BILL & MELINDA GATES foundation

Progress Narrative

Use this form to provide updates to your foundation program officer regarding progress made toward achieving your project's stated outputs and outcomes.

The Progress Narrative must be submitted in Word, as PDFs will not be accepted.

General Information

Investment Title	NextGen Phytosanitation: Rapid Elimination of Viruses from RTB Plants for Crop Improvement and Seed Systems		
Grantee/Vendor	International Potato Center		
Primary Contact	Dave Ellis	**Investment Start Date**	September 29th, 2016
Feedback Contact[1]	Dave Ellis	**Investment End Date**	September 30th, 2020
Feedback Email[1]	d.ellis@cgiar.org	**Reporting Period Start Date**	October 1st, 2018
Program Officer	Jim Lorenzen	**Reporting Period End Date**	September 30th, 2019
Program Coordinator	Amy Pope	**Reporting Due Date**	November 30th, 2019
Investment Total	$2,385,906.00	**Opportunity/Contract ID**	OPP1130216
Scheduled Payment Amount (If applicable)	$529,900.00		

[1] Feedback Contact/Email: The full name and email of the contact whom foundation staff queries for various surveys.

Submission Information

By submitting this report, I declare that I am authorized to certify, on behalf of the grantee or vendor identified on page 1, that I have examined the following statements and related attachments, and that to the best of my knowledge, they are true, correct and complete. I hereby also confirm that the grantee or vendor identified on page 1 has complied with all the terms and conditions of the Grant Agreement or Contract for Services, as applicable, including but not limited to the clauses contained therein regarding Use of Funds, Anti-Terrorism, Subgrants and Subcontracts, and Regulated Activities.

Date Submitted	December 9th, 2019	**Submitted by Contact Name**	Maria Teresa Bellido
		Submitted by Contact Title	Grants and Contracts Manager
		Submitted by Contact Email	m.bellido@cgiar.org
		Submitted by Contact Phone	+51 1 349 6017 Ext 3013

Progress and Results

1. Progress Details

Provide information regarding the current period's progress toward achieving the investment outputs and outcomes as well as the work planned or anticipated for the next period. In addition, submit the Results Tracker with actual results as requested.

SUMMARY

The project has been going smoothly and making substantial progress despite a well-planned request and approval of a no-cost extension (NCE) into a fourth year. Another factor was the unfortunate departure of a partner, University of Wisconsin-Madison (UW-M), due to the principal investigator (PI) leaving UW-M and neither the university nor the PI being able to continue with the project. These changes provided a unique opportunity for a favorable redirection of the project in this reporting period. The NCE was requested and noted in the last progress report by University of Helsinki (UofH). In the review of this it was recognized that all partners could benefit from an NCE and project timelines were therefore adjusted accordingly. The untimely loss of UW-M as a project partner prompted a detailed reexamination of their deliverables; fortunately, all deliverables but one (high throughput screening to identify novel antiviral compounds) could be met. Unspent funds from the withdrawal of UW-M from the project were redirected to finish deliverables associated with the QuickChip at the International Potato Center (CIP) and to expand experiments aimed at phytosanitary cleaning of sweetpotato, yam, and cassava material in the greenhouse prior to introduction into *in vitro* at CIP and the International Institute of Tropical

Agriculture (IITA). Overall, with a revised deliverable schedule for years 3 and 4, the project is advancing well with minor redirections and good progress by the remaining three partners.

The effects of the growth of meristems by reducing the macronutrients (full, half-, and quarter-strength macronutrients), and several other *in vitro* parameters were tested.

- In sweetpotato a reduction of the macronutrients in Murashige-Skoog (MS) medium generally favored growth of sweetpotato meristems, although darkness influenced survival. Early indications are that it does not result in a higher conversion to plants. The use of liquid medium, however, has a drastic effect on sweetpotato, potentially cutting in half the time needed for regeneration.
- In yam full-strength MS was generally better: a dark period appears to favor regrowth slightly and activated charcoal has a positive effect on meristem growth.
- In cassava reducing the MS nutrients had no favorable effect and a normal photoperiod without darkness appears to be more favorable. There was no measurable advantage to the addition of activated charcoal, nor did adjusting the hormone ratios have a beneficial effect.

Experiments for the *in vitro* application of antivirals (ribavirin, lactoferrin, salicylic acid) are ongoing as are experiments with the greenhouse application of chemotherapy and thermotherapy. All initial experiments have been set up and pending results are expected in mid-2020.

Small RNA sequencing and assembly (sRSA) continues to look very promising. Experiments completed or ongoing in year 3 included small-RNA isolation experiments, incorporation of an internal quality control to monitor variation in the process, determining the relative sensitivity of sRSA with varying sweetpotato viruses, and the continued comparison of sRSA with conventional indexing. Discussions with the United Kingdom Accreditations Service (UKAS) for incorporation of sRSA in CIP's routine phytosanitary cleaning process have been initiated.

Research continued with chemical inhibitors found to inhibit sweet potato chlorotic stunt virus (SPCSV) RNase III activity. A non-destructive plant assay has been developed based on quantified photosynthetic performance from chlorophyll fluorescence (ChlF) imaging and leaf thermography from thermal infrared (TIR) imaging among sweetpotatoes. Differences among different treatments observed from ChlF and TIR imaging were related to virus accumulation and distribution in sweetpotato.

The VirusDetect software has been reprogrammed. It can now be run on a Windows format, making it more user friendly.

The QuickChip technology was transferred to CIP, where capacity building for its use has been completed and it is fully functional at the laboratory level. Field verification will proceed in year 4.

INDIVIDUAL PROJECT PROGRESS REPORTS

In vitro: sweetpotato (CIP)

Reduced macronutrient levels

The effects of the growth of sweetpotato meristems by reducing the macronutrients (full, half-, and quarter-strength macronutrients) as well as the elimination of NH_4 from the *in vitro* medium was assessed. In general, a reduction of the macronutrients in MS medium favored growth of sweetpotato meristems. Meristems grown on quarter-strength macronutrient MS medium showed a significantly higher average regrowth rate (whole plantlet regeneration) of 37.5%, compared with the control full macronutrient treatment (21.0%) and half-strength macronutrient concentration (30.9%). No statistical differences were observed between quarter- and full-strength medium without ammonium (32.7%) (Table 1). Interesting, although the control treatment showed a significantly higher average survival rate of 67.5% compared with the quarter-strength macronutrient medium (57.1%), many of those failed to develop into complete *in vitro* plants. Quarter-strength medium also has significantly more roots and leaves than do the controls—41.5% versus 26.4%—plantlets with roots and 3.6 versus 2.6 leaves per plantlet, respectively. However, as previously observed, there was some genotypic specificity, with 1 of 9 accessions (CIP 420865) having the highest regrowth rate (65.6%) with full strength MS (control) and half-strength macronutrient. Virus-infected material showed a significantly higher median survival (60%) compared with the clean virus-free material (40%). However, no significant differences were recorded in plantlet regeneration, the most important variable. Based on these promising results, we will extend the experiment to a more diverse set of 45 accessions (e.g., mini-core collection) to confirm that a quarter-strength macronutrient concentration is superior for a larger range of genotypes.

Table 1. Average survival, regrowth, rooting rate, and number of leaves from plants derived from meristems from sweetpotato accessions cultured on four different culture media (full-, half, quarter-strength MS macronutrient concentration and full-strength MS replacing NH_4NO_3 by KNO_3), ~6-months (27 weeks) after meristem excision.

Culture medium	Survival rate (%)	±	SE	Regrowth rate (%)	±	SE	Rooting rate (%)	±	SE	No. of leaves	±	SE
Full-strength MMB*	67.5[a]	±	1.9	21.0 [C]	±	2.2	26 .4 [y]	±	2.3	2.6 [g]	±	0.1
1/2-strength MMB*	65.4 [a]	±	1.9	30.9 [B]	±	2.4	35.4 [x]	±	2.6	3.2 [ß]	±	0.1
1/4-strength MMB*	57.1 [b]	±	2.1	37.5 [A]	±	2.2	41.5 [x]	±	2.3	3.6 [a]	±	0.1
MMB (- NH_4NO_3 + KNO_3)	69.8 [a]	±	1.8	32.7 [A B]	±	2.3	41.6 [x]	±	2.5	3.4 [a ß]	±	0.1
Total	**65.0**	**±**	**1.0**	**30.5**	**±**	**1.2**	**36.2**	**±**	**1.2**	**3.2**	**±**	**0.1**

Note: Different letters indicate statistically significant differences for the Kruskal-Wallis multiple comparison test on the median values ($p<0.05$). The table shows the mean values with its standard deviation (SE). [*MMB: meristem media Batatas]

Dark treatments

Preliminary (single replication) results from an experiment to investigate the effect of various dark treatments (darkness for first, second, and fourth week) on meristem growth found no significant differences between the treatments for plantlet regeneration (31.7–41.7%), rooting rate (35.0–41.7%), or the number of leaves per plant (3.2–4.1). However, a late dark treatment (7 days of darkness during the fourth week post-meristem excision) resulted in a significantly higher survival rate (95.0%) (Table 2). Additional replications of the dark treatments are ongoing. Meristems were grown with four different exposure periods to darkness and assessed 8 weeks after excision. The following darkness conditions were tested: (a) no darkness (control), (b) first 7 days in darkness, (c) from day 8 to 14 in darkness, and (d) from day 28 to 35 in darkness. Table 2 shows the average values of virus-clean (HS2) and virus-infected (HS0) material.

Table 2. Average survival, regrowth, rooting rate and number of leaves on plants derived from meristems of five sweetpotato accessions grown under different dark treatments.

Darkness period (meristem culture)	Survival rate (%)	SE	Regrowth rate (%)	SE	Rooting rate (%)	SE	No. of leaves	SE
No darkness (control)	80.0 [b]	4.8	**40.0 [A]**	8.7	38.3 [x]	9.6	3.2 [□]	0.5
First 7 days in darkness	83.3 [b]	3.5	**41.7 [A]**	11.2	41.7 [x]	11.2	4.1 [□]	0.5
From day 8 to 14 in darkness	90.0 [a b]	3.7	**36.7 [A]**	8.5	38.3 [x]	8.3	3.4 [□]	0.5
From day 28 to 35 in darkness	95.0 [a]	2.6	**31.7 [A]**	9.1	35.0 [x]	9.1	3.8 [□]	0.4
Total	**87.1**	**2.0**	**37.5**	**4.6**	**38.3**	**4.6**	**3.6**	**0.2**

Note: Different letters indicate statistically significant differences for the Kruskal-Wallis test on the median values ($p<0.05$). The table shows mean values with respective standard errors (SE).

Liquid medium

Preliminary (single replication) results from an experiment to investigate the effect of a shaking liquid medium culture resulted in significantly higher average plantlet formation (68.3%) from meristems compared with the control solid medium treatment (36.7%) (Fig. 1). A similar tendency was observed for rooting (68.3% vs. 36.7%) and the number of leaves (5.9 vs. 3.6). These are very exciting results because if the preliminary results are confirmed, a substantial improvement of the meristem culture protocol will be achieved and the time from meristem to a regenerated plant reduced from 27 to 11–19 weeks!

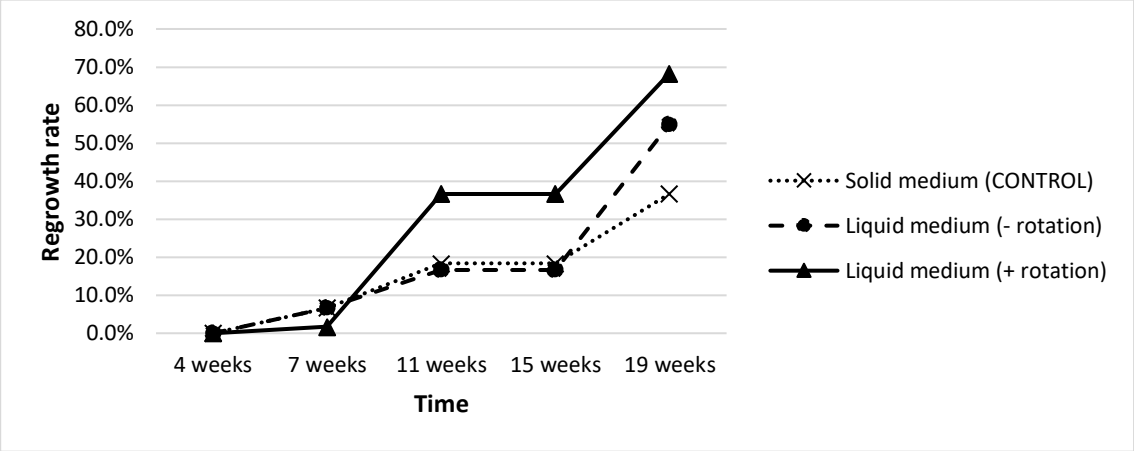

Figure 1. Average regrowth rate of meristems of five sweetpotato accessions grown on three different culture media. Regrowth was assessed at 4, 7, 11, 15, and 19 weeks after meristem excision.

Thermotherapy of greenhouse plants prior to introduction into in vitro

Virus-infected plants from six accessions were multiplied in the greenhouse and 6-week old plants were then placed for 4 weeks at 36±1°C (control plants were left in ambient greenhouse conditions). After 3 weeks of thermotherapy, the apical shoots were removed to promote growth of axillary buds. Following the 4-week thermotherapy treatment, plants were divided into ~2-cm segments (one apical and three stem segments), surface sterilized, and grown *in vitro* in routine sweetpotato *in vitro* medium. *In vitro* plants are currently growing and, although preliminary (15 weeks), the plants coming from the thermotherapy treatment have a more rapid regrowth rate (54.2–55.6%) than the control treatment (40.3–45.8%) (Fig. 2)**.** Polymerase chain reaction (PCR) diagnostics of the *in vitro* plants to determine whether the thermotherapy treatment eliminated the viruses will be done by the end of 2019, as will continued observations on plantlet regeneration.

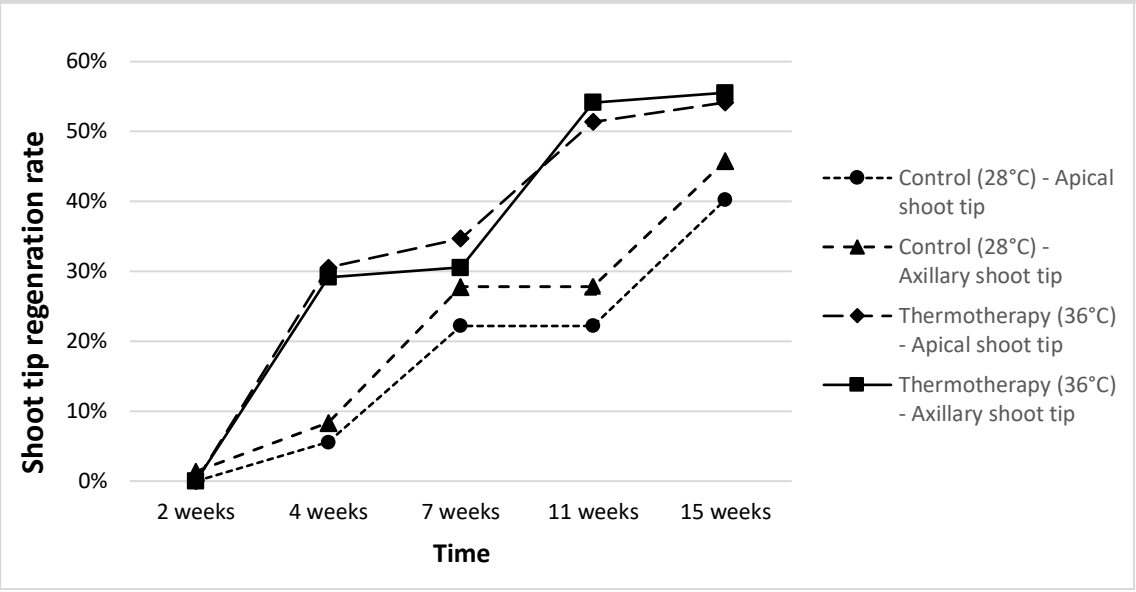

Figure 2. Average regrowth rates of apical and lateral shoot tips, introduced from greenhouse plants treated with thermotherapy (36°C) or not (control, 28°C). The graph shows the average regrowth values of shoot tips of six sweetpotato accessions from week 2 to week 15 after excision.

Topical application of antivirals on greenhouse plants
Virus-infected *in vitro* plants from six accessions were transplanted to the greenhouse, and at 8 weeks the apical stem segment was removed. Approximately 2 weeks later, four weekly sprayings of antivirals (or controls) were initiated: (a) salicylic acid (70 mg/L), (b) lactoferrin (1,000 mg/L), (c) Control 1 (water + tween 0.02% [v/v]), and (d) Control 2 (only water). After the second spraying, the apical stem segment was again removed. One week following the four weekly sprayings, plants were divided into ~2-cm segments (one apical and three stem segments), surface sterilized, and grown in routine sweetpotato *in vitro* medium. Results on virus elimination efficiency of this experiment will be available in early 2020.

In vitro *comparison of the application of antivirals versus chemotherapy*
Salicylic acid (5 mg/L) and ribavirin (20 mg/L) were filter-sterilized prior to placement in solid tissue culture medium containing newly isolated nodal segments. Lactoferrin (500 mg/L) precipitated in solid medium and was therefore added to liquid instead of solid medium. After 4 weeks, shoot tips (1.2–1.5 mm) were excised and cultured by routine *in vitro* methods. In a separate experiment, *in vitro* plants were exposed to thermotherapy for 4 weeks at 36±1°C prior shoot tip excision. Data analysis was complicated because sample sizes of regenerated plants were highly variable between accessions and treatments. Suspected issues with PCR multiplexing confounded the analysis of the results. For future experiments, targeted virus-specific PCR will thus be done. In summary, there were no statistical differences observed between the five treatments for the virus elimination rate (% of negative samples); however, CIP 420169, infected with SPFMV, showed a significantly higher virus-cleaning rate than the other accessions. CIP 401114 (Begomovirus) could not be cleaned with any of the applied treatments (0% cleaning rate).

In vitro yam (IITA)

Results of all the experiments, performed in triplicate, will be analyzed after taking the final observations in early 2020.

Reduced macronutrient levels
The regrowth rate of yam meristems was tested on different concentrations of MS macronutrients (full-, half-, quarter-, and eighth-strength MS). After 1 month it could already be observed that regrowth was better on the full- and half-strength MS.

Dark treatments
The effect of a longer dark interval (all yam meristems are given a 24-h dark treatment immediately after meristem excision) versus a 12-h photoperiod was tested to determine whether darkness could enhance yam meristem regrowth. Three treatments were tested: 12-h photoperiod as a control, 7 days of darkness, and 14 days of darkness before placing meristems in a 12-h photoperiod. Observations after 12 months showed that the meristems maintained in the dark for 7 days had a slightly better regrowth rate than meristems exposed to a 24-h dark period.

Activated charcoal
Different concentrations of activated charcoal (AC) (0, 0.5 mg/L, 1 mg/L, and 2 mg/L) were added to the culture media to determine its effect on *in vitro* yam meristem regrowth. After 1 month, it could be observed that the addition of AC had a positive effect on yam meristem regrowth rate, especially with higher concentration of AC.

Antioxidants and auxin/cytokinin ratio

Experiments have been set up and are ongoing with antioxidants (glutathione and ascorbic acid) and different auxin/ cytokinin ratios to continue the improvement of the yam meristem regrowth protocol.

In vitro cassava (IITA)

Results of all the experiments, performed in triplicate, will be analyzed after taking the final observations in early 2020.

Reduced macronutrient levels
Culturing the cassava meristems on different strength of the MS mineral-based culture medium (full-, half-, quarter-, and eighth-strength MS) was also carried out. Preliminary results of this experiment concluded that reducing the strength of the MS had no favorable effect on the cassava regeneration process. In terms of timeline, the full-strength MS will be kept as the routine recipe for cassava meristem regrowth.

Dark treatments
The test of different light regimes, as with yam, were tested on cassava meristems. It was observed that the continuous 12-h photoperiod was more favorable for cassava meristem regrowth for most of the tested accessions.

Activated charcoal
The addition of AC at different doses (0, 0.5 mg/L, 1 mg/L, and 2 mg/L) to the cassava meristem regrowth culture medium did not show any favorable effect as the regrowth rate between the treatments and the control was not significantly different.

Antioxidants
The addition of two antioxidants at different doses (glutathione at 0.049 and 0.074 mg/L and ascorbic acid at 25 mg/L and 40 mg/L) to the cassava meristem regrowth culture medium did not show any favorable effect as the regrowth rate between the treatments and the control was not significantly different.

Auxin/cytokinin rations
The hormone balance between auxin (NAA) and cytokinin (BAP) in the cassava meristem regrowth culture medium was tested by changing the concentrations and ratios of auxin (NAA 0.2 mg/L, BAP 0.075 mg/L, and GA3 0.02 mg/L) and cytokinin (NAA 0.1 mg/L, BAP 0.15 mg/L, and GA3 0.02 mg/L). For regrowth it was observed that cassava meristem cultured on the control treatment (normal hormone levels) had an equivalent regrowth rate compared with those cultured onto media on which hormone balance has been changed.

Apical versus axillary buds
Routinely, only apical meristems are sampled for cassava *in vitro* establishment. For shortening the time needed to produce cassava *in vitro* plant material via meristem culture, the effect of the source of the meristem explants from apical and axillary buds is being evaluated.

Yam and cassava (IITA)

In vitro *comparison of the application of antivirals*
To test the effect of different antiviral agents to suppress virus multiplication and phytosanitize plantlets, *in vitro* materials were confirmed for viruses—namely PCR for cassava mosaic begomoviruses (CMBVs) and real time (RT)-PCR for yam mosaic virus (YMV), yam mil mosaic virus (YMMV), and PCR for yam badnaviruses (YBV) in yam and cassava, respectively). Three antiviral agents were used: ribavarin (0, 10, 20, and 25 mg/L), lactoferrin (0, 100, 200, 500, and 1,000 mg/L), salicylic acid (0, 5, 10, 20, and 50 mg/L) filter-sterilized (0.22 µm) into MS medium. For each accession, 7 micro-cuttings were cultured onto each dose of the treatments for a total of 455 plants. These experiments were replicated three times. The cultures were maintained under 12-h photoperiod and 25–27°C temperature. After 4 weeks, 78%, 89%, and 88% of shoots survived, respectively, with exposure to the lactoferrin, salicylic acid, and ribavirin treatments. Loss of plantlets was due to necrosis, especially when treated with higher lactoferrin doses. Virus indexing is pending to assess virus elimination efficiency.

Topical application of antivirals on greenhouse material

Application of lactoferrin (Apolactoferrin, Life Extension, Quality supplements and Vitamins, Inc., Florida USA) was tested to assess the anti-viral effect on ACMV- and EACMV-infected cassava. Two of the three genotypes of cassava were naturally infected with ACMV (TME1962) and EACMV (TME4217), whereas cultivar TME168 was virus-free. Three concentrations (250 mg/L, 500 mg/L, and 1,000 mg/L) of lactoferrin solution and 20 mL of lactoferrin solution were applied to the root zones of individual plantlets 48 h after transplanting; the same volume of sterile distilled water was applied to the root zones of control plants. The treatment was repeated for 4 weeks. Plants were assessed every 2 weeks for foliar symptoms of cassava mosaic disease (CMD). Data were collected on plant height and CMD incidence and severity every 2 weeks for the next 6 weeks post-transplanting in the screenhouse. Samples were indexed for ACMV and EACMV by PCR. Plants of TME 1962, which were infected by ACMV, recovered from infection as plants were asymptomatic and virus was undetected in PCR, as of the last test 6 weeks after treatment. However, the plants of the EACMV-infected genotype, TME4217, remained symptomatic and tested positive to EACMV by PCR. The treatment had no noticeable effect on the healthy control (TME168).

Actigard® is a commercial product reported to stimulate systemic acquired resistance in plants. Actigard was tested against CMD suppression in cassava stem cuttings of CMD infected (with ACMV) cassava cv. NR8082 treated by soaking for 10

min in 250 mg/L Actigard solution, then stems dried at room temperature for 8–12 h and then planted in pots in a screenhouse. Stems treated in an aqueous solution of Karate (active ingredient lambda-cyhalothrin) (4 ml/L) + mancozeb (7 g/l) in water and only water were used as controls. Plants were monitored for CMD symptom severity and growth by measuring plant height. Leaf samples were tested by PCR for ACMV and EACMV virus confirmation. In a separate experiment, 12-week-old plants were sprayed with three concentrations of Actigard, 50 mg/L, 150 mg/L, and 250 mg/L and distilled as a control. Stem treatments had no effect on CMD severity, whereas foliar sprays of Actigard resulted in yellowing on sprayed plants. The newly emerging leaves on several plants, however, showed recovery from CMD symptoms. Recovered leaves when tested in PCR were negative to ACMV, but no recovery was observed in plants sprayed with 50 mg/L and 250 mg/L of Actigard and water control. This experiment is still underway.

sRSA: sweetpotato (CIP)

Six different RNA extraction protocols (see 2018 report for details on the different protocols) were tested for detecting five different viruses resulting in the decision to use the E.Z.N.A miRNA kit as the standardized method for further RNA extractions. The E.Z.N.A miRNA kit has resulted in the most reproducible and clearest results to date, and simultaneously reduced operational steps reducing opportunities for contaminations to occur.

The comparison of small RNA sequencing against large RNA sequencing was also finalized for the same five viruses. It was found that small RNA sequencing was much more sensitive and reliable than large RNA sequencing in the case of sweetpotato viruses. Thus, we will maintain small RNA as our principal approach.

In addition, several experiments were performed to include quality control measures into the library preparation process:

1. Nine artificial, small RNA spike-in sequences were designed, ordered, and included in libraries as an internal quality control for the library preparation process. The spike-in sequences were designed to show no similarity with any known sweetpotato-infecting virus or sweetpotato genome sequences and were 21nt in length, with one 24nt in sequence. The last four nucleotides on each end were random to avoid any ligation bias due to nucleotide type at the siRNA extremes. Spike-in sequences were added at eight different concentrations, with fivefold difference between each of them in five different ranges, from 0.002–1.25 to 1.25–97656 amol (10–18 moles) per library prep. Results showed that far fewer spike-in sequences were recovered by sequencing than expected—likely due to losses occurring during the RNA extraction process (spike-in was added to the buffer before extraction to control the whole process). A smaller percentage of molecules was captured by the library prep process than estimated. Thus, the minimum amount of spike-in sequence to be added to obtain reliable recovery of at least one spike-in sequence is around 500–1,000 amol. Spike-in amounts were adjusted to this minimum in subsequent libraries.
2. A control sample, *Capsicum baccatum* infected with an endornavirus, was included as 1 of the 48 samples to control for contamination between samples. This worked well; however, the average depth/million of the endornavirus was rather low (~10–30x) even in the control itself. This limited the sensitivity of detection of contamination, thus a better control virus which produces more siRNAs (depth/million >100) in the host would be required. Some candidates will be tested in the next sequencing runs.
3. Options were included in the new VirusDetect for Windows software to automatically analyze spike-in, and control sequence and have worked well.

Two groups of plant materials totaling 97 samples have been evaluated for direct comparison of sRSA and conventional indexing. Overall, 57 samples were found positive by both methods and 24 virus-negative by both methods. Fourteen accessions were found positive by sRSA, whereas negative for standard indexing. However, all of these were associated with partial- and low-depth genome coverage of detected viruses (Begomovirus in one group, and mostly SPFMV in the other group) and likely represent contaminations (Fig. 3). This indicates adjustments are needed for coverage cutoffs for these situations. We will analyze in more detail how to achieve this. Note that these samples were not yet run with the *C. baccatum* endornavirus control, which should allow adjustments based on contamination levels. Two accessions recorded symptoms in indicator plants but were negative for sRSA—as well as enzyme-linked immunosorbent assay and PCR tests. These will need to be analyzed in more detail to understand whether symptoms were caused by a pathogen or other sources.

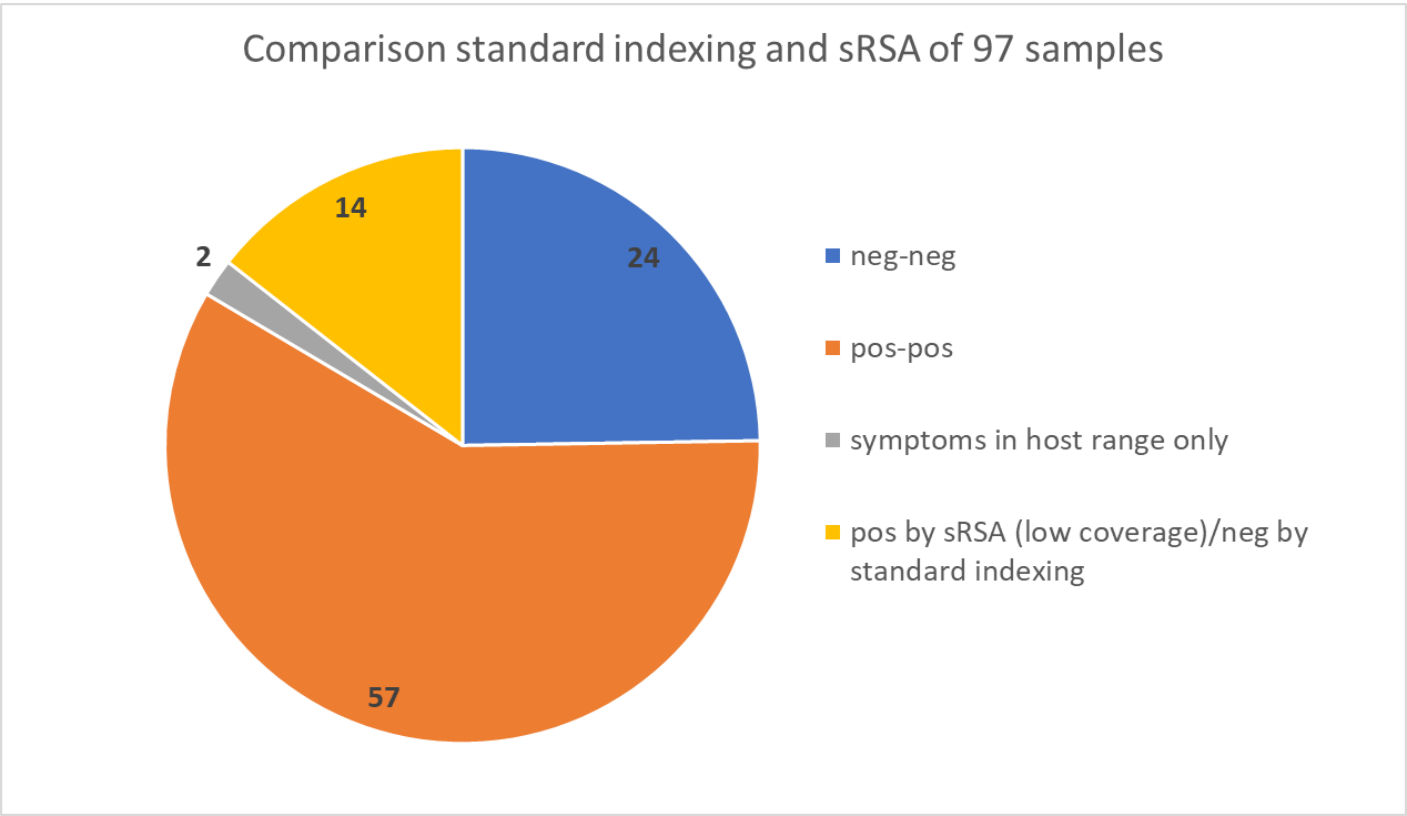

Figure 3. Number of samples testing positive (pos) or negative (neg) in the side-by-side comparison of sRSA with conventional indexing in 97 sweetpotato samples.

Another additional 170 samples have been processed and sequenced for side-by-side comparison of sRSA and conventional indexing prior to thermotherapy and meristem isolation. The analysis of these samples is ongoing and should be completed by the end of 2019. These same 170 samples will be tested once again side-by-side after virus cleanup.

An experiment was performed to determine the sensitivity of sRSA through a dilution series of eight accessions infected with the most important sweetpotato viruses and was repeated three times at different combinations (Table 3). Results showed that some viruses were consistently identifiable through a 128-time dilution, sensitivity for SPCSV detection was lost at 16- to 32-fold dilution in two of the repetitions, whereas potyvirus (single infection) detection was lost at 32- to 64-fold dilution in those same replicates. All viruses were detected up to 128-fold dilution in the third replication. The differences in detection limit in the different replications probably reflect variability in the quality of the library preps and highlight the importance of having internal controls as is being developed with spike-in sequences.

Table 3. Results from three repetitions of the sensitivity of detection of sweetpotato viruses by sRSA in a 1- to 128-fold dilution series

dilution factor:	1	2	4	8	16	32	64	128
REP 1								
Sample	sRSA.	sRSA.	sRSA.	sRSA.	sRSA.	sRSA.	sRSA.	sRSA.
A	Begomo, SPVCV	Begomo, SPVCV, SPCV	Begomo, SPVCV, SPCV, SPCSV	Begomo, SPVCV, SPCV, SPCSV, SPV2	Begomo, SPVCV, SPCV, SPCSV, SPV2	Begomo, SPVCV, SPCV, SPV2	Begomo, SPVCV, SPCV	Begomo, SPVCV
B	SPCV							
C	NEG	SPCSV						
D	SPCSV							
E	SPVCV	SPVCV, SPV2	SPVCV, SPV2, Begomo					
F	SPV2							
G	Begomo	Begomo						
H	NEG							
Loss of detection of:						SPCSV	SPV2	SPCV
REP 2								
B	SPCV	SPCV	SPCV, Begomo, SPV2	SPCV, Begomo, SPCSV, SPV2, SPVCV	SPCV, Begomo, SPV2, SPVCV	SPCV, Begomo, SPVCV	SPCV, Begomo, SPVCV	Begomo, SPVCV
C	NEG							
F	SPV2	SPV2						
G	Begomo							
D	SPCSV	SPCSV, SPVCV	SPCSV, Begomo, SPVCV					
E	SPVCV							
H	NEG	Begomo, SPVCV						
A	Begomo, SPVCV							
Loss of detection of:					SPCSV	SPV2		SPCV
REP 3								
A	Begomo, SPVCV	Begomo, SPVCV	Begomo, SPCV, SPV2, SPVCV	Begomo, SPCV, SPV2, SPVCV, SPCSV	Begomo, SPCV, SPV2, SPVCV, SPCSV	Begomo, SPCV, SPV2, SPVCV, SPCSV	Begomo, SPCV, SPV2, SPVCV, SPCSV	Begomo, SPCV, SPV2, SPVCV, SPCSV
E	SPVCV							
B	SPCV	SPCV, SPV2						
F	SPV2							
C	NEG		SPCSV					
G	Begomo							
D	SPCSV	SPCSV						
H	NEG							

During the 2019 CIP audit by UKAS for ISO 17025 accreditation, we discussed the needs for getting sRSA ISO accredited and UKAS was informed that this will be applied for during the first half of 2020. Accreditation of sRSA will require the submission of documentation forms, including standard operating procedures (SOPs) and evidence that the method is fit for purpose. Included in these SOPs is one for data management, which is still needed. Another potential challenge is that since sequencing is outsourced, UKAS needs to verify how this is managed from an accreditation point of view. Our current provider has all their processes ISO-certified, but we would rather be approved generically for any ISO-certified sequencing provider, to avoid becoming tied to only one provider.

In summary, continued research, time, and further due diligence for ISO is needed prior to being able to recommend a switch to sRSA as our primary diagnostic method for sweetpotato. Experiments with sensitivity of sRSA and spiking, we feel, will aid greatly in confirming confidence of sRSA as a diagnostic methodology.

sRSA, yam, and cassava (IITA)

sRSA libraries were constructed for 98 samples (51 yam, 19 cassava, 13 *Musa*, 2 *Nicotiana benthamiana,* 3 cocoyam, 5 maize, and 1 cowpea) as per the protocol detailed in Annex 1. First set of 48 libraries was analyzed (10 cassava, 2 *N. benthamina*, 1 Musa, 3 cocoyam, and 32 yam samples) and summary results are presented in Annex 2. The major viruses detected in yam were YMV and badnaviruses. Samples that previously tested negative to viruses remained virus-free

except the detection of contigs matching with several flexiviruses (Ampelovirus, Foveavirus, Carlavirus, and Potexvirus). Trace levels of YMV were detected in several libraries, indicating potential low-level contamination. Bioinformatic analysis is ongoing.

VirusDetect (UofH/CIP)

There was significant progress with VirusDetect in year 3 as it has been completely reformatted to be Windows (VirusDetect-Windows; VDW) compatible, thus making it much more user friendly (Fig. 4). (VDW can be downloaded from the following websites: https://research.cip.cgiar.org/virusdetect/ or http://potpathodiv.org/vd_download.html.) In addition, VirusDetect can now process both large and small RNA datasets, perform sequence trimming and cleaning, and fastQC (quality analysis). It provides for optional inclusion of spike-in and contamination control sequences. If spike-in and control sequence options are chosen, the software now reports data on the total and normalized number of spike-in sequences that are detected in each sample that can serve as a library quality control. It also provides a number for percentage of contamination occurring from the control sample that can be considered to determine thresholds for considering plants positive for viruses. Improvements are still intended based on feedback in training courses and limited funding has been provisioned for that during the NCE period.

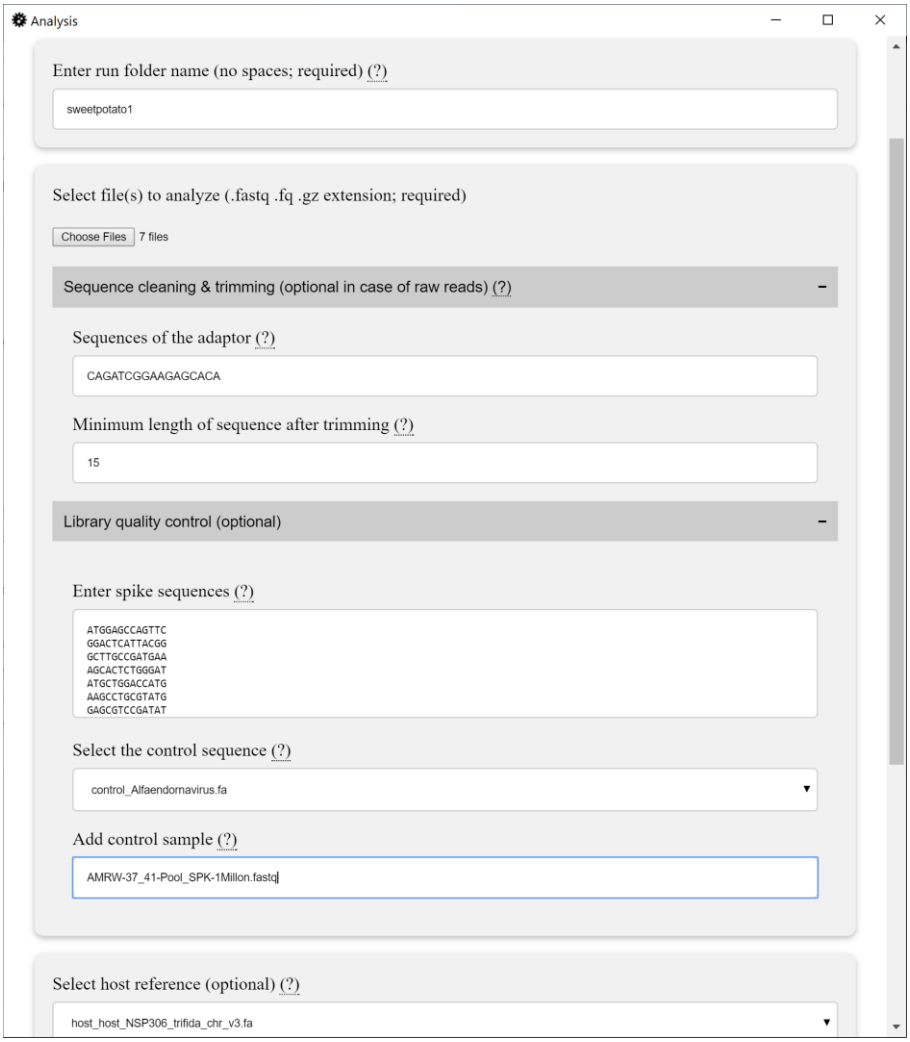

Figure 4. Screenshot of VDW interface.

High-throughput screening of RNase3 inhibitors (UofH)

Work continued in planta with chemical inhibitors found to inhibit SPCSV RNase III activity. To identify the inhibitors and look at plant-virus interactions, a nondestructive screen based on a high-throughput image-based plant phenotyping platform was used. Results indicated a correlation between morphology and virus accumulation in the plants, as measured by ChlF of photosystem II (PSII) and TIR imaging. The findings were further validated at the molecular level by monitoring related gene expression. Our study showed that the ChlF- and TIR-based imaging systems can be used for distinguishing the severity of viral symptoms in sweetpotato.

This nondestructive image-based plant phenotyping platform was also used to study plant-virus synergism in sweetpotato by comparing four virus treatments with two healthy controls.

By monitoring physiological and morphological effects of viral infection in sweetpotato over 29 days, we quantified photosynthetic performance from ChlF imaging and leaf thermography from TIR imaging among sweetpotatoes. Moreover, the differences among different treatments observed from ChlF and TIR imaging were related to virus accumulation and distribution in sweetpotato. These findings were further validated at the molecular level by related gene expression in both photosynthesis and carbon fixation pathways.

This study validated for the first time the use of ChlF- and TIR-based imaging systems to distinguish the severity of virus diseases related to SPFMV and SPCSV in sweetpotato. In addition, we demonstrated that the operating efficiency of PSII and photochemical quenching were the most sensitive parameters for the quantification of virus effects compared with maximum quantum efficiency, non-photochemical quenching, and leaf temperature.

Other highlights included:

- Validation of a fluorescence resonance energy transfer-based, high throughput screening, which can be used not only for RNase III of SPCSV but also other RNase IIIs.
- Submitted one scientific publication: Wang, L., Poque, S., and Valkonen, J.P.T. Forthcoming. Phenotyping viral infection in sweetpotato using high-throughput chlorophyll fluorescence and thermal imaging platform. *Plant Methods* (accepted for publication on October 10, 2019).
- Inhibition verification in plants grown in soil has been made for inhibitor candidates selected for RNase III of SCPSV, using the parameters determined in the phenotyping study. Based on the results from the screening and affinity assays at the molecular level, and validation of test plants, two scaffold clusters from five compound candidates were identified. These are good candidate inhibitors which could be used to develop antiviral drugs targeting to RNase III. A manuscript is in preparation.
- During the 12 months reported here, we have used an image-based high-throughput plant phenotyping platform to study sweetpotato-SPVD interactions in a nondestructive manner to observe physiological and morphological differences, photosynthetic performance, and leaf thermography. Relative gene expression in photosynthesis and carbon fixation pathways was assessed as were viral accumulation and distribution among sweetpotatoes infected with four virus treatments and two healthy (non-virus infected) controls. Hence, we now have a system to measure accurately the effect of putative RNase III inhibitors for alleviating SPVD.

Taken together, our results on virus inhibitors suggests that certain compounds provide an effect which we expected. For these studies we are running the plant phenotyping system.

<u>High-throughput screening of novel antiviral compounds (UW-M)</u>

This element of the project has been discontinued due to the loss of UW-M in the project, the fact that it was not deemed possible with the limited time and budget remaining, and the sophisticated nature of the research involved for this to be subcontracted to another party. Hence, presented here is the progress to date for this element of the project.

The original approach was to build a plant model system consisting of infecting *Nicotiana benthamiana* protoplasts with tobacco mosaic virus (TMV) expressing a fluorescent protein (Fig. 5).

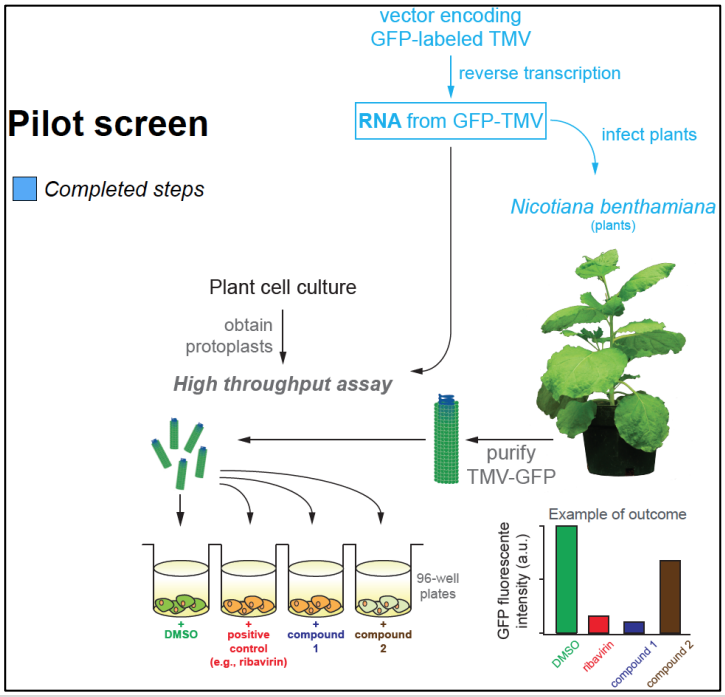

Figure 5. Schematic diagram of the proposed system for high throughput screening of novel putative antiviral compounds in plants.

The first screen consisted of the development of a 96-well plate in which each well contained a unique small molecule at a final concentration of 50 uM. Molecules that inhibited viral entry or replication would result in wells that had low fluorescence compared with control wells lacking a small molecule (negative control). Positive controls would entail the use of small molecule antivirals with broad-spectrum activity against viruses (e.g., ribavirin). Compounds that gave a statistically significant signal above control would be rescreened and then counter-screened against secondary and tertiary *in vitro* assays to confirm that activity arises from blocking infection rather than other phenotypes. Although *Nicotiana* is unrelated to yam, sweetpotato, and cassava, we felt that (1) it represented the highest chances of successfully developing a plant-based assay and (2) success might yield a collection of small molecule inhibitors of which at least one had activity against plant viruses that infect cassava, yam, and sweetpotato. Our choice of using TMV for the assay is due to its role as a model plant virus and its broad host selectivity, which we anticipated might also translate into the highest probability of success with the assay.

During the first 2 years of the project, the screen was systematically developed by working through and optimizing each step outlined in Figure 5. First, several tobacco mosaic virus-GFP (TMV-GFP) constructs were obtained and made viral RNA of TMV-GFP recombinantly. However, the method proved unsuccessful in infecting plants—via leaf-based methods of introducing the virus—to replicate and amplify the virus and yield many viral particles for the assay. Hundreds of different experiments were tried with different plants (beyond *Nicotiana*), different methods of infecting plants, and different infection conditions. Yet we were unable to get reproducible replication. At this point the project switched to *Agrobacterium*-mediated transformation of whole *N. benthamiana* plants using *Agrobacterium tumefaciens* strains carrying plasmid coding for TMV-GFP. This approach was successful by infecting leaves directly with a suspension of bacteria; however, the infection yield was low and thus a switch was made to turnip mosaic virus-GFP TuMV-GFP (which is and has a very wide range of hosts) and provided a much higher yield of infected plants. Major issues sanitizing viral particles isolated from plants to avoid transmitting other organisms that interfere with the infection assay were initially encountered, though they were eventually solved. We were able to infect dozens of plants, extract intact viral particles from plants to get high yields of TuMV-GFP for our screen, and store the virus for screening assays.

With TuMV-GFP in-hand, focus turned to the infection assay. Although the initial proposal was to infect *Nicotiana* protoplasts, after working with them the project broadened its plant-host range to include *Arabidopsis* and demonstrated variable success of infecting protoplasts using isolated TuMV-GFP particles. The project eventually narrowed host range down to four key cell lines (*Arabidoposis* T87W, *Arabidoposis* T87J, *Arabidoposis* PSB-D, and *Nicotiana* BY-2) and explored *in vitro* infection under a matrix of different conditions. Conditions for infecting *Arabidoposis* T87J in cell culture were developed for which no protoplasting was required. But despite heavy optimization, the project was never able to get the S/N high enough for a high throughput assay. Research continued with this strain and BY-2 protoplasts (both of which showed the most promising results); however, after months of optimization, the S/N remained too low for screening.

If future research will be pursued using this approach, it is very likely that optimization may be enhanced by switching the fluorophore to a fluorescent protein that is red-shifted in its spectrum. It is likely that plant cells accumulate flavochromes (from flavin degradation), which absorb/emit between 480 and 512 nm and hence mask viral replication. Cyan or yellow fluorescent proteins would be excellent choices to switch to, and initial work was started to engineer TuMV-CFP and TuMV-YFP when the project was terminated at UW-M. Thus, there was no time to create and test these constructs.

QuickChip (CIP)

When UW-M left the project in 2019, the QuickChip was almost ready for testing. The work as of May 2019 done at UW-M included:

1. The pre-identification of relevant viruses and the development of recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP) assays, or other isothermal assays.
2. Testing both LAMP and RPA assays in the QuickChip format, demonstrating that it can reproducibly achieve S/N ratios of 2–10.
3. Testing the viruses related to this project (Table 4), the RPA assays worked very well on the chip, with low to moderate S/N ratios for LAMP assays.

Table 4. Viruses tested for efficacy in the QuickChip format

Plant host	Viral species	Genomic material	Detection method
Sweet potato	SPFMV	ssRNA(+)	LAMP
Sweet potato	SPVC	ssRNA(+)	LAMP
Sweet potato	SPVG	ssRNA(+)	LAMP
Sweet potato	SPV2	ssRNA(+)	LAMP
Sweet potato	SPCSV	ssRNA(+)	LAMP
Sweet potato	SPLCV	ssDNA	LAMP
Yam	YMV	RNA	LAMP/RPA
Yam	YMMV	RNA	RPA
Cassava	CBSV	RNA	LAMP
Cassava	UCBSV	RNA	LAMP
Cassava	ACMV	DNA	RPA
Cassava	EACMV	DNA	RPA

The testing consisted of the following: (1) testing *in vitro* synthetic gBlocks of DNA using primers and probes designed specifically for detecting the viral sequences; (2) testing extracted viral RNA or DNA isolated from plant leaves in the lab using a laser-based fluorescence imager (Typhoon Imager, GE); (3) testing extracted viral RNA or DNA isolated from plant leaves in the lab using a bench-top reader that is an early prototype of the portable readers; and (4) testing leaves from infected plants using the portable readers.

CIP and Dr. Douglas Weibel from UW-M were meanwhile discussing how the QuickChip phase of the project could be finished. In the end, the university shipped all the equipment and consumables on hand necessary to produce the chips, how to design new chip layouts, how to run the devices, and interpret the results, as well as two prototype readers to CIP. This enabled CIP to finish the testing of the QuickChip. Dr. John Crooks (UW-M) traveled to CIP to build the capacity there to run the assays and test the QuickChip on material in the field. The readers were tested with sweetpotato leaf extracts and purified RNA extracted from SPFMV, SPCSV, and begomovirus-infected sweetpotato plants. Dr. Crooks also worked with CIP staff to solve the hurdles with low S/N ratios for the LAMP assays.

As of the writing of this progress report (September 30th, 2019), the lab testing of the QuickChip is almost complete and field testing in Peru is anticipated to start early in 2020. One challenge is that the QuickChip reader needs to be attached to a computer, and there appears to be additional programing needed to enhance the functionality of the app associated with the QuickChip.

Y1 (2017) Capacity-Building Activities

1. Weblink presentation on high throughput sequencing for virus detection at the “Taller Regional De La Convención Internacional De Protección Fitosanitaria (CIPF) Para América Latina” in August 2018. Representatives of all regional NPPOs attended (~ 30 people).
2. Work presented at the “30th Technical Consultation among Regional Plant Protection Organizations (RPPOs)” in Lima, Peru, October 2018; about 20 representatives of RPPOs from all over the world attended.
3. Work presented at the RTB-ISTRC in Cali in October 2018, attended by ~50 people from various countries.
4. Work presented at Sweetpotato Speedbreeders meeting (Mozambique, June 2019).
5. Outreach activities in the reporting period mainly done through distribution of flyers and incorporation of sRSA virus indexing as part of the seminars on clean seed production and virus indexing.
6. Several courses in the new VDW have been provided using complementary funding, besides the one in Helsinki in June, each time including improvements based on feedback from participants:
    a. September 2019: a library preparation and data analysis (using VDW) course at was given at Kenya Plant Health Inspection Service (KEPHIS), Muguga, Kenya, for six KEPHIS staff funded by the SASHA2 project using technology and knowhow partially from the NextGen project.
    b. A course on VDW for CGIAR genebank GHUs and European NPPOs was provided at Bioversity’s genebank in Gembloux, Belgium, in November 2019, funded by the Genebank platform.
    c. We trained 31 participants (14 females) from Peru, Ecuador, Colombia, Venezuela, and Argentina at a

workshop at CIP-Lima in VirusDetect in April.

Y2 PIs Interactions

1. No formal face-to-face meetings took place between the PIs in year 3 of the project, although bi- and trilateral meetings between all PIs occurred throughout the year at various venues when more than one of them was present.
2. Four Skype meetings were held among the PIs in year 3 in addition to continuous contact vis email.

2. Project Adjustments

For each outcome or output that is behind schedule or under target, explain what adjustments you are making to get back on track.

As detailed in the 2018 technical report, one major project adjustment was the request for and implementation of an NCE for the project into a fourth year. This request took longer than expected to enact and was not finalized until well into this reporting period. We are happy to report that the NCE is now fully implemented, all funds are reallocated, and all partners are working on the revised delivery schedule.

The other major adjustment for the project was the notification in March 2019 of UW-M's intent to relinquish Award No. OPP1130216, entitled NextGen Phytosanitation: Rapid Elimination of Viruses from RTB Plants for Crop Improvement and Seed Systems," because both PIs (Drs. Douglas Weibel and Ti-Yu Lin) for the project were no longer employed by the university. This came as a relative surprise, and we determined that it was not possible to work out the logistics of reallocation of project deliverables (the QuickChip) and terminating deliverables (high throughput screening of novel antiviral compounds) within the limited time and budget remaining. Other factors were the sophisticated nature of the research, which could not easily be subcontracted to another party, and the reallocation of unspent funds from the screening project to other relevant and needed research areas for the project.

Below is a brief summary of the changes that occurred due to the relinquishment of the award by UW-M:

1. The QuickChip technology was developed to the point where field testing was almost ready to start. Equipment purchased and necessary for further development and field testing were transferred from UW-M to CIP in Lima, and the post-doc working on the project at UW-M (Dr. John Crooks) was hired as a consultant to transfer the technology to CIP and complete the field testing of the QuickChip technology in Peru. The technology transfer has been completed in this reporting period, and QuickChip will be field-tested in Peru in 2020. With this transfer of the technology to CIP, we expect to fully meet the deliverables of the QuickChip portion of the project, except for the field testing of the QuickChip in Africa.
2. Although progress was made on the development of a high throughput screening method for novel antiviral compounds, this progress did not reach the point where it could be continued by anyone else at UW-M, anyone on the project team, nor any researcher outside of the project because of the lack of easily transferability due to the specialized nature of technology and the short timeframe remaining in the project. Thus, we are terminating this portion of the project and have reallocated funds from this deliverable to support other deliverables of the project, specifically further research in the application of antivirals on greenhouse plants to develop a method to clean material prior to or during the isolation into *in vitro* process. This reallocation is in support of Output 1.5: *An antiviral therapy protocol for increased efficiency and flexibility to produce virus-free plants*.
3. The reallocated funds will be directly used to expand our small-scale study to investigate the feasibility of greenhouse treatment with antivirals and/or thermotherapy to eliminate or reduce virus titer in plants prior to isolation into *in vitro*. The termination of UW-M as a partner in the NextGen Phytosanitary project, and the inability of the project to continue the development of a high throughput screening program for novel antiviral compounds, has necessitated, as well as enabled, the reevaluation of some project objectives and deliverables. One overriding goal of the project has been to develop a methodology to streamline phytosanitary cleaning of cassava, yam, and sweetpotato. With this goal in mind, we reallocated a portion of the unspent UW-M funds toward a larger effort targeted at elimination of viruses in propagules going into *in vitro*. This will allow testing of a broader range of genotypes of all three crops on more individuals per genotype. Greenhouse plants will be sprayed with antiviral compounds or exposed to thermotherapy prior to isolation into *in vitro*. Plants regenerated *in vitro* will then be screened to determine whether these methods eliminated the viruses.

3. Geographic Areas to Be Served

Provide the most updated list of countries and sub-regions/states that have benefitted or will benefit from this work and associated dollar amounts. If areas to be served include the United States, indicate city and state. Reflect both spent and unspent funds. Add more rows as needed. More information about Geographic Areas to Be Served can be found here.

Location	Foundation Funding– Total Project
Nigeria	$700,000
Ghana	$50,000
Benin	$50,000
Uganda	$100,000
Tanzania	$100,000
Kenya	$100,000
Mozambique	$ 50,000
Other countries W. Africa	$200,000
Other countries in sub-Saharan Africa	$200,000
Global	$835,906

4. Geographic Location of Work

Provide the most updated list of countries and sub-regions/states where this work has been or will be performed and associated dollar amounts. If location of work includes the United States, indicate city and state. Reflect both spent and unspent funds. Add more rows as needed. More information about Geographic Location of Work can be found here.

Location	Foundation Funding
CIP, Peru—Lead organization	$1,074,108
IITA, Nigeria	$673,427
University of Helsinki, Finland	$322,038
University of Wisconsin–Madison, Madison, WI	$316,333

5. Feedback for the Foundation

Provide one to three ways the foundation has successfully enabled your work so far. Provide one to three ways the foundation can improve.

We gratefully appreciate the support received from the foundation as this year the project was in uncharted territory. First by the request from one partner, growing to the other partners for an NCE, and then by the relinquishing of the award by one of the partners. While the foundation was supportive, our exact understanding of the process, including what paperwork was needed, was puzzling at times. Additionally, as the prime, CIP needed prior written approval for both the NCE and the reallocation of funds prior to notifying partners. This process was a relatively slow process, causing some delays in allowing partners to proceed.

6. Global Access and Intellectual Property

If your funding agreement is subject to Intellectual Property Reporting, please click the following link to complete an Intellectual Property (IP) Report.

If not, please acknowledge by typing "N/A": __N/A__

To delegate permissions to another member of your project team or for any questions regarding the Intellectual Property Report, please contact GlobalAccess@gatesfoundation.org.

7. Regulated Activities

Do you represent that all Regulated Activities[1] related to your project follow all applicable safety, regulatory, ethical and legal requirements? Please mark with an "X":

__N/A__ **N/A (no Regulated Activities in project)**

_____ **Yes**

_____ **No (if no, please explain below)**

Are any new Regulated Activities[1] planned which were not described in any documents previously submitted to the foundation? Please mark with an "X":

_____ **No**

_____ Yes (if yes, please explain below)

1 Regulated Activities include but are not limited to: clinical trials; research involving human subjects; provision of diagnostic, prophylactic, medical or health services; experimental medicine; the use of human tissue, animals, radioactive isotopes, pathogenic organisms, genetically modified organisms, recombinant nucleic acids, Select Agents or Toxins (www.selectagents.gov), Dual Use technology (http://export.gov/regulation/eg_main_018229.asp), or any substance, organism, or material that is toxic or hazardous; as well as the approvals, records, data, specimens, and materials related to any of the forgoing.

Financial Update

The purpose of the Financial Update section is to supplement the information provided in the "Financial Summary & Reporting" sheet in the foundation budget template, which reports actual expenditures and projections for the remaining periods of the grant. This section is a tool to help foundation staff fully understand the financial expenditures across the life of the project. Together, the Financial Update section and budget template ("Financial Summary & Reporting" sheet) should provide a complete quantitative and qualitative explanation of variances to approved budget.

Note: If you are using an older version of the budget template, this information could be in a different location in your template.

1. Summary

Briefly describe how total project spending to date compares against the budget and how your assumptions may have changed as the project progressed.

The project recorded a consolidated cumulative expenditure for years 1–3 of $1,645,588, resulting in an overall burn rate of 69% of the approved NCE/reallocated project budget of $2,385,906. Therefore, the total carryover reallocated to the Period 4 NCE (October 2019–September 2020) is $740,318.

CIP reported a total cumulative expenditure of $574,024 out of $819,127 budget, resulting in an overall burn rate of 70%, leaving a budget balance of $245,103 carried forward and reallocated in the year 4 NCE period to finance the delayed activities from year 3.

IITA cumulative expenditure reported (equal to actual disbursements) stood at $439,513 against a total budget of $638,702, resulting in an overall burn rate of 69% in years 1–3. The carry-over funds have been reallocated to the year 4 NCE period (October 2019–September 2020) for the implementation of the pending activities that the partner was unable to accomplish in year 3.

The UofH, with a budget of $300,539, reported a total expenditure (based on actual disbursements) of $101,076 for years 1–3, with an overall low burn rate of 34% carrying over an amount of $199,463 to the year 4 NCE.

The UW-M had a revised total budget of $316,333 based on the reported expenses after it withdrew from the project. This necessitated the adjustments and reallocation of the unspent funds from the screening project to the other relevant and needed research areas of the project in the NCE period.

A summarized table reflecting the above comments is shown below.

Consolidated Financial Summary - Year 1,2,3				
Budget Category	**Approved Budget**	**Accumulated Expenditure (US$)**	**Balance (US$)**	**Burn Rate (%)**
Personnel	445,633	405,302	40,331	91
Travel	27,265	14,425	12,840	53
Consultant	33,000	18,500	14,500	56
Equipment	-	-	-	-
Other Direct Costs	313,229	135,797	177,432	43
Subtotal	819,127	574,024	245,103	70
Sub Awards				-
IITA	638,702	439,513	199,189	69
University of Helsinki	300,539	101,076	199,463	34
University of Winconsin	316,333	316,333	-	100
Subtotal	1,255,574	856,922	398,999	68
Indirect costs	311,205	214,642	96,563	69
Grand total	2,385,906	1,645,588	740,318	69

2. Latest Period Variance

Provide explanation for any cost category variances outside the allowable range. Explain causes, consequences for the project, and mitigation plans if relevant. Report whether approval for the variance has been obtained from your Program Officer.

Note: "Latest period variance" compares actuals to previous projections for the period. See "Financial Summary & Reporting" sheet in the foundation budget template for calculated variance. If you are using an older version of the budget template, this information could be in a different location in your template. Allowable variance is defined in your grant agreement.

Period 3 financials stood at a total expenditure of $478,115 against a total approved budget reallocation of $684,254, resulting in a carry-over of $206,139 to the period 4 NCE.

The overall burn rate for the period is 70%, with 96% spent under CIP's budget. A negative variance of 12% is 2% higher than the agreed limit and has been noted under the consultant budget category. An approval budget reallocation request has been submitted by CIP for this adjustment.

While IITA reported an expenditure level of $272,967 (based on actual disbursement for the period), no disbursement was made to the University of Helsinki during this period; thus, no expenditure was recorded on their behalf. They have been using carry-over funds from the previous period

IITA had a higher burn rate of 137%, resulting in a negative variance of 37%. This is beyond the allowable limit because they had to finance several activities, hence more disbursement was made as compared with the approved reallocated budget for the period.

Period 3 financial summary is as below.

Current Financial Summary - Period 3				
Budget Category	**Approved Budget**	**Period 3 Expenditure (US$)**	**Balance (US$)**	**Burn Rate (%)**
Personnel	112,831	115,147	(2,316)	102
Travel	11,433	10,933	500	96
Consultant	16,484	18,500	(2,016)	112
Equipment	-	-	-	-
Other Direct Costs	38,150	26,693	11,457	70
Subtotal	178,897	171,273	7,624	96
Sub Awards				-
IITA	199,189	272,967	(73,778)	137
University of Helsinki	199,462	-	199,462	-
University of Winconsin	-	(46,209)	46,209	-
Subtotal	398,999	226,758	171,893	57
Indirect costs	106,706	80,084	26,622	75
Grand total	684,254	478,115	206,139	70

3. Total Grant Variance

Provide explanation for any cost category variances outside the allowable range. Explain causes, consequences for the project, and mitigation plans if relevant. Report whether approval for the variance has been obtained from your Program Officer.

Note: "Total grant variance" compares actuals plus current projections to the budget. See "Financial Summary & Reporting" sheet in the foundation budget template for calculated variance. If you are using an older version of the budget template, this information could be in a different location in your template. Allowable variance is defined in your grant agreement.

Consolidated Grant Summary - Year 1,2,3				
Budget Category	**Approved Budget (US$)**	**Accumulated Expenditure (US$)**	**Budget Variance(US$)**	**Variance (%)**
Personnel	445,633	405,302	40,331	9
Travel	27,265	14,425	12,840	47
Consultant	33,000	18,500	14,500	44
Equipment	-	-	-	-
Other Direct Costs	313,229	135,797	177,432	57
Subtotal	**819,127**	**574,024**	**245,103**	**30**
Sub Awards				-
IITA	638,702	439,513	199,189	31
University of Helsinki	300,539	101,076	199,463	66
University of Winconsin	316,333	316,333	-	-
Subtotal	**1,255,574**	**856,922**	**398,999**	**32**
Indirect costs	311,205	214,642	96,563	31
Grand total	**2,385,906**	**1,645,588**	**740,318**	**31**

As outlined above, most categories had variances outside the allowable range, with the overall burn rate standing at 31%. This was partly due to the minimal project start-up process than anticipated in the initial stages of the project as well as partners' implementation challenges. UofH had a low overall spending level due to unforeseen technical requirement/ complication of the project which needed additional time to produce reliable data and conclusions, but IITA had delays in obtaining needed supplies and in *in vitro* tissue growth, which delayed progress in experiments needed to complete the deliverables. CIP also had delays in tissue growth that affected confidence in meeting the deliverables. As discussed above, UW-M withdrew from the project, necessitating major adjustments in finalizing QuickChip-related activities as well as the continued technical oversight of the program.

Proposed Forecasting

We hereby note at this stage that the Period 4 NCE has already been approved by the program officer. But as detailed also in the financial report, a CIP submitted a further request for a budget reallocation regarding the need to finalize the reallocation of UW-M's work and unspent funds after their withdrawal from the project in order to accomplish one of the deliverables assigned to them in the project 1. Further development of the QuickChip technology through field-testing with viruses of sweetpotato in the field and 2. the development of a high throughput screening method to screen novel antiviral compounds in sweetpotato cassava and yam, which as noted will not be accomplished.

A referenced summarized proposed period 4 budget forecast reallocation for this adjustment is indicated in the table below.

The proposed Period 4 Budget Projection - NCE Period					
Category	**Year 1 Executed ($)**	**Year 2 Executed ($)**	**Year 3 Executed ($)**	**Proposed Year 4 Projection - NCE($)**	**Total ($)**
Personnel	96,511	193,644	115,147	110,379	515,681
Travel	-	3,492	10,933	5,000	19,425
Consultant	-	-	18,500	18,500	37,000
Equipment	-	-	-	20,000	20,000
Other Direct Costs	60,791	48,313	26,693	35,000	170,797
Subtotals	**157,302**	**245,449**	**171,273**	**188,879**	**762,903**
Sub Awards					
IITA	166,546	-	272,967	233,914	**673,427**
University of Helsinki	101,076	-	-	220,962	**322,038**
University of Winconsin	194,762	167,780	(46,209)	-	**316,333**
Subtotal	**462,384**	**167,780**	**226,758**	**454,876**	**1,311,798**
Indirect Cost	92,953	41,605	80,084	96,563	311,205
Total	**712,639**	**454,834**	**478,115**	**740,318**	**2,385,906**

4. Sub-awards (if applicable)

Use the chart to provide the name(s) of the sub-grantee(s) or subcontractor(s), actual disbursement for this reporting period, total disbursement to date from the primary grantee to sub-awardee, total spend to date by the sub-awardee and total contracted amount.

Note: The total of actual disbursements for this reporting period should equal the actual Sub-awards expenses reported on the "Financial Summary & Reporting" sheet in the foundation template for this reporting period. If you are using an older version of the budget template, this information could be in a different location in your template.

Organization Name	Actual Disbursement for this Reporting Period ($)	Total Disbursed from Primary Awardee to Sub to Date ($)	Total Sub-Awardee Spent to Date ($)	Total Contracted Amount ($)
IITA	272,967	439,513	439,513	638,702
UofH	0	101,076	101,076	300,538
UW-M	(46,208.76)	316,333	316,333	316,333

5. Other Sources of Support (if applicable)

List and describe any sources of *in-kind* project support or resources received in the reporting period.

Note: Names of the other sources of funding and their contributions (U.S.$) should be included in the budget template on the "Financial Summary & Reporting" sheet in the foundation budget template in the Funding Plan table. If you are using an older version of the budget template, this information could be in a different location in your template.

N/A

Describe how interest earned and/or currency gains were used to support the project.

The interest is being accrued until the end of the project. With donor's authorization, these additional funds will be used for the benefit of the project.

Annex 1. Diagnostics testing using sRSA and VirusDetect. Tissues of test samples were extracted using EZNA sRNA isolation kit and used for sRSA library preparation as per the standard protocol. Libraries were sequenced at FASTERIS (Switzerland) and sequences were analyzed using VirusDetect, after subtracting with host genome libraries.

									VirusDetect, after host subtraction		
S/N	**Crop**	**Sample ID**	**PCR Indexing status**	**Qubit values (ng/µl)**	**Cleaned Reads**	**Removed Host Reads**	**Contigs Without host sRNA subtraction**	**Contigs after host sRNA subtraction**	**Virus/Viroid detected**	**Possible Novel Viruses**	**Genus (Novel Viruses)**
1	Yam	TDa-1131	YMMV	3.99	5333508	2851571	556	805	Yam Badna, YMV, YMMV, Dasheen Mosaic virus,	Little cherry virus 2	Ampelovirus
2	Yam	TDa-1131	YMMV, Badna	5.22	3429107	2470337	397	561	Yam Badna, YMMV? YMV? Soybean blistering mosaic virus	Dasheen Mosaic virus	Potyvirus
3	Yam	TDa-1171	Carlavirus, Badna	3.45	3337345	2274738	262	181	Yam Badna, YMV,	Dasheen Mosaic virus, YMMV	Potyvirus
4	Yam	TDa-1183	YMMV, Badna	2.78	3402479	1795452	372	269	Yam Badna, YMMV? YMV	? Dasheen Mosaic virus	Suspected
5	Yam	TDa-1272	Carlavirus, YMMV	3.82	4245132	2717333	517	518	Yam Badna, YMMV, Dasheen Mosaic virus, YMV	Ederberry carlavirus B, Red clover vein mosaic virus, Shallot latent virus	Carlavirus
6	Yam	TDa-1272	Carlavirus, YMMV, YCMV	3.02	4035886	2291241	520	349	Yam Badna, YMMV, Dasheen Mosaic virus, YMV, Garlic latent virus, Rubus canadensis virus, Asian prunus virus, Apple stem pitting virus	Chocolate Lily virus A, Panas virus Y	NA, Potyvirus
7	Yam	TDr-1504	Carlavirus, YMV, YCMV	3.99	6116864	3472997	485	680	Yam Badna, YMV, Dasheen Mosaic virus, Cowpea mild mottle virus	Apricot latent virus, Apple stem pitting virus, Grapevine rupestris stem pitting-associated virus	Foveavirus
8	Yam	TDr-1504	Carlavirus, YMV, YCMV, Badna	2.1	2400802	1587226	355	310	Yam Badna, YMV,	YMMV, Dasheen Mosaic virus	Potyvirus
9	Yam	TDr-1512	Carlavirus, YMV, YCMV, Badna	0.052	286805	184411	179	309	Yam Badna, YMV,	Elderberry Carlavirus C	Carlavirus
10	Yam	TDr-1512	Carlavirus, YMV, YCMV, Badna	1.97	2283379	1630445	344	686	Yam Badna, YMV, Carlavirus,	Dasheen Mosaic virus	Potyvirus
11	Yam	TDr-1545	Carlavirus, YMV, YCMV, Badna	0.451	250744	187298	288	167	Yam Badna, YMV	Grapevine rupestris stem pitting associated virus	Foveavirus
12	Yam	TDr-1608	Carlavirus, YMV, YCMV, Badna	0.45	1318891	676653	205	230	Yam Badna, YMV	Nerine latent virus, Elderberry carlavirus A, E Grapevine	Carlavirus, Foveavirus

									VirusDetect, after host subtraction		
S/N	**Crop**	**Sample ID**	**PCR Indexing status**	**Qubit values (ng/µl)**	**Cleaned Reads**	**Removed Host Reads**	**Contigs Without host sRNA subtraction**	**Contigs after host sRNA subtraction**	**Virus/Viroid detected**	**Possible Novel Viruses**	**Genus (Novel Viruses)**
										rupestris stem pitting associated virus	
13	Yam	TDr-1608	Carlavirus, YMV, YCMV, Badna	5.17	9557186	4717759	NA	1277	Badna, YMV, Dasheen Mosaic virus? YMMV	Cowpea mild mottle virus, Nerine latent virus, Elderberry carlavirus B, Apple stem pitting virus, Rupestris stem pitting - associated virus	Carlavirus, Foveavirus
14	Yam	TDr-1642	Carlavirus, YMV, YCMV, Badna	4.31	6809860	3138700	715	584	Yam Badna, YMV, Dasheen Mosaaic virus	Elderberry carlavirus A, Apricot latent virus, Apple scar skin viroid	Carlavirus, Foveavirus, apscaviroid
15	Yam	TDr-1744	Carlavirus, YMV, Badna	3.97	11066637	5116011	NA	1968	Badna, YMV, Dasheen Mosaic virus, Garlic latent virus? YMMV	Peach chlorotic mottle virus, Elderberry carlavirus A, C Nerine latent virus, Lettuce chlorosis virus	Foveavirus, Carlavirus, Crinivirus
16	Yam	TDr-1811	Carlavirus, YMV, YCMV, Badna	3.4	4543337	2547416	661	1460	Yam Badna, YMV	Dasheen Mosaic virus, Yam Latent virus, Nerine laten virus, Elderberry carlavirus B, C, E, Apple stem pitting virus	Potyvirus, Carlavirus, Foveavirus
17	Yam	TDr-1841	Carlavirus, YMV, YCMV	3.01	2564379	1858999	333	246	Yam Badna, YMV	American hop latent virus, lderberry carlavirus A, Grapevine rupestris stem pitting associated virus,	Carlavirus, Foveavirus
18	Yam	TDr-1846	Carlavirus, YMV, YCMV, Badna	3.25	2233869	858099	312	195	Yam Badna, YMV	Elderberry Carlavirus A, Potato virus H, Plum pox virus	Carlavirus, Potyvirus
19	Yam	TDd-3104	Badna	5.42	2157108	1479545	305	452	Badna, YMV,	Garlic virus A, E, X, Shallor virus X, Dasheen Mosaic virus	Allexivirus, Potyvirus
20	Yam	TDd-3110	Badna	8.32	4096246	3101745	447	762	Badna, YMV, Dasheen Mosaic virus	Garlic virus A, E, X, YMMV	Allexivirus, Potyvirus
21	Yam	TDd-3110	Badna	2.35	2371550	1890452	180	262	Badna? YMV	Garlic virus E, X, Shallot virus X,	Allexivirus,

									VirusDetect, after host subtraction		
S/N	**Crop**	**Sample ID**	**PCR Indexing status**	**Qubit values (ng/µl)**	**Cleaned Reads**	**Removed Host Reads**	**Contigs Without host sRNA subtraction**	**Contigs after host sRNA subtraction**	**Virus/Viroid detected**	**Possible Novel Viruses**	**Genus (Novel Viruses)**
22	Yam	TDr 89/02665	YMV inoculated	2.69	4284732	1118731	202	198	Yam Badna, YMV	Dasheeen Mosaic virus	Potyvirus
23	Cassava	CR36-5	CsCMV suspected	1.58	1165985	1070847	52	67	None	? YMV	Suspected
24	Cassava	CR36-5	CsCMV suspected	0.869	519545	446889	58	68	None	? YMV	Suspected
25	Yam	TDr 89/02665(6)	Carlavirus, Badna	1.14	343072	215454	162	190	Badna	? YMV	Suspected
26	Yam	TDr 89/02665(6)	Carlavirus, Badna	0.563	155205	112223	19	60	Badna	None	NA
27	Cassava	Cassava treated (127)	No information	2.02	1257589	1105558	111	84	None	? YMV	Suspected
28	Cassava	Cassava treated (1055)	No information	4.12	2709488	2497918	125	134	None	? YMV	Suspected
29	Cassava	Cassava treated (1117)	No information	1.67	462941	395640	86	79	None	? YMV	Suspected
30	N. benth	TDr 89/02665	YMV inoculated	1.13	89505	NA	64		*YMV*	*No host subtraction*	
31	N. benth	TDr 89/02665	YMV inoculated	0.451	27376	NA	37		*YMV*	*No host subtraction*	
32	Yam	TDr 89/02665	YMV inoculated	1.33	1015059	352310	121	104	Badna, YMV	None	NA
33	Yam	TDr 89/02665	YMV inoculated	2.36	990618	402943	193	209	Badna, YMV,	Dasheen Mosaic virus	Potyvirus
34	Yam	TDr 89/02665	YMV inoculated	1.1	600474	182805	149	103	Badna, YMV	None	NA
35	Yam	TDr 89/02665	Healthy	2.1	622519	498425	206	251	Badna, YMV	? Dasheen Mosaic virus	Suspected
36	Yam	TDr 89/02665	Healthy	1.02	50622	28906	36	36	None	? YMV	Suspected
37	Yam	TDr 95/19177	Healthy	0.519	23695	14979	25	24	None	None	NA
38	Yam	TDr 95/19177	Healthy	1.38	230168	159327	162	135	Badna	? YMV	Suspected
39	Cassava	Cassava treated (1038)	No information	0.797	214299	180747	76	72	None	? YMV	Suspected
40	Cassava	Cassava treated (198)	No information	0.465	179426	156529	47	45	None	? YMV	Suspected
41	Cassava	Cassava treated (182)	No information	0.301	37511	31191	5	7	None	None	NA
42	Cassava	Cassava treated (1044)	No information	0.349	162421	136474	6	29	None	? YMV	Suspected

									VirusDetect, after host subtraction		
S/N	**Crop**	**Sample ID**	**PCR Indexing status**	**Qubit values (ng/µl)**	**Cleaned Reads**	**Removed Host Reads**	**Contigs Without host sRNA subtraction**	**Contigs after host sRNA subtraction**	**Virus/Viroid detected**	**Possible Novel Viruses**	**Genus (Novel Viruses)**
43	Cassava	Cassava treated (1196)	No information	0.354	95662	69898	35	35	None	? YMV	Suspected
44	Yam	TDr 95/19177(21)	Carlavirus, Badna	0.593	187588	137148	178	259	Badna	? YMV	Suspected
45	Cocoyam	Field	Mosaic	2.71	271641	NA	257		*Dasheen Mosaic virus*	*No host subtraction*	
46	Cocoyam	Field	Mosaic	2.07	2987047	NA	144		*Dasheen Mosaic virus*	*No host subtraction*	
47	Cocoyam	Field	Mosaic	0.514	238281	NA	181		*Dasheen Mosaic virus*	*No host subtraction*	
48	Banana	Prata Ana	BanMMV	0.999	548295	487680	56	78	None	? Bam? YMMV	Suspected
? Low reads											

Annex 2. Summary of second set of sRSA libraries pending for analysis

S/N	Crop	Infection status by PCR/symptoms	Sample ID	Total match reads	Clean Reads after adaptor trimming	% Clean reads
1	Musa	BSV	sRNA1.fastq	870823	157946	18.1
2	Musa	BSV	sRNA2.fastq	274744	102915	37.5
3	Musa	BSV	sRNA3.fastq	175129	44971	25.7
4	Musa	BSV	sRNA4.fastq	4304527	2677375	62.2
5	Musa	BSV	sRNA5.fastq	566108	134393	23.7
6	Musa	BSV	sRNA6.fastq	1187557	779918	65.7
7	Musa	BSV positive, BBTV?	sRNA7.fastq	4405931	1108549	25.2
8	Musa	BSV positive, BBTV?	sRNA8.fastq	319563	70669	22.1
9	Musa	BSV	sRNA9.fastq	409944	37683	9.2
10	Musa	BSV	sRNA10.fastq	1037949	470628	45.3
11	Musa	Healthy	sRNA11.fastq	390759	97056	24.8
12	Musa	BSV	sRNA12.fastq	2267547	805788	35.5
13	Cassava	Healthy	sRNA13.fastq	6055009	3489955	57.6
14	Cassava	Healthy	sRNA14.fastq	3088941	1394471	45.1
15	Cassava	Healthy	sRNA15.fastq	5733627	1982850	34.6
16	Cassava	Healthy	sRNA16.fastq	2502878	1389463	55.5
17	Cassava	Healthy	sRNA17.fastq	696595	385286	55.3
18	Cassava	Healthy	sRNA18.fastq	895435	505225	56.4
19	Cassava	Healthy	sRNA19.fastq	6151931	381911	6.2
20	Cassava	Healthy	sRNA20.fastq	9853879	988257	10.0
21	Cassava	Healthy	sRNA21.fastq	1059272	257327	24.3
22	Cassava	Healthy	sRNA22.fastq	935660	224909	24.0
23	Cassava	CsCMV suspected	sRNA23.fastq	610637	291947	47.8
24	Yam	Healthy	sRNA24.fastq	3607558	104064	2.9
25	Yam	Healthy	sRNA25.fastq	2269044	1996890	88.0
26	Yam	Healthy	sRNA26.fastq	2591723	1096350	42.3
27	Yam	Healthy	sRNA27.fastq	14569221	5015416	34.4
28	Yam	YCMV	sRNA28.fastq	2987831	713501	23.9
29	Cowpea	Unknown	sRNA29.fastq	5760057	1349109	23.4
30	YAM	Unknown	sRNA30.fastq	4199850	1763920	42.0
31	Yam	Unknown	sRNA31.fastq	445867	264142	59.2
32	Yam	Unknown	sRNA32.fastq	426647	163983	38.4
33	YAM	YMV, Carlavirus	sRNA33.fastq	14895801	9129398	61.3
34	YAM	Healthy	sRNA34.fastq	7993488	4048634	50.6
35	YAM	YMV, YCMV, Carlavirus	sRNA35.fastq	12029338	3443771	28.6
36	YAM	Healthy	sRNA36.fastq	5897352	1430214	24.3
37	YAM	No symptoms	sRNA37.fastq	325865	104152	32.0
38	YAM	No symptoms	sRNA38.fastq	3478503	427194	12.3
39	YAM	Mild symptoms	sRNA39.fastq	*Data download issue*		
40	YAM	Mild symptoms	sRNA40.fastq	12157782	5895442	48.5
41	YAM	Severe symptoms	sRNA41.fastq	2074062	1231910	59.4
42	YAM	Severe symptoms	sRNA42.fastq	10939380	6070197	55.5
43	YAM	No symptoms	sRNA43.fastq	1848073	167219	9.0
44	Maize	Severe symptoms	sRNA44.fastq	10267029	5666156	55.2
45	Maize	Severe symptoms	sRNA45.fastq	2242489	1313543	58.6
46	Maize	Severe symptoms	sRNA46.fastq	8911476	6713866	75.3
47	Maize	Mild symptoms	sRNA47.fastq	7008099	5024452	71.7
48	Maize	Mild symptoms	sRNA48.fastq	7030937	4368298	62.1

For Foundation Staff to Complete

Analysis (required if contingent payment or PO assessment differs from grantee/vendor assessment)

Progress Analysis

Include analysis of significant project variances and key learnings that may inform portfolio discussions for progress against the strategic goals.

Budget and Financial Analysis

Include analysis of unexpended funds or over expenditures. Refer to the Unexpended Grant Funds Policy for options available when recommending how to handle unexpended grant funds, or reach out to your primary contact in GCM.

Scheduled Payment Amount	$
Carryover Amount	$
Recommended Payment Amount	$

Approver Comments (if applicable)

Name	Title	Date Approved

Comments

Other issues: Despite the text, the budget seemed to be more underspent than in year 1.

For “Breakdown among additional dimensions,” there was nothing allocated for QuickChip. Why?

I should have asked this last year, but was there really no travel in year 1? Is CIP travel in year 2 accurately and completely listed?