A study on the Cause of a Sweet Potato Virus Disease in Barbados

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Abstract

In 2001 a severe, yield -limiting problem that resembles somewhat the sweet potato virus disease (SPVD) was observed in sweet potatoes in the southern parishes of Barbados. The Plant Pathology Department and the International Potato Center (CIP) investigated this syndrome. One of the most important virus diseases of sweet potato (*Ipomoea batatas)* is SPVD, which is caused by a synergistic effect of Sweet Potato Feathery Mottle potyvirus (SPFMV) and sweet potato chlorotic stunt crinivirus (SPCSV). However, other virus combinations may cause symptoms that resemble SPVD. The Nitrocellulose Membrane Enzyme Linked Immunosorbent Assay (NCM-ELISA) was used as the method of detection of the viruses present. The study showed that SPFMV, SPVG and LSU-2 were the major viruses present. Where SPVD-like symptoms occurred, the presence of SPFMV and other viral combinations were noted. In order to reduce the spread and further impact of this new disease, authorities will have to institute domestic quarantine and other disease management strategies. This is the first report of a SPVD- like disease on sweet potato in Barbados.

Key words: sweet potato virus disease, SPVD, sweet potato, *Ipomoea batatas,* Barbados

Introduction

Sweet potato (*Ipomoea batatas*) is considered one of the main staple food crops in Barbados and is planted throughout the year. It has also been identified as one of the crops to be used in achieving food security. Over the five-year period 1997-2001 (Agricultural Planning Unit, 2003, pers. comm.,) the crop produced a yearly average of 2 million kilograms

Reports of a virus-like disease affecting the sweet potato crop first surfaced during a particulary severe dry season in 2001. The affected plants were concentrated in the eastern parishes of St. Philip and St. John, and the central parishes of St. Michael and St. George. A reduction in yields of fields in these areas was associated with foliage symptoms that included chlorosis, mottling, leaf reduction and deformation and stunting. Other symptoms observed in the fields were swollen leaf veins and raised grooves on the potato tubers. Hither to this there had not been records of any viral problems occurring on sweet potato. Norse (1974) does not describe any viruses affecting sweet potato in Barbados.

There are over 20 viruses known to affect sweet potato worldwide (Moyer and Salazar, 1989; Brunt *et al* 1996) and these are thought to be responsible for more than 50% of the yield losses occurring in sweet potato production (Hahn, 1979; Ngeve and Bouwkamp, 1991). In most cases these viruses occur as mixed infections and tend to be specific to members of the Convolvulaceae family (Moyer and Salazar, 1989). Moreover, it has been shown that these viral mixtures or interactions may lead to the occurrence of a synergistic effect which result in more severe damage to the crop than would be expected if an individual virus was present (Gutierrez *et al*, 2003).

SPVD is thought to be the result of one such synergistic interaction between sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV) (Gibson *et al* 1998; Karyeija *et al*, 2000). This virus complex was first described by Sheffield (1953) in East Africa but the first reported presence of the disease is thought to have been around 1939 in the region now known as The Democratic Republic of Congo (Carey *et al,* 1999). At present, SPVD can be found throughout East and West Africa, the USA, Israel, China, Taiwan, Brazil Argentina and Peru (Ames *et al,* 1996)

SPVD is one of the more devastating diseases that affect sweet potato causing severe reduction in yields but apparently having no effect on the quality of the potato tuber (Gibson *et al.,* 1998, Hahn, 1979; Milgram, Cohen and Loebenstein, 1996). The disease is characterised by symptoms including vein clearing or mosaic, stunting, leaf reduction and deformation (Ames *et al.,* 1996; Clarke and Moyer, 1988; Gibson *et al.*, 1998). These symptoms were similar to those noticed on the infected crop in Barbados.

The diagnostic serological tool used in this work was the Enzyme Linked Immunosorbent Assay on Nitrocellulose Membrane (NCM-ELISA), which was developed by the International Potato Center (CIP) (Abad and Moyer, 1992). This test is equally as sensitive as the double antibody sandwich ELISA, in addition, the membranes can be stored for weeks after the samples are applied before the test is done locally or shipped to another location (Salazar and Jayasinghe, 2001).

The aim of this study was to determine whether or not SPVD was the cause of the problem presently affecting the sweet potato crop in Barbados.

Materials and Methods

The NCM-ELISA kits (including antisera) provided by CIP, were used to test the samples for the presence of SPFMV, SPCSV, SPMSV (Sweet potato mild speckling virus), SPMMV (Sweet potato mild mottle virus), SwPLV (Sweet potato latent virus), SPCFV (Sweet potato chlorotic fleck virus), SPCaLV (Sweet potato caulimovirus) and C-6 (new flexuous rod virus) (Gutierrez *et al*. 2003). Tests were conducted for two other viruses at the CIP using NCM-ELISA. These were SPVG (Sweet potato G potyvirus) and LSU-2, a new potyvirus shown to infect sweet potato in the USA (Salazar, 2003, Pers comm.).

Sampling

Ten fields were sampled within the areas where the problem was initially seen. The crops in these fields ranged in age from three weeks to three months. A total of 148 samples were taken. Leaves were taken from the top and center of both suspected and symptom-less plants and placed in a small plastic bag and labeled.

NCM-ELISA Protocol

The protocol used was based on the one described by Salazar and Jayasinghe (2001).

Two leaf disks of approximately 1cm in diameter were cut from the leaf samples using small glass tubes of 1cm in diameter. The disks were then ground in 2ml of extraction buffer (0.2% sodium sulphite in TBS) using a small test tube. Samples were left for approximately 30 minutes at room temperature (approximately 25° C). The nitrocellulose membranes were numbered and immersed in TBS. The membranes were then placed onto two dry Whatman #4 filter papers and left to stand for about 1-2 mins. A micropipette was then used to dispense 20ul of the clarified supernatant onto the membranes. They were then placed onto a dry filter paper and allowed to dry for 30 minutes at room temperature (approximately 25° C).

Protocol for serological development

1. The membranes were placed in a clean plastic container in blocking buffer solution (2% milk powder in TBS, containing 2% Triton X-100) and incubated whilst agitating for 1hour.
2. The blocking solution was decanted and the membranes washed quickly with TBS and the antibody solution (2% milk powder in TBS containing the antibodies at an appropriate concentration) was added. The membranes were incubated under agitation overnight at room temperature (approximately 25° C.)
3. Antibody solution was discarded and membranes washed with TBS-Tween 4 times, for 3 minutes each.
4. 30ml of the conjugated antibody solution (2% milk powder in TBS containing alkaline phosphatase-conjugated goat-anti-rabbit) was added to each membrane, covered and incubated with agitation for 1 hour.
5. The conjugate solution was discarded and membranes washed as in step 3.
6. 25ml of substrate solution [nitrobenzene-tetrazolium-NBT and 5-bromo-3-chloroindolyl phosphatase-BCIP in substrate buffer (0.1M Tris, 0.1M NaCl, 5mM $MgCl_2.6H_2O$, pH 9.6)] was added to membranes and left at room temperature for 30 minutes to 1 hour. The membranes were washed 3 times with distilled water for about 5-10 minutes to stop any further development of color.

Results

Table 1 and Table 2 provide a breakdown of the viruses detected in the survey carried out.

Of the 16 samples that showed SPVD–like symptoms, SPFMV and LSU-2 were detected as mixtures in 11 of them. SPFMV, SPVG and LSU-2 were detected as mixtures in two samples whilst a mixture of SPFMV, LSU-2 and SPCSV was detected in one sample. A mixture of four viruses (SPFMV, SPVG, LSU-2 and SPCSV) was detected in one sample. In only one sample, which showed the SPVD-like symptoms, was a virus or virus mixture not detected.

Two samples reacted only to SPFMV, one sample showed symptoms of vein clearing and the other being symptom less. Only five samples tested positive for SVPG alone. None of these plants showed any symptoms.

No samples tested positive for LSU-2 alone or SPVG and LSU-2 as a mixture. The symptoms of stunting and mottling were observed in the samples that tested positive for a mixture of the SPVG, LSU-2 and SPCSV viruses. SPCSV was detected alone in six samples. The symptoms ranged from stunting, mottling to the presence of chlorotic spots. In three of these samples however no symptoms were observed, this included the sample of the volunteer sweet potato (*Ipomoea batatas*). One sample of wild *Ipomoea spp*. reacted to both LSU-2 and SPCSV viruses. This plant showed no symptoms. SPMMV, SwPLV, SPCFV, SPMSV, SPCaLV and C-6 were not detected in any of the samples tested

Table 1: Incidence of viruses detected in sweet potato samples with SPVD-like symptoms

SPCSV	SPFMV	LSU-2	SPVG	Incidence (%)
	+	+		69
	+	+	+	10
+	+	+		7
+	+	+	+	7
-	-	-	-	7

Table 2. Association of viruses to symptoms

SPVG	LSU-2	SPCSV	SPMV	Symptoms
+	+	+		Stunting, mottling
		+		Stunting, mottling, chlorotic spots, no symptoms
	+	+		No symptoms
+				No symptoms
			+	Vein clearing, no symptoms

Discussion

This is the first time that SPVD-like symptoms have been seen in Barbados in addition to the detection of SPCSV strains, SPFMV, SPVG and LSU-2 on their own and in mixtures. Carey *et al* (1999) stated that this disease was only present when the symptoms were matched by the detection of the SPFMV and SPCSV mixture in samples. This criterion was not satisfied by the results from samples tested in Barbados with these symptoms. Hence the synergistic effect of SPFMV and SPCSV may not be the cause of the SPVD-like symptoms observed in Barbados.

It has been shown though that other viral synergistic interactions can cause diseases similar in symptomology to SPVD, for instance chlorotic dwarf disease (SPFMV, SPMSV and SPCSV) (Di Feo *et al*, 2000). Therefore, the seed program for Barbados, from which farmers could obtain clean planting material.

Further work is required to properly identify the viruses affecting sweet potatoes in Barbados.

fact that most of the samples with the disease symptoms (approximately 69%) reacted positively for both SPFMV and LSU-2 could indicate one of these interactions.

The possibility that the samples showing SPVD-like symptoms are caused by other viruses not yet identified must be considered. Further work is needed in this area.

With respect to the presence of the other viruses, not much has been reported in the literature on SPVG and LSU-2 (both relatively new viruses). However, it has been shown that SPCSV strains on their own can cause yield reduction of up to 34% (Carey *et al*., 1999; Gutierrez *et al*., 2003).

Since the high incidence of viruses in sweet potato is due primarily to the fact that infected cuttings are planted (Gutierrez *et al*., 2003), there is an urgent need to develop a virus-free

Gibson, R. W., Mpembe, I., Alicai, T., Carey, E. E., Mwanga, R. O. M., Seal, S. E., and Vetten, H. J. 1988. Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathol. 47: 95-102.*