International Rules for Seed Testing 2024

Validated Seed Health Testing Methods

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

Including changes and editorial corrections adopted at the Ordinary General Meeting 2023 in Verona, Italy

Effective from 1 January 2024

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.

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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
Version 1.5, 2021-01-01: Sample size revised
Version 1.6, 2024-01-01: Sample size revised

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 size (total number of seeds to be tested) and subsample size depend on intended use, the maximum acceptable infection level and the analytical sensitivity of the method. The minimum recommended working sample size is 1000 seeds and the maximum subsample size must be 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

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.

1.5 Baermann funnel or Oostenbrink dish can be used as alternative extraction methods (EPPO).
2. Examination
2.1 Pour water sample from the beaker into a counting dish.
2.2 Allow the sample to stand for at least 20 min to allow any nematodes to settle to the bottom of the counting dish.
2.3 Count both juveniles and adults of *A. besseyi*, in the counting dish under the dissecting microscope (magnification ×50)(see General methods).
3. Confirmation/identification of suspect nematodes
3.1 Confirm the identification at a higher magnification of ×1000.
3.2 *A. besseyi* is a bisexual nematode (males are common): females (0.62–0.88 mm) are usually slightly longer than males (0.44–0.72 mm). The body is slender with a slightly offset lip region, stylet 10–13 µm long. Lateral fields with four incisures. Excretory pore near anterior edge of the nerve ring. Vulva transverse with slightly raised lips, usually between 65 % and 75 % of the body length. Spermatheca elongated oval, filled with sperm. Post-uterine sac is short, the length of the post uterine sac measures 2.5–3.5 times the width of the anal body (Hunt, 1993; EPPO, 2017). Tail conoid, length measuring 3.5–5 times the width of the anal body, armed with three to four mucronate processes (Fig. 1, 2). Compare with positive control.
See Hockland (2001) or EPPO (2017) for an overview of morphologically comparable *Aphelenchoides* species or Sánchez-Monge *et al*. (2015) for an overview of plant-parasitic *Aphelenchoides* species.

General methods

Reporting results: The result of a seed health test should indicate the scientific name of the pathogen detected and the test method used. When reported on an ISTA Certificate, results are entered under 'Other Determinations'.
The report must indicate the number of seeds tested.
In the case of a negative result (pathogen not detected in any subsample), the results must be reported as 'not detected'.
In the case of a positive result, the report must indicate the mean number of nematodes per subsample and the number of positive subsamples out of the total number tested.

Quality assurance

Critical control points (CCP)

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

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ISTA (2007). Proposal for a new method for the detection of *Aphelenchoides besseyi* Christie in *Oryza sativa* L. seeds. *Method Validation Reports*. International Seed Testing Association, Bassersdorf, Switzerland.

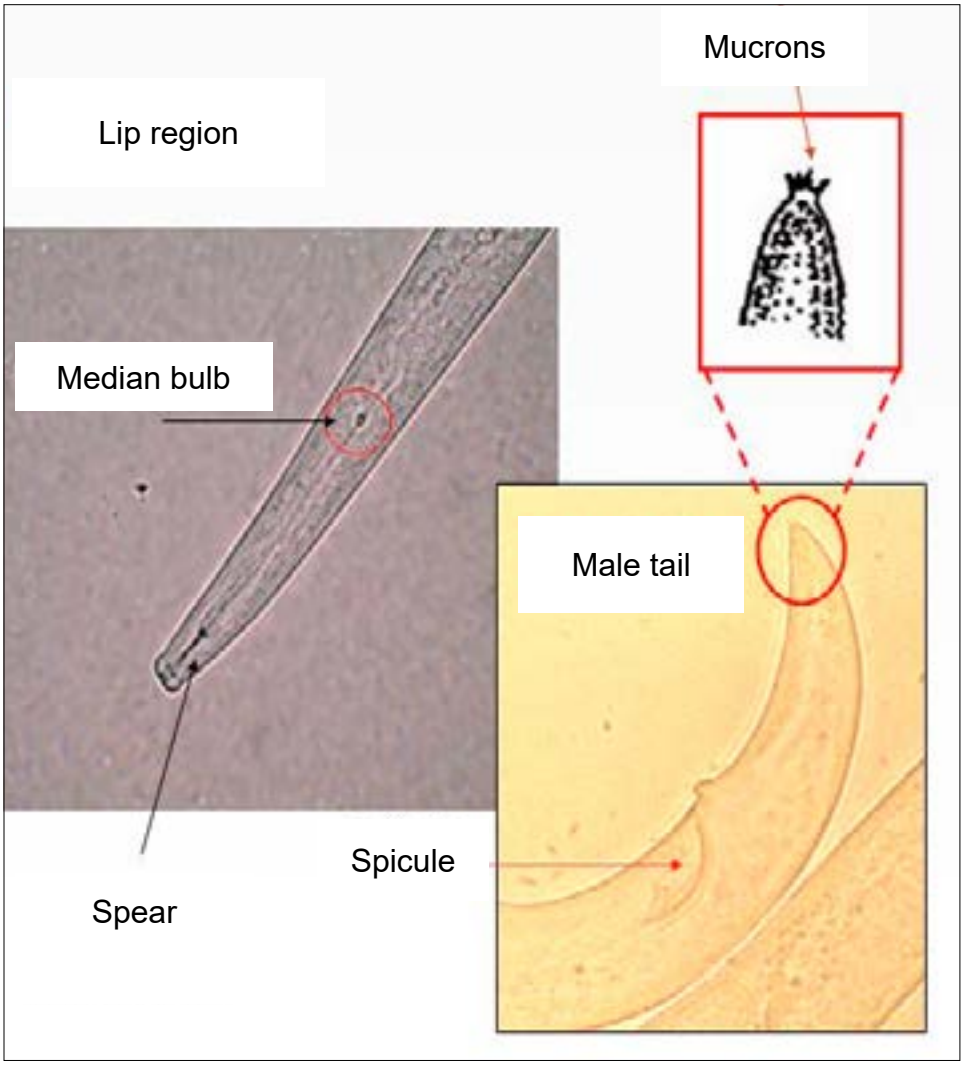

Figure 1. *Aphelenchoides besseyi* showing details of the lip region and male tail (taken from Allen, 1952).

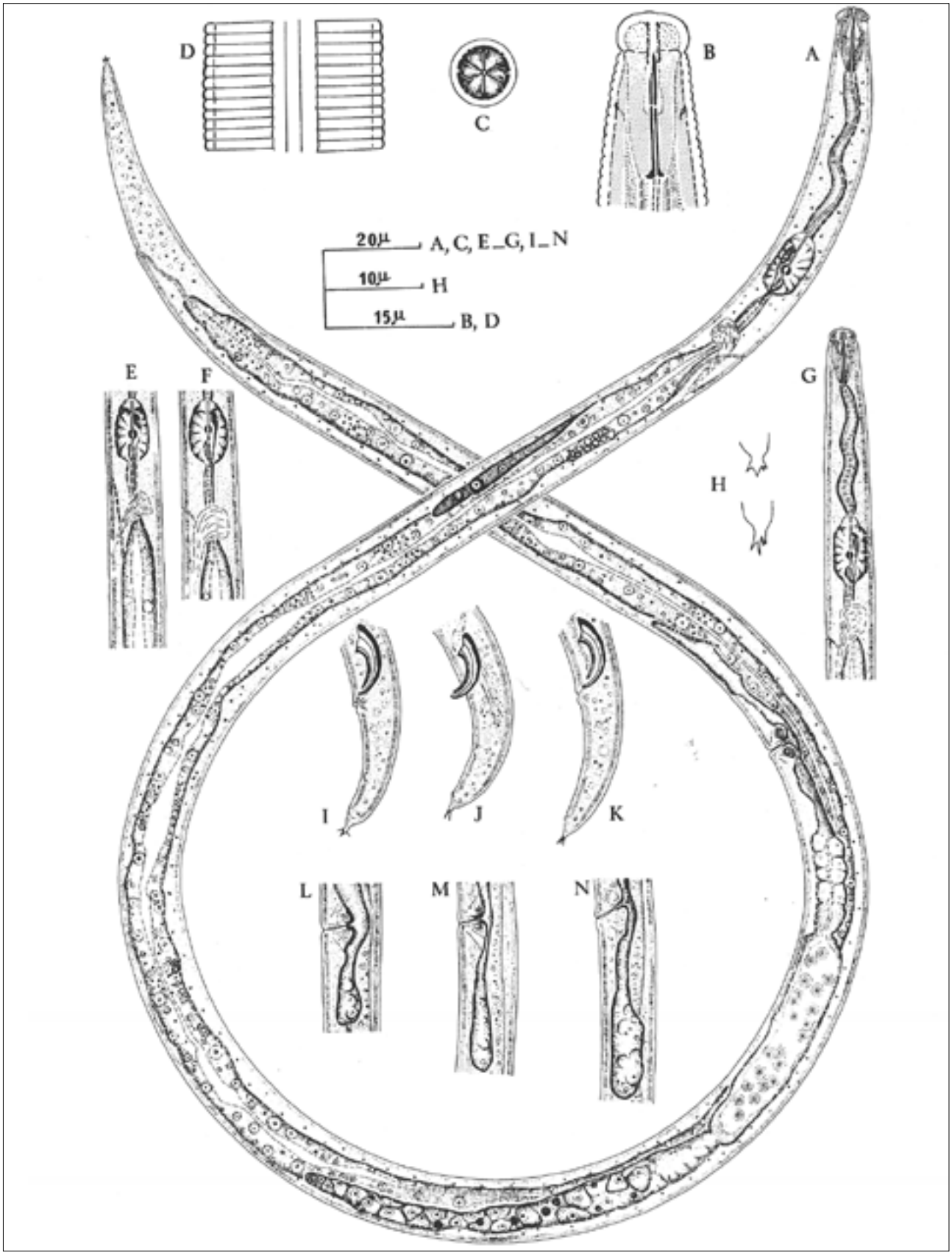

Figure 2. *Aphelenchoides besseyi*: female (A); female head end (B); female *en face* view (C); lateral field (D); variation in excretory pore position (E, F); male anterior end (G); female tail termini variation (H); male tail ends (I–K); post uterine sac variation (L–N) (after Hunt, 1993).