Department of Agriculture and Fisheries Qld

Australian sweetpotato pathogen testing procedures

April 2018

For Horticulture Innovation Australia (Hort Innovation) Ltd
Project VG13004 – Innovating new virus diagnostics and plant bed management in the Australian sweetpotato industry

Horticulture Innovation Australia

Australian Sweetpotato Growers Inc

Queensland Government

This publication has been compiled by Sandra Dennien of Horticulture and Forestry Sciences, Department of Agriculture and Fisheries.

This review has been prepared as part of the Hort Innovation project VG13004 “Innovating new virus diagnostics and planting bed management in the Australian Sweetpotato Industry”. This project has been funded by Horticulture Innovation Australia Limited using the vegetable industry levy and funds from the Australian Government. It will be continually updated throughout the project as new information comes to hand.

Contents

Introduction

During the last decade the provision of pathogen tested (PT) planting material and associated research on seed bed technology and agronomy has been instrumental in improving Australian productivity of leading to rapid industry expansion. Australian growers now produce the world's highest yields per hectare, currently worth upwards of $90M (ASPG). Recent research revealed that the use of PT planting vines or sprouts of optimal length produced via seedbed technology with incorporation of best practice planting techniques and agronomy, have improved yields by up to 80% (Coleman et al. 2006).

Due to the vegetative nature of on farm propagation, plants are exposed to pest and disease pressure over generations, leading to a build-up of pests and pathogens within planting material. Yields generally decline over at least 2 seasons in Australia, so growers purchase new PT material every one to two years to avoid economic losses. PT seed is produced in an area well isolated from commercial sweetpotato production but is partly field produced, so there is a need to regularly test material to ensure freedom from virus infection. Any successful PT scheme is therefore dependent upon accurate virus detection to ensure material is free from known viruses and ongoing capacity to remove those viruses.

The ability to detect and identify current and potentially new viruses is essential to the Australian sweetpotato industry, emphasised by the establishment of the Horticulture Innovation Australia (Hort Innovation) project VG13004 'Innovating new virus diagnostics and planting bed management in the Australian Sweetpotato Industry'. This project, a collaboration of (and co-funded by) the Australian sweetpotato industry, represented by Australian Sweetpotato Growers Inc. (ASPG), Hort Innovation and the Queensland Government, demonstrates an ongoing commitment to Australian sweetpotato industry productivity and biosecurity.

Effective virus testing requires a comprehensive knowledge of viruses present in country as well as any potential exotic virus threats, an understanding of the sweetpotato farming system and the applied technical ability to rapidly and accurately detect them. Recent work funded as part of ACIAR projects SCMN/2004/071 and PC 2011/053 detected Sweetpotato leaf curl virus (SPLCV) and other virus species in Australian germplasm collections that had been assembled in the early 2000s (Hughes 2010).

The Recently established US Sweetpotato National Clean Plant Diagnostic Network (NCPN) outlines minimum virus testing protocols specific to sweetpotato (*NCPN* sweetpotato n.d.) for viruses found in country. The International Potato Centre (CIP) in Peru also has standardised protocols to detect sweetpotato viruses occurring in their part of the world and other countries of interest. Whilst some virus species are common to all countries, other viruses occurring in Australia are not covered within the NCPN or CIP protocols.

This document outlines the standardised methodology currently used by the VG13004 project team at the Department of Agriculture and Fisheries Queensland's (DAF Qld) Gatton Research Facility (GRF) to: sample sweetpotato growing areas; provide growers with information on the virus status of their infield planting material; provide virus indexing capacity to the Australian clean seed scheme and maintain sample and long-term diagnostic integrity. The complex nature of sweetpotato viruses, differing levels of cultivar tolerance, low and changing titres, interactions between virus species and host plants, diverse strains and lack of visual symptoms, confounds accurate virus detection in this crop. For these reasons the current Australian PT system relies on the following combination of complimentary diagnostic procedures.

1. Phyto-diagnostics, to detect the presence of sweetpotato viruses including new and unknown or "novel" viruses using biological indicator plants. The indicator plant *Ipomoea setosa* is highly sensitive to most sweetpotato infecting viruses, therefore leaf symptoms produced are more apparent than those observed on sweetpotato plants. This also facilitates rapid increases in virus titres, enhancing downstream diagnostics such as NCM-ELISA and PCR.

2. Serology using a standard Nitrocellulose membrane enzyme linked immunosorbent assay (NCM-ELISA) test kit manufactured by the International Potato Center, Peru (CIP) to test for 10 known sweetpotato infecting viruses, (C-6, CMV, SPCaLV, SPCV, SPCFV, SPCSV, SPFMV, SPLV, SPMMV, SPMSV and SPVG). A prerequisite for the test is to use material that is first grafted onto *I. setosa,* as the virus concentration is higher in the indicator species and the test is affected by inhibitors present in sweetpotato sap (Fuentes pers comm.).

3. Molecular detection using, quantitative or real time PCR (qPCR). Using *I. setosa* or *Ipomoea batatas* (sweetpotato) tissue samples. The whole reaction is contained within the instrument, resulting in less chance of contamination of work areas. This is currently under development as there are limited proven published assays available for sweetpotato viruses.

These protocols form the basis for inducting new and casual staff into the diagnostic process at GRF, as well as providing an updatable document for recording and improving sweetpotato industry diagnostic procedures. It builds on the methodology detailed in Dennien et al. (2013); in the VG13004 Virus management in Australia Literature Review (2018), and the experiences of other virus diagnosticians throughout Oceania and the rest of the world.).

Table 1. Sweetpotato viruses reported from Australia

Family	Genera	Anacronym	Species	Type	Structure
Potyviridae	Potyvirus	SPFMV (Gibb 1993)	Sweetpotato feathery Mottle virus Strains O, RC and EA	ssRNA +	flexuous filaments
		SPV2 / IVMV / SPVY (Tairo 2006)	Sweetpotato virus 2. Syn: Ipomoea vein mosaic virus / Sweetpotato virus Y		
		SPVC (Tairo 2006)	Sweetpotato virus C		
Betaflexiviridae	Carlavirus	SPCFV (Jones 2007)	Sweetpotato chlorotic fleck virus	ssRNA +	flexuous filaments
Geminiviridae	Begomovirus	SPLCV (Hughes 2010)	Sweetpotato leaf curl virus	ssDNA	 twinned icosahedra
Caulimoviridae	Cavemovirus	SPCV (Norkunas 2009)	Sweetpotato collusive virus	dsDNA-RT	 icosahedra
Phytoplasma	*Candidatus aurantifolia*	SPLL syn: SPLL4 (Davis 2003)	Sweetpotato little leaf	phytoplasma	

Sample collection

Samples of sweetpotato vines and/or storage roots are collected and stored in a zip-lock bag (one sample per bag) and labelled with the collectors name and date. Samples need to be kept cool and if more than two days away from the diagnostic facility, cuttings are wrapped with moistened paper towel, before being placed into the zip-lock bags. Bags containing roots are not be sealed. All samples collected are entered into the sweetpotato virus survey registry, used to create a grower sample database to track symptoms and virus testing results. The registry records growers' names, date collected, location of farm, species and cultivar of sample, cropping stage (plant bed, commercial crop, weed or regrowth), description of symptoms if present and an allocated accession number.

Sample preservation and maintenance

Because the herbaceous indicator species used has a restricted growing period, it is currently required that cutting samples be maintained as living plants, until herbaceous indexing can take place. Usually September is the earliest this indexing can be undertaken at GRF continuing throughout summer until April.

Samples (vine cuttings and storage roots) are potted in 100 mm pots containing modified pasteurised UC mix (one-third washed river sand, one-third peat and one-third perlite, with the addition of blood and bone, dolomite, hydrated lime, potassium nitrate, potassium sulphate, superphosphate and trace elements as per Dennien et al. (2013). The potted samples are labelled with the grower's name and the accession number. They are then placed into a quarantine mesh insect-screened 'quarantine' bench, within an insect-proof glasshouse, and regularly hand watered as required.

Quarantined plants are sprayed with insecticides and miticides at fortnightly intervals. Application is done in the late evenings, so as not to cause foliage burn. After establishment (4-6 weeks), the quarantined plants are relocated to an insect-proof quarantine mesh screened bench, within a larger insect-proof quarantine meshed igloo, and base-watered using an automatic micro-irrigation system. Precautionary sprays for mite and insect control are continued at fortnightly intervals with igloo access strictly limited to prevent inadvertent insect incursions.

Preservation of plant tissue

As a back up to the live plant samples held at GRF, tissue is obtained from collected sweetpotato plant samples as well as *I.setosa* indexed samples for preservation using 2 methods: RNAlater™ (to preserve RNA) and drying over silica gel (to preserve DNA). Between 14 to 28 days PG, three leaves from each pair of *Ipomoea setosa* grafted plants and the respective sweetpotato plants as well as known positive and negative control plants are collected as per the above NCM-ELISA protocol.

A total of 6 to 8 leaf discs are cut from the mid-rib sections of the collective three leaves as per above tissue sampling method. Leaf discs are placed into new, labelled 2 ml tubes to which five volumes of RNAlater™ are added making sure that all leaf tissue was totally submerged. Leaf discs in RNAlater™ are kept at 4°C for 24 hours, with RNAlater™ removed the next day as per manufacturer's instructions. Leaf discs (samples) are catalogued, added to the sample database and stored at -20°C for downstream RNA extraction.

At the same time, whole leaves are also collected in labelled paper bags and dried over silica gel within 24-48 hours. Once leaves are fully dried, leaf samples are catalogued, added to the sample database and stored for future extraction in their respective paper bags within food grade vacuum seal bags. A small amount of silica gel is added to the bags to both ensure complete moisture removal and as storage monitoring tool as a colour change would indicate a break in the airtight seal.

Diagnostic protocols

1. Phyto-diagnostics

The universal Indicator plant *Ipomoea setosa* (Brazilian morning glory) is widely recognised as a highly sensitive indicator plant and is used globally to detect the presence of sweetpotato infecting viruses. *I. setosa* plants express symptoms readily when infected with virus/es (Fuentes 2005). Moyer and Salazar (1989) suggest that almost all viruses that infect sweetpotato also infect *I. setosa*. While symptom expression and severity can vary markedly between cultivars, depending on the viruses involved, the use of *I. setosa* provides a "level playing field" for symptom expression and many sweetpotato viruses are often present in sweetpotato plants in low titres. The sensitivity of this indicator plant to virus infection, facilitates rapid multiplication of viruses and consequently increased virus titres. This increase in titre also

facilitates downstream serological and molecular testing such as NCM-ELISA and conventional PCR, with the additional advantage that *I. setosa* plants do not contain inhibitors that impede diagnosis.

Due to the nature of this technology, phyto-diagnostic techniques do not always provide indications as to which virus species may be responsible for symptoms produced. Phyto-diagnostic indexing as part of the Australian PT protocol is conducted by experienced and highly skilled staff at GRF as some virus symptoms can be transient (Potyviruses), difficult to discern at lower titres (SPLCV) and mixed infections can result in multiple symptom expression.

Biological indexing using *I. setosa* is the first step in the Australian sweetpotato pathogen testing (PT) process at GRF. Seeds of *I. setosa* plants for use in sweetpotato virus indexing are produced annually at GRF in a quarantine-meshed, insect-proof igloo, dedicated to indicator-plant seed production. Ripened seeds are stored in a refrigerator in an airtight container to which a small net bag of silica gel was added to remove moisture. Currently, there is no published data to suggest that any sweetpotato infecting viruses are seed transmitted either in *I. setosa* or *I. batatas*.

Method

Seeding of biological indicator plants commences annually at GRF during September, in preparation for phyto-diagnostic indexing that is performed from October to April. This is the period when suitable weather conditions occur in south east Queensland. This is because *I. setosa* growth is optimal at 25°C and favours increasing day length and warmer night time temperatures [symptom expression is affected at higher or lower temperatures, (Fuentes pers. comm.)].

Pots are seeded within a screened area, inside an insect-proof glasshouse. Individual *I. setosa* seeds are sown into 100 mm pots filled with new pasteurised modified UC potting mix as per Dennien et al. (2013). *I. setosa* seeds readily germinate in Queensland and therefore pre-germination seed treatments are not required. Pots are placed under automatic micro-sprinklers with irrigation intensity adjusted as daytime temperatures increase during summer. Germination is usually evident after two to seven days, depending on temperature. After germination, Osmocote®, a granular, slow-release fertiliser, is applied at the rate of five grams (one teaspoon) per 100 ml pot, as per the product label. Access to this area is limited to staff working on the project, to prevent possible insect incursions within the glasshouse. Graft inoculation of seedlings occurs when the first two true leaves are fully expanded, usually at three to five weeks after germination (depending on temperature).

Graft inoculation

Plants to be virus indexed should be grown for at least 3 months, prior to testing, to allow virus titres to build up (Dennien 2013). Vine sections incorporating 3-5 nodes are removed from both the proximal (tip) and distal ends of collected plants to be tested. This is because the movement of virus particles within sweetpotato plants is largely unknown but is suspected to vary with virus involved.

Figure 1. End cleft and side veneer grafts, completed side veneer graft and *I. setosa* seedlings.

Two grafts are performed on each *I. setosa* plant, one end-cleft graft, and one side-veneer graft (Figure 1). Two *I. setosa* plants are grafted as above with cuttings (single node) from each collected sample. Five *I. setosa* plants are recommended to be grafted to check for presence of virus in plants undergoing pathogen testing. Numerous proven positive controls for a range of viruses previously detected in Australia, as well as pathogen-tested negative controls, are also grafted to a pair of *I. setosa* plants, during each round of virus indexing, for symptom comparisons.

Newly-grafted plants in the pots are covered with plastic bags. The pots are placed into large, shallow trays lined with plastic sheeting and filled with water to a depth of 10 mm on the floor of the glasshouse, where the plastic-covered plants are out of direct sunlight and exposed to cooler temperatures (Figure 2). Twice-weekly checks are performed, to ensure plants have sufficient water, but are not waterlogged.

Figure 2. Lower left, grafted plants covered with plastic bags and right, placed on benches. S. Dennien, DAF Qld.

At four days post grafting post grafting (PG), plastic bags are lifted slightly, depending on ambient glasshouse temperature, with all plastic bags removed at seven days PG. Cylindrical trellises are constructed from plastic garden trellis and cable ties (90cm x 15-20cm) and attached to weld-mesh covered nursery benches with cable ties. Individual grafted plants are then placed into individual 200 mm pot trays, with each replicated group of five plants (for each sample to be virus indexed) arranged around adjacent to a single trellis to aid in foliar symptom observation.

Grafted plants are hand watered twice weekly as required depending on ambient glasshouse temperatures over the 28 to 42 day PG testing period (56 to 70 days PG for Phytoplasma). Aquasol®, liquid foliar fertiliser is applied with a watering can to the base of plants only, at fortnightly intervals to ensure optimal growth as per Dennien et al. (2013).

As *I. setosa* vines grow they are trained onto the plastic trellises. Fortnightly Insecticides and miticides are applied during the late afternoon using a fogger (producing fine droplets) to minimise spray damage to indicator plant foliage, to avoid confusion between any potential leaf damage and virus symptoms. As maximum temperatures can regularly exceed 40°C from late December to January at GRF, shade cloth covers fitted to the glasshouse are deployed, and evaporative air coolers are employed to reduce daytime temperatures within the glasshouse.

Symptom evaluation

Initial regular monitoring is essential to capture early leaf symptoms, especially those of potyviruses, which can often develop rapidly, and then disappear just as rapidly within the first 14 days PG. To capture transient symptoms, indicator plants are observed twice weekly until 21 days post grafting PG, then weekly until 42 days PG.

Plants with suspected phytoplasma infections undergoing indexing are observed for 56 to 70 days PG. Symptom data is recorded weekly and each group of replicated *I. setosa* plants are photographed. Recorded data includes the number of surviving grafts and a range of symptoms (Figure 3) such as mottling, vein-clearing, chlorosis, chlorotic spots, chlorotic flecks, necrosis, leaf cupping/dishing, leaf roll, rugosity, stunting, leaf deformation, leaf balling, flowering and symptom spacial occurrence.

At the conclusion of biological indexing (around 42 days PG), all plant material including roots is removed from pots and trellises and placed into freezer bags, and frozen for several days, before being disposed of via domestic rubbish collection. Soil is stockpiled as waste not to be reused for virus indexing and checked regularly for any emergent of *I. setosa* seedlings as *I. setosa* is classed as a prohibited weed. Any seedlings emerging from used soil are immediately destroyed. Pots are cleaned in soapy water, then soaked in a 4% bleach solution and left to air dry. Plastic trellises are sprayed with 70% alcohol and left empty for at least seven days prior to the next round of biological indexing.

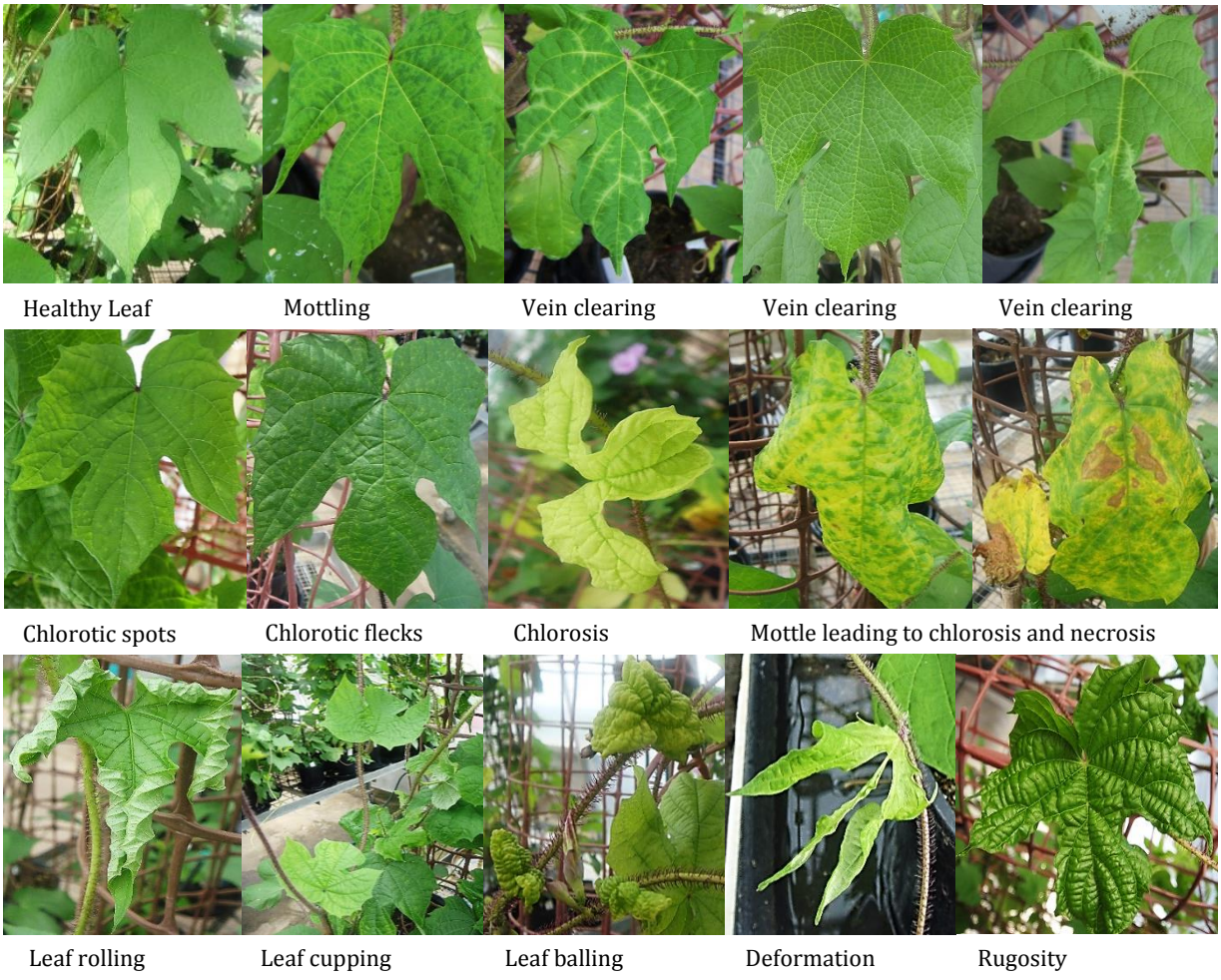

Figure 3. Visual virus symptoms on *I. setosa,* S. Dennien, DAF Qld.

2. Serological diagnostics

The second step in the virus indexing process is serological indexing using a sweetpotato virus specific antibody test to detect proteins on the virus particle coating. A specific detection antibody conjugated to an enzyme, produces a reaction, resulting in a colour change. Nitrocellulose membrane, enzyme linked immunosorbent assay (NCM-ELISA) is the main serological method currently used to detect viruses in sweetpotato This test is purchased from the International potato centre (CIP) in kit form and contains antibodies for 10 known sweetpotato infecting viruses (C-6, CMV, SPCV, SPCFV, SPCSV, SPFMV, SPLV, SPMMV, SPMSV and SPVG). It is recommended that infected material is first grafted on *I. setosa,* as the virus concentration will be higher in the indicator species and the test is affected by inhibitors present in sweetpotato sap (Fuentes pers comm.).

NCM-ELISA does not necessarily require a laboratory and can be completed in a shorter period of time by placing antibodies on membranes for three hours instead of overnight. It also has another very important advantage: the samples can be spotted on the nitrocellulose membrane and stored for several months before continuing with the test, or it can be sent to another laboratory for testing. This test can identify up to 10 known viruses, requires minimal laboratory equipment, and comes in a ready to use kit form and 96 samples can be processed at a time.

NCM-ELISA is not as sensitive as PCR, may not detect very low virus titres, takes 24 hours to complete and only contains antibodies for 10 viruses. There are no antisera currently available for SPLCV and as sweetpotato sap interferes with the test's efficiency, sap samples from *I. setosa* grown under optimal conditions are required.

Method

Between 14 and 21 days PG, three leaves were removed from one plant out of each replicated group of grafted *I. setosa* plants as per Dennien et al. (2013). Gloves should be worn and changed regularly and leaf removal should ideally be performed as early as possible in the morning prior to the commencement of photosynthesis. One symptomatic leaf is selected from each region of the plant; top, middle and base to allow for differences in virus distribution within plants.

If symptomatic leaves were absent then asymptomatic leaves were used. The three leaves from each plant were collected into new, labelled zip lock bags and kept cool in transit to the laboratory where the NCM-ELISA assay was performed as per kit instructions and as per Dennien et al (2013). A positive result is indicated by a purple colour change to sample blots (Figure 4).

Figure 4. Sample area for leaf discs, adding antibodies, positive results indicated by colour change, S. Dennien, DAF Qld.

3. Molecular diagnostics

Real time PCR or quantitative polymerase chain reaction qPCR and RT-qPCR works in the same way as PCR, but measures DNA/cDNA amplification as it occurs by capturing and measuring fluorescence released by fluorescently-tagged probes. By comparing the cycle threshold (Ct) values of samples of unknown DNA concentration with a series of sample dilutions from a known DNA concentration, the amount of DNA in an unknown reaction can be accurately determined. qPCR results can be obtained faster and with less variability than standard PCR due to sensitive fluorescent chemistry and elimination of post-PCR detection procedures.

A qPCR run time is 1.5 to 2 hours, is able to be multiplexed (multiple targets run at once) and is high throughput, excluding sample preparation and extraction. The whole reaction takes place inside the instrument therefore PCR products are contained, resulting in less chance of contamination of work areas. qPCR is up to 100 times more sensitive than conventional PCR (Wilson 2014). The test is also quantitative as it measures the amount of DNA/cDNA produced.

qPCR requires expensive equipment and currently there are limited published primer/probe sets available for sweetpotato viruses. Sequencing of other viruses is needed along with primer probe design to detect a wider range of sweetpotato viruses using this method (Fuentes 2012; Boonham et al. 2013; Fuentes 2014; Wilson 2014).

Method

As per the previous tissue sampling method for NCM-ELISA, whole leaves are collected between 14 and 21 days PG. Three leaves are removed from one plant out of each replicated group of grafted *I. setosa* plants as per Dennien et al. (2013). Gloves should be worn and changed regularly and leaf removal should ideally be performed as early as possible in the morning prior to the commencement of photosynthesis.

Between 14 to 28 days PG, three leaves from each pair of *Ipomoea setosa* grafted plants and the respective sweetpotato plants were collected as per the above NCM-ELISA protocol and taken to the laboratory for DNA and RNA extraction. One disc is cut from each of these three leaves from the midrib area using the large end of a disposable 1 ml pipette tip. A new pipette tip is used for each plant sample. Leaf discs (3 to 6 in total) or dried tissue sections (far north Qld samples) are placed into 2 ml safe-lock tubes and ground with stainless steel beads, using a Bullet Blender Storm™ tissue homogeniser (Next Advance).

DNA extraction is performed at GRF using either Dneasy™ plant mini kit (Qiagen), or Magjet™ plant genomic DNA kit (Thermo Scientific) as per manufacturer's instructions. RNA extraction is performed using Rneasy™ plant mini kit (Qiagen) or the Isolate II™ RNA plant mini kit (Bioline). Other laboratories may prefer to use to CTAB method. A Qubit 2.0™ fluorimeter (Life Technologies) was used to quantify extracted DNA and RNA and samples were catalogued, added to the sample database and stored at -20°C for downstream qPCR.

Extraction of DNA and RNA from leaf tissue

Between 14 - 28 days PG, three leaves from each group of *Ipomoea setosa* grafted plants and the respective sweetpotato plants were collected as per the above NCM-ELISA protocol and taken to the laboratory for DNA and RNA extraction. One disc was cut from each of these three leaves from the midrib area using the large end of a disposable 1 ml pipette tip. A new pipette tip was used for each plant sample. Leaf discs (3 to 6 in total) or dried tissue sections (far north Qld samples) were placed into 2 ml safe-lock tubes and ground with stainless steel beads, using a Bullet Blender Storm™ tissue homogeniser (Next Advance).

DNA extraction was performed using either Dneasy™ plant mini kit (Qiagen), or Magjet™ plant genomic DNA kit (Thermo Scientific) as per manufacturer's instructions. RNA extraction was performed using Rneasy™ plant mini kit (Qiagen) or the Isolate II™ RNA plant mini kit (Bioline). A Qubit 2.0™ fluorimeter

(Life Technologies) was used to quantify extracted DNA and RNA and samples were catalogued, added to the sample database and stored at -20°C for downstream qPCR.

qPCR and qRT-PCR

All qPCR and qRT-PCR assays are performed on a StepOnePlusTM (Applied Biosystems) real time quantitative PCR system, using optical 96 well plates or 8 tube strips and include both no template controls and known negative sweetpotato plant controls.

The following published qPCR protocols were used in the detection of sweetpotato viruses.

- SPLCV: To detect Sweetpotato leaf curl virus (SPLCV), published protocols as per Barkley (2011) were used with known positive (SPLCV) and negative control plants.
- SPFMV and SPV2/IVMV: Detection of Sweetpotato feathery mottle virus (SPFMV) along with the closely related Sweetpotato virus 2 (SPV2), syn: Ipomoea vein mosaic virus (IVMV) is performed using published protocols as per Kokkinos and Clark (2006), and known SPFMV positive and negative control plants.
- SPVG: Detection of Sweetpotato virus G (SPVG) was also performed using published protocols as per Kokkinos and Clark (2006) and known SPVG positive and negative control plants.
- SPCSV: Published protocols for the detection of Sweetpotato chlorotic stunt virus (SPCSV) as per Kokkinos and Clark (2006) and Fuentes (2012), were used with known SPFMV positive and negative control plants.
- qPCR Protocols For Sweetpotato collusive virus (SPCV) and Sweetpotato chlorotic fleck virus (SPCFV) are currently undergoing review and efficacy testing against known positive and negative controls, prior to publication.

Acknowledgements

Ms Dennien is particularly indebted to Segundo Fuentes from the International Center for Potato Research (CIP), Peru and Dr Chris Clark from Louisiana State University for their expert sweetpotato virology advice. Thank you to Dr Amit Sukal from the Secretariat of the Pacific Community, SPC, Fiji for his assistance with virology protocols and Michael Hughes DAF Q for editing this document.

Bibliography

Barkley, N., Pinnow, D., Wang, M., Ling, K.S., Jarret, R., 2011. Detection and classification of SPLCV isolates in the US sweetpotato germplasm collection via a real-time PCR assay and phylogenetic analysis. *Plant Disease* **95**, 1385-1391.

Beetham, P., Mason, A., Beetham, P., 1992. Production of pathogen-tested sweet potato. Australian Centre for International Agricultural Research.

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., Mumford, R., 2013. Methods in virus diagnostics: from ELISA to next generation sequencing. *Virus research*.

Clark, C.A., Davis, J.A., Mukasa, S.B., Abad, J.A., Tugume, A.K., Cuellar, W.J., Fuentes, S., Kreuze, J.F., Tairo, F.D., Gibson, R.W., Valkonen, J.P.T., 2012. Sweetpotato Viruses: 15 Years of Progress on Understanding and Managing Complex Diseases. *Plant Disease* **96**, 168-185. https://doi.org/10.1094/PDIS-07-11-0550

Coleman, E, Maltby, J, McCrystal, R, O'Donnell, W & Playford, C, 2006, 'Developing smooth skin, easy to peel sweetpotatoes'. Final Report for HAL Project VG02114. Queensland Department of Primary Industries.

Dennien,S., Homare, D., Hughes, M., Lovatt, J., Coleman, E., Jackson, G., 2013. Growing healthy sweetpotato: best practices for producing planting material, ACIAR Monograph No. 153. Australian Centre for International Agricultural Research, Canberra.

Dennien, S. 2015. Sweetpotato virus detection: Literature review April 2018. ASPG website link.

Dennien, S. Hughes, M., Coleman, E., O'Donnell, W., 2016 'Virus detections in sweetpotato from Australia's east coast 2004-2014'. Poster presented at IHTS Cairns, November 2016. In publication.

Fuentes, S., 2012. Real-time PT-PCR for diagnosis of sweetpotato chlorotic stunt and sweetpotato feathery mottle virus.

Fuentes, S., 2014. New Diagnostic methods for sweetpotato virus indexing.

Hughes, M., Final Report, 2010. Reducing pest and disease impact on yield in selected PNG sweetpotato production systems. ACIAR final report.

Hughes M., 2014 Annual report, Indentifying appropriate strategies for reducing virus and weevil losses in sweetpotato production systems in Papua New guinea and Australia. ACIAR.

International potato centre n.d., Sweetpotato, viewed 10 January 2018, https://cipotato.org/crops/sweetpotato/sweetpotato-cont/

Kokkinos, C. D., Clark, C.A. 2006. Real-Time PCR Assays for Detection and Quantification of Sweetpotato Viruses *Plant Disease* **90** (6), 783-788.

Love, SL, Rhodes, BB & Moyer, JW, 1987, Meristem-tip culture and virus indexing of sweetpotatoes', Practical Manuals for Handling Crop Germplasm, International Board for Plant Genetic Resources (IBPGR), Rome (Italy).

National clean plant network sweetpotato (NCPN), 'Minimum virus testing protocols for sweetpotato', http://ucanr.edu/sites/ncpnsweetpotato/

Wilson, C.R., 2014. Applied Plant Virology. CABI International, USA.