International Rules for Seed Testing 2027

Validated Seed Health Testing Methods

7-011: Detection of *Pyricularia oryzae* in *Oryza sativa* (rice) seed

Including changes and editorial corrections adopted at the Ordinary General Meeting 2026 in Calgary, Canada

Effective from 1 January 2027

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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7-011: Detection of *Pyricularia oryzae* in *Oryza sativa* (rice) seed

Host: *Oryza sativa* L.
Pathogen(s): *Magnaporthe grisea* (Hebert) Barr (imperfect state *Pyricularia oryzae* Cavara), syn. *P. grisea*

Prepared by: ISTA-PDC Method Validation Subcommittee

Authors: ISTA-PDC Method Validation Subcommittee

Revision history

Version 1.0, 2000-07-13
Revised 2001-11-20: J. Sheppard, V. Cockerell
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 1
Version 1.4, 2017-01-01: Reporting results revised
Version 1.5, 2021-01-01: Sample preparation changed to Sample size and paragraph revised
Version 1.6, 2024-01-01: Sample size revised
Version 1.7, 2027-01-01: Flowchart added in Methods

Background

This method was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 12 and revised in 1981 by S. B. Mathur, Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark. 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 *Pyricularia 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.)

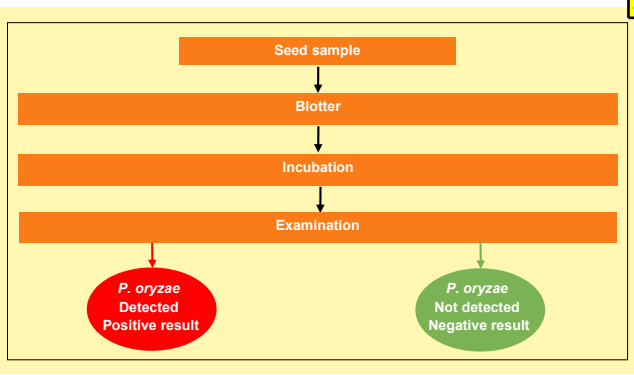
Sample size

The sample size (total number of seeds to be tested) depends on intended use, the maximum acceptable infection level and the analytical sensitivity of the method. The minimum sample size should be 400 seeds.

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

Methods



1. Pretreatment: None.
2 Blotter method:
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 alternating cycles of 12 h darkness and 12 h light, preferably NUV. 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 *P. oryzae*. Generally, the fungus produces small, inconspicuous, grey to green colonies on glumes (Fig. 1), consisting of short, delicate, conidiophores carrying clusters of conidia at their tips. The growth rarely covers the whole seed. In doubtful cases confirmation may be made by examining conidia at ×200 magnification. Conidia are typically obpyriform (Fig. 2), hyaline, truncated with a short tooth at the base, 2-septate, usually with a pointed acute apex, 20–25 µm × 9–12 µm.

Notes: For correct identification of *P. oryzae* on seed, it is essential that the seed is very carefully examined under a stereomicroscope between ×25–50 magnification. Care must be taken not to confuse *Pyricularia* growth with that of a common saprophyte, *Cladosporium*. Clusters of a few conidia with acute tips, on short pale conidiophores, viewed under stereomicroscope, are diagnostic for *Pyricularia*. In *Cladosporium*, the numerous conidia are grouped as in a 'brush' on comparatively long, dark conidiophores. In doubtful cases, conidia must be examined at higher magnifications, ×200–400. Compare with positive control.

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in results within or between tests are 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 Table 5B Part 1 of Chapter 5 of the ISTA Rules, or Table G1 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. 12, S. B. Mathur, 1981.

Handbook of Seed Health Testing, Series 3, 1966, *Proceedings of the International Seed Testing Association*, **30**, 1045–1115.

Mahdi, M. T. (1968). Comparative study for evaluating methods for detecting *Pyricularia oryzae* Cav. on rice seed. *Proceedings of the International Seed Testing Association*, **33**, 603–607.

Mathur, S. B. (1973). *Report on Comparative Seed Health Testing of Rice and Maize conducted by the members of the Working Group on Tropical and Subtropical Cereals.* Presented at the Fourteenth International Workshop on Seed Pathology, held in Minneapolis, U.S.A., 37 p. Mimeograph. Copenhagen.

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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., Lambat, A. K. & Mathur, S. B. (1970). Seed Health testing of rice III. Testing procedures for detection of *Pyricularia oryzae* Cav. *Proceedings of the International Seed Testing Association*, **35**, 157–163.

Ou, S. H. (1972). *Rice Diseases*. Commonwealth Mycological Institute, Kew, Surrey, England. 368 pp.



Figure 1. Growth of *P. oryzae* on rice glumes.



Figure 2. Conidia and conidiophores of *P. oryzae*. ×750.

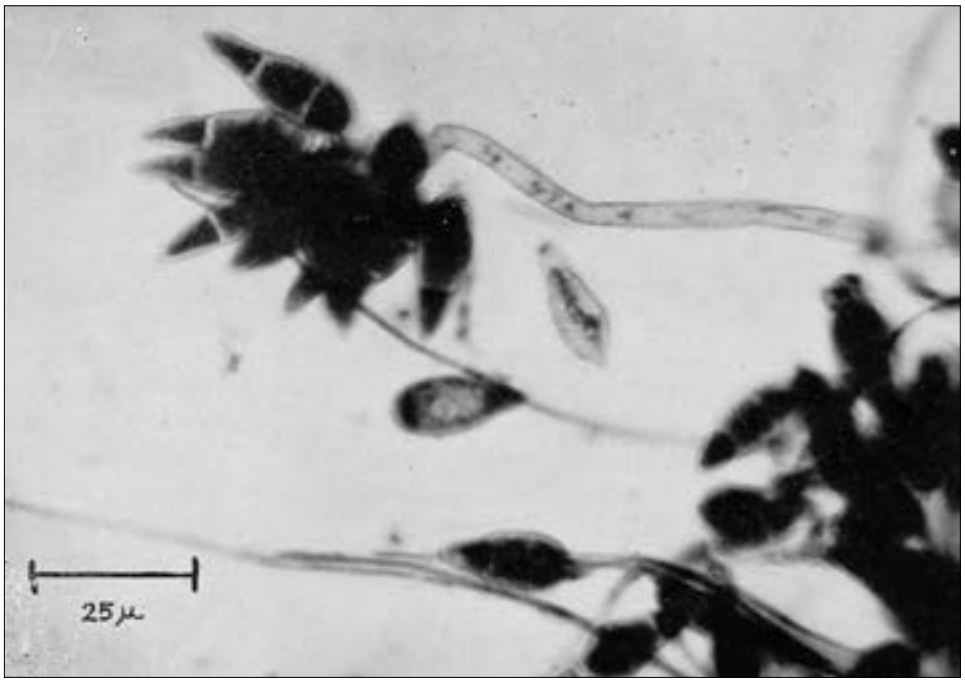


Figure 3. Conidia and conidiophore.

Suzuki, H. (1930). Experimental studies on the possibilities of primary infection of *Pyricularia oryzae* and *Ophiobolus miyabeanus* internal of rice seeds. *Annals of the Phytopathological Society of Japan,* **2,** 245–275. (*Review of Applied Mycology*, **9**, 556).

Validation references

Studied in international comparative testing: 1960, 1963, 1964, 1973 und 1978.