosphate (KH_2PO4) mixed with 1 % celite (SiO_2) in double-distilled water and adjusted to pH 7.5.

Graft transmission of CBSV

In the graft transmission experiments, 12–26 days were required to attain infection of all the test stock, suggesting that the method is the most efficient with respect to the rate of virus transmission. Initially, CBSV-free susceptible plants developed characteristic leaf vein chlorosis and necrotic blotches typical of CBSD in less than one month after graft inoculation. Earlier detection of the virus *ca.* 12 DAI was recorded in grafts that involved infected rootstock and virus-free scion compared to the reciprocal combination (Table 1). Likewise, less time was required for symptom expression, 20 DAI compared to the 28 DAI for the infected scion to virus-free rootstock. Shorter periods of *ca.* 4-weeks were required to attain 100 % incidence in the infected rootstocks grafted to the virus-free scions compared to the five weeks in the virus-free rootstocks grafted to the infected scions (Figure 3).

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 15 No. 4
September 2015
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

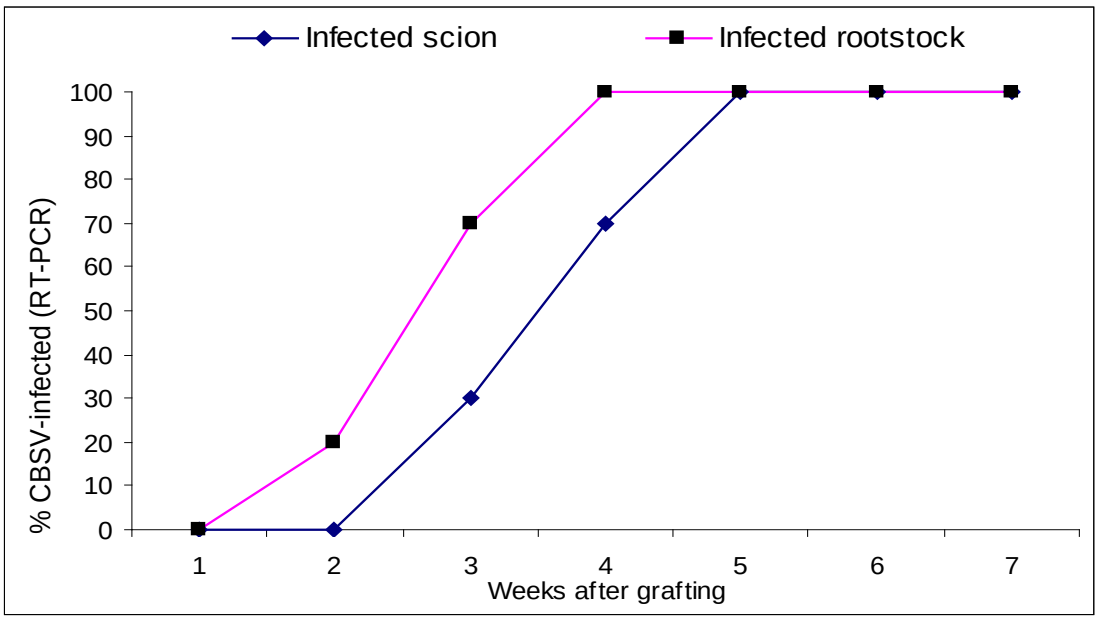

Figure 3: Relationship between the methods and time taken to CBSV detection in cv. Albert

Generally, the experimental results suggested that a maximum of five weeks were required to attain 100 % incidence with either of the graft techniques used. Leaf symptoms were recorded earlier (two weeks) in infected rootstock-virus free scion grafts compared to the reverse *ca* four weeks. The observed leaf symptoms included necrotic blotches (Figure 4A) and feathery chlorosis. Despite the variation in time to attain a severity score exceeding 2, symptoms gradually increased with time for both graft techniques.

***A**: White circle indicating numerous chlorotic blotches on leaves in graft transmission with CBSV-free scion on CBSV-infected rootstock*

***B:** Brown chlorotic spots (shown by white arrows in the white circle) on a plant leaf infected through cutting tools.*

Figure 4: Observable CBSV leaf symptoms from two CBSV transmission experiments in grafting (A) and infected cutting tools (B)

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 15 No. 4 September 2015
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Transmission of CBSV by seeds

None of the seedlings developed from seeds obtained from CBSD affected mother plants exhibited the disease symptoms. All plants tested negative for CBSV in RT-PCR even after repeated tests for more than six months. The CBSD symptoms were not observed in any of the seedlings even after repeated experimentation.

Transmissions of CBSV to seeds and cuttings through infected debris

None of the seedlings which were sown in root debris from CBSV-infected plants became infected. Neither CBSD symptoms nor virus detection by RT-PCR signified the presence of the virus in the experimental plants. Although four of twenty four plants (17 %) exposed to debris:soil ratios of 1:2 and 2:2, exhibited chlorotic leaf symptoms that were somewhat similar to those of CBSD, none of them tested positive to the virus even after repeated testing in a triplicate RT-PCR. Plants exposed to the debris:soil ratio of 3:1 hardly sprouted and those that did died a week after sprouting. In pre-soaking experiments that involved CBSV-infected root debris in water at all tested dilution rations, none of the planted cuttings sprouted. The same results were recorded even after repetition of the experiment with reduced exposure of the cuttings (between six and twelve hours). Therefore, no CBSV-infection data were obtained from these experiments.

CBSV transmission by cutting tools

Six of 27 (22 %) test plants were infected by cutting tools. However, only 2 of the 6 CBSV-positive plants developed disease symptoms (Figure 4B). Irregular-shaped chlorotic spots were observed and later coalesced to form blotches. A relatively long time was required (113 DAI and 132 DAI) to first detection of the virus and development of CBSD symptoms, respectively. Infections were confirmed by RT-PCR.

Transmission of CBSV by leaf harvesting

One of 15 plants (7 %) in the leaf harvesting experiment became infected with the virus. The infected plant developed characteristic CBSD symptoms of feathery chlorosis which started in tertiary veins extending to cover the secondary and primary veins. The CBSD symptomatic plant tested positive with RT-PCR.

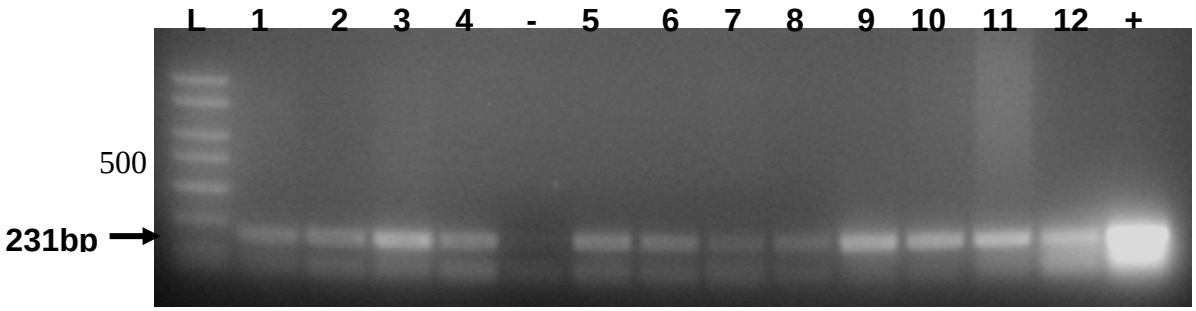

Figure 5: Agarose gel electrophoresis of RT-PCR amplified products (231bp CBSV coat protein gene fragment) for samples from CBSV transmission experiments using CBSV-specific primer pair CBSV 10F and CBSV 11R

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 15 No. 4 September 2015 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Confirmation of CBSV infection in inoculated tissues

The target PCR band of approximately 231bp was amplified successfully in all infected samples using the CBSV coat protein gene-specific primers (Figure 5), thereby confirming the presence of the virus. Based on band strengths, the virus was detected strongly in graft transmission and in buffer SB compared to the other transmission methods tested. Despite the slight variation in band strength, the virus was positively detected in the infected samples.

DISCUSSION

Sap transmission of CBSV

In sap transmission, significantly higher transmission rates of CBSV and earlier expression of CBSD symptoms was achieved with infected leaves extracted in buffer SB (54 %) compared with buffer SA (29 %). Based on the composition of the two buffers, buffer SA included bentonite and glycine, but the pH was adjusted to pH 9.0 unlike the pH 7.5 of buffer SB. Bentonite has been reported to improve the infectivity of the potato spindle tuber viroids [26]. However, the role of glycine in enhancing virus infectivity seems limited apart from being a conjugate inhibitor of the B6 protein [27]. With bentonite as a component of the buffer, greater efficiency was expected from buffer SA compared to buffer SB, but the reverse was realized. The difference in molar concentration between the two buffers (0.05M for buffer SA and 0.1M for buffer SB) possibly contributed to the variation in infectivity. The higher molarity in buffer SB probably increased its efficiency compared to buffer SA. The buffer pH may also affect the infectivity efficiency. Since buffer SB had its pH adjusted to 7.5 unlike pH 9.0 of the buffer SA, that factor could be vital to the infectivity of CBSV.

Graft transmission of CBSV

Graft inoculation was the most efficient and effective of the techniques assessed because 100 % transmission was attained. The relatively shorter time required for the virus detection and symptoms expression when the CBSV-free scion was grafted onto an infected root stock suggests this technique to be the best for transmission studies. Earlier detection of CBSV by this grafting technique also suggest that CBSV titre was higher in the lower parts of the plants particularly the roots compared to the shoots. This is consistent with the classical study on the nature of virus movement in plants as demonstrated by Samuel [15] with *Tobacco mosaic virus* (TMV). The author demonstrated that after infection and replication, TMV particles were systemically translocated through phloem to lower parts of the tomato plant and re-distributed to the youngest leaves and the rest of plant shoots. Unlike in other forms of CBSV inoculation that were tested, the expressed symptoms were clear and typical of CBSD. The findings in this study are consistent with the CABRI [28] findings, that grafting is an effective way of transmitting virus strains that are not readily or not at all mechanically transmissible to susceptible host plants.

Seed transmission of CBSV

Cassava brown streak virus was not transmitted through seeds which support earlier findings [21]. The lack of CBSV transmission through seeds derived from infected

mother plants suggest that the morphology of the reproductive organs of cassava does not allow entrance and survival of CBSV in the embryo. According to Carroll [29], non-seed transmitted viruses are not detected in pollen or embryos. In contrast, there could be some inhibitors of virus infection of seeds as reported by Gunnery and Datta [30]. In their studies, a low molecular weight RNA was isolated from barley embryos that specifically inhibited the initiation of protein synthesis. Such a molecule was suggested to play a role in inhibiting virus synthesis and seed transmission in some species. The lack of transmission of the virus through seeds would support conventional breeding for resistance to CBSD, through selective crossing. The ease with which breeder seeds may be exchanged regionally and internationally without risk of transmitting CBSV is also an added benefit of the information gained through this study.

Transmissions of CBSV to seeds and cuttings through infected debris

The inability of CBSV to be spread from infected root debris is important in ensuring that newly established cassava plants are not at risk from the remains of the previous crop. Virus-free cuttings of cassava can therefore be planted in a field previously grown with CBSV-infected material with no apparent risk of carry-over of virus inoculum. The inability of the cuttings to sprout after pre-exposure to infected debris suggests the likely presence of inhibitive chemicals that acted allelopathetically on the cuttings. The lack of any sign of fungal infections on the pre-soaked cuttings discounted the possibility of fungi being a cause of cutting deaths. However, more detailed studies are required to confirm this observation.

Transmission of CBSV by cutting tools and leaf harvesting

The infection rates of 22 % for cutting tools and 7 % for leaf harvesting suggested that farming agronomic practices can make an appreciable contribution to the spread of CBSV. For the first time, it was demonstrated that CBSV can be transmitted through normal agronomic operations. CBSV transmission to only one plant through leaf harvesting was too low to warrant scientific conclusion. However, given the small number of the test plants the contribution of leaf harvesting could be more substantial. Further studies with many more test plants would be needed to justify the contribution of leave harvesting to CBSV spread. Although the transmission efficiency was relatively low, the cumulative contribution could be significant because of the multiplication rate of cassava in which each cutting can produce an average of ten cuttings per annum [31].

Conclusively, the current study demonstrated that sap transmission of CBSV is achievable when the inoculum is obtained from the youngest symptomatic leaves of infected plants with buffer pH adjusted to 7.5. New transmission of the virus is neither expected from seeds nor infected plant debris. This may allow for the possibility of continuous cropping, especially in areas where the area of productive land is limiting. Exchange of seed genetic materials for breeding can be done without fear of spreading CBSV to disease-free areas. Graft transmission of the virus is the best technique for indexing and detection of the virus in any infected stock of CBSV-susceptible plants. Best and quickest results are achieved when susceptible virus-free scions are grafted onto infected rootstocks. Cutting tools, such as knives, used in preparation of cassava cuttings and harvesting of cassava leaves for use as vegetable may contribute to the spread of CBSD.

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 15 No. 4
September 2015
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

ACKNOWLEDGEMENTS

Authors are grateful to the International Institute of Tropical Agriculture (IITA) for funding the study. Dr. I. Ingelbrecht of IITA, Ibadan is acknowledged for supplementing the CBSV-free tissue culture experimental materials. Special thanks to Dr. J. Ndunguru of ARI-Mikocheni, for the technical guidance. We also appreciate the technical support on field works from Dr. G. Mkamilo, Messrs. E. Gama and H. Julius of Naliendele Research Institute. The cassava growing farmers in Mtwara peri-urban are acknowledged for allowing us free collection of seeds and cuttings from their CBSV-infected plants.

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 15 No. 4 September 2015 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Table 1: Summary of non-vector transmission and infectivity of CBSV

Treatment	Replicates	Time to CBSV detection	Time to symptoms	Number of Symptomatic plants	CBSV Positive (RT-PCR)	Infected plants as per RT-PCR (%)
Sap inoculation						
Buffer A	3	28 - 122 DAI	30 - 35 DAI	4	7/24	16.7
Control	3	0	0	0	0/3	0.0
Buffer B	3	27 - 124 DAI	28 - 68 DAI	8	13/24	54.1
Control	3	0	0	0	0/3	0.0
Grafting (scion)	N/A	26 DAI	28 DAI	10	10/10	100
Control	N/A	0	0	0	0/2	0.0
Grafting (rootstock)	N/A	12 DAI	20 DAI	10	10/10	100
Control	N/A	0	0	0	0/2	0.0
Seeds from infected	6	0	0	0	0/180	0.0
Seeds on debris:soil						
ratio 0:4	3	0	0	0	0/15	0.0
ratio1:3	3	0	0	0	0/15	0.0
ratio 2:2	3	0	0	0	0/15	0.0
Cuttings on infected root debris : soil						
ratio 0:4	3	0	0	0	0/12	0.0
ratio1:3	3	0	5 months	1*	0/12	0.0
ratio 2:2	3	0	6 months	3*	0/12	0.0
ratio 3:1	3	N/A	N/A	N/A	N/A	-
Cuttings pre-soaked						
(infected root debris : sterile water)						
ratio 0:4	N/A	0	0	0	0/4	0.0
ratio1:3	N/A	N/A	N/A	N/A	N/A	-
ratio 2:2	N/A	N/A	N/A	N/A	N/A	-
ratio 3:1	N/A	N/A	N/A	N/A	N/A	-
Leaf harvesting	3	74 DAI	110 DAI	1	1/15	6.7
Control	N/A	0	0	0	0/2	0.0
Cutting tools	3	113 DAI	132 DAI	2	6/27	22.2
Control	N/A	0	0	0	0/3	0.0

* CBSD-like symptoms but not confirmed by RT-PCR. Control refers to the plants to which the respective treatments were not applied. DAI; days after inoculation, N/A; not applicable

REFERENCES

1. **Legg JP and JM Thresh** Cassava virus disease in Africa. Plant virology in sub-Saharan Africa. 2003; 517-552pp. http://www.iita.org/info/virology/pdf_files/517-552.pdf. Accessed August, 2011.

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

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

4. **Mbanzibwa DR, Tian YP, Tugume AK, Mukasa SB, Tairo F, Kyamanywa S, Kulaya A and PTJ Valkonen** Genetically distinct strains of *Cassava brown streak virus* in the Lake Victoria Basin and the Indian Ocean Coastal areas of East Africa. Archives of Virology 2009; **154**: 353-359.

5. **Mbanzibwa DR, Tian YP, Tugume AK, Patil BL, Yadav JS, Bagewadi B, Abarshi M. M, Aliai T, Changadeya W, Mkumbira J, Muli MB, Mukasa SB, Tairo F, Baguma Y, Kyamanywa S, Kullaya A, Maruthi MN, Fauquet CM and JPT Valkonen** Evolution of cassava brown streak-associated viruses. Journal of General Virology, 2011; **92**:974-987.

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

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

8. **Hillocks RJ and DL Jennings** Cassava Brown Streak Disease: a review of present knowledge and research needs. *Intern. J. Pest Man.* 2003; **49(3)**: 225-234.

9. **Jones WO** Manioc in Africa. Stanford University Press. Stanford Connecticut, 1959.

10. **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.

11. **Hillocks RJ** Cassava virus disease and their control with special reference to southern Tanzania. *Integr. Pest Man. Rev*. 1997; **2**: 125-138.

12. **Hillocks RJ, Raya MD and MJ Thresh** The association between root necrosis and above-ground symptoms of brown streak virus infection of cassava in southern Tanzania. *Intern. J. Pest Man.* 1996; **42**: 285-289.

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

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

15. **Samuel G** The movement of *Tobacco mosaic virus* within the plant. *Ann. Appl. Biol.* 1934; **21**: 90-111.

16. **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 Dis.* 2007; **91(1)**: 24-29.

17. **Lister RM** Mechanical transmission of cassava brown streak virus. Nature 1959; **1983**: 1588-1589.

18. **Storey HH** Report of the Plant Pathologist. Annual Report from E. Afr. Agric. Res. Stat. 1939.

19. **Hillocks RJ, Raya MD and MJ Thresh** Factors affecting the distribution, spread and symptoms expression of cassava brown streak disease in Tanzania. *Afr. J. Root Tuber Crops* 1999; **3(2)**: 57-61.

20. **Kanju, EE, Mtunda KJ, Muhana M, Raya MD and NM Mahungu** Management of Cassava Brown Streak Virus Disease in Tanzania. Pp 66-69. In: Legg JP and Hillocks RJ (Eds) Cassava brown streak virus disease: Past, Present and Future. Proceedings of an international workshop, Mombasa, Kenya, 27-30 October, 2002. Natural Resources International Limited, Aylesford, Uk. 2003; 100.

21. **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.

22. **Lennon AM, Aiton MM and BD Harrison** Cassava Viruses from Africa. Scottish Crop Res. Inst. Annual Report for 1985. 1986; 168.

23. **Anonymou**s Methods of grafting and budding fruit trees. Communication and Educational Technology Services, University of Minnesota Extension, 1993. http://www.extension.umn.edu/distribution/horticulture/components/DG0532c.html. Accessed May, 2006.

24. **Rothenberger RR and CJ Starbuck** Grafting. MU Extension, Dept. of Horticulture. University of Missouri, 2002. http://extension.missouri.edu/xplor/agguides/hort/g06971.htm. Accessed May, 2006.

25. **Rwegasira GM** Aspects of Epidemiology of Cassava Brown Streak Virus Disease in Tanzania. PhD Thesis. University of the Witwatersrand, South Africa. 222, 2009.

26. **Grasmick ME and SA Slack** Detection of potato spindle tuber viroid in true potato seed by bioassay on Rutgers tomato. *Americ. J. Pot. Res.* 1987; **64(5)**: 235-244.

27. **Anonymous** Compositions comprising nicotinylalanine and an inhibitor of glycine conjugation or vitamin B6, 1999. http://www.freepatentsonline.com/5916906.html --Accessed May, 2009.

28. **CABRI** Grafting of virus infected plant material to healthy host plants. Laboratory procedures for plant cell viruses. The CABRI Consortium 1999-2003, 2004. http://www.cabri.org/guidelines/plant_virus/pcv202ap3.html. Accessed July, 2010.

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

30. **Gunnery S and A Datta** An inhibitor RNA of translation of barley embryo. *Biochem. Bioph. Res. Comm.* 1987; **142**: 383-388.

31. **Otoo JA** Rapid multiplication of cassava. IITA Research Guide 1996; 51. http://www.iita.org/cms/details/trn_mat/irg51/irg51.htm Accessed August, 2006.