



NAPPO

North American Plant Protection Organization
Organización Norteamericana de Protección a las Plantas

NAPPO Conference Call Report

NAPPO Conference Report

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| Expert Group: | Seeds-ToBRFV | |
| Location: | Video conference – Zoom meeting | |
| Date: | January 18, 2022 | |
| Chairperson | Beatriz Xoconostle (CINVESTAV) | |
| Participants: | | |
| Vessela Mavrodieva (APHIS – PPQ) | Geoffrey Dennis (APHIS – PPQ) | Jennifer Nickerson (CFIA) |
| Daniela Alejandra Bocanegra Flores (SENASICA) | Huimin Xu (CFIA) | Samantha Thomas (US Industry) |
| Ed Podleckis (APHIS – PPQ) | Nancy Osterbauer (APHIS – PPQ) | Ángel Ramírez Suárez (SENASICA) |
| Marlene Ortiz (MX Industry) | Rick Dunkle (US Industry) | José Manuel Cambrón Crisantos (SENASICA) |
| Brooke Zale (APHIS – PPQ: Observer) | Nedelka Marín-Martínez (NAPPO) | Stephanie Bloem (NAPPO) |
| Maribel Hurtado (NAPPO) | Alonso Suazo (NAPPO) | |
| Summary | | |
| Project: | <i>A pilot for the harmonization of diagnostic protocols for seed pests focuses on ToBRFV.</i> | |
| General comments: | Welcome remarks by the NAPPO TD and the Chairperson. The TD agreed to take notes and write the report. EG approved recording this videoconference for report purposes. | |
| Item 1: | Questions and answers on the data collection platform. | |
| Consensus: | <p>Geoffrey Dennis:</p> <ul style="list-style-type: none"> Indicated that no formal requests to access the data collection platform have been submitted to PPQ. Informed that permissions to access the platform will be granted once the form is submitted and approved. Informed that changes will be made to the portal format to include the ISHI-Veg protocol with two viral gene targets. These amendments will be completed by the end of February 2022. A tab specially designed for the additional ampliset will be included. <p>The TD encouraged EG members to request access to the platform.</p> | |
| Item 2: | Proposal to develop a document for the results analysis and framework. | |

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| <p>Consensus:</p> | <p>Geoffrey Dennis proposed to get subgroup 2 together to work on the details of the formulas and the definitions that will be used for the analyses. The idea is to produce a document that will be shared with the EG for feedback. Geoff indicated that some details in the validation analysis should be addressed, and it is recommended that the EG solves these issues before data collection starts. Examples of this are the number of replicates used in the validation categories such as repeatability and limit of detection (% positive at a specific concentration of interest) and sensitivity (% positive over range being observed).</p> <p>Geoff indicated that he will produce a first draft of the document to circulate among validation experts in all three NAPPO countries.</p> |
| <p>Item 3:</p> | <p>Updates on panels work</p> |
| <p>Consensus:</p> | <p>PPQ provided the following updates:</p> <ul style="list-style-type: none"> • Seed samples: <ul style="list-style-type: none"> ○ Two seed lots of both infected and ToBRFV-free Tomato and Pepper seeds have been evaluated. These seeds are ready for the panels. ○ One of each lot will be selected for the development of panels. ○ Titers and homogeneity of virus levels were determined in two lots of infected (ToBRFV +) seeds. ○ A new lot of infected seeds with the required viral concentration for this study was created by mixing virus-free and virus-infected seeds from the homogenized seed lot. ○ Tested all five methods as described and provided by all countries in five-point dilution curves to make sure the methods detect the target genes. Positive virus detection was obtained using all detection methods that will be assayed. ○ Tests were also performed using the 18S endogenous target gene in RT-PCR (used in the SENASICA protocol) with positive virus detection. ○ Forms have been created to track all the work and ensure traceability. ○ Individual samples can now be produced. Work will start with healthy seeds and then with the infected seeds. <p>Decision: Mexico informed they read the validation data provided by PPQ in December 2021 to provide their position on the use of SqMV or NAD5 as internal controls in the ISHI Veg protocol. Mexico indicated that SqMV is regulated in Mexico and therefore cannot be used as control but <u>agreed to use NAD5 as an internal control.</u></p> <ul style="list-style-type: none"> • Transcripts and reagents: |

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| | <ul style="list-style-type: none"> ○ Reproducibility on the transcripts synthesis is OK. ○ Concerns: Stability of the transcripts during transportation. Stability tests will be done in collaboration with Kevin Ong at TAMU. ○ Transcripts have been sent in different storage conditions to track stability at regular time intervals. This will also be monitored when the panels are distributed. ○ A list of reagents, probes, and primers has been compiled and will be sent to Stephanie Bloem (NAPPO) for purchasing. Aliquoting of reagents for each panel will be done at the PPQ lab. ○ It has been checked that the fluorescent probes that will be purchased are correctly detected by the equipment of participating laboratories. ○ Tests will be done before shipping the aliquots to the participating labs to check for purity and to make sure that reagents, probes, and primers are working as expected. <ul style="list-style-type: none"> ● Permits: <ul style="list-style-type: none"> ○ The ED will assist in transporting the panels to Mexico. ○ Canada is working on the official import permits to move virus-infected seeds to Canada. Jennifer Nickerson indicated that an import permit will be needed if samples are shipped from the US to the participating laboratories in Canada. If samples are shipped from the US to the Charlottetown laboratory, a permit to move the samples across provinces will be needed. Incomplete forms will not be taken into consideration and the permit rejected. Jennifer Nickerson will send the information to Samantha Thomas with the specific details needed for the import permit. Discussions will continue. ○ Mexico indicated that SENASICA will provide an authorization letter to allow the person moving the material to Mexico to introduce the material in the country to be used for research purposes. ● Others: <ul style="list-style-type: none"> ○ Randomization of samples for the panels will be done in Kevin Ong's lab. |
| Item 4: | Supplementary materials not provided (possibility of financing by each country: optical tubes, optical boxes, filter pipette tips, gloves) |
| Consensus: | Notes: <ul style="list-style-type: none"> ● Budget was prepared taking into consideration that some labs will need additional financial assistance to buy supplementary materials. ● Laboratories in the US were informed they will receive financial support to purchase supplementary materials. ● Mexico indicated that SENASICA will cover the cost of supplementary material for participating labs in Mexico. |

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| | <ul style="list-style-type: none"> Each laboratory should make a list of the additional material and work with NAPPO for financial assistance if needed. Mexico requested to get an estimated list of supplementary material that will be needed. Beatriz volunteered to calculate the number of plastic supplies necessary for the ring assay. |
| Item 5: | Proposals for panel shipment dates, test period, and record of results. |
| Consensus: | <p>Notes:</p> <ul style="list-style-type: none"> Terms of references (ToR): Ed Podleckis informed that a ToR document is being prepared and estimated to complete this work in three weeks. The document will be sent to the Secretariat for translation and shared with the EG in both languages for feedback prior to submitting the document to the EC for approval. Master document. The ED informed the EG that the NAPPO Secretariat is preparing a document with all the information that has been provided by the EG. This document, when completed, will be shared with Beatriz Xoconostle to review. The reviewed document will be shared with the EG in English first and then in Spanish. <p>Additional notes:</p> <ul style="list-style-type: none"> For the laboratory work, consider that we are still under COVID regulations. |
| Other subjects | The chairperson informed the group about the EUPHRESKO report on the harmonization of protocols for detection of ToBRFV and encouraged the EG to read the report because it contains information of relevance to this project. |

Next Steps

| Responsible Person | Action | Date |
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| NAPPO Secretariat | Schedule a call with the Seeds SG 2 to discuss a proposal to develop a document for the results analysis and framework. | |
| Geoffrey Dennis | Write a first draft of the proposed result analysis and framework document and send it to the Secretariat for translation and sharing with the EG. | |
| APHIS PPQ | Send a list of reagents and information on where to purchase them to the ED. NAPPO will purchase the reagents, probes, and primers and send them to PPQ for aliquoting and panel preparations. | |
| Jennifer Nickerson | Send an email to Samantha Thomas providing details on what is needed to complete the import permit to move infected seeds from the US to Canada. | |
| Samantha Thomas | Provide details to Jennifer Nickerson to complete the import permit to move infected seeds to Canada. | |
| Beatriz Xoconostle | Provide a list of supplementary materials that will be needed for the participating labs in MX. | |
| Ed Podleckis | Complete the ToR draft to share with the EG before it is submitted to the NAPPO EC for approval. EG to | |

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| | provide feedback. | |
| NAPPO Secretariat (TD and SO) | Complete “Master Document” with all the information that has been provided by the EG. The English version of the document will be shared with the EG first. | |
| Next Meeting | | |
| Location: | Video conference - Zoom | |
| Date: | TBD. The Secretariat will send a poll to the EG. | |
| Proposed Agenda Items | | |
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