RECOMMENDATION

Appendix 1

TECHNICAL JUSTIFICATION OF THE FORMAL OBJECTION

[1] Draft Annex to ISPM 27 – Tomato spotted wilt virus, Impatiens necrotic spot virus and Watermelon silver mottle virus (2004-019)

[2] 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.

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

[4] 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.

[5] The draft of the protocol with some suggestions for adaptation of the text is provided in Appendix 2.

[6] Furthermore, reading the text again, our expert noticed parts that could deserve improvement. We realize that these comments come at a late stage but they could be taken into account for a future revision of the protocol. There are presented below and suggestions are made in the attached Appendix 2:

[7] 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.

[8] 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:

[9] 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]

[10] 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 2.