



NAPPO

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

NAPPO Conference Call Report

Expert Group:	Seeds_ToBRFV-SG3	
Location:	Videoconference – MS Team Meetings	
Date:	October 7, 2021	
Chairperson/Presidente	Beatriz Xoconostle (CINVESTAV, MX)	
Participants:		
Norma González (CENAM, MX)	Nancy Osterbauer (APHIS-PPQ)	Ángel Ramírez (SENASICA)
Mercedes Herrera (CENAM, MX)	Jennifer Nickerson (CFIA)	Samantha Thomas (US industry)
Melina Pérez (CENAM, MX)	Maribel Hurtado (NAPPO)	Ed Podleckis (APHIS – PPQ)
Vessela Mavrodieva (APHIS – PPQ)	Marlene Ortiz (MX Industry)	Huimin Xu (CFIA)
Daniela Alejandra Bocanegra Flores (SENASICA)	Eduardo Garrido (INIFAP)	Nedelka Marín-Martínez (NAPPO)
Geoffrey Dennis (APHIS PPQ)	Alonso Suazo (NAPPO)	
Summary		
Project:	A pilot for harmonization of diagnostic protocols for seed pests focused on ToBRFV.	
Item 1:	National Metrology Institute of Mexico (CENAM in Spanish) proposal on the development of reference material for the NAPPO Seed project.	
Consensus:	<p>Dr. Norma González (CENAM) provided an overview of the roles and mission of CENAM. Following a detailed description of the processes and roles of CENAM, Dr. González presented a proposal to the NAPPO EG to produce reference material that could be used in the ring tests.</p> <p>Dr. González:</p> <ul style="list-style-type: none"> • Indicated that all reference material will need to be defined first (solid, matrix or solution) and will be produced following international guidelines. The production process will also vary depending on how the material is defined. • Provided detailed information on how the reference material will be prepared, packed, homogenized and how the homogeneity and stability will be determined. <p>CENAM's proposal:</p> <ul style="list-style-type: none"> • Develop two types of reference material <ul style="list-style-type: none"> ○ Material in Matrix form: <ul style="list-style-type: none"> ▪ Positive, infected tomato seed powder 	

	<ul style="list-style-type: none"> ▪ Negative, healthy tomato seed powder ▪ Positive, infected pepper seed powder ▪ Negative, healthy pepper seed powder ○ Calibration material (synthetic linearized DNA plasmid of ToBRFV) to establish the calibration curve for PCR protocols. <ul style="list-style-type: none"> • To produce the calibration material described above, NAPPO should provide the plasmid. • Estimation of reference material production time and cost is indicated below: <table border="1" data-bbox="686 485 1510 1041"> <thead> <tr> <th>Material</th> <th>Estimated time (month)</th> <th>Estimated cost (US \$)</th> </tr> </thead> <tbody> <tr> <td>Tomato and pepper seed controls (positive and negative)</td> <td>14</td> <td>43,000.00</td> </tr> <tr> <td>Linearized DNA plasmid of ToBRFV</td> <td>12</td> <td>22,000.00</td> </tr> <tr> <td>Preparation and packing of material</td> <td>1</td> <td></td> </tr> <tr> <td>Determination of stability and homogeneity</td> <td>1</td> <td></td> </tr> </tbody> </table> <ul style="list-style-type: none"> • The TD thanked members of CENAM for the information provided and indicated the EG will discuss the details provided by CENAM and decide on the next steps as a group. • EG members indicated the EG has decided to use whole seeds and not ground seeds although recognized the benefits of using ground seeds for the sample homogenization process but not for the sample stability. The EG also indicated that the ISHI-Veg method uses ground seeds. • CENAM also indicated they have worked on other type of reference material on tomato and citrus tristeza virus. CENAM also indicated this will be the first time they will work on reference material with RNA virus and RNA transcripts. 	Material	Estimated time (month)	Estimated cost (US \$)	Tomato and pepper seed controls (positive and negative)	14	43,000.00	Linearized DNA plasmid of ToBRFV	12	22,000.00	Preparation and packing of material	1		Determination of stability and homogeneity	1	
Material	Estimated time (month)	Estimated cost (US \$)														
Tomato and pepper seed controls (positive and negative)	14	43,000.00														
Linearized DNA plasmid of ToBRFV	12	22,000.00														
Preparation and packing of material	1															
Determination of stability and homogeneity	1															
Item 2:	Ring test workflow diagrams															
Consensus:	<p>Jennifer Nickerson provided an overview of the workflow diagrams for the processes and logistics necessary for the ring tests to facilitate the discussions. The diagrams were shared with the EG for feedback.</p> <p>Processes diagram: Feedback from the EG was provided to produce a new version to share with the EG. Relevant feedback includes:</p>															

	<ul style="list-style-type: none"> • Controls and samples should be “<i>blind</i>” to reduce bias among participating labs during the ring tests. • Separate the information for reagents into primers and probes. • Add primers and probes for the ISHI-Veg protocol. <p>Logistics diagram: An outline of the logistics involved in each process was presented as a flowchart including the logistics involved with the seed and sample preparation, reagents, and panel preparation. This flowchart was created to facilitate future discussions with the expert group.</p>
Item 3:	Equipment in participating labs
Consensus:	The SG Chairperson asked members of the EG to make sure the participating labs have the necessary equipment for the detection of the fluorochromes used in the protocols and to provide a list of the equipment.
Item 4:	Seed panels
Consensus:	<p>The SG Chairperson asked for an update on the list required for the seed panels. APHIS PPQ indicated that this is a work in progress and provided the following remarks:</p> <p>Samples:</p> <ul style="list-style-type: none"> • Sample A (tomato analytical sample, positive): Will probably require an in-vitro transcript. • Sample B (tomato seed, positive): Enough material available from Industry and APHIS PPQ. APHIS PPQ has a highly “infested” lot with the virus from which dilutions can be made. • Sample C (Seed + ToMV): 2 different concentrations will be needed and there is enough material available. Two options: <ul style="list-style-type: none"> ○ In-vitro transcripts ○ Seed lots with two different concentrations • Negative material (Healthy tomato and pepper seeds): Difficult to find but need to find seeds infected with ToMV. <p>Controls:</p> <ul style="list-style-type: none"> • Some controls are the same as the sample material. Enough material is available for all the controls.
Item 5:	Data collection, storage, and analysis
Consensus:	<p>Geoffrey Dennis (APHIS – PPQ) provided a demo of the USDA National Animal Health Laboratory (NAHL www.nahl.org) portal developed for the ToBRFV project. Geoff indicated that this portal for the ToBRFV is still under development.</p> <p>Additional notes:</p> <ul style="list-style-type: none"> • PowerPoint files with instructions on how to use the portal were sent to the NAPPO Secretariat for distribution to the

	<p>EG.</p> <ul style="list-style-type: none"> • Instructional material (PPT files) and a registration form that will need to be used by participating labs will have to be distributed to all participating labs. • Laboratory staff that will participate in the ring tests need to fill out the “Access Request Form” or “NAHL form and should be sent to Geoffrey Dennis. • Laboratory registration is done through the website. Participating labs should click on the “PT” tab within the “LabDir” tab in the NAHL website. • The ring test will be listed under the PT tab. • Documents necessary for the ring test can be added to the portal. • Geoff provided a demo on how to input and report data into the portal. He also indicated that data can be exported into other platforms like MS Excel. • Geoff indicated that the system still has some bugs to fix and suggested to EG member to fill the forms after these technical issues are resolved.
--	--

Next Steps

Responsible Person	Action	Date
NAPPO Secretariat	Translate and share instructions on how to use the NAHL portal for data collection, storage, and analysis and the system registration form for participating labs to have access to the system.	
EG members	Provide feedback on the processes and logistics workflow.	

Next Meeting

Location:	Videoconference
Date:	November 9 from 2:00 to 4:00 pm EST

Proposed Agenda Items

1. Communicate NAPPO decision of using reference material prepared by the EG
2. Request from the industry: Protection of intellectual property on seed varieties employed in the assay
3. Feedback on the protocols shared with the EG
4. Comments on the use of the portal for data entry, storage, and analysis
5. Description of positive controls
6. Assignment of tasks according to the workflow, calendar of activities
7. Other business.