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

NAPPO Conference Call Report

Expert Group:	Seeds-ToBRFV	
Location:	Zoom meeting – Videoconference	
Date:	March 19, 2021	
Chairperson	Ed Podleckis (APHIS – PPQ)	
Participants:		
Pamela Ross (CFIA)	Nancy Osterbauer (APHIS – PPQ)	Vessela Mavrodieva (APHIS – PPQ)
Geoffrey Dennis (APHIS – PPQ)	Kevin Ong (TA&MU)	José Manuel Cambrón Crisantos (SENASICA)
Jessica Berenice Valencia Luna (SENASICA)	Daniela Alejandra Bocanegra Flores (SENASICA)	Ángel Ramírez Flores Suarez (SENASICA)
Rick Dunkle (US Industry)	Samantha Thomas (US Industry)	Mario Puente Raya (MX Industry)
Huimin Xu (CFIA)	Patricia Abad (APHIS – PPQ)	Stephanie Bloem (NAPPO)
Nedelka Marín-Martínez (NAPPO)	Alonso Suazo (NAPPO)	Patricia McAllister (CFIA)
Summary		
Project:	A pilot for harmonization of diagnostic protocols for seed pests focused on ToBRFV.	
General comments:	• The Chairperson and the NAPPO TD welcomed and thanked members for joining the call. • Agenda approved as presented. • The TD agreed to take notes and prepare the conference call report.	
Item 1:	Subgroup 1 updates – Vessela Mavrodieva (APHIS – PPQ)	
Consensus:	The following updates were provided: • Subgroup reviewed the existing 5 protocols including 3 with conventional PCR and 2 with RT-PCR. • All participant labs will run all five of the selected protocols • All assays for the ring test will be direct seed tests and not tests conducted on vegetative plant material from germinated seeds. • The EG will need to prepare an equipment list needed to run the tests. • Group needs to provide more information about the ring tests to the participating labs before the labs confirm their interest to participate in the ring tests. • Subgroup is discussing the costs associated with the	

	assays and cost sharing to reduce cost for each lab. • Each country needs to provide the cost of each reaction per test.
Item 2:	Subgroup 2 updates – Geoffrey Dennis (APHIS – PPQ)

Consensus:	The following updates were provided:
	• The subgroup discussed the “materials” to use for the tests including
	  ○ Two analytical samples using synthesized targets
	  ○ One positive diagnostic seed lot
	  ○ Negative tomato and negative pepper seed controls
	  ○ Positive process control
	  ○ Two negative process controls
	  ○ No template controls
	  ○ RNA calibrator for RT-PCR
	• One lab has been able to make the synthesized targets.
	• Subgroup continues to work on the details of the validation design.
	
	Additional highlights and notes:
	
	• The chairperson indicated that the entire group should be in agreement with all the parameters presented by both subgroups.
	• The group agreed that each selected participating lab will test all five selected protocols.
	• Mexico informed that labs in Mexico with good international reputation have confirmed their interest in participating in the ring tests but indicated that financial assistance will be needed.
	• The ED indicated that NAPPO can provide some financial assistance for the ring tests. The industry (ASTA and AMSAC) also indicated that they may be able to financially contribute.
	• An estimate of how much funding is needed was requested by the ED to determine how much NAPPO can contribute.
	• Samples (test materials):
	  1. Analytical sample with the target added to it and no seed material. No extraction or seed grinding involved. This will be needed to form a baseline and is referred to as “sample A”. This sample is a healthy seed RNA spiked with a plasmid or transcript.
	  2. Positive tomato seed.
	  3. Similar to “Sample A” but instead of being spiked with the target it will be spiked with a cross-reacting species.
	  4. Negative tomato seeds

	5. Negative pepper seeds • Types of controls depending on the assay used: 1. Positive process control – Positive seed 2. Two negative process control: one with tomatoes seed and the other with pepper seeds. 3. No template control. 4. Calibrator for the RT-PCR assay. This consist of purified RNA and buffer to keep the RNA stabilized. • Sample numbers: 1. Considering that 8 labs will participate, and each lab will test each of the 5 assays, the estimated total per lab is 299 reactions or five 96-well plates. This will be done by two technicians per lab. 2. Sample numbers is important to estimate costs of running the tests. 3. The number of RNA extractions is much less than the number of PCR reactions. 4. Geoffrey Dennis will share a spreadsheet with the EG with detailed information on the sample numbers and types. • Number of laboratories and technicians per laboratory required. Geoffrey Dennis indicated that: 1. Eight (8) labs is the minimum number expected based on international standards. The number is important to calculate the reproducibility and selectivity of the test. 2. Any number less than 8 reduces the statistical power. • Labs in each country. 1. **US**: two public labs identified but there is interest from private labs to participate too. The US can have between 3-5 laboratories. 2. **Mexico:** Confirmed two research labs and the lab from the National Reference Center; total of 3 labs. 3. **Canada:** Confirmed three labs: two labs from CFIA and one from the U. of Guelph. 4. Canada noted that it is important that if a private lab participates in the ring tests, it should be accredited based on the ISO standards. 5. Mexico indicated that it is preferable if the EG does not consider private labs in the first phase of this project but agrees that in the future, when private labs could be considered, it is important that they are accredited based on the ISO standard. 6. US labs are all accredited and are part of the National Seed Health System (private or government labs). • Participation of labs outside the NAPPO region.

	1. US indicated that labs in Australia and New Zealand could also participate if accepted by NAPPO countries. If labs from these countries participate in the ring tests, they will not require funding from NAPPO. 2. EG members agreed that a private lab accredited by the NPPO should be allowed to participate in the ring tests. 3. Canada has no objection with the participation of labs outside the NAPPO region. Participation of additional labs will benefit the reliability of the ring tests, but the logistics associated with it will be a problem, for example, sample preparation and distribution. Additional labs will also increase the costs. 4. Mexico has no objection with the participation of labs outside the NAPPO region but indicated that it is important the labs are accredited by the corresponding phytosanitary entities (NPPOs). 5. Participation of labs outside the NAPPO region can result in a multiregional collaboration. 6. An official communicate will be needed if other labs outside the region will participate. The EG will have to make a recommendation for consideration by the NAPPO Executive Committee.
Item 3:	Next meeting
Consensus:	The EG agreed the next meeting will be a joint subgroups 1 and 2 meeting. A meeting with the entire group will be scheduled following the SG1 and SG2 meeting.
Other subjects	EG consensus
Consensus:	The EG was in agreement with the following points: 1. Selected protocols (5). 2. Samples and controls to be used for the 5 selected protocols. 3. Selected labs will test all five protocols. 4. Participating labs should be accredited by the respective phytosanitary entity. 5. Laboratories outside the NAPPO region can participate in the ring tests but need to be accredited by the NPPO. 6. The participation of labs outside the NAPPO region should be proposed by the EG and authorized by the NAPPO EC. 7. Upon authorization from the NAPPO EC, participating labs outside the NAPPO region will get an official communication from NAPPO.

Next Steps		
Responsible Person	**Action**	**Date**

Next Meeting		
Location:	Zoom meeting – Video conference	
Date:	April 14 from 1:00 to 2:00 pm EST	
Proposed Agenda Items		
1.		
2.		