



## Serological detection of virus diseases of sweet potato in Kenya

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### ABSTRACT

**Objective:** To identify virus diseases attacking sweet potato in the major production areas in Kenya.

**Methodology and results:** A total of 220 symptomatic and 108 asymptomatic sweet potato vines were collected from farmers' fields, established in an insect-proof screenhouse and tested for viruses by nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA). The viruses detected were *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato mild mottle virus* (SPMMV) and *Sweet potato chlorotic fleck virus* (SPCFV). SPFMV was the most prevalent virus and the most widespread, detected in 67 and 20% of the symptomatic and asymptomatic plants, respectively. SPCSV was the second most common and it was detected in 64 and 13% of the symptomatic and asymptomatic plant samples, respectively. SPMMV was present in 12% of the symptomatic plant samples. SPCFV was rare, being detected in only 4% of the plant samples. *Cucumber mosaic virus* (CMV), *Sweet potato latent virus* (SwPLV), *Sweet potato caulimo-like virus* (SPCaLV), *Sweet potato mild speckling virus* (SPMSV) and C-6 virus were not detected in any of the samples assayed. SPFMV and SPCSV were detected in all the 15 districts that were surveyed, whereas SPMMV and SPCFV were detected in 9 and 4 districts, respectively. Five different virus complexes were detected in the samples assayed. Dual infection with SPFMV and SPCSV was the most common multiple infection and was detected in 52 and 12% of the symptomatic and asymptomatic plants, respectively.

**Conclusion and application of findings:** This study has provided a quantitative assessment of co-occurrence of viruses in sweet potato plants in Kenya, and highlights the importance of developing resistance specifically targeting SPCSV in either conventional or non-conventional breeding programs as a means of virus disease management.

**Key words:** Sweet potato, symptoms, virus, survey, Kenya

Citation: NYABOGA EN, ATEKA EM and BULIMO WD, 2008. Serological detection of virus diseases of sweet potato in Kenya. *Journal of Applied Biosciences* 7: 222 – 229.

### INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is an important starchy tuberous root crop grown in many tropical and subtropical regions of the world. About 75% of African sweet potato production occurs in East Africa, especially around Lake Victoria, where it is a basic subsistence crop mainly grown by women (Gibson & Aritua, 2002). The crop grows well

under varying agro-ecological conditions in Kenya, from coastal lowlands to altitudes of about 2000m in the central highlands. Considerable production is realized in six provinces namely Nyanza, Western, Rift Valley, Eastern, Central and Coast (Qaim, 1999).



Sweet potato has several advantages that enhance its potential role in combating food shortages and malnutrition occasioned by population growth and pressure on land (Woolfe, 1992). The crop has a short growing season and thus it can fit into many different cropping systems; it has a high productivity per unit area, performs well in infertile soils, is relatively drought-insensitive and can be harvested gradually over an extended period (Karyeija *et al.*, 1998).

Productivity of sweet potato is greatly constrained by pests and diseases, the most important being viruses (Fughe, 2007). Depending on cultivar, infecting virus, stage of infection and whether the crop is infected with a single or multiple viruses, viral diseases may cause up to 100% yield loss (Ngeve & Bouwkamp, 1991).

Several viruses have been reported to infect sweet potato in Africa. These include the *Sweet potato feathery mottle potyvirus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato latent virus* (SwPLV), *Sweet potato caulimo-like virus* (SPCaLV), *Cucumber mosaic virus* (CMV), *Sweet potato virus Y*, *Sweet potato virus G* and *Sweet potato leaf curl virus* (SPLCV) (Hahn, 1997; Geddes, 1990; Wambugu, 1991; Mukasa *et al.*, 2003; Is Hak *et al.*, 2003; Ateka *et al.*, 2004; Tairo *et al.*, 2004).

## MATERIALS AND METHODS

**Survey of sweet potato viruses:** A survey for viruses was conducted in the major sweet potato growing areas in Kenya during the months of July to September 2005. The districts surveyed included Kakamega, Bungoma and Busia in Western province; Siaya, Migori, Nyando and Kisii in Nyanza province; Nyeri, Kiambu and Muranga in Central province; Embu, Mbeere and Makueni in Eastern province; and Kwale and Kilifi in Coast province. Sweet potato fields with a 3 to 5-month-old crop were sampled along rural roads or paths at approximately 5km intervals.

A total of 220 symptomatic and 108 asymptomatic vine cuttings of plants were collected from the fields. The sampled vines were transferred to the Kenya Agricultural Research Institute (KARI)-

There have been two previously reported surveys of viruses infecting sweet potato in Kenya. The identification by serology of seven viruses in a survey of sweet potato in Kenya (Wambugu, 1991) now appears unlikely and could have resulted from false positives. In more recent surveys, only four viruses have been detected in sweet potato in Kenya (Ateka *et al.*, 2004). These comprise of the potyvirus *Sweet potato feathery mottle virus* (SPFMV), the crinivirus *Sweet potato chlorotic stunt virus* (SPCSV), the ipomovirus *Sweet potato mild mottle virus* (SPMMV) and *Sweet potato chlorotic fleck virus* (SPCFV) for which the genus *Carlavirus* has been proposed (Aritua *et al.*, 2003). These viruses often occur in multiple infections in the field with the most commonly encountered combination being that between SPFMV and SPCSV. This dual infection is responsible for the severe sweet potato virus disease (SPVD) (Mukasa *et al.*, 2006).

Understanding the diversity of combinations/associations between different viruses has implications for virus diagnosis, epidemiology and the implementation of control measures. This paper reports the relative frequency of occurrence of viruses identified in a survey encompassing all the major sweet potato growing areas in Kenya.

Biotechnology Center for establishment in the greenhouse. This allowed observation of symptom development on all the sampled vines in a similar environment before carrying out serological analysis. Plants were sprayed regularly with insecticides against aphids and whiteflies to avoid virus spread among plants.

**Serological analysis:** A disc (1 cm in diameter) was taken from a leaf at the top, middle and lower part of the stem from each plant and used for serological testing of viruses using the nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) (Gibb & Padovan, 1993). Polyclonal antibodies specific to SPFMV, SPCSV, SPMMV, SPCFV, SPCaLV, C-6, SwPLV, CMV and SPMSV, as well as NCM strips



spotted with sap from virus-positive and non-infected control plants obtained from the International Potato Center (CIP, Lima, Peru) were used. The development

of a purple colour on the sample spots confirmed virus-positive samples (Gutierrez *et al.*, 2003).

## RESULTS

**Incidence of virus infection:** Fifty eight percent of the 328 samples tested positive for at least one virus. Of the symptomatic plant samples, 79% reacted with antisera to one or more viruses, with the frequency of detection being highest in samples obtained from Nyanza province and the neighboring Western province. The frequency of detection was lowest in the Coast and Eastern provinces (Table 1). Of the 108

asymptomatic plant samples collected and assayed, only 19 (18%) reacted with antisera for at least one virus. Viral diseases were widespread in most of the provinces surveyed with frequencies of detection ranging from 54 to 93% and 5 to 58% in the symptomatic and asymptomatic plant samples, respectively.

Table 1: Proportion of symptomatic and asymptomatic sweet potato plant samples that tested positive for at least one virus when assayed serologically by NCM-ELISA.

Province	Symptomatic plants		Asymptomatic plants	
	Plants assayed	Positive for one or more viruses (%)	Plants assayed	Positive for one or more viruses (%)
Western	38	92	12	58
Nyanza	86	93	28	17
Central	25	76	28	14
Eastern	18	55	21	09
Coast	53	54	17	05

SPFMV, SPCSV, SPMMV and SPCFV were detected in the symptomatic sweet potato plants, whereas three viruses SPFMV, SPCSV and SPMMV were detected in asymptomatic plant samples from the five provinces (Table 2). The frequency of detection was higher in symptom bearing than in asymptomatic samples. SPFMV was detected in samples from all the districts surveyed (Fig. 1; Table 2). A total of 146 (67%) symptomatic and 19 (18%) asymptomatic samples reacted with the SPFMV antibodies, making it the most frequently detected virus.

SPCSV was the second most frequently detected virus, being detected in 141 (64%) and 12 (11%) of the symptomatic and asymptomatic plants, respectively. Similar to SPFMV, SPCSV was detected in samples from all the districts (Fig. 2). SPMMV was detected in 26 (12%) symptomatic and 3 (3%) asymptomatic plants collected from all the areas except Kwale, Kilifi, Embu, Mbeere, Murang'a and Kiambu districts (Fig. 2). SPCFV was detected in 4% of the symptomatic samples and was not detected in any of the asymptomatic plant samples. SPCFV had the most restricted distribution being found in only four districts of

Migori, Kisii, Kakamega and Nyeri (Table 1). CMV, SwPLV, SPCaLV, C-6 and SPMSV were not detected in any of the assayed plant samples. Only 47 (21%) of the apparently diseased plants did not react with any antisera, although the symptoms resembled those caused by viruses. Of the asymptomatic plants, 82% did not react with any antisera.

Thirty two symptomatic plants (15%) were infected by a single virus, whereas 141 (64%) were infected with two or more viruses. In contrast, 6 (5%) and 12 (11%) asymptomatic samples were infected by single and mixed viruses, respectively. SPFMV was the most common in single infections being in 21 (10%) of the symptomatic and 5 (4%) of the asymptomatic plant samples (Fig 1). SPCSV was the second most commonly detected single virus being in 9 (4%) and 1 (1%) of the symptomatic and asymptomatic plants, respectively. SPMMV was also prevalent in single infections with 2 (1%) symptomatic plants being infected. There were no single infections of SPCFV.

Five different viral disease complexes were detected in the assayed plant samples (Fig. 1). SPFMV + SPCSV (=SPVD) was the most common



combination, occurring in 117 (52%) and 12 (11%) of the symptomatic and asymptomatic plant samples, respectively (Fig 1). SPCSV + SPMMV was the second most prevalent dual infection and was detected in 4 (2%) of the symptomatic plants but was absent in the asymptomatic plants. A mixed infection of SPFMV and SPMMV was rare, being detected in only 1 (1%) of the symptomatic plants collected from Bungoma district of Western province. Triple infections involving SPFMV,

SPCSV and SPMMV were observed in 11 (5%) symptomatic plants, whereas a mixed infection involving four viruses (SPFMV, SPCSV, SPMMV and SPCFV) was detected in 8 (4%) symptomatic plants. Unlike in the symptomatic plants, mixed infections were extremely rare in the symptomless plants and only one virus complex involving SPFMV and SPCSV was detected in the symptomless plants.

**Table 2:** Proportion of asymptomatic (A) and symptomatic (S) sweet potato plant samples from the five provinces of Kenya reacting positive for different viruses.

Province	District	Viruses detected									
		No. of samples		SPFMV		SPCSV		SPMMV		SPCFV	
		A	S	A	S	A	S	A	S	A	S
Western	Kakamega	8	20	63	100	25	90	13	15	0	5
	Bungoma	2	10	50	64	0	25	50	20	0	0
Nyanza	Busia	2	8	50	13	50	38	50	25	0	0
	Kisii	10	34	20	82	20	71	0	21	0	12
	Migori	4	16	25	88	25	88	0	19	0	19
	Nyando	13	28	8	93	8	93	0	14	0	0
Central	Siaya	1	8	33	75	33	100	0	13	0	0
	Nyeri	5	17	40	35	40	41	0	6	0	6
	Murang'a	10	5	20	20	20	20	0	0	0	0
Eastern	Kiambu	13	3	8	33	8	33	0	0	0	0
	Embu	2	7	0	42	0	42	0	0	0	0
	Mbeere	4	2	25	0	25	50	0	0	0	0
Coast	Makueni	15	9	7	44	7	44	0	20	0	0
	Kwale	10	40	0	60	0	60	0	0	0	0
	Kilifi	7	13	14	23	0	23	0	0	0	0

**Virus-like symptoms and diseases observed:** The virus-like symptoms became pronounced in the leaves of young shoots 2 weeks after establishment in the screen house. The most commonly observed symptoms were chlorotic spots, mottling, general chlorosis, leaf clearing, leaf distortion, mosaic, purpling, stunting, thinning and vein chlorosis (Fig. 2) with the frequency of each symptom varying with the cultivar. Symptoms on plants that were co-infected with several viruses were typically more severe than on plants infected with a single virus. Sweet potato plants that tested positive only for SPFMV had characteristic vein clearing symptoms (Fig. 2C), vein feathering and chlorotic spots, although some sweet potato cultivars exhibited no symptoms (Fig. 2B).

Symptoms associated with SPCSV infected plants included purpling (Fig. 2A) and yellowing of the lower and middle leaves and general overall stunting of

the plants. Plant samples that were seropositive for both SPFMV + SPCSV showed severe symptoms including leaf distortion, leaf narrowing, stunting of the plant and purpling of older leaves (Fig. 2F). In single SPMMV infections, the sweet potato plants expressed mostly leaf mottling, mild interveinal chlorosis symptoms (Fig. 2E) and vein yellowing (Fig. 2G). In mixed infections with SPCSV the plants were thin and stunted thus presenting SPMMV as an important virus in Kenya. The sweet potato plants infected with SPFMV and SPMMV had only chlorotic spots on leaves (Fig. 2H). The plants that were infected with SPFMV, SPCSV and SPMMV were all weak, stunted, and their leaves had a wavy edge and a chlorotic mottle. Symptoms of mixed infections involving the four viruses detected resembled those of SPVD but were slightly more severe. Multiple infections, especially those



involving SPCSV, were associated with severe symptoms.

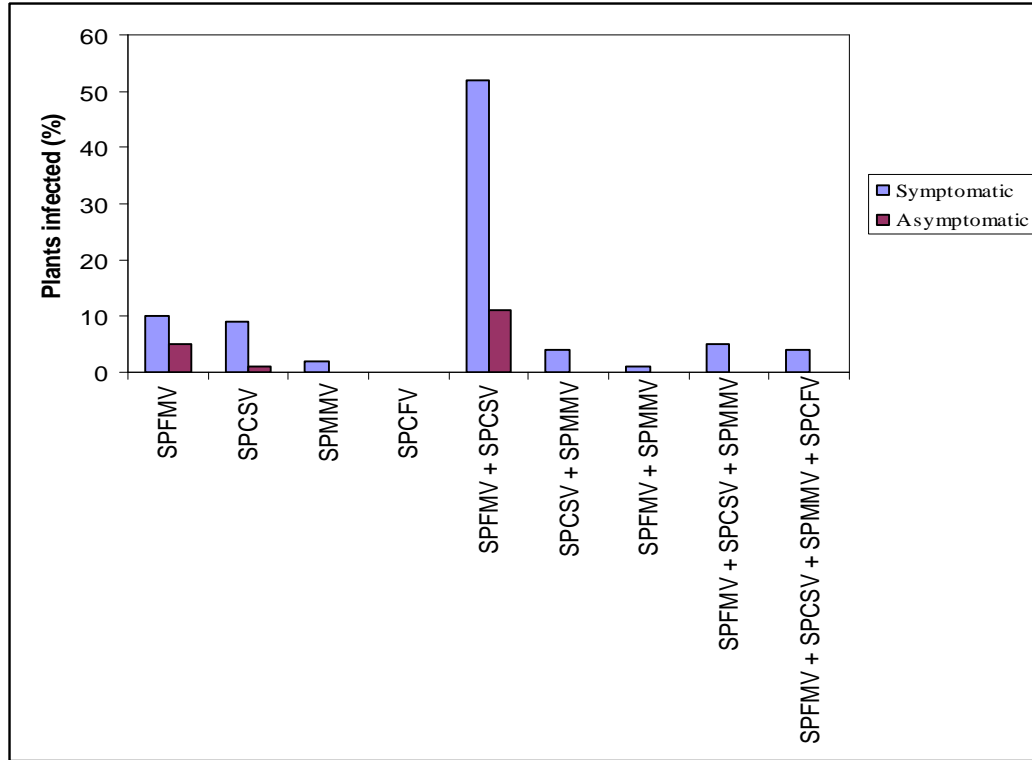


Figure 1: Proportion of single and mixed virus infections detected by nitrocellulose membrane enzyme-linked immunosorbent assay in symptomatic and asymptomatic sweet potato plants in Kenya.

## DISCUSSION

Four viruses namely SPFMV, SPCSV, SPMMV and SPCFV were detected in sweet potato plants collected from farmers' fields in major growing areas of Kenya. The most common and widespread was SPFMV. This concurs with previous reports that SPFMV occurs everywhere sweet potato is grown (Moyer & Salazar, 1989; Sakai *et al.*, 1997). It has been reported that SPFMV on its own causes mild or no symptoms in East African sweet potato cultivars (Gibson *et al.*, 1997). This observation was confirmed in this study, with a substantial proportion of samples from asymptomatic plants reacting with antiserum to SPFMV.

SPCSV was the second most prevalent virus and was detected both in single and mixed infections, with very severe symptoms being observed in mixed infections. This observation forms the basis of a proposition that SPCSV is the most important virus that infects sweet potato in Kenya. SPCFV has a narrow distribution and is rarely encountered, which is in agreement with previous reports from Kenya and other

countries in East Africa (Mukasa *et al.*, 2003; Ateka *et al.*, 2004; Tairo *et al.*, 2004).

The same types of viruses that were identified in this study, i.e. SPFMV, SPCSV, SPMMV and SPCFV have also been detected in a similar survey in Uganda (Mukasa *et al.*, 2003) and Tanzania (Tairo *et al.*, 2004). The widespread occurrence of SPFMV as compared to the other three viruses might be related to the way farmers select their planting materials. Since sweet potato plants that are singly infected with SPFMV exhibit mild or no symptoms, farmers may not be able to distinguish and exclude such SPFMV-infected cuttings from the planting materials they select for the next crop, thereby maintaining this virus. An additional explanation could be the relative abundance of its aphid vectors over the whitefly vectors of SPCSV (Schaefer & Terry, 1976).

Multiple virus infections in sweet potato are a common phenomenon (Gibson *et al.*, 1998; Karyeija *et al.*, 2000). SPFMV and SPCSV often occurred together to produce the severe disease SPVD (Gibson *et al.*,



1998; Mukasa *et al.*, 2003). In this study it was observed that SPVD, the disease caused by simultaneous infection with SPFMV and SPCSV is severe and widespread in Kenya. These results agree with findings from previous surveys in Uganda (Mukasa *et al.*, 2003) and Tanzania (Tairo *et al.*, 2004), where

co-infections of SPFMV and SPCSV were observed to be common. The widespread occurrence of SPVD in Kenya could be related to the practice of farmers using vines from their existing gardens as planting materials, and without sanitary control thus facilitating spread of the disease.

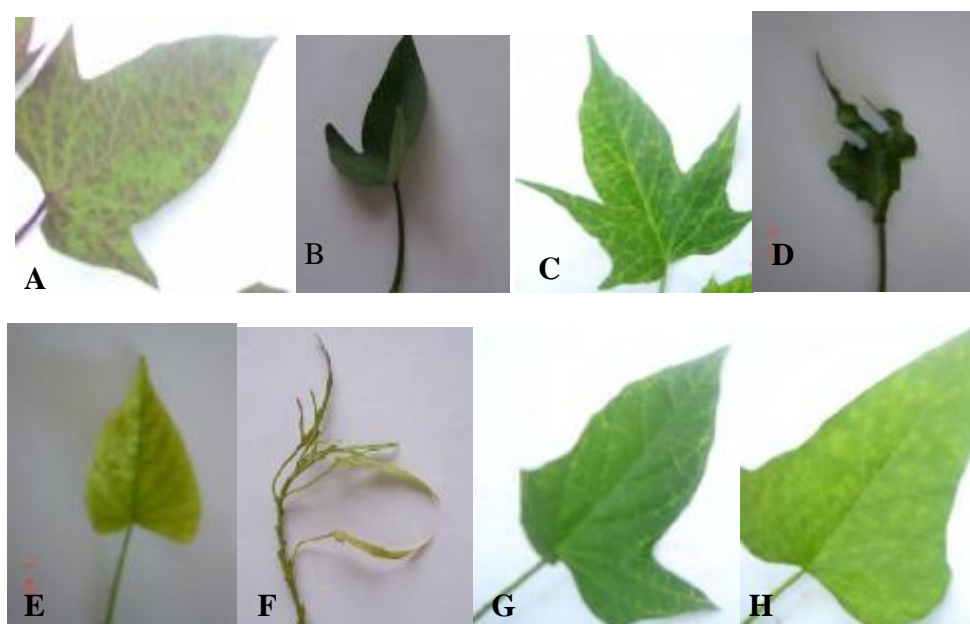


Figure 2: Virus symptoms observed on sweet potato plants collected from five provinces of Kenya and established in the screen house. (A) purpling of leaves in plants infected with SPCSV, (B) symptomless leaves of plants infected with SPFMV, (C) vein clearing in leaves of plants infected with SPFMV, (D) deformed leaves of plants infected with SPFMV and SPCSV, (E) interveinal chlorosis in leaves of plants infected with SPMMV, (F) chlorotic, small deformed leaves in plants infected with SPFMV and SPCSV, (G) severe symptoms in plants infected with SPFMV, SPCSV, SPMMV and SPCFV, (H) chlorotic spots on leaves of plants infected with SPFMV and SPMMV.

Other viral disease complexes were also observed, which invariably involve SPCSV. SPMMV occurred most frequently in mixed infections with SPCSV than alone, as was observed in a previous study in Uganda (Mukasa *et al.*, 2003), although it was not reflected as a commonly found co-occurrence in sweet potato plants. The co-occurrence of these viruses may be due to mixed transmission of the two viruses by their common whitefly vector (*Bemisia tabaci*) (Hollings *et al.*, 1976). The two viruses were only detected in symptomatic plants but it is not known whether synergism exists in co-infected plants. SPMMV also occurred in complex with SPCSV and SPFMV, thus confirming earlier reports (Ateka *et al.*, 2004; Mukasa *et al.*, 2004). Our results reinforce previous findings of severe symptoms being associated with co-infections with multiple viruses (Gibson *et al.*, 1998; Di

Feo *et al.*, 2000; Mukasa *et al.*, 2003; Ateka *et al.*, 2004; Tairo *et al.*, 2004). However, it should be noted that not all severe symptoms on sweet potato are due to synergistic effect of mixed infections (Salazar & Fuentes, 2001).

Almost 79% (173) of the 220 symptomatic plants tested positive with at least one of the virus-specific antisera used, which suggests that the four viruses detected are largely responsible for the virus diseases of sweet potato in Kenya. Several symptomatic plants (21%) did not react with any antisera used although the symptoms resembled those caused by viruses. It is possible that more viruses or virus-like agents than the nine viruses tested in this study infect sweet potato in Kenya. The other possible explanations are the presence in the plant tissue of phenolic compounds, latex and inhibitors that adversely



affect the serological detection and symptoms caused by non-viral factors.

Some symptomless plants (17%) also reacted positively with one or more of the antisera, which might be due to the ability of the plants to tolerate the effects of virus infection. However, SPFMV and SPCSV, the most common combination of mixed infection was detected in only 13 symptomless plants, confirming that SPVD symptoms are fairly severe (Gibson *et al.*, 1998; Karyeija *et al.*, 2000).

Virus diseases were highest in Nyanza and Western provinces, as previously reported by Ateka *et al.* (2004). In Western and Nyanza provinces, all year round production combined with piece-meal harvesting prolongs the period crops are retained, thus providing a reservoir of virus-infected sweet potato plants from which vectors can transmit viruses to new crops. Farms in Eastern, Central and Coast provinces had low virus disease incidences. These provinces have a markedly different cropping system from the provinces in the Lake Victoria basin, and sweet potato is not continuously grown throughout the year. The low disease incidences in these provinces could partly be

explained by the discontinuous production cycles of sweet potato that make it less likely for viruses to be spread between crops.

This study has provided a quantitative assessment of co-occurrence of viruses in sweet potato plants in Kenya, taking into account the major growing areas. SPCSV was associated with very severe symptoms in mixed infections with the viruses detected. This finding highlights the importance of targeting resistance to SPCSV in either conventional or non-conventional breeding programs as a means of virus disease management.

Our findings do not rule out the possibility that other, as yet unknown, or less characterized viruses not detected in this study might occur in the surveyed areas since 21% of plants established in the screen house showed virus-like symptoms but were sero-negative with the antibodies used. Further studies are required to identify the cause of symptoms in these plants.

**Acknowledgements:** The authors would like to acknowledge USAID for funding this work. The views expressed are the authors' and not those of USAID.

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