International Rules for Seed Testing
2020

Validated Seed Health Testing Methods

7-025: Detection of *Aphelenchoïdes besseyi* in *Oryza sativa* (rice) seed

Including changes and editorial corrections adopted at the Ordinary General Meeting 2019, Hyderabad, India

Effective from 1 January 2020
Validation reports

See References. Copies are available by e-mail from the ISTA Secretariat at ista.office@ista.ch.

Please send comments, suggestions or reports of problems relating to this method to the ISTA Seed Health Committee, c/o ISTA Secretariat.

Disclaimer

Whilst ISTA has taken care to ensure the accuracy of the methods and information described in this method description, ISTA shall not be liable for any loss or damage, etc. resulting from the use of this method.

Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during weighing out of ingredients. It is assumed that persons carrying out this test are in a laboratory suitable for carrying out microbiological procedures and familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic techniques. Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.

Note on the use of the translations

The electronic version of the International Rules for Seed Testing includes the English, French, German and Spanish versions. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.
7-025: Detection of Aphelenchoides besseyi in Oryza sativa (rice) seed

Host: Oryza sativa L.
Pathogen(s): Aphelenchoides besseyi Christie

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Revision history
Version 1.0, 2007-10-08
Version 1.1, 2013-01-01: Definition of sample size
Version 1.2, 2014-01-01: Addition of positive control
Version 1.3, 2017-01-01: Sample and subsample sizes changed; Reporting results revised
Version 1.4, 2020-01-01: Revision of text on extraction and identification criteria based on review by Prof. Gerrit Karssen and Corinne Sarniguet

Background
White tip disease of rice (Oryza sativa L.) caused by Aphelenchoides besseyi Christie (1942) is widely distributed in all rice-growing areas (Fortuner & Williams, 1975). A. besseyi is a seed-transmitted nematode and therefore important from the point of view of quarantine (Gergon & Mew, 1991). The European and Mediterranean Plant Protection Organization (EPPO) has published a simple method to test rice seeds in order to detect A. besseyi in seed lots for quarantine purposes (EPPO, 1998). Until now a standardised method for detecting and estimating numbers of A. besseyi has never been presented to ISTA. Using dehulled seeds for the extraction of the nematodes resulted in an increased number of nematodes compared to the existing EPPO method (Giudici et al., 2003). The suitability of this method for the detection of A. besseyi was confirmed in the peer validation study for this method.

Treated seed
This method has not been validated for the determination of A. besseyi on treated seed. Seed treatments may affect the performance of this method. (Definition of treatment: any process, physical, biological or chemical, to which a seed lot is submitted. See 7.2.3.)

Sample size
The sample (total number of seeds tested) and subsample size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum recommended sample size is 1000 seeds. In any case, the maximum subsample size is 250 seeds.

Materials
Reference material: reference cultures or other appropriate material
Mill: Husker TR-120 (Kett Electric Laboratory, Japan) or equivalent
Containers: beakers 45 mm diameter
Counting dish: any standard nematode counting dish (e.g. De Grisse dish 90 mm diameter)
Sieves: nylon, with meshes of 0.25 mm
Incubator: operating at 25 ±2°C
Microscopes: dissecting microscope, magnification ×50; high-power microscope, magnification ×1000

Sample preparation
The test is carried out on a working sample obtained as described in section 7.4.1 of the ISTA Rules.

Methods
Critical control points are indicated by CCP.
1. Extraction
   1.1 Dehull the seeds by using a mill with a 1 mm distance between the rolls (CCP).
   1.2 Fit a nylon sieve, with a mesh of 0.25 mm, into a beaker of 45 mm diameter and transfer kernels and hulls onto the nylon sieve. Fill this beaker with 20 ml of water.
   1.3 Leave the beaker undisturbed for 24 h at 25 ±2°C.
   1.4 Remove the sieve from the beaker and squeeze it gently to remove excess liquid.
### Quality assurance

#### Critical control points (CCP)

Clean the mill between each sample to prevent cross contamination (Step 1.1).

#### References


Validation references


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**Figure 1.** *Aphelenchoïdes besseyi* showing details of the lip region and male tail (taken from Allen, 1952).
Figure 2. *Aphelenchoides besseyi*: female (A); female head end (B); female *en face* view (C); lateral field (D); variation in excretory pore position (E, F); male anterior end (G); female tail termini variation (H); male tail ends (I–K); post uterine sac variation (L–N) (after Hunt, 1993).