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Detection of *Tomato brown rugose fruit virus* (ToBRFV) in Tomato and Pepper Seed by SE-qPCR

Addendum Validation report, April 2024

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Addendum validation report 'Detection of *Tomato brown rugose fruit virus* (ToBRFV) in Tomato and Pepper Seed by SE-qPCR'.

This document serves as an addendum to the ISHI validation report for the detection of *Tomato brown rugose fruit virus* (ToBRFV) in tomato (*Solanum lycopersicum*) and pepper (*Capsicum annum*) seed by SE-qPCR published in March 2020 on the ISF website. The data presented in the validation report showed that the SE-qPCR assay is fit for purpose based on experimental data for the analytical specificity, analytical sensitivity, selectivity, repeatability and reproducibility of the method. However, due to limited available infected material at the time of testing, only two laboratories were able to conduct the reproducibility experiments. In this addendum, follow-up work to assess the reproducibility of the method is highlighted.

The reproducibility of a method is the degree of similarity in results when the method is performed across laboratories with replicate seed subsamples (ISHI, 2020). Reproducibility can be estimated by calculating concordance (Langton *et al.*, 2002). Concordance is based on the probability of finding the same test results for identical test materials between laboratories. Concordance of at least 90% is considered as an indicator of good reproducibility.

The ISHI ToBRFV SE-qPCR primers were included in a test performance study (TPS) organized in the framework of the Euphresco project "Validation of diagnostic tests for the detection and identification of *Tomato brown rugose fruit virus* (ToBRFV) in tomato and pepper seeds" (Euphresco, Project 2019-A-327, Giesbers *et al.*, 2022). The sample set consisted of 30 tomato seed samples (four high infected, 20 medium infected, six ToBRFV-negative) and 10 pepper samples (five medium infected, five ToBRFV-negative) of 1,000 seeds each. The datasets from 16 participants of the TPS were included in their analysis of the ISHI ToBRFV SE-qPCR assay. Results of the TPS showed that the ISHI SE-qPCR assay allows the reliable and correct diagnosis of ToBRFV in tomato and pepper seeds, with a concordance of 97.7% for the tomato seed samples, and 96.3% for the pepper seed samples. These results confirm that the reproducibility of the ISHI ToBRFV SE-qPCR assay for the detection of ToBRFV in tomato and pepper seed is fit for purpose.

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