SHORT COMMUNICATION

SURVEY OF SWEET POTATO VIRUSES IN WESTERN KENYA AND DETECTION OF *CUCUMBER MOSAIC VIRUS*

S.A. Opiyo[1], E.M. Ateka[2], P.O. Owuor[1], L.O.A. Manguro[1] and H.W. Karuri[3]

[1]Department of Chemistry, Maseno University, P. O. Box 333, Maseno, Kenya
[2]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, P.O. Box, 62000-00200, Nairobi, Kenya
[3]Kenya Agricultural Research Institute, Biotechnology Centre, P. O. Box 14733 00800, Nairobi, Kenya

SUMMARY

Sweet potato is an important food crop worldwide, but several pests and diseases limit its production. In eastern Africa, virus-induced diseases rank second to weevils in causing yield reduction. Symptomatic sweet potato cuttings (327) were collected from Nyanza and Western Provinces in western Kenya in 2009. The samples were tested for *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato mild mottle virus* (SPMMV), Sweet potato chlorotic fleck virus (SPCFV), *Sweet potato latent virus* (SPLV), Sweet potato caulimo-like virus (SPCa-LV), *Cucumber mosaic virus* (CMV), C-6, *Sweet potato virus G* (SPVG) and *Sweet potato mild speckling virus* (SPMSV) using nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA). SPFMV, SPCSV, SPCFV, SPMMV and CMV were detected and 89% of the samples as a whole were found to be infected. SPFMV was detected in all infected samples followed by SPCSV (55%). Multiple infections were detected in the majority of the samples (80%) and the most common dual infection was with SPFMV and SPCSV (52%). The occurrence of CMV was low (5%) but was confirmed by RT-PCR with amplification of a 670 bp coat protein gene fragment from total RNA. This is the first record of CMV in sweet potato in Kenya.

Key words: Sweet potato, CMV, NCM-ELISA, RT-PCR, diagnosis, survey.

Sweet potato (*Ipomoea batatas* L.) is an important food crop worldwide. About 75% of African sweet potato production is concentrated in East Africa, especially around Lake Victoria, where it is a basic subsistence crop, grown mainly by rural women near their homes to feed their families (Kapinga *et al.*, 1995; Gibson *et al.*, 1997; Gibson and Aritua, 2002). The crop is dependable since it is drought tolerant and acts as food security and famine relief crop during famine periods (Karyeija *et al.,* 1998). In the last decade, the importance of sweet potato has increased greatly in many African regions due to frequent droughts and prevalence of pests and diseases which adversely affect the production of staple food crops such as maize, cassava and banana (Thottappilly *et al.*, 1993; Otim-Nape *et al.*, 2000; Tushemereirwe *et al.*, 2004).

Despite its high potential for food security, production of sweet potato is constrained by pests and diseases (Carey *et al.*, 1997; Karyeija *et al.,* 1998; Gibson and Aritua, 2002; Aritua *et al.,* 2007). In Africa, infection by different viruses rank second to weevils in causing yield reduction in this crop (Geddes, 1990). Some of the viruses known to infect sweet potato include *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato latent virus* (SPLV), Sweet potato caulimo-like virus (SPCa-LV), *Cucumber mosaic virus* (CMV), Sweet potato virus Y (SPVY), C-6 (Untiveros *et al.,* 2007), *Sweet potato virus G* (SPVG), *Sweet potato mild speckling virus* (SPMSV) and *Sweet potato leaf curl virus* (SPLCV) (IsHak *et al.*, 2003; Mukasa *et al.,* 2003; Ateka *et al.,* 2004; Tairo *et al.,* 2004; Miano *et al.*, 2006). SPFMV, SPCSV, SPMMV, SPCFV and SPLCV were previously detected in some parts of East Africa (Miano *et al.*, 2006). However, since there is little information on the distribution of sweet potato viruses in western Kenya, this study aimed at determining the present distribution of viruses in the crops of this area.

In western Kenya, sweet potato is widely grown in Bungoma, Busia, Kakamega, Teso and Vihiga districts (Western Province) and in Homa Bay, Kisii, Kisumu, Rachuonyo and Siaya districts (Nyanza Province), in all of which a survey was conducted. Sweet potato fields with a 3- to 5-month-old crop were randomly sampled along rural roads or paths at approximately 2-5 km intervals. A total of 327 vines obtained from symptomatic plants were collected and transferred to Jomo Kenyatta University of Agriculture and Technology (JKUAT), Department of Horticulture where they were planted in

Corresponding author: S.A. Opiyo
Fax: +057.351220/351221
E-mail: sylvopiyo@yahoo.com

15-20 cm diameter pots in sterile soil, in an insect-proof screenhouse. Plants were sprayed regularly with insecticides against aphids and whiteflies to avoid virus spread among them.

Sweet potato samples were subjected to serological assays for viruses using nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) kits as described by Gibb and Padovan (1993). One centimeter-diameter disc was taken from a leaf at the top, middle and bottom of the stem from each plant and used for serological testing (Gibb and Padovan, 1993). Polyclonal antisera specific to SPFMV, SPCSV, SPMMV, SPCFV, SPMSV, SPCa-LV, SPLV, C-6, SPVG and CMV as well as negative and positive controls were obtained from the International Potato Center (CIP, Lima, Peru). The development of a purple color on nitrocellulose membrane confirmed virus positive samples (Gutierrez *et al.*, 2003).

Total RNA was extracted from the leaves of samples determined to be infected with CMV from the serological analysis and from one healthy control. Fresh young leaf tissue (100 mg) was ground to fine powder in liquid nitrogen using motar and pestle. Total RNA was extracted from the leaves using RNeasy Plant Mini Kit (Qiagen, USA) according to the manufacturer's instructions. The integrity of the extracted RNA was evaluated by electrophoresis in 1% agarose gel stained with ethidium bromide. Total RNA was used to amplify a CMV coat protein (CP) sequence fragment by RT-PCR using CMV-specific primers, 5'-GCCGTAAGCTGGATGGAC AA- 3' (forward) and 5'-TATGATAAGAAGCT TGTTTCCG-3' (reverse) (Wylie *et al.,* 1993). The primers were synthesized by Sigma Life Sciences Corporation (Germany).

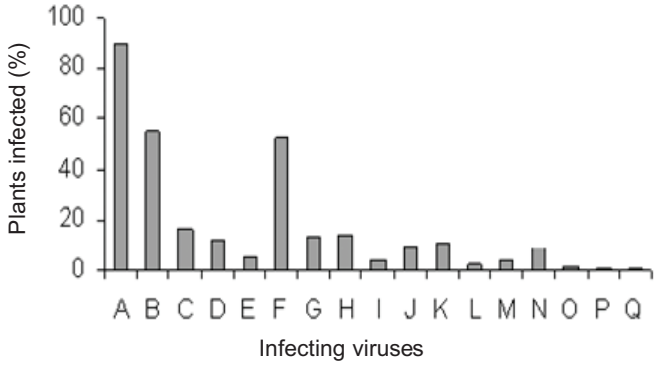

Fig. 1. Proportion (%) of single and mixed virus infections detected by nitrocellulose membrane enzyme-linked immunosorbent assay in symptomatic sweet potato plants in Western Kenya. A. *Sweet potato feathery mottle virus* (SPFMV); B. *Sweet potato chlorotic stunt virus* (SPCSV); C. *Sweet potato mild mottle virus* (SPMMV); D. *Sweet potato chlorotic fleck virus* (SPCFV); E. *Cucumber mosaic virus* (CMV); F. SPFMV+SPCSV; G. SPFMV+SPMMV; H. SPFMV+SPCFV; I. SPFMV+CMV; J. SPCSV+SPMMV K. SPCSV+SPCFV; L. SPCSVV+CMV; M. SPCFV+SPMMV; N. SPFMV+SPCSV+ SPMMV O. SPFMV+SPMMV+ SPCFV; P. SPFMV+SPCSV+CMV; Q. SPFMV+ SPCSV+SPMMV+SPCFV.

Reverse transcription (RT) was performed in an 18.8 µl reaction mixture using Omniscript Reverse Transcription Kit (Qiagen, USA). Three µl of total RNA (concentration 1 µg/µl), 0.2 µl of reverse primer, 0.3 µl of random primer and 9.5 µl of RNase-free H_2O were incubated at 70ºC for 10 min, then cooled in ice. For first strand cDNA synthesis, 2 µl of 10x strand buffer, 0.3 µl of 5 mM dNTP mix, 0.5 µl of Omniscript Reverse Transcriptase (Qiagen, USA), 0.5 µl of RNase inhibitor and 2.5 µl of RNase-free H_2O were added to the reaction tube and the mixture further incubated at 42ºC for 1 h. PCR was performed using the Taq PCR Master Mix Kit (Qiagen, USA). PCR reaction mixture (20 µl,) consisted of 10 µl of PCR Master Mix (containing $MgCl_2$, dNTP mix, Taq polymerase and PCR buffer), 4 µl of Q-Solution (Qiagen, USA), and 0.3 µl each of forward and reverse primer, 1 µl of cDNA (1 µg/µl) and 4.4 µl of RNase-free H_2O. PCR was carried out using the following cycling conditions: one cycle at 95ºC, 5 min for initial denaturation followed by 35 cycles 96ºC, 5 sec for denaturation, 6 ºC, 5 sec for primer annealing and 72ºC, 30 sec for extension and a final extension at 72ºC for 1 min. PCR products were analyzed by electrophoresis using 1% agarose gel stained with ethidium bromide and DNA bands were visualized under UV light.

Results (Table 1) shows the proportion of samples by district that reacted positive to at least one of the virus-specific antisera used in the serological tests. Out of the 327 samples tested, 89% were infected whereas 11% gave negative results. Samples from Western and Nyanza Provinces showed 94% and 84% infection, respectively. Samples from Bungoma, Busia, Kakamega, Kisii and Rachuonyo were totally infected, whereas those from Siaya (Nyanza Province) showed the lowest infection rate (60%).

Five viruses namely SPFMV, SPCSV, SPMMV, SPCFV and CMV were detected by serology (Table 2). SPFMV, which was the most widespread, was found in all the 10 districts surveyed with frequencies ranging from 60% to 100%, and an average infection rate of 89% (293 of 327 samples). SPCSV ranked second as it occurred in 179 (55%) samples. SPMMV, SPCFV and CMV had incidences of 17%, 12% and 5%, respectively. In particular, SPMMV was recorded in 9 of 10 districts, SPCFV in 8 of 10 districts whereas CMV, which was the least widespread, was detected in 4 of 10 districts, i.e. Bungoma (14%), Teso (11%), Kisii (10%), and Rachuonyo (15%).

SPFMV, SPCSV, SPMMV, SPCFV and SPMSV had previously been recorded from sweet potato in Kenya (Ateka *et al.,* 2004; Miano *et al.,* 2006; Nyaboga *et al.,* 2008). The widespread occurrence of SPFMV in the region is in agreement with the notion that that this virus

Table 1. Proportion of samples per district that reacted positive for one or more viruses.

Province	District	Samples tested (No.)	Plants positive to one or more viruses (%)
Western	Bungoma	36	100
	Busia	33	100
	Kakamega	27	100
	Teso	27	85
	Vihiga	30	78
	Mean	30	93
Nyanza	Homa Bay	30	90
	Kisii	27	100
	Kisumu	30	70
	Rachuonyo	57	100
	Siaya	30	60
	Mean	36	84

Table 2. Proportion (%) of sweet potato samples that reacted positive for different viruses.

Province	District	Samples tested (No.)	SPFMV	SPCSV	SPMMV	SPCFV	CMV
Western	Bungoma	36	100	92	17	25	14
	Busia	33	100	91	9	18	0
	Kakamega	27	89	44	10	10	0
	Teso	27	89	56	33	0	11
	Vihiga	30	90	50	10	10	0
	Mean	30	94	67	16	13	5
Nyanza	H/bay	30	90	50	10	5	0
	Kisii	27	100	44	11	6	10
	Kisumu	30	70	50	20	20	0
	Rachuonyo	57	100	42	32	21	15
	Siaya	30	60	30	0	0	0
	Mean	36	84	43	15	10	5

occurs virtually wherever sweet potato is grown, including countries in tropical and sub-tropical as well as temperate regions (Moyer and Salazar, 1989; Salazar and Fuentes, 2001). The widespread occurrence of SPFMV (89%) as compared with the other four viruses detected can depend on the way farmers select planting materials. Since sweet potato plants that are singly infected with SPFMV exhibit mild or no clearly visible symptoms (Gibson *et al*., 1997), farmers may not be able to exclude SPFMV-infected cuttings from the planting materials they visually select for the next crop, thereby maintaining this virus.

Over 80% of the samples tested showed presence of mixed infections. The most common infection combination was that of SPFMV+SPCSV and was detected in 52% of the samples (Fig. 1). Other dual infection combinations were SPFMV+SPMMV (13%), SPFMV+SPCFV (14%), SPFMV+CMV (4%), SPCSV+SPMMV (9%), SPCSV+SPCFV (10%) and SPCFV+ SPMMV (5%). The most widespread triple infection detected was by SPFMV+ SPCSV+ SPMMV which occurred in 9% of the samples. Other triple infection combinations were SPFMV +SPCSV+SPCFV (2%) and SPFMV+ SPCSV+ CMV (1%). The most complex virus infection involving four viruses (SPFMV+ SPCSV+SPMMV+SPCFV) was detected in 3 samples (1%).

The high frequency of dual infection by SPFMV and SPCSV concurs with previous findings (Mukasa *et al*., 2003; Ateka *et al.,* 2004; Tairo *et al*., 2004; Miano *et al.,* 2006; Nyaboga *et al.,* 2008). In fact, co-infection of SPFMV and SPCSV causes sweet potato virus disease (SPVD), the most economically important disease of sweet potato (Gibson *et al*., 1998; Mukasa *et al*., 2003). This viral mixture leads to a synergistic effect that re-

sults in more severe damage to sweet potato than it would occur if the crop were infected by any of the two viruses (Gutierrez *et al*., 2003). All CMV-infected plants carried SPFMV. Also this finding is in agreement with previous reports (Cohen *et al.*, 1988), suggesting a synergistic co-existence between the two viruses.

Of the 327 symptomatic samples tested, 89% were infected by at least one virus. Such a high infection incidence could be attributed to the piece-meal harvesting and continuous cropping which are practiced in the region, thereby providing a continuous reservoir of the infecting pathogens. Since the high incidence of viruses in sweet potato is mainly due to the use of infected planting materials (Gutierrez *et al.*, 2003), there is an urgent need to develop in Kenya a programme for the production of a virus-free plants from which farmers could obtain clean planting materials for vegetative propagation. Although samples for testing were selected based on the presence of virus-like symptoms, 11% of them did not react with any of the ten virus-specific antisera used in this study. This suggests the presence of new still unknown viruses or of variants of known viruses that are not recognized by the antibodies used. More assays targeting viruses other than those tested in this study is necessary.

Total RNA extracted from 12 samples that were CMV-positive when tested by NCM-ELISA, were subjected to RT-PCR using CMV-specific primers to confirm that the positive reaction did not result from contamination or was not an artifact. In all cases, amplicons of the expected size (670 bp) were obtained from the serologically positive samples but not from CMV-free controls. To the best of our knowledge, this is the first report of CMV from sweet potato in Kenya.

ACKNOWLEDGEMENTS

The authors acknowledge CIDA, AU/NEPAD, BecANet and ILRI for funding this research. The Director of the Institute of Biotechnology Research (IBR), Jomo Kenyatta University of Agriculture and Technology (JKUAT) is also acknowledged for allowing this work to be carried out in his laboratory.

REFERENCES

Ateka E.M., Njeru R.W., Kibaru A.G., Kimenju J.W., Barg E., Gibson R.W., Vetten H.J., 2004. Identification of viruses infecting sweet potato in Kenya. *Annals of Applied Biology* **144**: 371-379.

Aritua V., Barg E., Adipala E., Vetten H.J., 2007. Sequence analysis of the entire RNA genome of a sweet potato chlorotic fleck virus isolate reveals that it belongs to a distinct carlavirus species. *Archives of Virology* **152**: 813-818.

Carey E.E., Gichuki S.T., Ndolo P.J. Turyamureeba G., Kapinga R., Lutaladio N.B., 1997. Sweet potato breeding for Eastern, Central and Southern Africa: An Overview. *Proceedings of the 4th Triennial Congress of the African Potato Association, Pretoria 1997*: 89-93.

Cohen J., Loebenstein G., Spiegel S., 1988. Infection of sweet potato by Cucumber mosaic virus depends on the presence of Sweet potato feathery mottle virus. *Plant Disease* **72**: 583-585.

Geddes A.M.W., 1990. The relative importance of crop pests in sub-Saharan Africa. *Natural Resources Institute Bulletin,* No. 36**.**

Gibb K.S., Padovan A.C., 1993. Detection of sweet-potato feathery mottle potyvirus in sweet-potato grown in northern Australia using an efficient and simple assay. *International Journal of Pest Management* **39**: 223-228.

Gibson R.W., Aritua A., 2002. The perspective of sweet potato chlorotic stunt virus in sweet potato production in Africa: A review. *African Crop Science Journal* **10**: 281-310.

Gibson R.W., Mwanga R.O.M., Kasule S., Mpembe I., Carey E.E., 1997. Apparent absence of viruses in symptomless field-grown sweet potato in Uganda. *Annals of Applied Biology* **30**: 480-490.

Gibson R.W., Mpembe I., Alicai T., Carey E.E., Mwanga R.O.M., Seal S.E., Vetten H.J., 1998. Symptoms, etiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathology* **47**: 95-102.

Gutierrez D.L., Fuentes S., Salazar L.F., 2003. Sweet potato virus disease (SPVD): Distribution, incidence and effect on sweet potato yield in Peru. *Plant Disease* **87**: 297-302.

IsHak J.A., Kreuze J.F., Johansson A., Mukasa S.B., Tairo F., Abo El-Abbas F.M., Valkonen J.P.T., 2003. Some molecular characteristics of three viruses from SPVD-affected sweet potato plants in Egypt. *Archives of Virology* **148**: 2449-2460.

Kapinga R.E., Ewell P.T., Jeremiah S.C., Kileo R., 1995. Sweet potato in Tanzanian Farming and Food Systems. Implications for Research. International Potato Center (CIP) Sub-Saharan Africa Regional Office, Nairobi, Kenya, and Ministry of Agriculture, Dar-es-Salaam, Tanzania.

Karyeija R.F., Gibson R.W., Valkonen J.P.T., 1998. Resistance to sweet potato virus disease (SPVD) in the wild East African *Ipomoea* spp. *Annals of Applied Biology* **133**: 39-44.

Miano D.W., LaBonte D.R., Clark C.A., Valverde R.A., Hoy M.W., Hurtt S., Li R., 2006. First report of a begomovirus infecting sweet potato in Kenya. *Plant Disease* **90**: 832.

Moyer J.W., Salazar L.F., 1989. Viruses and virus-like diseases of sweet potato. *Plant Disease* **73**: 451-455.

Mukasa S.B., Rubaihayo P.R., Valkonen J.P.T., 2003. Incidence of viruses and virus-like disease of sweet potato in Uganda. *Plant Disease* **87**: 329-335.

Nyaboga E.N., Ateka E.M., Bulimo W.D., 2008. Serological detection of virus diseases of sweet potato in Kenya. *Journal of Applied Biosciences* **7**: 222-229.

Otim-Nape G.W., Maruthi M.N., Thresh J.M., 2000. The incidence and severity of cassava mosaic disease in Uganda. 1990-1992. *Tropical Science* **38**: 25-37.

Salazar E.L., Fuentes S., 2001. Current Status of major virus diseases of sweet potatoes. *Proceedings International Workshop on Sweet Potato Cultivars Decline Study, Miyakonojo 2001*: 14-19.

Tairo F., Kullaya A., Valkonen J.P.T., 2004. Incidence of viruses infecting sweet potato in Tanzania. *Plant Disease* **88**: 916-920.

Thottappilly G., Dahal G., Lockhart B.E.L., 1993. Viruses and virus diseases of maize in tropical Africa. *Plant Pathology* **42**: 492-509.

Tushemereirwe W.K., Kangire A., Kubiriba J., Nakyanzi M., Gold C.S., 2004. First report of *Xanthomonas campestris* pv. *musacearum* on banana in Uganda. *Plant Pathology* **53**: 802-807.

Received December 14, 2009
Accepted March 22, 2010

Untiveros, M., Fuentes, S., Salazar, L.F. 2007. Synergistic interaction of *Sweet potato chlorotic stunt virus* (Crinivirus) with carla-, cucumo-, ipomo-, and potyviruses infecting sweet potato. *Plant disease* **91**: 669-676.

Wylie S., Wilson C.R., Jones R.A.C., Jones M.G.K., 1993. A polymerase chain reaction assay for Cucumber mosaic virus in lupin seeds. *Australian Journal of Agricutural Research* **44**: 41-51.