International Rules for Seed Testing 2019

Validated Seed Health Testing Methods

7-010: Detection of *Bipolaris oryzae* in *Oryza sativa* (rice) seed

Including changes and editorial corrections adopted at the Ordinary General Meeting 2018, Sapporo, Japan

Effective from 1 January 2019

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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7-010: Detection of *Bipolaris oryzae* in *Oryza sativa* (rice) seed

Host: *Oryza sativa* L.

Pathogen(s): *Bipolaris oryzae* (Breda de Haan) Shoem., syn. *Drechslera oryzae*, syn. *Helminthosporium oryzae* Breda de Haan (Perfect state *Cochliobolus miyabeanus* (Ito & Kurib.) Drechsler ex Dastur, syn. *Ophiobolus miyabeanus* Ito & Kuribayashi)

Authors: ISTA-PDC Method Validation Sub-committee

Revision history

Version 1.0, 2000-07-13
Revised 2001-11-20 J. Sheppard
Reprinted 2003
Version 1.1, 2008-01-01: Treated seed revised; Reporting results revised
Version 1.2, 2014-01-01: Clarification of blotter preparation and incubation; addition of positive control
Version 1.3, 2016-01-01: New Figure 3
Version 1.4, 2017-01-01: Reporting results revised
Version 1.5, 2018-01-01: Changes to the taxonomic names of fungi

Background

This method was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 11. The method was incorporated into the newly revised *Annexe to Chapter 7* in 2002 from the 1999 edition of the ISTA Rules. The method was reviewed by the ISTA-Seed Health Committee in 2006 (Cockerell & Koenraadt, 2007) with the recommendation to accept for a further five years.

Treated seed

This method has not been validated for the determination of *Bipolaris oryzae* on treated seed. Seed treatments may affect the performance of the method. (Definition of treatment: any process, physical, biological or chemical, to which a seed lot is subjected, including seed coatings. See 7.2.3.)

Materials

Reference material: reference cultures or other appropriate material
Media: blotters (filter paper), e.g. Whatman No. 1 or equivalent
Petri dishes: When sowing density is given by a number of seeds per Petri dish, a diameter of 90 mm is assumed.
Incubator: capable of operating in the range 22 ±2 °C. To stimulate sporulation, alternating 12 h periods of darkness and near-ultraviolet light (NUV) during incubation are recommended. The recommended source is the black light fluorescent lamp (peak at 360 nm) but daylight fluorescent tubes are satisfactory.

Sample preparation

The test is carried out on a working sample of 400 seeds as described in Section 7.4.1 of the ISTA Rules.

Methods

1. Pretreatment: None.
2. Plating:

2.1 Place three layers of 90 mm filter paper in each plate and soak with sterile distilled/deionised water. Drain away excess water.

2.2 Aseptically place 25 seeds, evenly spaced, on the surface of the filter paper in each dish.

2.3 Positive control (reference material): Aseptically place seeds in an appropriate number of plates to obtain the reference culture, or plate a reference culture on media. The number of plates required will depend on the level of contamination of the positive control seed lot.

3. Incubation: 7 days at 22 °C in NUV in 12 h light/12 h dark cycle. If the filter paper dries out during incubation, add an appropriate amount of sterile distilled/deionised water onto the paper, usually after 3 days of incubation. Avoid touching the seeds as adding water can cause cross-contamination.

4. Examination: Examine each seed at ×12–50 magnification for conidia of *B*. *oryzae*. Conidiophores of the fungus are produced on the seed coat and also on light grey aerial mycelium covering whole or part of the seed, giving a fluffy appearance. The fungus may occasionally spread on to the blotters. In doubtful cases confirmation may be made by examining conidia at ×200 magnification. Conidia are crescent-shaped 35–107 µm × 11–17 µm (Fig. 1) light brown to brown, widest in the middle or below the middle and tapering to rounded ends. Compare with positive control.

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in result within or between tests is sufficiently wide as to raise doubts about the accuracy of the results. Suitable tolerances, which can be applied to most direct seed health tests, can be found in Tables 5B of Chapter 5 of the ISTA Rules, or in Miles (1963).

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), the results must be reported as 'not detected'.

In the case of a positive result, the report must indicate the percentage of infected seeds.

Quality assurance

Critical control points (CCP)

None listed.

References

The following references are extracted from the *ISTA Handbook of Seed Health Testing*, Working Sheet No. 11, 1964.

Azeemudin, Soraya & Ponchet, J. (1961). Isolement de *Piricularia oryzae* (Br. Cav.) et de *Helminthosporium oryzae* Breda de Haan à partir de semences de riz *Oryza sativa* L. *Annis. Epiphyt.,* **12**, 141–147.

Miles, S. R. (1963). Handbook of tolerances and of measures of precision for seed testing. *Proceedings of the International Seed Testing Association*, **28** (3), 525–686.

Neergaard, P. & Saad, A., (1962). Seed health testing of rice. A contribution to development of laboratory routine testing methods. *Indian Phytopathology,* **15**, 85–111.

Validation references

Studied in international comparative testing: 1960, 1963, 1964.

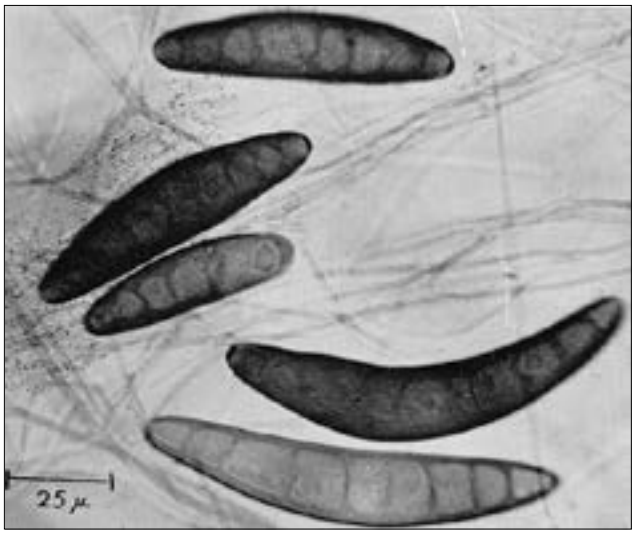

Figure 1. Conidia.

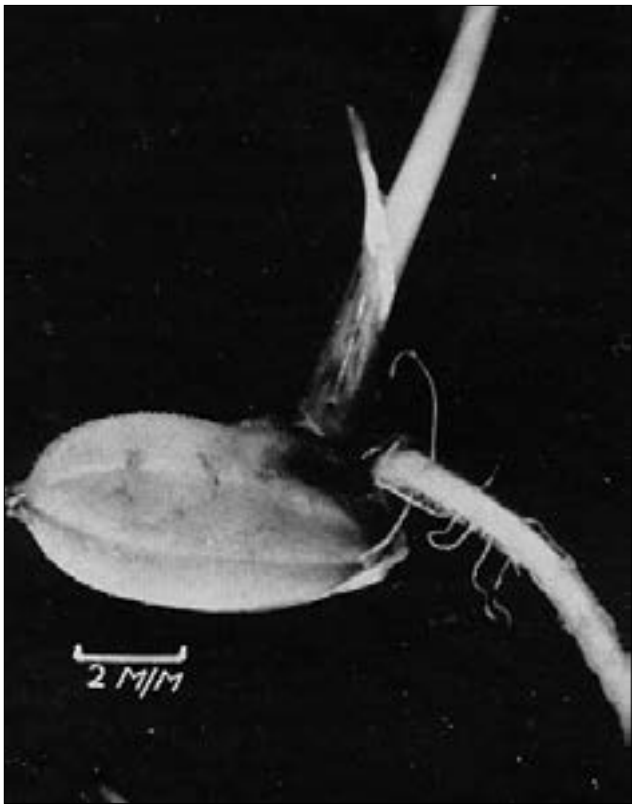

Figure 2. Seedling in blotter test, lesion on coleoptile.

Figure 3. Conidiophores and conidia on rice seed.