



NAPPO

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

NAPPO Conference Call/Meeting Report

Expert Group:	Seeds-ToBRFV - Subgroup 3	
Location:	Zoom meeting - Videoconference	
Date:	December 8, 2021	
Chairperson	Beatriz Xoconostle (CINVESTAV)	
Participants:		
Jessica Berenice Valencia Luna (SENASICA)	Stephanie Dubon (APHIS – PPQ)	Eduardo Garrido (INIFAP)
Stephanie Bloem (NAPPO)	Ángel Ramírez Suárez (SENASICA)	Geoffrey Dennis (APHIS - PPQ)
José Manuel Cambrón Crisantos (SENASICA)	Daniela Alejandra Bocanegra Flores (SENASICA)	Vessela Mavrodieva (APHIS – PPQ)
Huimin Xu (CFIA)	Edward Podleckis (APHIS – PPQ)	Nancy Osterbauer (APHIS – PPQ)
Brooke Zale (APHIS – PPQ)	Snezana Haymes (APHIS – PPQ)	Nedelka Marín-Martínez (NAPPO)
Alonso Suazo (NAPPO)		
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:	APHIS PPQ S&T Plant Pathogen Confirmatory Diagnostic Laboratory - Presentation	
Consensus:	<p>Dr. Snezana Haymes provided an overview and detailed information on the history and roles of the APHIS PPQ S&T Plant Pathogen Confirmatory Diagnostic Laboratory including:</p> <ul style="list-style-type: none"> • The type of services provided: confirmatory and routine diagnostic of pathogens of regulatory concerns, method development and validation of approved protocols and proficiency testing (production and development of panels and reference materials). • Collaboration and work with international organizations. • Panel preparation, validation (Determination of sample homogeneity and stability), instruction forms and distribution. • Tissue processing and DNA sample preparation. 	

	<p>Following Dr. Haymes presentation, M.Sc. Brooke Zale provided an overview on the work that has been done on the development of seed preparation and transcript samples for the NAPPO ToBRFV ring test. M. Sc. Zale also:</p> <ul style="list-style-type: none"> • Indicated that transcript system provided by Mexico has been reproduced. The Maryland laboratory synthesized and purified the transcripts in preparative amounts for the ring test. • Outlined the process for batches validation. • Explained the process for QC and document control. Batches with high standard variation are usually rejected.
Item 2:	Use of SqMV as spike control and 18S as internal control
Consensus:	<p>Each country provided feedback on the use of SqMV as spike control and 18S as internal control as follows:</p> <p>US:</p> <ul style="list-style-type: none"> • Will use the 18S as internal control in a separate reaction as described in the SENASICA protocol. • Will use the NAD5 as internal control with the ISHI Veg protocol, instead of the SqMV. If SqMV were to be used, it would have to be additionally provided to the laboratories. Because each laboratory will use their own RNA extraction protocol, and the use of SqMV is a control for RNA purification, the use of SqMV is not mandatory. • Indicated that the National Seed Health has conducted validation test of the ISHI Veg protocol using NAD5 and SqMV and determined there is no difference in the results when one or the other control is used. <p>Mexico:</p> <ul style="list-style-type: none"> • Concerned about using only NAD5 as an internal control because it is not how it was established for the ISHI Veg protocol. • Requested validation results that show no significant differences in the results when NAD5 is used as a control in the ISHI Veg protocol. <p>Canada:</p> <ul style="list-style-type: none"> • Supported the position of using only NAD5 as an internal control. <p>Consensus: The US agreed to get the validation data using NAD5 as internal control with the ISHI Veg protocol and will share this information with Mexico before the next videoconference call.</p>
Item 3:	ISHI Veg protocol – Duplex or triplex
Consensus:	<p>The subgroup chairperson asked each country about their position with respect to using a duplex or triplex reaction for the ISHI Veg protocol.</p> <p>US:</p> <ul style="list-style-type: none"> • Prefers a duplex reaction. • The data collection platform has been set for a duplex

	<p>reaction.</p> <ul style="list-style-type: none"> • Modifications to the data collection platform will be required if a triplex reaction is used. This will require a significant amount of time. <p>Canada:</p> <ul style="list-style-type: none"> • ISHI Veg has been validated using both a duplex or triplex reaction. • Canada supports the group decision and considers that a duplex reaction is appropriate. <p>Mexico:</p> <ul style="list-style-type: none"> • Prefers the use of a triplex reaction as stated in the ISHI Veg protocol. <p>All:</p> <ul style="list-style-type: none"> • Share information by e-mail to support the use a duplex reaction and discuss and agree before the next meeting.
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Next Steps

Responsible Person	Action	Date
US	Share the validation data using NAD5 as internal control with the ISHI Veg protocol.	Before next meeting

Next Meeting

Location:	Videoconference – Zoom meeting
Date:	TBD

Proposed Agenda Items

1. Description on the validation data using NAD5 as internal control with the ISHI Veg protocol.
2. Comments on the Euphresco project report