



International Rules for Seed Testing
Annexe to Chapter 7: Seed Health Testing Methods



7-011: Detection of *Pyricularia oryzae* on *Oryza sativa* (Rice)

Published by: International Seed Testing Association (ISTA), Bassersdorf, Switzerland
2008

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 damage etc., resulting from the use of this method.

- Crop:** *Oryza sativa* (Rice)
- Pathogen:** *Magnaporthe grisea* (Hebert) Barr [teleomorph]
Pyricularia oryzae Cavara syn. *Piricularia grisea*
[anamorph]
- Prepared by:** ISTA-PDC Method Validation Working Sub-committee
- Revision History:** Version 1.0 July 13, 2000
Revised 20.11.2001 J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.1 2008-01-01
“Treated seed” revised; “Reporting results” revised
- Submitted by:** ISTA-PDC Method Validation Working Sub-committee

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 & Koenraad, 2007) with the recommendation to accept for a further five years.

Studied in International Comparative Testing: 1960, 1963, 1964, 1973 and 1978.

Safety Precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during preparation of media, autoclaving and weighing out of ingredients. It is assumed that this procedure is being carried out in microbiological laboratory by persons familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic technique. Dispose of all waste materials in an appropriate way (e.g. autoclave, disinfect) and in accordance with local safety regulations.

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)

Materials

- | | |
|---------------------------|---|
| Reference Material | - The use of reference cultures or other appropriate material is recommended when ever possible. |
| Media | - Blotters (filter paper) |
| 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 <i>black light</i> 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.

Method

1. *Pretreatment*
None.
2. *Blotter Method*
On water soaked blotters in Petri dishes. Place 25 seeds in each dish.
3. *Incubation*
7 days at 22 °C in alternating cycles of 12 h darkness and 12 h light, preferably NUV.
4. *Examination*
Examine each seed at x12-50 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 $\times 200$ magnification. Conidia are typically obpyriform (Fig. 2), hyaline, truncated with a short tooth at the base, 2-septate, usually with a pointed acute apex, $20\text{--}25\ \mu\text{m} \times 9\text{--}12\ \mu\text{m}$.

Notes:

For correct identification of *P. oryzae* on seed, it is essential that the seed is very carefully examined under a stereoscopic microscope between $\times 25\text{--}50$ 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 stereoscopic microscope, 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, $\times 200\text{--}400$.

General Methods (common to many test procedures)

1. 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 the *Handbook of Tolerances and Measures of Precision for Seed Testing* by S.R. Miles (*Proceedings of the International Seed Testing Association* 28 (1963) No 3, p 644).

2. 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 International Seed Analysis Certificate*, results are entered under Other Determinations.

Quality Assurance

Critical Control Points

None listed

References

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 12, S. B. Mathur, 1981.

Handbook on Seed Health Testing, Series 3, 1966, Proc. int. Seed Test. Assoc., 30, 1045-1115.

Mahdi, M. T. (1968). Comparative study for evaluating methods for *detecting Pyricularia oryzae* Cav. on rice seed. Proc. int. Seed Test. Assoc., 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.

Mathur, S. B. (1978). Report on the Working Group on Tropical and Subtropical Crops. In the Report on the Sixteenth International Workshop on Seed Pathology, ISTA-PDC, Karlsruhe. 63 p. Mimeograph, Karlsruhe.

Neergaard, Paul, Lambat, A. K. and Mathur, S. B. (1970). Seed Health testing of rice III. Testing procedures for detection of *Pyricularia oryzae* Cav. Proc. int. Seed Test. Ass., 35, 157-163.

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

Suzuki, H. (1930). Experimental studies on the possibilities of primary infection of *Pyricularia oryzae* and *Ophiobolus miyabeanus* internal of rice seeds. Ann. phytopath. Soc. Japan, 2, 245-275. (Rev. appl. Mycol., 9, 556).



Fig. 1. Growth of *P. oryzae* on glumes. $\times 100$



Fig. 2. Conidia and conidiophores of *P. oryzae*. x750



Fig. 3. Conidia and conidiophore