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

NAPPO Conference Call Report

Expert Group:	Seeds-ToBRFV Subgroup 3	
Location:	Videoconference – Zoom meeting	
Date:	August 20, 2021	
Chairperson	Beatriz Xoconostle (CINVESTAV, MX)	
Participants:		
Samantha Thomas (US industry)	Jennifer Nickerson (CFIA)	Maribel Hurtado (NAPPO)
Geoffrey Dennis (USDA-APHIS-PPQ)	Edward Podleckis (USDA-APHIS-PPQ)	Kevin Ong (TAMU)
Marlene Ortiz (Mexican industry)	Vessela Mavrodieva (USDA-APHIS-PPQ)	Stephanie Bloem (NAPPO)
Nedelka Marín-Martínez (NAPPO)	Alonso Suazo (NAPPO)	Ángel Ramírez (SENASICA)
Summary		
Project:	A pilot for harmonization of diagnostic protocols for seed pests focused on ToBRFV.	
Item 1:	Source of seeds and RNA.	
Consensus:	The Subgroup Chairperson: • Provided a list of participating labs in Canada and Mexico and requested members from the US to send the list of laboratories in the US to update the database. • Inquired about the availability of control material for the ring tests. US members indicated that: ○ Mexico and US industry and PPQ have seeds available. ○ PPQ has seeds that can be used as controls. ○ It is necessary to determine who is going to get those samples and define what a sample is. It was also indicated that laboratories need to have the protocols to better estimate how many seeds will be needed. ○ Work needs to be done on seeds to characterize the seeds and to ensure uniformity with the samples (uniformity in the level of infestation etc.). Kevin Ong and Vessela Mavrodieva indicated the labs in TA&M and PPQ can help with the characterization of the samples. Ángel Ramírez indicated that SENASICA can help but with the protocol they are currently using. Working with other protocols will take longer. Mr. Ramírez indicated that CENAM (National Center for Metrology) could do the work in Mexico (evaluate	

	seeds to determine levels of infestation). CENAM will need the seeds from the EG. Vessela suggested she can work with Kevin Ong developing a list of what is needed for the panels. o Mr. Ramírez proposed CENAM to prepare candidate reference materials (positive and negative controls, RNA, etc.) but it will cost an estimated US $52,260 for each one of reference materials. Samantha Thomas indicated that the US industry is willing to do the characterization of seed material (homogeneity and viral content)at no cost. o EG members suggested to invite members of o CENAM to join the EG discussions and provide additional details on the sample preparations and costs. o Consider having panels for labs to practice before the actual ring tests are implemented. The US industry indicated they have seeds available and if needed, panels can be made for labs to test the protocols prior to the ring tests.
Item 2:	Permits
Consensus:	The Chairperson: • Reminded the EG that seeds, and RNA will be moved across NAPPO countries. • Indicated that Mexico has already provided details of the permits required to move that material. • Inquired about the permits required in the US and Canada. US indicated that permits with a specific period are required to move any plant material into the US. No permits are needed to move RNA.
Item 3:	Protocols
Consensus:	The Chairperson: • Indicated that: • A draft manual contains o Two protocols for RNA extraction (in case a laboratory needs them, as this step won't be harmonized). o Three protocols for conventional RT-PCR o Two protocols for qRT-PCR have been included in the document (guidelines for labs) • Informed that a document with detailed information on each protocol has been written but needs additional information on some protocols to complete it. • Requested information on some protocols needed to complete the document. Marlene Ortíz will provide it. • Indicated the EG should calculate the amount and types of reagents to use (including primers) taking into consideration the number of labs and protocols. The

	spreadsheet created by Geoffrey Dennis (APHIS PPQ) could be used as a reference. • Indicated that it is also important to determine the equipment needed in each participating lab, especially the thermocyclers used for the PCR reactions. Vessela Mavrodieva, Kevin Ong, and Ángel Ramírez agree to work on this task.
Item 4:	Data collection, storage, and analysis.
Consensus:	Geoffrey Dennis (APHIS PPQ) indicated that • PPQ has an online portal for data entry, storage, and analysis. • A module for the NAPPO ring tests has been developed within the portal. • A 30 min demo will be provided during the next call with the EG. • A special permit can be granted to the NAPPO Scientific Officer who will be assisting in the data storage and analysis.

Next Steps

Responsible Person	Action	Date
Jennifer Nickerson	Provide a flowchart with the steps required for the seed panels.	
Vessela Mavrodieva, Ángel Ramírez, and Jennifer Nickerson	Check with their participating labs the type of PCR equipment they have for compatibility.	
Vessela Mavrodieva and Kevin Ong	Develop a list of what is needed for the seed panels.	
Marlene Ortiz	Provide additional information on the selected protocols to Beatriz.	

Next Meeting

Location:	A poll will be sent to the EG to schedule the next videoconference.
Date:	TBD

Proposed Agenda Items

1. Presentation by CENAM on the preparation of candidates of reference material (20 min, 10 min for questions and comments).
2. Explanation of the flowchart with the steps required for the seed panels. Presented by Jennifer Nickerson.
3. List of lab equipment available in the participant laboratories, emphasis in compatible PCR equipment. Presented by Vessela Mavrodieva, Ángel Ramírez, and Jennifer Nickerson
4. List of what is needed for the seed panels. Presented by Vessela Mavrodieva and Kevin Ong
5. Demo presentation by Geoffrey Dennis (APHIS PPQ) of the online portal for data entry, storage, and analysis (20 min, 10 min for questions and comments).
6. Request from the industry: Protection of intellectual property on seed varieties employed in the assay (Request of restricted use: for diagnostics only, to limit seed distribution).
7. Assignment of tasks according to the workflow
8. General comments