ARSF2 Final Report *October 2022*

Project Details

Project code	011 - **Winnie Maso - PNG**
Project name	**A reliable loop-mediated isothermal amplification (LAMP) technique for detection of Sweetpotato Virus G (SPVG)**
Project leader	Winnie Maso
Location	PNG, Highlands region, Aiyura
Organisation	National Agricultural Research Instiute
Mentor/Collaborator	Sandra Dennien, Jean Bobby
Date submitted	15/03/2023
Have you completed your project (Yes/No)	*Note: You may be required to submit another final report if a substantial amount of work remains to be done and the final project payment may be delayed until your project is completed.*
If project not completed, when will it be completed?	<Insert date DD/MM/YYYY>

Note: *The purpose of this report is:*

1. *A formal project report to assess the success of the project and the impact of the ARSF project funding.*
2. *Provide insights into the impacts the ARSF project has had on the experience and career development of the person awarded the project, and the other team members.*
3. *Provide material that can be used to develop a coffee-table book that will highlight the ARSF projects.*

Summary

INSTRUCTIONS: Provide a summary of the key aspects of your project, under the following headings, that can be shared with the ARSF program's stakeholders. **The total length should be no more than 2 pages.**

Commercial sweetpotato (*Ipomea batatas*) production is increasing in Papua New Guinea's Highlands provinces and transported to sell in markets of major towns in country. The use of healthy planting is necessary to maintain quality yields because sweetpotato viruses are a major production constraint. These are initially spread by propagating infected planting materials, and can result in significant yield reductions. Sweetpotato virus G (SPVG) is commonly detected either in single or dual infections with Sweetpotato Feathery Mottle Virus (SPFMV) in PNG sweetpotato. Safe movement of planting materials across various production sites requires a reliable, simple and cost-effective detection method to complement PNG's current viruses' management protocol. A single step reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for SPVG to support commercial production in Papua New Guinea was developed during my Master of Philosophy program at the University of Queensland in 2020. Research showed that RNA extracted from Flinders Technology Association (FTA) cards (a nucleic acid preservative) indicated strong promise for SPVG detection using LAMP technology in PNG. Further testing of fresh sweetpotato/indicator plant leaves was required before it can be deployed, however, COVID-19 affected the field work travel to perform rapid detection of SPVG in PNG sweetpotato samples used in the study. Furthermore, SPVG is not present in Australia hence, research activities for rapid detections were not completed. This proposed study was intended to enable rapid detection of SPVG using novel LAMP technology by complementing the standard ELISA-based testing in the sweetpotato pathogen testing scheme in PNG.

For this SPVG LAMP assay to be more robust, further evaluation of fresh leaves of I. setosa (grafted with sweetpotato scions) was necessary to validate a reliable LAMP detection method for SPVG in PNG.

Rationale and objectives *(Very briefly describe the reason for doing this project and what were its objectives)*:

Rationale

Preserved RNA from FTA card samples were screened using the LAMP technology and promising results of SPVG detection were obtained. However, for field use, this assay required validation using fresh leaf samples (sweetpotato/grafted indicator plants) from sweetpotato in PNG. Therefore, a rapid SPVG LAMP detection protocol would determine its efficacy in the fight against SPVG infections of sweetpotato, provide safe movement of sweetpotato planting materials and prevent biosecurity risks between PNG and Australia. Moreover, this study extension would fully complement the existing ELISA-based protocol by providing a rapid, cost effective and reliable method of SPVG detection.

Objective

Validation of a SPVG LAMP assay using grafted indicator plants of field derived sweetpotato.

Methods *(Very briefly describe what your project did)*:

Sample preparation

A total of 47 sweetpotato varieties were randomly selected from NARI sweetpotato collections. Nodes from these plants were grafted on to sweetpotato virus indicator plants called Ipomea setosa. Symptomatic leaves associated with sweetpotato virus G and sweetpotato feathery mottle virus were recorded. Samples were processed and tested using the NCM-ELISA kits. Sweetpotato varieties that tested positive for SPVG and SPFMV were further subjected to RT-qLAMP analyses using an alkaline PEG 200 buffer. This APEG buffer enables quick nucleic acid extraction that would otherwise require plant nucleic acid extraction kits.

The isothermal condition was 65 °C for 30 mins and 95 °C for annealing where amplified LAMP products of the assays were evaluated by their time to reaction (TTR). A positive detection was considered if a TTR was ≤25:00 mins, an indetermined detection was ≥25:00 mins while a negative detection had no amplification.

Results *(Describe the key findings from your project, and comment on the extent to which the project achieved its intended aims)*:

Weird and indetermined reactions (>25 mins TTR) were seen in RT-qLAMP SPVG assay using APEG buffer. The weird detections showed the assay to be non-specific when using the APEG 200 buffer to perform quick extractions.

Firstly, weird reactions may be due to inactivation of viruses during sample processing time. Chomczynski & Rymaszewski 2018 found inactive pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus* after 15-min exposure to the alkaline PEG reagent at room temperature. They did not test viruses hence, Wanjala et al. 2021 tested viruses immediately after processing samples and produced positive reactions and this was seen in SPFMV detections. Apparently, this was not seen in SPVG detections therefore, the RT-qLAMP SPVG assay may require nucleic extractions using a Qiagen extraction kit before using this SPVG assay.

Secondly, PEG 200 was already opened in 2020 and weird reactions for both SPVG and SPFMV assays in further tests indicated poor stability of PEG 200. To conclude, this SPVG LAMP assay is not ready for onsite detection as it requires total RNA extraction before RT-qLAMP is performed.

Impact and implications of your results *(Describe the impact your project has had or will have on the systems you were investigating):*

LAMP technology is suitable for rapid detection and complements PNGs current virus management protocols. However, there are challenges involved in the logistics of acquiring molecular grade LAMP products from overseas manufacturers.

Results from this study showed the relevance of using LAMP technology in performing diagnostic tests that would benefit R&D programs in PNG. Appropriate pathogen detection tool like LAMP technology has enhanced continued support for commercial sweetpotato activities in PNG.

Detailed report

Results

INSTRUCTIONS: Report in detail the findings from the project. This is the new knowledge and understanding that you have generated.

Sweetpotato (*Ipomea batatas*) is a staple crop in Papua New Guinea (PNG) with three-quarters of this crop produced in the Highlands provinces for household consumption. Traditionally, surplus sweetpotato are sold at local roadside or nearby market stalls. In the early 2000s, growing more sweetpotato became an income opportunity which saw movement of sale from the Highlands to other coastal markets in PNG (Chang & Spriggs 2008). Like other crops, sweetpotato is vulnerable to diseases, and viruses in particular pose risks of reduced yields (Loebenstein 2009; Clark & Hoy 2006).

Viruses are widely considered to be of great economic importance in sweetpotato production globally (Clark et al. 2012; Gibson & Kreuze 2014). Yields of virus-infected sweetpotato plants are often reduced by as much as 80-90% (Carey et al. 1999; Davis & Ruabete 2010; Clark et al. 2012). Although viruses can be vectored by insects such as aphids (*Myzus persicae*, *Aphis gossypii*) and whiteflies (*Bemisia tabaci*) (Byamukama et al. 2004; Clark et al. 2012), infected propagation material is the primary means of viral spread (Moyer & Larsen 1991; Gibson et al. 1997; Mbanzibwa et al. 2014).

Sweetpotato virus G (SPVG) is a potyvirus that is closely related to, but distinct from the better-known *Sweetpotato feathery mottle virus* (SPFMV) and both viruses have been reported in mixed infections (Colinet et al. 1994; Souto et al. 2003; Ateka et al. 2007; Li et al. 2012). SPVG is common in PNG sweetpotato and is detected either in single or dual infections (ACIAR 2010). Therefore, screening prospective propagation material is a key tool for disease management.

The basis of sweetpotato virus management is the production and release of virus-free propagation material. In 2005, a pathogen-tested (PT) technology for sweetpotato production in PNG was introduced as a scheme under an initiative by the Australian Centre for International Agricultural Research (ACIAR) and Queensland Department of Agriculture and Fisheries (QDAF), in collaboration with the National Agricultural Research Institute (NARI). It is a comprehensive process that involves virus testing of planting materials as a key task to certify selected varieties for commercial production in the Highlands region (ACIAR 2009; Dennien et al. 2013).

Virus detection in sweetpotato is challenging because at least 30 viruses of sweetpotato are known (Clark et al. 2012). Adding to the complexity, some of these have multiple strains with differing epidemiologies and detection requirements (Dolores et al. 2012). Routine virus monitoring of planting materials is an essential process required to support a viable commercial scheme. Sweetpotato virus detection methods can include biological, serological and molecular approaches. Currently, a significant number of viral diagnostic tests employ detection kits developed by companies for testing specific viruses. For example, the International Potato Center (CIP) in Peru has been leading sweetpotato viral research and has developed serological tests using enzyme-linked immunosorbent assay (ELISA) as well as protocols for the detection of viruses using the polymerase chain reaction (PCR) (Salazar & Fuentes 2000). This universal CIP nitrocellulose membrane – enzyme-linked immunosorbent assay (NCM-ELISA) kit tests specifically for 10 sweetpotato viruses: *Sweetpotato feathery mottle virus* (SPFMV), *Sweetpotato mild mottle virus* (SPMMV), *Sweetpotato latent virus* (SPLV), *Sweetpotato chlorotic fleck virus* (SPCFV), *Sweetpotato mild mottle virus* (SPMSV), *Sweetpotato caulimo-like virus* (SPCaLV), *Sweetpotato C-6 virus* (SPC-6), *Sweetpotato virus G* (SPVG), *Sweetpotato cucumber mosaic virus* (SPCMV) and *Sweetpotato chlorotic stunt virus* (SPCSV). This kit has been deployed in the current PNG PT scheme.

When selecting a diagnostic test, it is necessary to consider costs, time, resources and techniques involved depending on the scope of work. Viruses are microscopic and it is difficult to determine if sweetpotato plants are infected by viruses until specific detection methods are used. For example, grafting is performed to transmit sap of sweetpotato planting materials onto healthy indicator plants such as Brazilian morning glory *(Ipomea setosa)* to view symptoms when they appear on their leaves. Virus screening using indicator plants is referred to as herbaceous indexing in the sweetpotato PT scheme. The herbaceous indexing and NCM-ELISA are practical detection methods employed in PNG to manage sweetpotato viruses. While these detection methods are reliable and cost effective, there is scope to modernise them with the addition of molecular diagnostics, which are typically more sensitive and much more rapid. A complementary detection method that is also reliable and simple to use must be considered to facilitate supply of field planting materials, particularly for commercial production sites. This requires rapid in-field tests for specific virus detection.

Molecular diagnostic approaches have developed rapidly in sweetpotato viruses (Clark et al. 2012). The availability of molecular detection methods has led to advances in sweetpotato virus knowledge (Clark et al. 2012). With commercial production, virus detection is a routine procedure to ensure safe movement of planting material across various sites. Although molecular diagnoses give rapid and specific reliable results, they can be prohibitively expensive

and complex in their format for many developing countries. However, there is scope to adopt suitable techniques to complement the current testing methods in PNG.

Loop-mediated isothermal amplification (LAMP) is a cost effective, reliable detection method that was developed in 2000 by the Eiken Company. It was designed to produce rapid results, and has been widely deployed in clinical tests (Notomi et al. 2000). It is now used by researchers in viral studies and can be performed directly in the field at a constant temperature (65° C) to detect specific target viruses using three pairs of primers (internal, external and loop primers) to generate amplification of DNA (Boonham et al. 2014).

A single step reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for SPVG to support commercial production in Papua New Guinea was developed at the University of Queensland in 2020. Total RNA extracted from Flinders Technology Association (FTA) cards (a nucleic acid preservative) indicated strong promise for SPVG detection using LAMP technology in PNG. However, further testing of fresh sweetpotato and or grafted indicator plant leaves was required before deploying the assay for rapid detection of SPVG in PNG sweetpotato.

Therefore, the focus of this study was aimed to validate rapid detection of SPVG using novel LAMP technology to complement the standard NCM-ELISA-based testing in the sweetpotato pathogen testing scheme in PNG.

Rationale and objectives *(Very briefly describe the reason for doing this project and what were its objectives)*:

Rationale

Preserved RNA from FTA card samples were screened using the LAMP technology and promising results of SPVG detection were obtained. However, for field use this assay required validation using fresh leaf samples (sweetpotato/grafted indicator plants) from sweetpotato in PNG in order to establish a rapid detection protocol. Therefore, a rapid SPVG LAMP detection protocol would determine its efficacy in the fight against SPVG infections of sweetpotato, provide safe movement of sweetpotato planting materials and prevent biosecurity risks between PNG and Australia. Moreover, this study extension would fully complement the existing ELISA-based protocol by providing a rapid, cost effective and reliable method of SPVG detection.

Objective

Validation of a SPVG LAMP assay using grafted indicator plants of field derived sweetpotato.

Methods *(Very briefly describe what your project did)*:

Sample preparation

Ipomea setosa seeds were germinated and seedlings raised to 4 weeks old. Sweetpotato varieties were grafted on to *I.setosa* seedlings where each I. setosa seedling was grafted with either top shoot, middle and basal scions of each sweetpotato variety. That is, each I. setosa plant was grafted with a shoot, middle and base scion from each sweetpotato plant. As controls, 1-2 I. setosa plants were grafted with pathogen-tested sweetpotato plants. Establishment of grafts took 1 week before viral symptoms started to appear on leaves of indicator plants. Visual observations were recorded onto data sheets (sweetpotato indexing record sheet) between 1-4 weeks for each data plants. Pictures of leaf symptoms for each plant were captured during data recording.

ELISA and LAMP analyses

Three rounds of grafts were made where 10mm discs from leaves of grafted *I. setosa* plants were stored on NCM in preparation for ELISA analyses. Following the same leaves collection method, the 2nd and 3rd round of grafted *I. setosa* were used in both NCM-ELISA and qRT LAMP SPVG assays. From each *I. setosa* grafted plants, a selection of 3 leaves (top, middle and base) from each plant were collected after 4 weeks. Off the collected leaves, 2 sets of leaf discs of 10-15 mm were cut out from the mid rib of same leaf and used simultaneously in ELISA and LAMP detection.

Total nucleic acid extraction

The isothermal condition was 65 °C for 30 mins and 95 °C for annealing where amplified LAMP products of the assays were evaluated by their time to reaction (TTR). A positive detection was considered if a TTR was ≤25:00 mins, an indetermined detection was ≥25:00 mins while a negative detection had no amplification. Real-time assays were performed using the LAMP Genie II instrument (Optigene) in a 25 µL reaction mixtures containing 15 µL of RT isothermal master mix (DR 004-RT 50) at a 1x concentration, 0.5 µL (200 nM) each external primer, 0.5 µL (2 µM) each internal primer, 0.25 µL (1 µM) each loop primer, 5.7 µL DNase/RNase Free pure water and 1 µL RNA. Following the respective manufacturer's instructions, The Optigene isothermal master mix (Cat. No. ISO-DR004) was resuspended for validating the SPVG assay in a one-step RT-qLAMP detection.

A SPFMV (F3: TACAACGTAAMCTTGACTGATATGAGT, B3: GTTATGTATATTTCTAGTAACRTCAGT, FIP: TGCRGCTGCYTTCATCTGYAWWTGTGGATATGCATTTGATTTYTAYGAGCT, BIP:

AAGAATGCGMRWAATCGGTTTGGGCCTCTCCGTCTCYTCTTCTT, FL: TTCTTTAGCACGTGYAGGKG, BL: TGGAYGGAAACGTCTCCAC) RT-qLAMP developed by CIP (PT workshop 2014) was used to cross-check against designed SPVG primers for specificity.

The SPVG (F3: CAACAATTCCAGATAGTAGAGG, B3: GTGCTCTTTTCTTACTATGTGT, FIP: CTTTGAGGGGTTGATGTCATTGTTT-GGTTGATACATCACAAATACCG, BIP: CACTGGGGCAAGAGATAGAGA-CCGTGGCACTATGAAAGT, FL: GGTCTCTACCTGGTGTGAAACT, BL: TGTGAATGCTGGTACAGTTGG RT-qLAMP was used to validate this assay.

A quick extraction method using an alkaline polyethylene glycol (APEG) by Chomczynski and Rymaszewski, 2006 was employed to perform RT-qLAMP SPVG and RT-qLAMP SPFMV assays. The APEG buffer was prepared by combining 60g PEG 200 (Sigma-Aldrich) with 0.93 mL of 2M KOH and 39 mL water and pH adjusted to 13.5. Three 1 cm diameter *I. setosa* leaves from sweetpotato grafts were cut using 1 cm test tube in extraction bags and mixed with 1 mL APEG buffer. The samples were ground using a test tube and left to stand for 1 min for sedimentation. The extracts were diluted 1:10 in nuclease free water (Integrated DNA Technologies) and used directly for RT-qLAMP SPVG and RT-qLAMP SPFMV assays.

Results *(Describe the key findings from your project, and comment on the extent to which the project achieved its intended aims)*:

Ipomea setosa symptoms & NCM-ELISA

A total of 47 nursery-derived sweetpotato varieties were randomly selected to screen for sweetpotato virus G (SPVG) and sweetpotato feathery mottle virus (SPFMV) (Table 1). Nodes were taken from each sweetpotato and grafted on single healthy plants of raised *Ipomea setosa* seedlings. Leaves of individual grafted plants were inspected fortnightly and symptoms associated with sweetpotato virus infections were recorded. Symptoms like vein clearing, mottling, chlorotic spots, cupping and necrosis were recorded (fig 1), collected on the 4th week and preserved on nitrocellulose membranes (NCM) for NCM- ELISA SPVG and NCM-ELISA SPFMV screening. Positive detections for SPVG and SPFMV from NCM-ELISA showed purple dots from field-derived sweetpotato (fig 2).

It was evident that vein clearing, mottling and chlorotic spots were symptoms associated with potyvirus infections like Sweetpotato Virus G (fig 1). Symptoms like cupping and necrosis were associated with Begomovirus infections as described by Dennien et al. 2013 and noted

for later screening. Specific detection of NCM-ELISA SPVG complemented symptoms seen on *I.setosa* (fig 2).

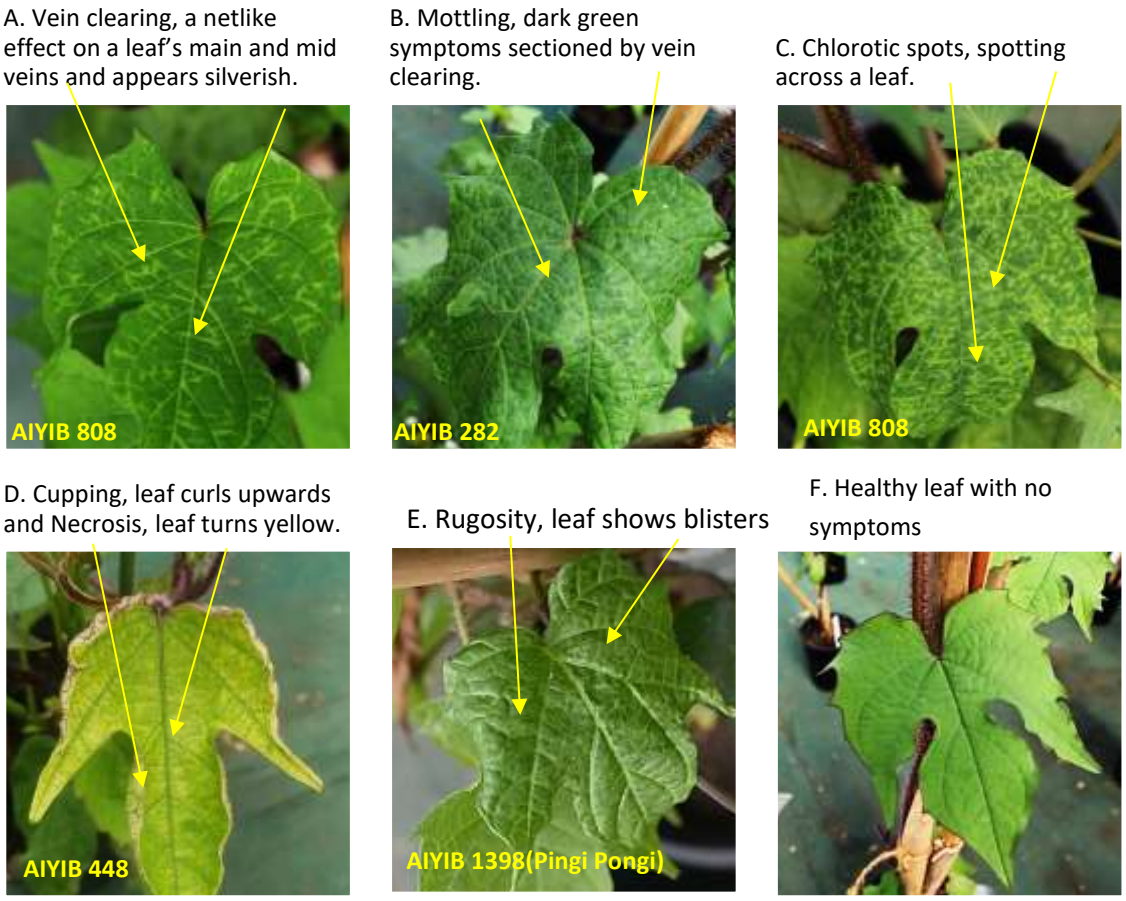


Figure 1. Five symptoms of sweetpotato virus infections that appeared at times of inspection on *I.setosa* from nursery-derived sweetpotato included A. Vein clearing, B. Mottling, C. Chlorotic spots, D. Cupping +Necrosis, Rugosity and F. Healthy (negative control).

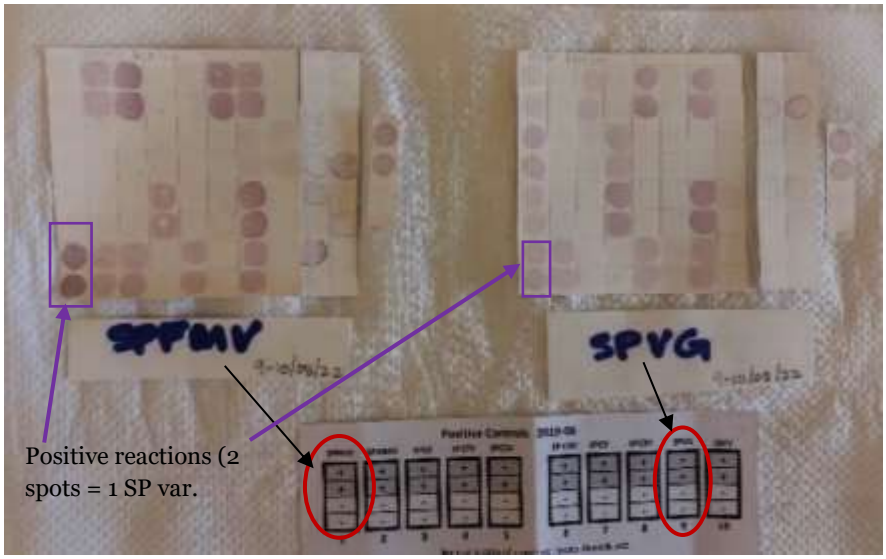


Figure 2: NCM-ELISA SPVG, NCM-ELISA SPFMV detections from nursery-derived sweetpotato at NARI-Aiyura. Positive (+) SPVG detections were seen in 15/47 sweetpotato of which 8/15 appeared in dual infections with SPFMV. Positive detections had purple spots appearing on the NCM.

In table 1, AIYIB 1398 (Pingi Pongi), AIYIB 681, AIYIB 808, AIYIB 228, AIYIB 957, AIYIB 446, AIYIB 175, AIYIB 448 had SPVG + SPFMV infections while AIYIB 282, AIYIB 1145, AIYIB 670, AIYIB 1014, AIYIB 475, AIYIB 1132 and AIYIB 443 had SPVG infections. SPFMV infections were detected in AIYIB 1182 (WHCK 005), AIYIB 1390 (Minj Purple), AIYIB 168, AIYIB 870, AIYIB 879 and AIYIB 712.

Table 1. NCM-ELISA of 47 randomly selected PNG sweetpotato were screened for SPVG and SPFMV. There were single (SPVG, SPFMV) and combined (SPVG+SPFMV) infections. Light purple indicates a single while the dark purple shading shows combined infections.

Sample no	Accession number	Material	Region	I.setosa symptoms	NCM-ELISA		Sample no	Accession number	Material	Region	I.setosa symptoms	NCM-ELISA	
		Ex situ	EHP		SPVG	SPFMV			Ex situ	EHP		SPVG	SPFMV
1	AIYIB 038	PGR	AIYURA	+	-	-	25	AIYIB 736	PGR	AIYURA	+	-	-
2	AIYIB 282	PGR	AIYURA	+	+	-	26	AIYIB 870	PGR	AIYURA	-	-	+
3	AIYIB 1398 PINGI PONGI	Grower	AIYURA	+	+	+	27	AIYIB 299	PGR	AIYURA	+	-	-
4	AIYIB 1213	PGR	AIYURA	-	-	-	28	AIYIB 1014	PGR	AIYURA	+	+	-
5	AIYIB 1182 WHCK 005	PGR	AIYURA	+	-	+	29	AIYIB 446	PGR	AIYURA	+	+	+
6	AIYIB 1390 MINJ PURPLE	Grower	AIYURA	+	-	+	30	AIYIB 475	PGR	AIYURA	+	+	-
7	AYIB 1165 WHAGI BESTA(PT)	Control	AIYURA	-	-	-	31	AIYIB 175	PGR	AIYURA	+	+	+
8	AIYIB 1145	PGR	AIYURA	+	+	-	32	AIYIB 1093	PGR	AIYURA	+	-	-
9	AIYIB 982	PGR	AIYURA	-	-	-	33	AIYIB 142	PGR	AIYURA	+	-	-
10	AIYIB 681	PGR	AIYURA	+	+	+	34	AIYIB 1212	PGR	AIYURA	+	-	-
11	AIYIB 792	PGR	AIYURA	+	-	-	35	AIYIB 753	PGR	AIYURA	+	-	-
12	AIYIB 670	PGR	AIYURA	+	+	-	36	AIYIB 676	PGR	AIYURA	+	-	-
13	AIYIB 718	PGR	AIYURA	+	-	-	37	AIYIB 722	PGR	AIYURA	+	-	-
14	AIYIB 808	PGR	AIYURA	+	+	+	38	AIYIB 448	PGR	AIYURA	+	+	+
15	AIYIB 149	PGR	AIYURA	+	-	-	39	AIYIB 843	PGR	AIYURA	+	-	-
16	AIYIB 228	PGR	AIYURA	+	+	+	40	AIYIB 801	PGR	AIYURA	+	-	-
17	AIYIB 629	PGR	AIYURA	+	-	-	41	AIYIB 642	PGR	AIYURA	+	-	-

18	AIYIB 447	PGR	AIYURA	+	-	-	42	AIYIB 225	PGR	AIYURA	+	-	-
19	AIYIB 1035	PGR	AIYURA	+	-	-	43	AIYIB 390	PGR	AIYURA	+	-	-
20	AIYIB 168	PGR	AIYURA	+	-	+	44	AIYIB 768	PGR	AIYURA	+	-	-
21	AIYIB 1036	PGR	AIYURA	+	-	-	45	AIYIB 1132	PGR	AIYURA	+	+	-
22	AIYIB 950	PGR	AIYURA	-	-	-	46	AIYIB 712	PGR	AIYURA	+	-	+
23	AIYIB 879	PGR	AIYURA	+	-	+	47	AIYIB 443	PGR	AIYURA	+	+	-
24	AIYIB 957	PGR	AIYURA	+	+	+		**Total**			**42/47**	**15/47**	**14/47**

RT-qLAMP SPVG and RT-qLAMP SPFMV

Following the NCM-ELISA SPVG and NCM-ELISA SPFMV detections, 15 sweetpotato varieties were further subjected to RT-qLAMP analyses. From the analyses, RT-qLAMP SPVG had weird reactions using APEG buffer (fig 3) except for RNA extracted sweetpotato (AIYIB 038 & AIYIB 1213) that had an indetermined detection that was ≥25:00.

To cross-check whether the APEG was producing the same reactions, the same sweetpotato were analysed using RT-qLAMP SPFMV assay. Positive detections were ≤25:00 mins and detected from 2/15 sweetpotato varieties (fig 4).

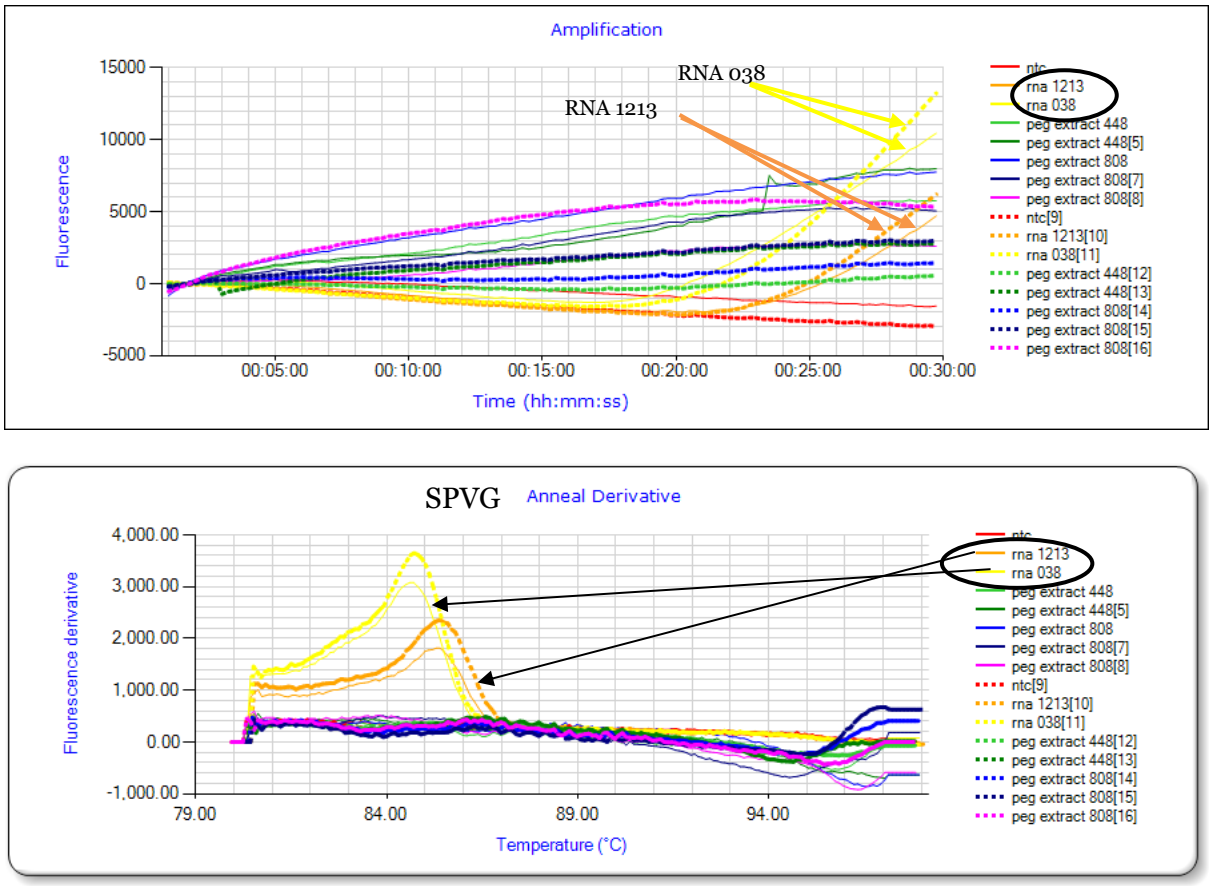
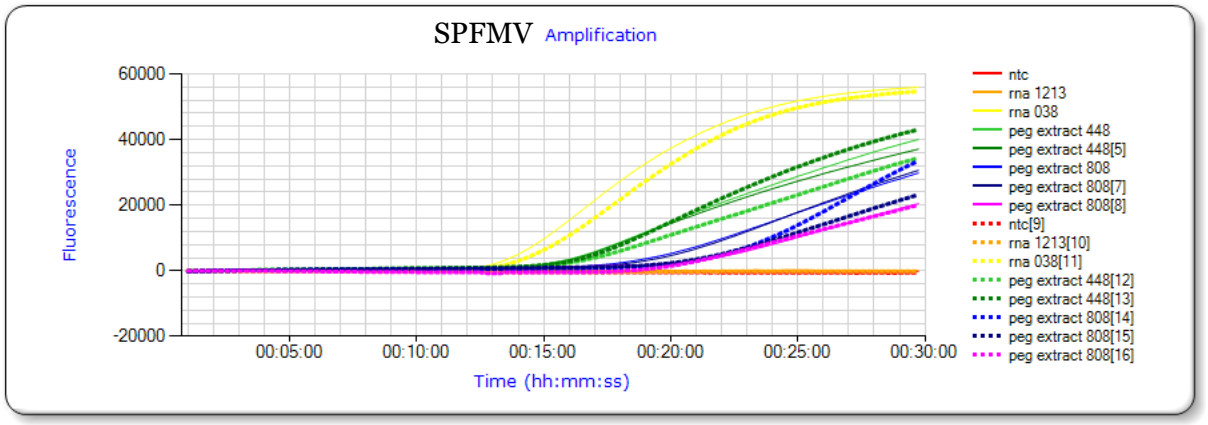


Figure 3. Validation test with SPVG assay. SPVG amplification and anneal derivative showed late reaction only for RNA extracted sweetpotato (AIYIB 1213 & AIYIB 038) while APEG RNA extracted sweetpotato showed zero amplification and annealing. Instead, weird amplification was seen in RT-qLAMP SPVG.



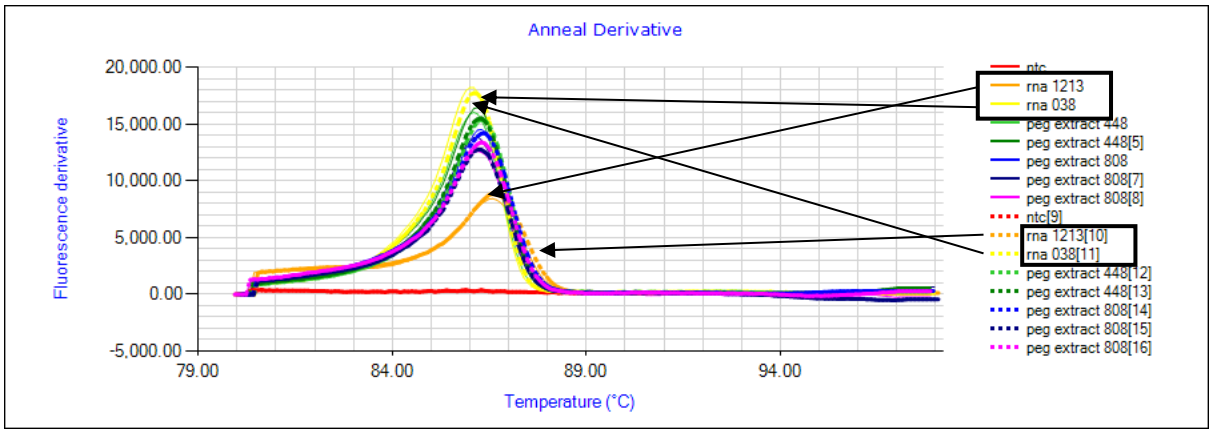


Figure 4. SPFMV amplification and anneal derivative showed positive reactions. However, RT-qLAMP also had positive reactions for RNA AIYIB 1213 & RNA AIYIB 038.

Table 2. Further evaluations with SPVG and SPFMV assay produced indetermined (~) amplifications using 6% APEG buffer.

Sample	**RT-qLAMP SPFMV using APEG quick extraction** **(n=4) TTR**		**RT-qLAMP SPVG using APEG quick extraction** **(n=4) (TTR)**			
No Template Control (LAMP)	-	-	-	-	-	-
AIYIB 1398- Pingi Pongi	~	~	~	~	~	~
AIYIB 1014	~	~	~	~	~	~
AIYIB 443	~	~	~	~	~	~
AIYIB 175	~	~	~	~	~	~
AIYIB 448	~	~	~	~	~	~

AIYIB 808	~	~	~	~	~	~
Negative control	~	~	~	~	~	~
Positive control	~	~	~	~	~	~

Conclusions

Weird and Indetermined reactions (>25 mins TTR) were seen in RT-qLAMP SPVG assay using APEG buffer. The weird detections showed the assay to be non-specific when using the APEG 200 buffer to perform quick extractions. There shouldn't be any weird reactions because a negative reaction shows a straight line along the Time (hh:mm:ss) and Temperature (˚C) axes during amplification and annealing as shown in Figure 4. Firstly, weird reactions may be due to the stability of viruses during sample processing time. Chomczynski & Rymaszewski 2018 found inactive pathogens like *Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus cereus* after 15-min exposure to the alkaline PEG reagent at room temperature. They did not test viruses hence, Wanjala et al. 2021 tested viruses immediately after processing samples and produced positive reactions and this was seen in SPFMV detections (fig 4). Apparently, this was not seen in SPVG detections therefore, the RT-qLAMP SPVG assay may require total RNA extractions using a Qiagen extraction kit before performing RT-qLAMP SPVG detections.

Secondly, due to the stability of PEG 200 as it was already opened in 2020, weird indetermined reactions were produced for both SPVG and SPFMV assays as seen in table 2. To conclude, RT-qLAMP SPVG assay is not suitable for onsite detection using APEG buffer.

Impacts and implications

INSTRUCTIONS: Describe how your project has already had, or will in future have, impact on agriculture/food systems or communities and whether it has implications for future research or future agriculture development, both locally (in the area where your project worked) or more broadly nationally or internationally).

Scientific impact and implications

This detection tool complements PNGs current virus management protocols thus will support safe movement of sweetpotato planting materials in PNG and its surrounding pacific communities. The approach of rapid SPVG qLAMP detection has been validated at Aiyura NARI, PNG. It has enhanced the capacity of NARI staff at Aiyura to perform molecular studies using the LAMP technology.

The study has showed that virus detection using this method is necessary in supporting commercial production in PNG and is practical for on site detection. However, the use of LAMP is dependant to challenges in acquiring molecular grade chemicals from international suppliers.

Products

INSTRUCTIONS: provide a list of all the products produced by your project under each of the headings below. For "published papers", include conference publications, provide a full standard reference format and indicate whether each publication is published or accepted or submitted or is being written or will be written in future. For "other communications", include reports, extension articles, fliers, popular press articles and social media

produced from or about the project. Where relevant, provide an internet link to each communication. For products that do not fit under other headings, provide information under the "other products" heading. Examples might be patents, Apps, software etc.

Published papers:

Is being written

Other communications:

The findings from the study were communicated through a PowerPoint presentation and discussed around a roundtable with NARI Aiyura staff and feedback was taken note. The title of the presentation was 'Validation of RT-qLAMP SPVG assay for rapid onsite detection'. The feedback was positive in the sense that the technology is useful for field detection and that it is an addition to virus management protocols in PNG.


Title - Validation of RT-qLAMP SPVG ass

Datasets:


GRAFTED SP LIST.docx


Sweetpotato virus record sheet.docx


ARSF2.xlsx


SPVG experiment report.pdf


SPFMV experiment report.pdf


LAMP protocol.docx

Other products:

Nil

Activities & Outputs not yet completed

*INSTRUCTIONS: Complete the following table for **each activity in the project that has not been completed** (do not complete this for the activities that have been completed). Use the same format as used in the quarterly progress reports. The activities should have the same numbering and title as the research and communications activities in your original Project Plan.*

Project activity 1: Procure ELISA kit (s) and LAMP diagnostic materials

Status	**Challenges**
Objective	To ensure research materials/items/equipment are made available
Activity dates	*Planned:* As soon as funds were available *Actual:* Oct 2022 and Dec 2022
Progress	Received LAMP reagent in November 2022 and concluded LAMP analyses in Dec 2022.
Outputs created	Rapid detection using SPVG assay validated and is suitable for lab use and not onsite detection

Copy and paste in additional research activity boxes as required

<Communications Activity <number>: give the number and descriptive title used in the project plan>

Status	**In progress** **Challenges** **Cancelled**
Objective	Research findings are communicated through report and or publication
Activity dates	*Planned:* October 2022 *Actual:* March 2023
Progress	Results obtained in December 2022 and findings to be communicated through report and or publication
Outputs created	X1 PowerPoint presentation to NARI Aiyura staff

Copy and paste in additional communications activities boxes as required

Challenges & Lessons Learned

INSTRUCTIONS: Complete the table below with any challenges that were encountered in the project and the lessons that were learned. Include any solution that you implemented.

Challenge	Lessons learned/solutions
Lack of accessing chemicals from suppliers	Some Molecular suppliers can be sourced from South east Asia other than NZ and Australia who can ship products to PNG without delay. Optigene in Australia is much more efficient as compared to Merck (previously Sigma-Aldrich). I encountered delays with Thermo Fisher and CIP Peru in bringing in NCM-ELISA kits and lab items to PNG for research purposes and had to borrow from other colleagues to complete research activities. Also communicating with Australian collaborator and NARI staff helped in bringing a chemical (PEG200) that was necessary to complete project activity 3, LAMP analyses using PEG extracts.
Poor understanding of NARI researcher (myself) with logistics when procuring biochemicals from overseas suppliers.	The NARI should have a system that enables researchers to order products that require certain logistical support. An example is Thermo- Fisher Scientific Limited that does not ship directly to a customer and that the customer must make arrangement with a freight forwarder. I learned to communicate with logistical services on the process involved in bringing chemicals.
Cost of molecular project materials	Molecular studies are costly but are more sensitive and precise in virus studies. I ran out of funds to freight lab items from Thermo Fisher Limited, NZ to NARI,PNG and must submit the final report before I receive remainder of project funds (25%) to complete payment and receive project materials.
<Insert>	<Insert>
<Insert>	<Insert>
<Insert>	<Insert>

Objectives that were not completed

Describe any of your original project objectives that you were not able to achieve. Briefly describe why they were not achieved and describe any lessons learned.

Project activity 3: Sampling and testing of sweetpotato leaves

Direct testing of sweetpotato leaves were not tested due to lack of PEG 200 buffer. The leftover used stock from Gatton Research Facility was used with grafted indicator plants. Further testing of sweetpotato leaves would have provided results for onsite detections other than use of grafted indicator plants. Moreover, testing sweetpotato leaves would have validated rapid onsite detection of SPVG infections in sweetpotato gardens.

Working during the COVID-19 pandemic

Describe the most important factor or factors that enabled this project to be undertaken during the pandemic.

This study was conducted in PNG and did not need travel.

The challenge was the logistics to bring in biochemicals from molecular suppliers. Other suppliers were able to process ordered project materials during the pandemic and this enabled completion of research activities. The communication was also crucial between the mentor and research during the pandemic and this enable successful completion of study.

Lessons for operating in a crisis

What have you learnt about conducting research in a crisis situation that could be useful to you or others in future?

It was noted that forward planning after documenting quarterly reports have helped to address challenges and improved on the opportunities that were discussed between the research teams, NARI, ARSF and Queensland Department of Agriculture and Fisheries- Gatton Research Facility.

Australian collaborator or mentor

The ARSF supported you with an Australian mentor or collaborator. Describe how this was useful to your project. Describe any difficulties you encountered in working with your collaborator/mentor. Are you likely to collaborate with your collaborator/mentor in the future?

Communication on project implementation was useful. Logistics between NARI and Australian chemical suppliers (MERCK) that do not ship to PNG was challenging. Australian collaborator provided chemical (PEG200) that supplier does not ship to PNG for project activity to be completed. Collaboration between NARI and Australian collaborator supported study completion and is likely to continue in future when necessary.

Personal and professional impacts

The following questions are intended to learn more about how the ARSF project affected you (the ACIAR alumnus awarded the project). The questions about your personal details will help us understand the impacts of the ARSF.

Personal details

Age: 40

Gender (female, male, non-binary): Female

Do you identify as having a disability? No

Current position in your organisation: Scientist-Tissue culture and pathology

Organisation

Describe any impacts your ARSF work has had on your organisation. For example, has it informed future work or influenced your organisation's policies or practices? If your work has had impact on other organisations please also describe that here.

The impact of this study contributes towards NARIs priority in achieving economic development of value chains like sweetpotato, banana and taro in PNG. Sweetpotato is increasingly popular in PNG domestic markets and the use of improved plant disease diagnostic approaches like the LAMP technology strengthens NARIs capacity to contribute effectively in crop production and food security in PNG.

Personal and professional

Describe any new skills that you or your team gained during the project, and explain how the project made this possible.

NARI Aiyura staff gained new information on use of LAMP technology and were introduced to the opportunities available in plant health diagnostics. The gained knowledge was that rapid tests complemented the current virus management for sweetpotato virus detection protocols in PNG.

Describe any impact that ARSF has had on your career so far and what impacts, if any, you believe it will have in future.

I believe the knowledge gained from this study is useful for my work in biotechnological applications because I can now confidently test plants using available techniques to certify planting materials, provide technical support in disease diagnostics and maintain high standard of tissue culture practices in PNG. I have trained lab casuals and tertiary students using this knowledge and am happy to hear stories of students who have joined organisations like Fresh Produce Development Agency after gaining knowledge under my supervision. The biological science department at the university of Goroka in Eastern Highlands province, PNG have vested interests in sending undergraduates to NARI Aiyura to undergo training and I think this will boost biotechnology benefits for PNG agriculture in future.

Photographs from the project

Please insert here, or attach to the email submitting your report, up to 10 photographs that help communicate what your project was about and the impact it had. Please include a short caption with each image that explains where and when it was taken, the names of everyone shown and what is happening in the image.

Using the novel qLAMP technology to validate RT-qLAMP SPVG assay at NARI Aiyura

Preparing the SPVG assay at NARI Aiyura to test sweetpotato in PNG using novel LAMP technology