



NAPPO

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

Conference Call Report

Expert Group:	Seeds ToBRFV	
Location:	Video conference – Zoom meeting	
Date:	April 20, 2022	
Chairperson	Ed Podleckis (APHIS – PPQ)	
Participants:		
Desmond Hammill (CFIA)	Maribel Hurtado (NAPPO)	Stephanie Bloem (NAPPO)
Rick Dunkle (US Industry)	Huimin Xu (CFIA)	Jessica Berenice Valencia Luna (SENASICA)
Jennifer Nickerson (CFIA)	Nancy Osterbauer (APHIS – PPQ)	Vessela Mavrodieva (APHIS – PPQ)
José Manuel Cambrón Crisantos (SENASICA)	Marlene Ortiz (Mexico Industry)	Daniela Alejandra Bocanegra Flores (SENASICA)
Alonso Suazo (NAPPO)	Stephanie Dubon (APHIS – PPQ)	
Summary		
Project:	<i>A pilot for the harmonization of diagnostic protocols for seed pests focuses on ToBRFV</i>	
General comments:	<p>Welcome remarks provided by the NAPPO Secretariat and the subgroup chairperson.</p> <p>The NAPPO TD will take notes and write the videoconference call report.</p> <p>EG members authorized recording the session for report purposes.</p>	
Item 1:	EG membership updates	
Consensus:	<p>New ad hoc EG member.</p> <p>Mr. Desmond Hammill (CFIA) was introduced to the EG as a new ad hoc member. EG Members introduced themselves followed by a welcome note from the NAPPO ED and TD.</p> <p>Election of new Chairperson.</p> <p>Ed Podleckis, the EG chairperson, announced his retirement from APHIS – PPQ at the end of April 2022. The ED thanked Ed for his contributions and leadership with the NAPPO Seeds EG and indicated that, following NAPPO protocols, the vice-chair should become the chair. Marlene Ortiz (Mexico Industry) accepted the nomination and the EG supported Marlene as the new EG chair.</p>	
Item 2:	Ring test updates: Amendments to PPQ protocol – Vessela Mavrodieva (APHIS -PPQ)	

<p>Consensus:</p>	<p>Vessela Mavrodieva (APHIS PPQ) indicated that amplifications were observed when NAD5 labelled with the fluorophore (fluorescent dye) VIC was used in reactions without host RNA in Protocol C. The data presented to the EG indicated that:</p> <ul style="list-style-type: none"> • In vitro transcripts samples are prepared for 3 regions in molecular grade water and no plant RNA amplification is expected, however, amplification was seen when using NAD5 marked with VIC and the ToBRF target is labelled with FAM. • There is no evidence of contamination with plant RNA in the reactions with protocol C when the internal control NAD5 was labeled with VIC • The unexpected amplification was probably due to signal bleeding which happens when the fluorophores used in a multiplex reaction have somewhat overlapping spectra, e.g. FAM & VIC. • The unexpected results are probably “artifacts” of the reaction. • Results using NAD5 probes labeled with the fluorophores ABY and Cy5 (used in protocol B) showed amplification with ABY but not with Cy5 in protocol C. • Seed samples tested with protocol C using NAD5/Cy5 produced comparable results. • Primer sets sequences used in protocol C are internal to those used for protocol B with forward primer sequences being shared by both sets and reverse primer sequences overlapping. <p>Based on the data presented, the U.S. requested to the EG members to provide feedback and indicate their position on the use of NAD5 ampliset labelled with Cy5 in protocol C as an internal control instead of NAD5 ampliset labelled with VIC as currently used in protocol C. The U.S. indicated that this will not compromise the outcome of the amplifications with protocol C but will require an adjustment to the written protocol and reagent packs for protocol C. Protocol validation was not necessary.</p> <p>The EG agreed and accepted the U. S. proposal. A consensus by all three NAPPO member countries was reached.</p>
<p>Item 3:</p>	<p>Test panels transportation to Mexico</p>
<p>Consensus:</p>	<p>The TD indicated that several options to transport the panels to Mexico were discussed in previous video conferences with the EG but a consensus on who will move the material to Mexico was not reached. EG members noted that Beatriz Xoconostle volunteered to this task. The TD will get confirmation from Beatriz Xoconostle to proceed with the next steps and NAPPO will coordinate this effort with Mexico. The ED indicated that NAPPO will cover the flight ticket.</p>

Next Steps		
Responsible Person	Action	Date
Next Meeting		
Location:	Video conference – Zoom meeting	
Date:	TBD. A poll will be sent to the EG.	
Proposed Agenda Items		
1.		
2.		
3.		