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Research Article

Evaluation of Sweet Potato Virus Occurrence and Distribution in Sweet Potato Farmer's Fields in Papua New Guinea

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Abstract

Sweet potato (*Ipomoea batatas* (L.) Lam.) is an important staple food crop in Papua New Guinea, however the production is been limited by pest and disease of which virus is a major constraint. There have been studies done on virus, but limited information is available on the occurrence and distribution of the known sweet potato viruses. Past work was mostly based on opportunistic observation on morphological symptoms with no detailed validation. In this study, aerial survey of virus infected sweet potato fields' (old and new) was conducted in 2013 in selected Provinces; namely Eastern Highlands, Western Highlands, Madang and Central. Using Nitrocellulose Membrane Enzyme-Linked Immunosorbent Assay (NCM-ELISA) test kit developed by Potato International Centre (CIP) Peru, symptomatic leaf 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), Sweetpotato Caulimo-like Virus (SPCa-LV), Cucumber Mosaic Virus (CMV), C-6, Sweet Potato Virus G (SPVG) and Sweet Potato Mild Speckling Virus (SPMSV). Vines and/or storage roots of the sample plants were collected and further screened via virus indexing using indicator plant Ipomoea setosa. The results revealed preliminary detection of additional viruses, SPMSV and Sweet Potato Chlorotic Stunt Virus (SPCSV) beside three other known viruses: SPFMV, SPVG and SPCaLV. The most prevalent virus is SPCaLV distributed in all sites followed by SPFMV, SPSMV, SPVG and SPCSV. Virus incidence severely observed in highlands sites compared to coastal lowlands and there's not much difference between old and new fields – confirming that the practices of farmers using planting material from old fields to new, viruses are transmitted as well. This study revealed wide range of viruses occurred and distributed across the farmer's fields which required the need of using sensitive diagnostic techniques for correct detection and the use of virus-free planting material as a management strategy.

Keywords: Sweet Potato; Virus; Incidence; Indicator Plant Ipomoea Setosa; NCM-ELISA; Occurrence; Distribution

Introduction

Sweet potato is the major root crop staple in Papua New Guinea (PNG), especially in the highlands region and it is also increasingly becoming important in coastal areas due to its agronomic superiority over the other crops, such as taro and yams. It provides the primary source of dietary energy for 60% of the population [1] however, over the years of production the crop has experienced continual yield decline caused mostly by pest and disease of which virus is a major constraint [2].

Virus-induced diseases are off concern at the moment because the crop is very sensitive to virus infection [3] and since virus is a systematic pathogen, it can persist and spread over successive crop cycles through vegetatively propagated materials [4].

Since early reports of suspected viral diseases of sweet potato in the United State of America [5] and in Eastern Africa [6], today there were at least 30 viruses recognized as pathogens of sweet potato worldwide [7]. In PNG there were six (6) confirmed viruses

detected to infect sweetpotato, albeit with very little information available on their occurrence and distribution [8,9]. These viruses include: Sweet Potato Feathery Mottle Virus (SPFMV), Sweet Potato Virus G (SPVG), Sweet Potato Mild Mottle Virus (SPMMV), Sweet Potato Ringspot Virus (SPRSV), Sweet Potato Chlorotic Fleck Virus (SPCFV) and Sweet Potato Caulimo-like Virus (SPCaLV). In addition, from a final report of an Australian Centre of International Agriculture Research (ACIAR) funded project SMCN/2004/071 "Reducing pest and disease impact on yield in selected PNG sweet potato production systems" implemented in 2006 to 2009 by National Agriculture Research Institute (NARI) and partners, Hughes [2] reported to confirm the presence of those viruses using recognized diagnostic techniques (mostly NCM-ELISA and virus indexing). Among those known viruses was a Sweet Potato Leaf Curl Virus (SPLCV) genus Begomovirus confirmed to be new to PNG. A worrying observation was the potential presence of Sweet Potato Chlorotic Stunt Virus (SPCSV) but that was only based on visual assessments. Pearson [10] stated that identification of virus diseases found in sweet potato was almost entirely attributed on the basis of the morphological symptoms expressed by the host plant. This may explain the absence of viruses in the Pacific Pest List database for sweet potato in PNG as few of the records have been authenticated as required for inclusion as a public record [11].

Sweet potato viruses are challenging to detect due to low titers and uneven virus distribution, presence of high concentration of inhibitors in sweet potato plants that interfere with serological or PCR-based methods, occurrence as mixed infections, and diverse strains. With use of reliable techniques, virus detection can be done rapid and precise and characterize to understand better the epidemiology of the disease (s) caused by these viruses, in order to develop an infectivity-based forecasting system and management strategies [7,12].

In this study, a survey was systematically conducted at seven (7) different sweet potato farming sites in PNG in conjunction with activities implemented by NARI as part of the European Union (EU) funded project (2012 to 2016) 'Generation and adaptation of improved agricultural technologies' aimed at establishing information on the occurrence and distribution of sweet potato viruses in new and old sweet potato farmer's fields.

Materials and Methods

Virus survey in farmers' fields

In 2013, a survey was conducted in seven selected sweet potato farming sites in PNG, namely Kopafo (Eastern Highlands Province), Kiripia and Alkena (Western Highlands Province), Murukanam and Derin (Madang Province), and Hisiu and Yule Island (Central Province). Two farmers per site were selected in which both their old fields (harvested and abandon) and new fields (more than 3 months old/unharvested) were surveyed. A total of 28 fields were surveyed in all the sites.

Initially, each famer's field was visually assessed for virus symptoms and recorded accordingly against a virus symptomatic descriptor. A total of 6 symptomatic leaves were sampled (totalled of 84 samples) following a strict diagonal sampling pattern in every fields. The samples were placed inside a zip lock bag, labelled by their locality and local names, packed in an esky and transported to a convenient location where samples were pre-processed by blotting the extracted tissue samples onto the respective membrane labelled against polyclonal antisera of specific viruses as described by Gibbs and Padovan [13]. The dried membranes were stored in an airtight container and once returned to NARI Momase Regional Centre (MRC) Biotechnology Laboratory in Bubia (Lae, Morobe Province); the samples were stored at 4ºC until further processing at once. All 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), Sweetpotato 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-link immunosorbent Assay (NCM-ELISA) developed by International Potato Centre (CIP), Peru. The development of a purple color on nitrocellulose membrane confirmed virus positive samples [14].

Virus indexing via indicator plant I.setosa grafting and NCM-ELISA test kit

At the same sample plants where the symptomatic leaves were extracted; storage roots and/or vine cuttings were collected respectively for further screening on station. A total of 45 samples were collected as storage roots where available but mostly vines

comprising a range of local varieties; Alkena and Keripia with 13 varieties, Kopafo 8, Murukanam 12, Dering 5, Yule Island 2 and Hisiu 6. At Momase Regional Centre (MRC) Bubia, they were established first in 2L size pots filled with sterilized top soil inside the screen house and later maintained in the field.

The testing of those varieties was done by first indexing onto the indicator plants Ipomoea setosa as described by Frison and Ng [15] then followed by confirmation testing using the NCM- ELISA kit. Grafting on indicator plants increases the virus titre in the target plant (scion) and allows for a more reliable detection of a putative virus in a sweetpotato sample compared to direct testing of leaves collected in the field [16].

Indicator plant were established by soaking viable seeds overnight to break seed dormancy. Fine sterilized top soil mixed with sand was filled into 2L size pots and placed inside the screen house. After 3 days the germinated seeds were transplanted into prepared pots with one seedling per pot. The plants were maintained well with adequate watering, staking and soil fillings before grafting of target samples at 2 weeks after planting. Two scions having a fully expanded leaf of the remaining 41 farmer's sweetpotato varieties (4 died during establishment) were side grafted onto healthy grown *I. setosa* plants (two per plant). Scalpels were sterilized with 70 % ethanol each time prior to cutting the scions to avoid cross-contamination of plants during the grafting process. The grafted joints wrapped with plumber's tape helped prevent desiccation. Short pegs were placed beside the grafted scions for support. Clear plastic bags were then placed over the grafted plants with pots completely watered. The plastic bags helped in maintaining humidity inside to encourage growth. Each grafted pot was labeled respectively and maintained inside the screen house. After five days the plastic bags were removed and kept open to sunlight and start observed for virus symptoms development. Couples of weeks later of virus symptom assessments, symptomatic leaves of *I. setosa* were sampled for serological assay using CIP NCM-ELISA kit. From the two pots of grafted sweetpotato varieties, three samples per variety were collected and placed in respective plastic bags and brought to the lab in an esky. The testing was then conducted as described previously [13].

Results

Virus incidence at farmer's fields

A wide range of sweetpotato virus symptoms were commonly observed indicating putative infection with a virus.

Virus detection from symptomatic sweet potato leaf samples

The results from the visual assessment of farmers' fields and the subsequent testing of samples using serology test are shown below (Table 1).

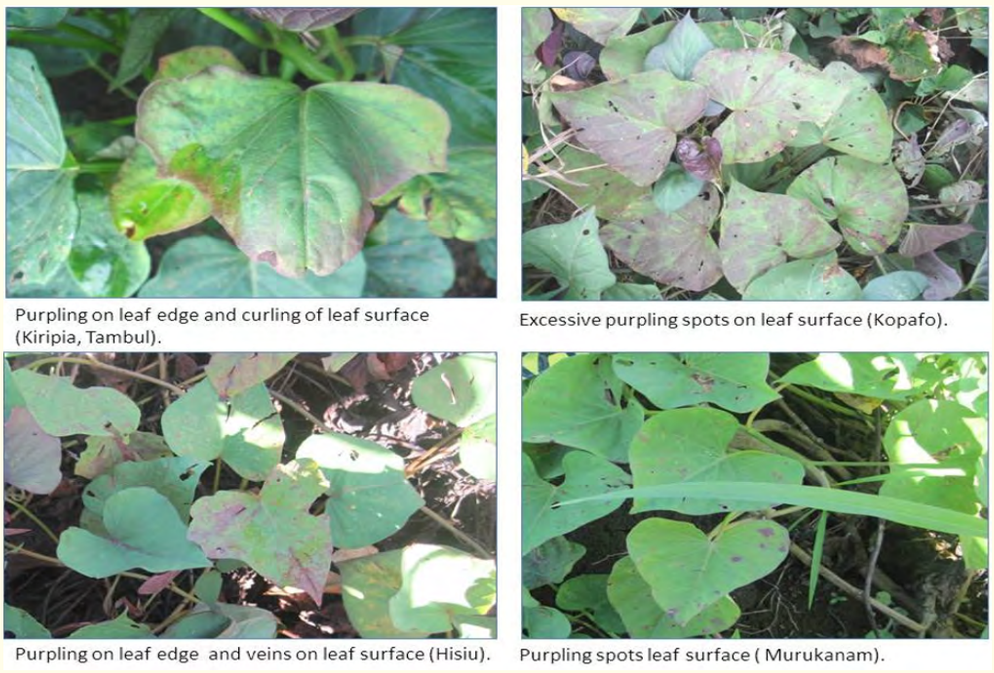

Figure 1: Virus symptoms observed at farmers' gardens.

Site	Date	Symptoms observed[1] -ref to key below	Suspected virus[2]	Confirmed virus using NCM-ELISA (old garden)	Confirmed virus using NCM-ELISA (new garden)	Faint reaction of viruses detected in both new or old garden
Kopafo	23/07/2013	1, 2*, 3, 7, 4, 5*	SPFMV, SPCFV, SPVG.	SPFMV	SPFMV	SPCMV, SPMSV SPCSV
Alkena and Kiripia		1, 2*, 3, 4, 5*	SPFMV, SPVG, SPCSV.	SPFMV	Negative	
Murukanam	07/09/2013	2*	SPFMV	Negative	SPCSV	SPFMV, SPMSV, SPCV, SPCMV SPCFV
Hisiu	09/10/2013	2*, 6	SPFMV, SPCSV.	Negative	Negative	
Yule Island		2*, 6	SPFMV, SPCSV.			
Derin	10/10/2013	Ns	SPCFV	Negative	Negative	

Table 1: Observed putative virus symptoms of sweet potato leaf samples and results of NCM-ELISA test of samples collected at farmers' fields in the seven sites.

[1]Key to virus symptoms: Leaf curling and up rolling (1); Purplish spots on leaf surface and near leaf edges (2); Leaf yellowing and yellowing spots over leaf surface (3); Yellowish spreading spots associated with vein chlorosis, (4); Excessive purpling of leaf surface (5); Stunting and yellowish spreading spots (6); Chlorotic spots appearing with flecked leaf surface (7). Ns = no clear symptom. Most commonly observed symptoms (*).

[2]SPFMV: Sweetpotato Feathery Mottle Virus; SPCFV: Sweetpotato Chlorotic Fleck Virus; SPCSV: Sweetpotato Chlorotic Stunt Virus; SPMSV: Sweetpotato Mile Speckling Virus; SPCV: Sweetpotato Caulimo-like Virus; SPCMV: Sweetpotato Cucumber Mosaic Virus.

Visual assessment has showed number of symptoms indicating the possible presence of different viruses at the respective sites such as Sweet Potato Feathery Mottle Virus, (SPFMV), Sweet Potato Chlorotic Fleck Virus (SPCFV), Sweet Potato virus G (SPVG) and Sweet Potato Chlorotic Stunt Virus (SPCSV). Results from the NCM-ELISA only showed clear reaction for SPFMV and SPCSV viruses. Fainted reactions for SPFMV, SPVG, and SPCFV viruses were observed in a number of samples.

Virus detection via virus indexing (grafting using Ipomoea setosa and NCM-ELISA test kit).

Results of the serological test of the samples grafted on *I. setosa* are summarized in table 2.

Sites	Farmer's local cultivar	Symptoms observed[1] (ref to key below)	Virus[2] as detected by NCM-ELISA									
Kopafo			FMV	MMV	LV	CFV	C-6	MSV	CV	CSV	SPVG	CMV
	Gimani GKA	3,7,8,14	+	-	-	-	-	-	-	-	+	-
	Efiyufae	3	-	-	-	-	-	+	-	-	-	-
	I Don't Care	3,7	-	-	-	-	-	-	-	+	+	-
	Whagi Besta	3,7,8,11,14,15	+	-	-	-	-	+	+	-	+	-
Alkena and Kiripia	Twisties	3,7,8,14,15	+	-	-	-	-	-	-	-	-	-
Murukanam	CC1	3,7,8,14,15	-	-	-	-	-	-	+	-	-	-
	CC2	3,7,8,14	-	-	-	-	-	-	+	-	-	-
	Kawok	3,7,8,14	-	-	-	-	-	-	+	-	-	-
	Finga	3,7	-	-	-	-	-	+	+	-	-	-
	Wanway	3,7,8,14	-	-	-	-	-	-	+	-	-	-

Table 2. NCM-ELISA test results via virus indexing using indicator plant, I.setosa of positively detected leaf samples (only) from the sites.

[1]Key to virus symptoms: Leaf curling and up rolling (3); Chlorotic spots appearing with flecked leaf surface (6); Deformed and brisky leaf surface, irregular shape (7); Leaf yellowing and yellowing spots over leaf surface (8); Yellowing of mid ribs main leaf veins (11); Yellowish spreading spots associated with vein chlorosis (14); Excessive feathery symptoms (15).

[2]SPFMV: Sweetpotato Feathery Mottle Virus; SPVG: Sweetpotato Virus G; SPCSV: Sweetpotato Chlorotic Stunt Virus; SPMSV: Sweetpotato Mile Speckling Virus; SPCV: Sweetpotato Caulimo-like Virus.

Most common symptoms observed after two weeks of grafting includes; leaf curling and up rolling, deformed and brisky leaf surface, irregular, leaf yellowing and yellowing spots over leaf surface and yellowish spreading spots associated with vein chlorosis. Sweet potato samples were detected positive with single and multiple virus infections respectively. Sweet Potato Virus G (SPVG) was commonly found in Kopafo followed by Sweet Potato Feathery Mottle Virus (SPFMV) and Sweet Potato Mile Speckling Virus (SPMSV) then Sweet Potato Caulimo-like Virus (SPCV) and Sweetpotato Chlorotic Stunt Virus (SPCSV). SPFMV was only found in Alkena and Kiripia. SPCSV was commonly found in Murukanam followed by SPMSV. No virus was found for Derin, Yule Island and Hisiu samples due to unsuccessful indexing of I. setosa grafts.

Discussion and Conclusion

This study has detected five viruses to occurred and distributed throughout the seven (7) sweet potato farming communities, namely Sweet Potato Feathery Mottle Virus (SPFMV), Sweet Potato virus G (SPVG), Sweet Potato Mile Speckling Virus (SPMSV), Sweet Potato Caulimo-like Virus (SPCV) and Sweetpotato Chlorotic Stunt Virus (SPCSV). Potyvirus (SPMSV) is confirmed to be new to PNG while SPFMV, SPVG, SPCV, SPCSV (fainted reaction) had been already reported before or picked up in virus testing conducted as part of ACIAR funded projects SMCN/2004/071 [2] SPCSV is off great concern because in co-infection with SPFMV, it causes the SPVD which can result in devastating yield decline as shown elsewhere [9]. Most viruses so far detected in PNG using NCM-ELISA kit and electronic microscopy only which have yet to be confirmed through Polymerase Chain Reaction (PCR) testing and sequencing of the genome in order to be published and confirmed as an authenticated record. It is likely that those viruses have been here for some time but there have not been any pest and disease surveys in a long time, so it remained undetected.

Aerial survey of virus infected sweet potato showed wide range of virus symptoms occurred and distributed throughout farming communities with Kopafo being observed commonly then in Alkena, Kiripia, Hisiu, Yule Island and Derin. This indicated high virus incidence in low soil temperature sites compared to the high soil temperature sites in the coastal lowlands with not much difference between old and new fields, confirming that the practices of farmers using planting material from old fields to new, viruses are transmitted as well.

Nitrocellulose Membrane Enzyme-link immunosorbent Assay (NCM-ELISA) results from symptomatic sweet potato foliage directly extracted from farmer's fields (Table 1) did not correspond with results obtained from virus indexing (grafting using *I. setosa* and NCM-ELISA test kit) (Table 2). Viruses were detected in both samples, but more was found via virus indexing (grafting using *I. setosa* and NCM-ELISA test kit). In addition, there were variable virus symptoms observed in the farmer's fields and most symptomatic leaves were sampled and tested, however, resulted only for Sweet Potato Feathery Mottle Virus (SPFMV) and Sweet Potato Chlorotic Stunt Virus (SPCSV), while fainted reactions suggested presence of other viruses (Table 1). Literature reviewed revealed that the virus titre in samples directly collected from the farmer's fields is low resulting in negative or only faint reactions [7,12] Grafting onto indicator plants *I. setosa* increases the virus titre in the target plant (scion) and allows for a more reliable detection of a putative virus in a sweetpotato sample compared to direct testing of leaves collected in the field [16]. Symptoms may also be caused by other pathogens, environment, pests, nutrition etc. and could be mistaken for virus infection – for example, purpling color of mature leaves caused by nutrient deficiency in the soil or plant maturity may confused with virus symptoms [17]. It is therefore better to avoid direct testing and instead do grafting on I. setosa followed by NCM- ELISA.

This survey has shown wide range of viruses occurred and distributed in the farmer's fields all throughout the sites. Because of limitations in the sampling and testing method there is need for a re-confirmation test for the preliminary results using more sensitive or advanced virus diagnostic techniques such as Polymerase Chain Reaction (PCR). The Sweet Potato Chlorotic Stunt Virus (SPCSV) in particular has to be confirmed with systematic approaches with such reliable technique for rapid detection and identification for timely management since its co-infection with other viruses such as Sweet Potato Feathery Mottle Virus (SPFMV) can cause severe yield reduction. It is also revealed the need of using virus-free planting material as a management strategy to contain the spread of virus diseases.

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