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

NAPPO Conference Call Report

Expert Group:	Seeds-ToBRFV	
Location:	Videoconference – Zoom meeting	
Date:	November 9, 2021	
Chairperson	Beatriz Xoconostle (CINVESTAV, MX)	
Participants:		
Jennifer Nickerson (CFIA)	Ed Podleckis (APHIS – PPQ)	Nancy Osterbauer (APHIS – PPQ)
Vessela Mavrodieva (APHIS - PPQ)	Geoffrey Dennis (APHIS – PPQ)	Kevin Ong (TAMU)
José Manuel Cambrón Crisantos (SENASICA)	Jessica Berenice Valencia Luna (SENASICA)	Angel Ramírez Suárez (SENASICA)
Eduardo Garrido (INIFAP)	Samantha Thomas (US Industry)	Stephanie Dubon (APHIS – PPQ)
Rick Dunkle (US Industry)	Stephanie Bloem (NAPPO)	Maribel Hurtado (NAPPO)
Nedelka Marín-Martínez (NAPPO)	Alonso Suazo (NAPPO)	
Summary		
Project:	*A pilot for the harmonization of diagnostic protocols for seed pests focuses on ToBRFV*	
General comments:	• Brief introduction and welcome remarks provided by the NAPPO Secretariat. • The NAPPO TD will take notes and write the videoconference call report. • EG members authorized recording the session for report purposes.	
Item 1:	Decision regarding the reference material for this project and the proposal presented to CENAM	
Consensus:	The NAPPO ED informed that a letter was sent to EG members from MX regarding the decision to consider CENAM's proposal to prepare the reference material for the project. The letter was latter sent to members from Canada and the United States. The Subgroup (SG) Chairperson provided additional details and thanked CENAM for their presentation and the information provided to the EG.	
Item 2:	Industry's request for intellectual property protection of seed varieties.	
Consensus:	The SG Chairperson proposed to use a document, signed by NAPPO on behalf of the participating laboratories, indicating that seeds for this project will be used for research purposes only. A sample document was presented.	

	The SG Chairperson will share a copy of this document with the EG
Item 3:	Feedback on the protocols shared with the EG
Consensus:	The SG Chairperson indicated the following criteria were used when considering the protocols to use: • Specificity (evaluated with ToMV, a close-related virus) • Reproducibility (detection in 10 different labs) • Repetitivity (detection in 2 or 3 replicates) • Sensitivity (Two viral concentrations: High and low) and • Stability of primers and probes. Feedback on the protocols: • One step required for the APHIS-PPQ and CFIA protocols (one multiplex) and two steps for the SENASICA protocol (two singleplex). • In the second assay of the SENASICA protocol 18S transcript is used as endogenous control to test the quality and quantity of the RNA. • The second assay in the SENASICA protocol was not taking into consideration when designing the panels. • US inquired about using the internal controls currently used in the APHIS-PPQ and CFIA protocols (nad5) as an alternative to the 18S (used in the SENASICA protocol) to determine the RNA quality RNA. • Mexico indicated that: o their protocol is optimized using the 18S and would prefer for the group to use the 18S control. Shifting to nad5 as an alternative to 18S will require revalidation of the SENASICA protocol. o The first step is to use the 18S control to check for nucleic acid quality and the second amplification is for the virus detection. • Protocols, reagents, and panel configuration will need to be modified (adjusted) if the group agrees to use the 18S control for the SENASICA protocol and the nad5 control for the other protocols. • ISHI-Veg protocol is a multiplex protocol and uses the Squash Mosaic Virus (SqMV) as a spike control that also provides information on the RNA quality and quantity (extraction efficiency). The group needs to decide if nad5 will be used as a control for this protocol in which case the primers and procedures are available or if SqMV will be used as an additional spike control in which case new primers will be needed. The decision to include or not the SqMV as spike control will also affect the data collection setup with the portal already set by APHIS PPQ, currently programmed to accept two values per sample • Regarding the ISHI-Veg protocol, the EG need to agree on whether a triplex or duplex will be done to make the

	necessary adjustment in the data collection system. The portal is currently set up for a duplex. The US proposed to put together a file with all the primers and options and requested the feedback from the EG. • Additional information was requested to the cDNA synthesis procedure in the SENASICA protocol, to provide more detailed information on the reagents used. EG members from SENASICA agreed to provide the requested information. Decisions and action items needed for the EG: • Use of SqMV as a spike control. • Feedback from SENASICA on the suggested addition for the cDNA synthesis process. • How is the group going to use the ISHI-Veg protocol: duplex or triplex.
Item 4:	Protocols document
Consensus:	The subgroup chairperson: • Indicated that protocols have been put in the same format in the document. • Noted that references for some protocols are missing. • Indicated that proposed additions to the SENASICA protocol will be incorporated in the document pending the approval and feedback that will be received from SENASICA. • Suggested that a flowchart prepared by Canada will be included at the beginning of the document to provide a visual representation of the document content. • Suggested to add a brief introduction to explain the ring tests process. • Suggested to include a list of materials that will not be provided to participating labs including pipet tips, tubes, etc. • Suggested to include precise instructions for each lab (what materials and reagents will be provided, what protocols they will use, expected results, and guidelines and instructions to enter data into the data collection system).
Item 5:	Panel information, reference lab and participating laboratories
Consensus:	PPQ presented a summarized table with information on what the participating laboratories will assay, what reference labs will provide, and the composition of panels. Relevant aspects in this table include: • "*Blind*" panel samples (three seed samples and two analytical samples) are indicated as A, B, C, D, and E. • Analytical samples are construct being prepared. • Four control samples (infested seeds) and a calibrator are included.

	• A non-template control is also included in each lab (molecular grade water).
	• For the positive tomato seed (Sample B):
	  ○ each participating lab will receive two bags containing each 1000 seeds from which there will be two RNA extractions.
	  ○ Five dilutions will be prepared from each RNA extraction.
	  ○ The number of PCR reactions in the table is per assay and not per sample.
	  ○ The number of replicates for each assay is additional information which will be included in a different table. PPQ will share a revised second table that includes the number of replicates with the EG.
	• PPQ indicated that the laboratory in Maryland has been preparing proficiency test panels material for more than 15 years and a presentation will be provided to the EG during the next videoconference call. In this presentation, the processes for panel preparation and the methods for validation and determination of sample stability will be described.

Item 6:	Data collection platform
Consensus:	PPQ indicated that work is being done to fix the problems with the data collection system. Geoffrey Dennis indicated that he could grant access to EG members interested in exploring the system. Those interested should send Geoff their names, email, and affiliation.
Item 7:	Positive controls
Consensus:	The subgroup Chairperson provided details on the positive controls to use for the ring tests including: • Primer designs for the targets used for the virus detection. • Vector used to clone the targets. • Sequences of recombinants plasmids. • *In vitro* transcription results with linearized plasmid after restriction digestion with *Bam*HI and digestion with DNase. • Material sent to the Maryland lab for evaluation. • Primers and probes were assayed and amplified as expected. The Chairperson will share the sequences of the transcripts.
Item 8:	Next steps
Consensus:	The EG will be working on the following: • List and amounts of reagents required based on modifications as determined from these discussions (Vessela Mavrodieva volunteer to do this task). • Preparation and aliquoting of reagents. The list of reagents will be sent to the NAPPO Secretariat for

	purchasing. Purchase and shipping information will be provided to NAPPO. • Reagents will be distributed with the seed bag and other material.

Next Steps		
Responsible Person	**Action**	**Date**
Beatriz Xoconostle	Share copy of legal document to address intellectual property value of seeds used for the project.	Already sent to NAPPO
Vessela Mavrodieva	Share file with information on number of replicates to use for the assays.	Already sent to GE
Beatriz Xoconostle	Share sequence of transcripts with Geoffrey Dennis.	Already sent to Geoff
Vessela Mavrodieva	Coordinate with Maryland lab manager a presentation for the EG to provide insights into the validation methods used and the panel preparation.	TBD

For discussion to reach consensus in the next conference call	
1	Use of SqMV as a spike control
2	ISHI – Veg protocol: Will the group use a duplex, or multiplex reaction.