International Seed Federation
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Detection of Pospiviroids in Tomato and Pepper Seed by SE-qPCR

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Developed by ISHI

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Crops: Tomato (*Solanum lycopersicum*), Pepper (*Capsicum annuum*)

Pathogens: Pospiviroids: *Citrus exocortis viroid* (CEVd), *Columnea latent viroid* (CLVd), *Pepper chat fruit viroid* (PCFVd), *Potato spindle tuber viroid* (PSTVd), *Tomato apical stunt viroid* (TASVd), *Tomato chlorotic dwarf viroid* (TCDVd), *Tomato planta macho viroid* (TPMVd).

Version: 2 (March 2023)

ISHI recommends using either the Naktuinbouw or NSHS method

A detection method for seven pospiviroids in tomato and pepper seed was initially approved by the National Seed Health System's (NSHS) Policy & Procedures Advisory Board in June 2020 (*So 6.1*).

Two detection methods for the same pospiviroids have also been published by the Naktuinbouw for tomato seed (*SPN-V043*) and pepper seed (*SPN-V044*).

PRINCIPLE

The NSHS and Naktuinbouw methods are based on the same principle:

- Detection of pospiviroids in tomato or pepper seed by a seed extract RT-qPCR (SE-qPCR). If no pospiviroid RNA is detected, the sample is considered free from the target pospiviroids.
- Total RNA is extracted from tomato or pepper seed by using a suitable kit designed to isolate and purify RNA. The possible presence of viroid RNA can be detected by the specific set of primers and labelled TaqMan probes in multiplex RT-qPCR assays. An internal amplification control (IAC) is used to control the quality of RNA extraction and to check for the presence of potential PCR inhibitors in the RNA extracts.

Sample and subsample size

The methods describe a sample size of 3,000 seeds divided into three subsamples of 1,000 seeds or six subsamples of 500 seeds.

Sample preparation

The NSHS method describes the use of either a Geno/Grinder or an IKA Mill to grind seeds (dry grinding). The Naktuinbouw protocol describes the use of a Geno/Grinder for pepper seed (dry grinding) and a BagMixer for tomato seed (wet grinding). If alternative equipment is used, the performance of the sample preparation equipment should be verified.

RNA extraction

The methods indicate that RNA should be isolated and purified from seed extracts with a suitable kit, both NSHS and Naktuinbouw methods cite the commercial names of suitable kits. Alternative kits can be used, the laboratory should verify the performance of RNA extraction kits during in-lab method implementation.

RT-qPCR

Several multiplex RT-qPCR mixes are described in the methods. The pospiviroid-specific primers and probes are identical in both the NSHS and Naktuinbouw methods (Table 1).

Table 1. Summary of RT-qPCR assays described in the NSHS and Naktuinbouw protocols.

Target	Primer/probe source
CEVd*	Monger *et al.*, 2010
CLVd	Monger *et al.*, 2010
PCFVd	Naktuinbouw, SPN-V043/SPN-V044
PSTVd/TCDVd/TPMVd	Boonham *et al.*, 2004
TASVd	Monger *et al.*, 2010
TPMVd	Botermans *et al.*, 2013; Naktuinbouw, SPN-V043/SPN-V044

* Optional in NSHS protocol.

METHOD VALIDATION

The RT-qPCR assays described above have been subject to several validation studies. The initial publications describe experiments designed to evaluate the analytical specificity of the qPCR assays (Boonham *et al.*, 2004 ; Monger *et al.*, 2010). The Naktuinbouw protocols were initially validated by Naktuinbouw for the detection PSTVd and TCDVd (Bakker *et al.*, 2015).

The NSHS method was developed by collaborators from Bayer Crop Science and the Iowa State University Seed Science Center and was approved by the National Seed Health System's Policy & Procedures Advisory Board (June, 2020). A validation report is available on request to NSHS.

NOTES

It should be noted that RT-qPCR assays are "indirect tests" which do not give information about viability/infectivity or pathogenicity. ISF has adopted a position paper about indirect tests (Indirect_seed_health_tests (worldseed.org)).

POSPIVIROID EPIDEMIOLOGY

The ISF regulated pest list database provides a scientific basis to regulating specific pest-crop combination and to limit the regulation of pests to those that are justified. The database is constructed on a thorough scientific assessment of whether seed is a pathway for the entry, establishment and spread of pests that are regulated (Pest Lists (worldseed.org)). According to this database, it is not certain that tomato seed is a pathway for CLVd, PCFVd, TASVd, TCDVd, TPMVd, and PSTVd. The database also indicates that it is not certain that pepper seed is a pathway for PSTVd and PCFVd, and that pepper is not known to be a host for CLVd.

LINKS TO CITED METHODS

SEED HEALTH TESTING METHODS – NSHS

Protocols | Naktuinbouw

REFERENCES

Bakker, D., Bruinsma, M., Dekter, R.W., Toonen, M.A.J., Verhoeven, J.Th.J. and Koenraadt, H.M.S. (2015). Detection of PSTVd and TCDVd in seeds of tomato using real-time RT-PCR. *EPPO Bulletin,* **45**, 14–21.

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Monger, W., Tomlinson, J., Boonham, N., Marn, M.V., Plesko, I.M., Molinero-Demilly,V., Tassus, X., Meekes, E., Toonen, M., Papayiannis, L., Perez-Egusquiza, Z., Mehle, N., Jansen, C. and Nielsen, S.L. (2010). Development and interlaboratory evaluation of real-time PCR assays for the detection of pospiviroids. *Journal of Virological Methods*, **169**, 207–210.

REVISION HISTORY

Version	Date	Changes (minor editorial changes not indicated)
1	February 2018	First version of the protocol.
2	March 2023	Pepper (*Capsicum annuum*) crop added. Recommendation to use NSHS method added. Text on sample and subsample sizes modified. Section *Restrictions on use* removed. Protocol presented in accordance with ISHI protocol guidelines.