IPPC Draft Diagnostic Protocol for *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV) (2004-019)

Standards Committee response to the objection received

(*Prepared by technical Panel on Diagnostic Protocols, approved by the Standards Committee (2017_eSC_May_13)*)

BACKGROUND

[1] The draft diagnostic protocol (DP) for *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV) (2004-019) was submitted to country consultation on February 2015. The Technical Panel on Diagnostic Protocols (TPDP) revised the draft and responded to member comments, and recommended to the Standards Committee for their approval.

[2] The SC approved the responses to member comments[1] and the draft DP to be submitted to the DP Notification Period from 1 July to 15 August 2016. During the notification period the draft DP received an objection from the European Union and its Member States (EU)[2]. The comments made by the EU were reviewed by the Technical Panel on Diagnostic Protocols (TPDP). The panel provided responses to the objection comments and the draft DP was revised accordingly. The TPDP recommended to the SC that the responses to the objection comments be approved, and the draft DP be approved again for a DP notification period. The SC approved the responses to the objection[3] and approved the draft DP for another DP notification period.

[3] The draft DP was submitted again to the DP notification period from 15 December 2016 to 30 January 2017. During this notification period the draft DP was objected again by the EU[4]. The EU provided the technical justification for the objection and proposals for the improvement of the text of the draft DP.

[4] The SC considered the concerns raised in the EU objection and provided the following responses.

SC RESPONSES TO THE EU CONCERNS

Concern No. 1:

[5] "The drafting group has addressed most of the issues raised in the formal objection on the previous draft of the protocol and we thank them for that. The primers described in the paper of Hassani-Mehraban *et al.* (2016) have been included. However, the non-validated species-specific primers for TSWV, INSV and WSMoV have been included instead of the generic primers for American clade 1 (TSWV, INSV) and Asian clade 1 (WSMoV) which were tested against a broad selection of tospoviruses. These generic primers are located in highly conserved regions of the tospovirus genomes, which, therefore, make them very suitable for detection of different isolates of the target viruses.

[6] The species-specific primers that are included in the latest draft of the protocol were only used for confirmation of the identity of the isolates, and for this reason no data are available on the performance (sensitivity, specificity, etc.) of these primers. Since these primer sets have not been

[1] Compiled comments with SC responses: https://www.ippc.int/en/publications/82652/

[2] Objection received during the DP Notification period from 01 July 2016 to 15 August 2016: https://www.ippc.int/en/publications/82787/

[3] SC responses to the objection received during the DP Notification period from 01 July 2016 to 15 August 2016: https://www.ippc.int/en/publications/83852/

[4] Link to the objection: https://www.ippc.int/en/publications/83990/

developed, optimised and validated for routine detection, they should not be included in a diagnostic protocol without further validation.

[7] Instead however, the generic primer sets, AM1-F/AM1-R can be used for detection of TSWV (~763 bp) and INSV (~762 bp), and AS-EA-F/AS1-R for detection of WSMoV (~367 bp), following the test protocols as described. Moreover, the sequences of these amplicons have been shown to allow (provisional) identification of these species, and will identify other species belonging to these clades as well. So, if it is decided to include primers from the paper of Hassani-Mehraban et al (2016) in the IPPC protocol, these generic primer sets should be included instead of the specific primer sets."

SC response:

[8] In addressing this comment of the EU the new PCRs and primers described by Hassani-Mehraban *et al.* (2016) for specific detection of TSWV, INSV and WSMoV were added to the DP, and recommended for both detection and identification. The generic primers of Hassani-Mehraban *et al.* (2016) were not added as the universal primers of Mumford et al (1996a) and Chen et al. (2012) were considered to be better suited for the purpose of this DP.

[9] The EU requested that the specific primers be deleted and rather the generic primers for American clade 1 (TSWV, INSV) and Asian clade 1 (WSMoV) be added instead as these were tested against a broad selection of tospoviruses. The appropriate changes have now been made in the draft DP.

Concern No. 2:

[10] "For the test described by Chen et al., 2012, no details are provided on the RT-PCR conditions and sizes of the amplicons. We are wondering if these details should not be included if this protocol is meant to assist laboratories in implementing diagnostic tests. Maybe these details can be provided by a laboratory that is currently using this test."

SC response:

[11] These details and relevant information have now been added to the draft DP.

Concern No. 3:

[12] "Concerning the description of the different test, it would be more logic to put for each test the information on primers and conditions and test results together. For example:

[13] Test 1 a, b, c [paragraphs 64 (a), 68 (b), 73 (c)]

- *Primers [a: 65, 66; b: 69, 70, 71, 72; c: 74, 75]*
- *RT and PCR (or RT-PCR) reaction [76, 77]*
- *Expected amplicons [78]*

[14] For Test 2 [67 + additional information to be added] and Test 3 [generic primers of Hassani-Mehraban et al., 2016 + additional information to be added] use similar order. See also suggestions in Appendix 3."

SC response:

[15] A recommendation was made that for the individual tests the information on primers, conditions of testing and test results be put together. Also, ensure that the order of the different aspects of the test is consistent. These changes have been made to the draft DP.

Concern No. 4:

[16] "The Tospovirus classification reference of Nichol et al., 2005 is outdated – the ICTV published a new version - Plyusnin et al., 2012."

SC response:

[17] This was replaced by the new reference of Plyusnin *et al.*, 2012, and cited as appropriate.