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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, 2019
Feedback Email[1]	d.ellis@cgiar.org	**Reporting Period Start Date**	September 29th, 2017
Program Officer	Jim Lorenzen	**Reporting Period End Date**	October 31st, 2018
Program Coordinator	Emily Zuberi	**Reporting Due Date**	November 30th, 2018
Investment Total	$2,385,906.00	**Opportunity/Contract ID**	OPP1130216
Scheduled Payment Amount (If applicable)	$828,688.00		

[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 of 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	November 28th, 2018	**Submitted by Contact Name**	Maria Teresa Bellido
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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

Considerable gains were made in the second year to catch up for the slow start of the project in 2017. *In vitro* multiplication, a shortcoming in Year 1 (Y1), has made considerable progress. Interestingly, modification of macronutrients in the culture medium for both sweetpotato and cassava had minor effects, and the largest variable remains the genotype of the accession. Interestingly in sweetpotato, virus-infected *in vitro* plants in general performed better than non-virus–infected plants in most experiments. The testing of antioxidants (glutathione and ascorbic acid) had little effect on the growth of cassava meristems; experiments are still ongoing with yam. Greenhouse spraying of salicylic acid and lactoferrin on sweetpotato, yam, and cassava are underway to determine the effect on virus elimination. *In vitro* application of ribavirin, lactoferrin, and salicylic acid are also underway. *In vitro* application of antiviral compounds is expected to be ready in early 2019 to initiate testing of novel antiviral compound identified in screening experiments at the University of Wisconsin–Madison. sRSA technology continues to be developed and tested against existing phytosanitary cleaning method. Experiments to make the method more user friendly, less expensive, and more efficient are ongoing, with a major step

involving the use of columns to extract the sRNA, thus eliminating the need to cut sRNA bands from the gel. VirusDetect continues to evolve and be the program of choice for identification of the sRNA fragments. Progress in the development of VirusDetect has been slow due primarily to the challenges of hiring a proficient bioinformatics student at the University of Helsinki. This challenge has been remedied and we expect the development of Windows-based interface to be developed for testing in Y3. This has not limited project advancement, however. Excellent progress has been made with the identification of sweetpotato chlorotic stunt virus (SPCSV) RNase3 inhibitors, and assays to detect inhibition have been successfully developed: 31 compounds have been selected for *in vitro* screening. The high-throughput screening (HTS) method for novel antiviral compounds has been developed, with the screening of up to 50,000 compounds planned for Y3. The QuickChip technology is progressing well with the development of 11 recombinase polymerase amplification and loop-mediated isothermal amplification (LAMP)-based assays against yam, sweetpotato, and cassava viruses (6 in sweetpotato, 2 in yam, and 3 in cassava). Field testing is planned for 2019. Two week-long capacity-building workshops on sRSA technology were given, one at the Crops Research Institute (CRI) in Kumasi, Ghana, and the other at the World Agroforestry Centre (ICRAF) in Nairobi. Both workshops were well attended by national program staff from the regions. The University of Helsinki has requested a no-cost extension (NCE) for a fourth year of the project to meet deliverables. Despite this request, the project is progressing well and has accomplished most goals on schedule.

INDIVIDUAL PROJECT PROGRESS REPORTS

1. *In vitro*: sweetpotato (CIP)
   As mentioned in the report for Y1, a liquid *in vitro* multiplication step has been incorporated into our sweetpotato plantlet production protocol; but it is still taking 18–22 weeks to produce enough plants for each experiment.

   Modification of macronutrient levels and deleting NH_4. The experimental design for the modification of macronutrient levels and the elimination of NH_4 from the *in vitro* culture medium consisted of three repetitions of 15 meristems from each of nine accessions (CIP 421581, CIP 420865, CIP 420796, CIP 420889, CIP 443681, CIP 441466, CIP 421809, CIP 105086, and CIP 430370). Each accession consists of two inventories, with and without viruses, to determine if viruses affect the *in vitro* response. Macronutrient concentrations included full- (100%), half- (50%), and quarter- (25%) strength macronutrients. Evaluations for meristem survival, rooting, and number of expanded leaves were done every 15 days. Results include:
   - Accession-specific responses to all treatments were the most significant.
   - Virus-infected tissue surprisingly had generally better meristem survival, were more variable in rooting, but showed no clear trend in overall meristem survival.
   - Changes in culture medium only had a significant effect on percentage of rooting.
   - In summary, the genotype effect is greater than treatment effect, and *in vitro* plant growth and survival are generally better if plants are infected with viruses than if plants are not.

   Treatment of greenhouse plants with antiviral compounds. Dosage response curves were done on individual greenhouse plants from six accessions ~2.5 months after transplanting from *in vitro* to the greenhouse, by spraying plants with 15 and 70 mg/L salicylic acid 4X and 1,000 mg/L lactoferrin until dripping at weekly intervals (day 1, 7, 14, and 21). At day 28 (1 week after final spraying), meristems were reintroduced *in vitro*. Results include:
   - Plants grew comparable to the controls with no toxicity effects after spraying with both concentrations of salicylic acid and the single dose of lactoferrin. Thus, 70 mg/L of salicylic acid and 100 mg/L of lactoferrin will be used for the final experiment.
   - The experiment will consist of three independent replications of six plants each from six accessions, plus six control plants (sprayed only with water + 0.02% v/v Tween 20). Virus load will be confirmed by polymerase chain reaction (PCR) in all plants prior to spraying and then again after 3 months growth *in vitro*.
   - Plants will be isolated the second week of September 2018, with the first spray application the second week of November.

   In vitro *application of antiviral compounds*. Dosage response curves were done by incorporation of 0, 10, 20, and 30 mg/L of ribavirin and 0, 5, 10, and 16 mg/L of salicylic acid filter sterilized into the culture medium. Nine uninodal explants each from five different accessions were placed on media for 1 month containing the antiviral compounds. Results include:
   - Salicylic acid—levels above 5 mg/L were detrimental to plantlet formation.
   - Ribavirin—levels above 20 mg/L were detrimental to plantlet formation.
   - An experiment with lactoferrin at 500, 1,000, and 1,500 mg/L showed that all levels of lactoferrin tested were detrimental for *in vitro* plant growth (vitrification and inhibition of root elongation) at these concentrations.
   - Experiments with 5 mg/L of salicylic acid and 20 mg/L of ribavirin were set up in three replications in mid-August with shoot tip isolation in mid-September. Results will be analyzed in December 2018.

2. *In vitro:* cassava, yam (International Institute of Tropical Agriculture [IITA])
   Ten yam (six *D. alata* and four *D. rotundata*) and 12 cassava accessions were selected for the experiments to improve meristem regrowth using manipulation of culture parameters. Systematically, 15 meristems per accession were excised for each treatment for both crops.

Effect of photoperiod on meristem regrowth. Photoperiod conditions for better meristem regrowth were tested using three conditions: (1) continuous exposure to light as a control, (2) 7 days in the dark followed by light exposure for 12 h each day, and (3) 14 days in the dark followed by light exposure for 12 h each day. Observations after 12 weeks showed that the yam meristems subjected to 7 days in the dark had better regrowth rate, equivalent to continuous photoperiod light conditions. The continuous 12 h light exposure was favorable to cassava meristem regrowth for the majority of the tested accessions.

Effect of growth media composition on meristem regrowth. Yam meristem regrowth was tested with different strengths of MS media: (1) full-strength MS, (2) half-strength MS, (3) quarter-strength MS, and (4) eighth-strength MS. Equivalent regrowth was observed after 12 weeks in the full- and half-strength media. Genotype influence was observed on regrowth rate regardless of the growth media. However, reducing the media strength had no effect on the cassava meristem regrowth. Therefore the existing method of using full-strength MS media will be continued. Experiments to test the influence of antioxidants (glutathione and ascorbic acid) and different auxin/cytokinin ratios on yam meristems are ongoing. In the case of cassava, the addition of two antioxidants at different doses (glutathione at 0.049 mg/L and 0.074 mg/L and ascorbic acid at 25 mg/L and 40 mg/L) did not enhance the meristem regrowth rate compared with the control. Routinely, only apical meristems are sampled for cassava *in vitro* establishment. An experiment has been initiated to assess the growth rate of meristem explants from apical and axillary shoots of cassava.

3. sRSA: sweetpotato (CIP)

Experiments were set up to increase the efficiency, cost, and reproducibility of RNA extraction in sweetpotato, with a focus on avoiding cutting RNA out of gels and the replacement of Superscript with cheaper reverse transcriptase. For selection of plant material, 16 accessions were selected based on past virus history with various viruses. In four of the accessions, the previously detected viruses were undetectable; this is both puzzling and unexpected, suggesting that it could be (1) a viral strain issue, (2) identity issues, (3) elimination of the virus in long-term *in vitro culture*, or (5) others. These plants will be indexed again to confirm virus status. Nevertheless, 12 accessions were confirmed virus positive and are being used for the study to determine the sensitivity of sRSA. Also, an experiment was performed comparing sRSA detection from *in vitro* and greenhouse plants from those accessions. An unexpected (never observed before) shift to smaller sizes of small RNA size was observed in the results, mostly from greenhouse plants. Whereas the viruses were still detected by VirusDetect in these samples, we suspect it might affect sensitivity (due to smaller size sequences).

For protocol robustness and improving efficiency and costs, in summary, all extraction methods worked although:

- CTAB appears to reduce coverage, but this could be compensated for by increased sequencing depth.
- Virus genome detection and coverage were good with all methods; column extraction performed the best.
- In comparing large RNAs with small RNAs, although all viruses were detected readily by sRNA at all sequencing depths, feathery mottle and begomovirus were not detected with large RNAs in all single infections of several cultivars. Additionally, SPCSV was detected better with large RNAs in one out of four cultivars tested. In conclusion, sRNAs seem to be overall more reliable for detection of sweetpotato viruses.
- For a quality control step, the following is being tested:
    - Include one sample per library with different known viruses to serve as positive and negative controls (pepper or bean virus)
    - Spike of siRNA with eight different nucleotides (21–24BP) with known concentrations and sizes to understand the quality of the process on sRNAs from extraction to library prep to sequencing.
- Validation of sRSA
    - A total of 170 accessions never tested before and going through routine ISO-certified phytocleaning procedure were multiplied *in vitro* and plants were subjected to sRSA prior to any viral elimination therapy (Sept./2018).
    - Other *in vitro* plants of the same accession proceeded through thermotherapy and meristem isolation followed by re-multiplication *in vitro*.
    - Plants from these treatments will then be analyzed by sRSA again (Jan./2019) and compared with results with from standard ISO-certified indexing.

4. sRSA, chemotherapy: yam and cassava (IITA)

1.2. Develop protocols for use of existing antiviral compounds to produce phytosanitary clean plants of yam and cassava

In vitro *chemotherapy*. Three chemical agents, ribavarin (RV), lactoferrin (LF), and salicylic acid (SA), were identified for antiviral treatment for *in vitro* use. RV is a nucleoside analog known to have an inhibitory effect on RNA viruses, whereas LF and SA are known to enhance host defense signaling, leading to mechanisms that interfere with virus replication and movement. *In vitro* cultures of virus-infected yam (yam mosaic virus [YMV]) and cassava (African cassava mosaic virus [ACMV]) and virus-free stocks of the same are being established. The chemical agents will be added directly into the media at the following concentrations along with the control (no treatment).

- RV—10 mg/L and 20 mg/L
- LF—250 mg/L, 500 mg/L, and 1,000 mg/L
- SA—100 µm and 500 µm

Nodal explants will be used as the tissue source in two replications of five plantlets per treatment. The implementation of this experiment was delayed due to the slow rate of bulking of the planting material. We expect to set up this experiment in November–December 2018.

Topical application of antiviral agents. LF and SA were applied on yam (TDr3381, TDr3468, TDr4376, and TDr4646) and cassava (IBA011368) plants to determine their antiviral effect on virus-infected plants*. In vitro* plants were collected from IITA's Genetic Resources Center based on their health status on 25 January 2018, multiplied using the semi-autotrophic hydroponic (SAH) method and later transplanted into pots in a screenhouse on 27 April 2018. All the plants were indexed for health status; virus presence was confirmed (YMV in yam and ACMV and East African cassava mosaic virus [EACMV] in cassava). LF and SA were applied on yam during the third week of August 2018. SA at 200 µm and 500 µm concentrations was sprayed on cassava leaves, and LA at 1.8 g/L and SA at 500 µm were sprayed on yam leaves. Control plants were sprayed with distilled water. Four plants were used for each treatment. Plants are being observed regularly for symptom remission and shoot growth. Plants sprayed with SA showed no difference from the control, whereas mild symptom remission was observed on yam plants sprayed with LA. This experiment is ongoing.

Regeneration of virus-free plants by a rescue procedure. Experiments were designed to excise nodal explants from different portions of the yam plants based on the presence or absence of virus symptoms (from symptomatic shoots, asymptomatic but virus positive shoots, and asymptomatic but virus negative shoots) to assess the possibility of generating virus-free plants from the apparently virus-negative tissue sources. This is based on the hypothesis of discontinuous distribution of virus within the shoots. The nodal explants were taken from 15 *D. alata* accessions and 35 *D. rotundata* accessions and established *in vitro*. The *in vitro* plants are due for virus indexing in December 2018.

1.3. Compare sRSA and conventional indexing methods

A total of 140 sRSA libraries were constructed using the method previously standardized at IITA. Cassava comprised 39 samples sourced from tissue culture (N = 23), SAH (N = 5), and screenhouse (N = 11); and 101 samples of yam comprising tissue culture (N = 66), SAH (7), and screenhouse (N = 28) (Annex 1.3.1). These include previously indexed samples tested as virus-infected or virus-negative, and untested samples. The small RNA from source tissues was extracted using EZNA kits and by the conventional method using Trizol reagent. Seventy samples were extracted using Trizol, 72 with the EZNA kit, and 15 samples with both methods. Extracted RNA was size-selected by gel elution of 21–24 nt dsRNAs; the same extractions were used for small RNA library preparation. Libraries of 48 tagged with unique identification tags were pooled for sRSA sequencing at FASTERIS (Switzerland). Three pooled sets were sent for sequencing. Sequencing resulted in reads of as high as 35.5 million to as low as about 500, leaving an average read at about 8 million per library. However, after adopter trimming, the read numbers reduced dramatically: the highest was 250,000 reads and lowest was 14 (Annex 1.3.2). Average reads after trimming reduced to 48,671—about 1% of the original library size. Trouble shooting indicated a high number of empty libraries, pointing to the loss of dsRNA during gel elution of sRNA. This issue was fixed by improvising the protocol to avoid the need for the gel elution step after sRNA isolation, and the staff were retrained in library preparation and sequencing. The next set of libraries is expected to yield better results. This was the first occasion of constructing libraries in-house and provided a good learning experience for the team.

Sequence analysis of libraries using the VirusDetect software indicated the presence of ACMV, EACMV, and Ivorian bacilliform cassava virus in cassava samples and YMV and badnaviruses in the yam samples. A few yam samples contained sequences of "betaflexiviruses." Owing to low reads the data were not analyzed further. Overall, there was good correlation between the sRSA and conventional diagnostics results (Annex 1.3.1).

5. VirusDetect (University of Helsinki)

There has been little progress in developing an improved VirusDetect program during Y2, although the use of VirusDetect has increased widely in different institutes and countries. The aspects to be improved in VirusDetect were described in the report from Y1: "*The VirusDetect platform has been integrated with the Chipster platform. The objective is to make VirusDetect more user-friendly, capable of running on laptops and with greater capacity and functionality. To achieve this, VirusDetect has been integrated into the Chipster enabling platform. This move has enabled the incorporation of many more user-friendly elements (simplified algorithms, less guesswork in calling sequence matches), but it is a relatively heavy program with many elements not needed for virus detection. Hence, the program can be complicated to download, particularly in areas where bandwidth is low (such as most of Africa). That said, it has been a huge advance for the capabilities of VirusDetect, but moving forward in Y2–Y3, the combining of these two pre-made platforms with much wider scope than needed for this project, needs to be streamlined, keeping the best components but eliminating unneeded elements. While the enhanced functionality is delivering the desired results, the Chipster platform contains many components that are not needed for VirusDetect and hence some modification of the platform in Y2 is warranted. Certain functions need to be developed and added to enable characterization of whole genomes of partially characterized or new viruses. Suggested changes moving forward include establishing parameters for minimum contig and kmer lengths to balance sensitivity and reliability and improve functions for reliability/stringency and setting parameters for overlap of reads for contig formation. The goal remains*

that by the end of 2018, VirusDetect will be a simplified, easier-to-use program without the need for huge computing or internet power to run it. Taken together, Chipster has been a major improvement and the goals of Y1 (2016–2017) have been achieved."

The rationale for the lack of advanced as was expected in Y2 in the development of VirusDetect has been due to difficulties in recruiting an expert to carry out the necessary final programming and testing steps. The position for an expert has been announced several times, but the very high demand of experts in this field and the short time nature of the project (4 months) were likely causes for the failure in recruitment. However, we remain confident that the matter is resolved as we have combined this position with other work to develop it into part of a doctoral project and have offered the position to a talented young scientist. She has expressed interest in finalizing VirusDetect as the first part of her PhD studies at the University of Helsinki, which will pay for the costs of the first 4 months directed to completion of the adjustments in VirusDetect. According to discussions in the annual meeting of the NextGen Phytosanitation project (8–9 Sept. 2018) in the Netherlands, CIP will continue covering the costs after 4 months with funding from other projects.

6. High-throughput screening of RNase3 inhibitors (University of Helsinki)

Background in brief (Y1): (1) An experimental system was set up for screening compounds for inhibition of viral RNase3 activity. (2) The three-dimensional structure of the catalytic site of RNase3 from SPCSV was modeled and 130,000 chemical compounds were tested *in silico* for their ability to dock to the catalytic site of RNase3. The number of chemicals to be tested *in vitro* was limited to 6,620 compounds predicted to dock to the catalytic site of RNase3. (3) Those 6,620 compounds were screened in the Finnish Institute for Molecular Medicine (FIMM) by the fluorescence resonance energy transfer (FRET) method, which resulted in 106 compounds having inhibitory activity on the catalytic activity of RNase3. Of these, 24 compounds had high activity and 12 showed 100% inhibition.

Progress in Y2: Expression and purification of the SPCSV RNase3 enzyme in larger amounts from *E. coli* was optimized. The 130,000 drug molecules from the High Throughput Biomedicine Unit of FIMM were screened *in silico* using a method targeting the catalytic site of RNase3 by Glid-docking using Schroedinger's Maetro software. RNase3 structural information was obtained by homologous modeling and was modified using Discovery Studio software according to the structural information from Protein Data Bank and SWISS-MODEL. The top 6,600 docking poses were selected to wet lab HTS with PHERAstarFS at FIMM. We designed a method based on FRET using catalysis characteristics of RNase3. Results showed that 112 compounds interfere significantly with RNase3 activity. To avoid false positive results (compounds binding to the substrate instead of the RNase3 enzyme), further affinity analysis between RNase3 and 99 of the 112 compounds were tested by MicroScale Thermophoresis (MST) (Monolith NT at the University of Helsinki, and SLEVITA SA in Poland). On the basis of FRET and MST screening results, 42 compounds were selected for a parallel affinity binding assay with surface plasmon resonance (BiacoreT100, UH). Using the results from the catalysis screening and affinity assay, 31 compounds were selected for an *in vitro* plant confirmation assay with quantitative reverse transcription PCR (qRT-PCR) to determine the effect of the compounds on virus accumulation (both SPFMV and SPCSV tested).

Sweetpotato cultures were established *in vitro*, including plants which were single-infected (SPFMV) and co-infected (SPCSV + SPFMV). The latter virus combination causes the severe sweet potato virus disease (SPVD). qRT-PCR was used to test virus distribution and concentration in the plants using primers targeting the coat protein regions of SPFMV or SPCSV. Preliminary experiments on *in vitro* plants with a number of the most efficient RNase3 inhibitors selected in the previous experiments are ongoing, yet early experiments show that plants tested with different inhibitors varied in virus concentration. These experiments also allowed the narrowing to 11 of the most promising inhibitor candidates.

During the initial *in vitro* screening, a few promising inhibitors of viruses were added to the plant culture medium. This resulted in a reduction of virus accumulation in some cases with no effect on root growth, suggesting that roots can absorb inhibitors with minimal to no damage.

The National Plant Phenotyping Facility (NaPPI) (https://www.helsinki.fi/en/infrastructures/national-plant-phenotyping/nappi-facilities) at our campus (Viikki) is available for documenting physiological and developmental effects on the plants. Preliminary phenotype testing on SPVD-affected sweetpotato plants using the NaPPI platform showed that the method is sensitive enough to recognize the difference between healthy, single virus-infected and double virus-infected plants, both at morphological and physiological levels. Hence, reduction of symptoms of SPVD following inhibitor treatment of plants grown in soil may be monitored by NaPPI in future experiments.

7. High-throughput screening of novel antiviral compounds (UW–Madison)

The project was delayed until 1 April 2017, due to the negotiation of an intellectual property agreement between CIP and UW–Madison (as mentioned above). Experiments using the initial project design with tobacco mosaic virus RNA and protoplasts were unsuccessful. After multiple iterations with different viruses and experimental approaches, we zeroed in on a turnip mosaic virus (TuMV)-based assay system that required the following steps: (1) build and/or acquire GFP-labeled TuMV strains; (2) set up an agro-infiltration protocol to infect Nicotiana plants; (3) collect fluorescently labeled viral particles; and (4) work out a protocol for infecting plant cell lines. We have successfully demonstrated the proof of principle using this approach and are currently creating and collecting more fluorescently labeled viral particles and performing pilot small-molecule screening assays on 1,000 small molecules (including known antivirals

as positive hits, e.g., RV) to determine a Z-score for the effect of compounds on virus replication in host cells. In Y1 we selected pilot libraries of small molecules at UW–Madison to initially screen; we have these libraries queued up and ready for screening experiments. In Y3 we will screen up to 50,000 small molecules from these libraries to identify potent antivirals, counter-screen and validate positive hits, and characterize their activity in field-based phytosanitation assays.

8. QuickChip (UW–Madison)

   The QuickChip phase of the project was also delayed in starting even though the QuickChip patent (USPTO 9,050,593) had been disclosed previously to the Foundation. A target list of 11 recombinase polymerase amplification and LAMP-based assays against yam, sweetpotato, and cassava (6 in sweetpotato, 2 in yam, and 3 in cassava) were identified and primers and probes were developed for all 11 assays. We obtained APHIS permit approval to import infected plant materials and isolated viral nucleic acid and to move them across state lines to our lab in Madison for testing. Through collaborators and academic labs, we obtained isolated viral nucleic acids for all 11 strains and collections of infected and healthy plant tissues (cuttings and leaves). We established plants in the greenhouse for testing and performed PCR tube-based assays for all viruses. We performed recombinase polymerization assays or LAMP assays for 7 of the 11 assays in tubes and began to move the assays on to QuickChips. We fabricated ~200 chips and worked on configuring/ training the software on our readers to quantify assay results. Assays for the 11 viruses are now being optimized and characterized (e.g., performing receiver operating characteristic curves) to enable the movement of the QuickChip into field-testing as well as the fabrication of another 400 chips for assays and capacity building.

Y1 (2017) CAPACITY-BUILDING ACTIVITIES

1. We participated in sRSA and VirusDetect workshops in CIP–Lima, where two IITA staff (Mrs. Patricia Ogunsanya and Mr. Masood Lateef) and one University of Ibadan MSc student, Mr. Ikechukwu Nnaji, attended.
2. The project presented a poster on NextGen phytosanitation project outputs at the 4th Global Cassava Partnerships in the 21st Contrary (GCP21) International Conference in Cotonou, Benin, 11–15 June 2018 (Annex 1.6.1).
3. Two training courses on sRSA and VirusDetect use for clonal crop virus indexing were co-sponsored by the NextGen Phytosanitary project. The courses offered hands-on training in sRSA library preparation and data analysis using VirusDetect software. Participants were allowed to use their own samples for processing and model data sets were given for virus detection using VirusDetect:
   - CRI in Kumasi, Ghana, 18–22 June 2018. Attending the workshop included 13 participants from the following organizations, CRI (9), the University of Cape Coast (2), CIP–Ghana (1), and ICRAF–Ghana (1). A subsequent grant from the Genebank Platform has provided follow-up supplies for CRI to implement lessons learned.
   - ICRAF in Nairobi, Kenya, 25–29 June 2018. This workshop was co-sponsored by the CGIAR Genebank Platform Germplasm Health Unit and supported the participation of scientists from national programs as well as CGIAR centers in Africa. Participants included two from Kenya Plant Health Inspectorate Service and one from the Kenya Agriculture and Livestock Research Organization.

Y2 PRINCIPAL INVESITGATORS (PIs) INTERACTIONS

1. We held an annual team meeting for project PIs at the BluRadisson Hotel, Schiphol Airport, on 8–9 September 2018.
2. PIs meeting during sRSA training courses and other opportunities when two or more PIs were at the same venue.
3. We hold regular constant Skype contacts between PIs.

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.

1. Development of VirusDetect has not progressed as expected during Y2; however, as mentioned above, we hope to meet all project deliverables in Y3.
2. **The University of Helsinki had a lower spending rate than originally expected and have requested an NCE of their remaining $143,699 from Y2 to Y3. An NCE of budgeted funds for Y3 into Y4 are due to the gained knowledge that the next steps of the research will be much more expensive than previous years**.

   In summary:
   a. The total research costs in Y1 ($10,891) and Y2 ($53,908) were $64,799. The budget for Y1 ($101,076) and Y2 ($107,422) together was $208,498. Hence, there should be $143,699 left from the budgets of project Y1 and Y2 (audited expenses for the University of Helsinki are pending confirmation by CIP).
   b. The reason for the lower consumption of funds were the following:
      i. FIMM, Helsinki, has not charged for their services (i.e., no costs for time of their technicians or use of the equipment); only the costs of consumables have been charged from our project.
      ii. The doctoral candidate Linping Wang obtained a stipend from the China Scholarship Council (CSC) until August 2018. Hence, this project has started to pay her salary only recently and she will be instrumental for

success in Y3–Y4 of the project.

iii. The Information Technology Center for Science in Finland (CSC) was interested in developing VirusDetect to a certain point. Therefore, it did not charge for their time in adding applications and functions to VirusDetect based on our requests.

iv. A postdoctoral scientist was requited to the project in January 2018 rather than September 2017. Hence, salary costs have been lower than originally planned.

c. **University of Helsinki is thus requesting an extension for their deliverables for the project through September 2020.**

i. In the first 2 years of this project, progress toward the deliverables was made as expected; however, the University of Helsinki now also realizes the lengthy time and expense required to produce reliable data and conclusions for the portions of the project they are accountable for.

ii. This includes more repetitions of experiments and the unforeseen need to test the same research question with two different experimental approaches. Hence, the studies have advanced slower but the quality of the results is better.

d. *No additional funding is needed*:

i. The University of Helsinki will use the **$143,699** (left from the budgets of project Y1 and Y2 yet the amount still needs to be audited by CIP) **in Y3**. The funding originally allocated to Y3 (**$102,040**) will be used in **Y4**.

ii. In this way, the funding left for the university will be used efficiently, with a maximal amount of relevant results for long-term efficacy of the project can be obtained for the project goal—improved control of SPVD.

e. **REVISED RESEARCH PLAN FOR Y3 AND Y4 FOR UNIVERSITY OF HELSINKI:**

i. **Y3:**

1. Under *in vitro* conditions, dose response experiments will be carried out on the 11 best performing candidate inhibitors from Y2 to identify the lowest effective dose of the inhibitors. Chemical inhibition of SPVD will be monitored in the greenhouse using hyperspectral imaging facilities from NaPPI) on campus (Viikki) for disease characterization and detection.
2. Efficiency of inhibitor uptake approaches will be compared between root absorption, foliar spraying, and vacuum infiltration. Validation of inhibitor uptake will be tested by liquid-chromatography mass spectrometry experiments and protein-ligand interactions by confocal microscopy. Validation at the molecular level will be achieved by molecular dynamics simulation to demonstrate the mechanism of interaction between candidate inhibitors and RNase3 using Schroedinger Maestro software in the supercomputer of CSC).

ii. **Y4:**

1. The two to four best inhibitor candidates will be resynthesized and subjected to catalysis screening and affinity assay. Co-crystallization of protein-ligand complexes will be attempted to determine the mechanism of inhibition. These experiments will be performed in the Crystallization Core Facility in the BioCenter of the University of Helsinki.
2. Effects of selected candidate inhibitor compounds on viral small RNA (sRNA) patterns in sweetpotato plants will be evaluated by sRSA. Influence of RNase3 inhibitors on changes in amounts of different classes of sRNAs, or total amounts of sRNAs, will provide evidence that RNase3 and the inhibitor candidates play roles in RNAi pathways.
3. Selected RNase3 inhibitors will be tested for toxicity to animal/human cells and for phytotoxicity in the Pharmacology department at the University of Helsinki.
4. Small-scale field experiments are planned in Uganda and Tanzania (possibly Kenya) with long-term collaborators in studies on sweetpotato viruses and their control.
5. If applicable, enquiries with the agrochemical industry and the Bill & Melinda Gates Foundation will be made regarding interest in project development and commercialization.

3. As mentioned above, the QuickChip phase of the project was delayed in starting due to the need to negotiate an intellectual property agreement between CIP and UW–Madison for the QuickChip technology (Patent 9,050,593). The realization of this patent delayed the signing of the final project papers to allow UW–Madison to initiate work on the QuickChip phase of the project. Although significant progress has been made in Y1 on the QuickChip, another 2.5 years are needed to fully meet the deliverables of the QuickChip. *Therefore, we are requesting an NCE and carry-over of the funds budgeted for the QuickChip to allow field testing of the QuickChip against sweetpotato, yam, and cassava viruses as well as capacity building and training of QuickChip on the African continent.* The NCE is being requested for the QuickChip as that phase of the project was only slated for Y1. We are fully confident that all deliverables for the QuickChip can be met by March 2019.

4. The delay in signing the final project papers also delayed the start of the antiviral screening project. To offset that delay, we have reorganized the team at UW–Madison working on this project to accelerate it in **Y2. Consequently, we are requesting that some of the budget for screening in Y2 be moved forward into Y3. We do not anticipate any delays in delivering the Y3 deliverables for this project.**

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 (U.S.$) – 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 SSA	$200,000
Global	$836,000

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 (U.S.$) (Year 2)
CIP, Peru—Lead organization	321,705
IITA, Nigeria	231,331
University of Helsinki, Finland	107,123
University of Wisconsin–Madison, Madison, WI	209,725

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.

ENABLING

1. Funding to enable technology to be developed, transferred, and operationalized
2. Technical support—project partnership
3. Resources for capacity building

IMPROVE

1. Provide more start-up time for project initiation
2. Guidance in management of the project

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 are in compliance with 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.

CIP
Y2 of this project provided significant opportunities to make up for the slower start in 2017. Funding has been adequate to meet the project deliverables, and progress against deliverables have been met. Interesting developments that have made us rethink the phytosanitation process for sweetpotato include experimental evidence that virus-infected plants may have an advantage *in vitro* over non-virus–infected plants and that the *in vitro* environment may help eliminate viruses. Both observations need to be tested further. Additionally, the strength of the genotype- (accession) specific response was surprising (although not unexpected).

IITA
As with CIP, Y2 of this project provided opportunities to catch up for the slow start in 2017. Funding enabled the project to meet deliverables. *In vitro* constraints have largely been overcome and the experiments are proceeding on schedule. It is interesting that the genotype- (accession) specific response also was a large variable in cassava and yam as it is with sweetpotato. Perhaps in general, we mistakenly assume the genotypic response of germplasm to be more consistent across the diversity seen in genebank collections.

UW–Madison
The 6-month delay in starting the project greatly postponed all experimentation, manufacturing of parts, and initiation of hiring the needed help. Continual discussions with the project lead ensured smooth project coordination during this time, but this delay greatly upset plans for Y1. Despite this, we foresee no major changes in deliverables other than everything being compressed into a 2.5-year timeframe.

- **QuickChip.** Owing to the later-than-expected start date, this effort was pushed back about 6 months into Y2 deliverables. In Y1 plant and nucleic acid samples for all three plants and 12 viruses were obtained. All plant diagnostic assays were developed and established in the lab. We are currently working on converting these to assays on the QuickChip platform. We expect to conduct the field-testing in Africa and complete the project in Y3.
- **Antivirals.** The discovery of new antivirals was also delayed. In Y2 the new experimental design with TuMV was established, the acquisition of key materials (Agrobacterium and viral constructs) was completed, and expertise with techniques of propagating and collecting viral particles was developed. In Y3 we aimed to close the gap to accomplish both the unmet Y2 deliverable as well as the proposed Y3 deliverables. To accomplish this, we are currently optimizing the high throughput screening assay to improve the S/N. We expect to complete the screening and provide

the top 10 antivirals in Y3.

Helsinki

Two major unexpected surprises were seen with the projects at Helsinki: the unexpected in-kind contributions from FIMM and CSC and the funding from China for Linping Wang, and the need for more in-depth analysis of the RNase3 inhibitors to confirm their mode of action. Additionally, we underestimated the effort it would take to find a bio-informatician who was willing to develop VirusDetect into a more user-friendly program. In the end, improvement of VirusDetect as a standalone project was not sufficiently attractive, and other projects needed to be developed to entice someone to work on this. Work with the RNase inhibitors, while hugely successful, has faced significant technical challenges. And though all of these have been dealt with, these challenges have highlighted a need to change experimental designs for this phase of the overall project.

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 or not 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.

Variances are negative because of a Y2 catch-up in research due to the slower-than-expected ramp-up of the project. Despite this, all partners except the University of Helsinki are expected to meet all deliverables on time for Y3.

In all cases, the balance from Y2 will be carried over into Y3 and will be used to ensure deliverables for the project.

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 or not 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.

N/A

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 (U.S.$)	Total Disbursed from Primary Awardee to Sub to Date (U.S.$)	Total Sub-Awardee Spent to Date (U.S.$)	Total Contracted Amount (U.S.$)
IITA	0	166,546	166,546	648,702
University of Helsinki	0	101,076	101,076	310,538
UW-Madison	167,780	362,542	194,762	487,890

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.

Privacy and Non-Confidentiality Notice

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