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

7-010: Detection of *Drechslera oryzae* on *Oryza sativa* (Rice)

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Crop: *Oryza sativa* (Rice)

Pathogen: *Drechslera oryzae* (Breda de Haan) Subram. & Jain, *Cochliobolus miyabeanus* (Ito & Kurib.) Drechsler ex Dastur [teleomorph] syn. *Ophiobolus miyabeanus* Ito & Kuribayashi, *Bipolaris oryzae* (Breda de Haan) Shoem., *Helminthosporium oryzae* Breda de Haan

Prepared by: ISTA-PDC Method Validation Sub-committee

Revision History: Version 1.0 July 13, 2000
Revised 20.11.2001 J. Sheppard
Reprinted 2003
Version 1.1 2008-01-01
"Treated seed" revised; "Reporting results" revised

Submitted by: ISTA-PDC Method Validation Sub-committee

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.

Studied in International Comparative Testing: 1960, 1963, 1964.

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 *Drechslera 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 ºC. 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.

Method

1. *Pretreatment*

   None
2. *Blotter*

   On water-soaked blotters in Petri dishes. Place 25 seeds in each dish.
3. *Incubation*

   7 days at 22 ºC in NUV in 12 h light/12 h dark cycle.
4. *Examination*

   Examine each seed at ×12–50 magnification for conidia of *D. 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.

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. 11, 1964.

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

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

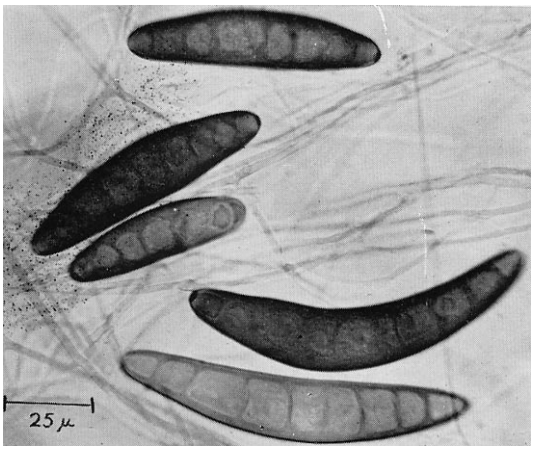

Fig. 1. Conidia

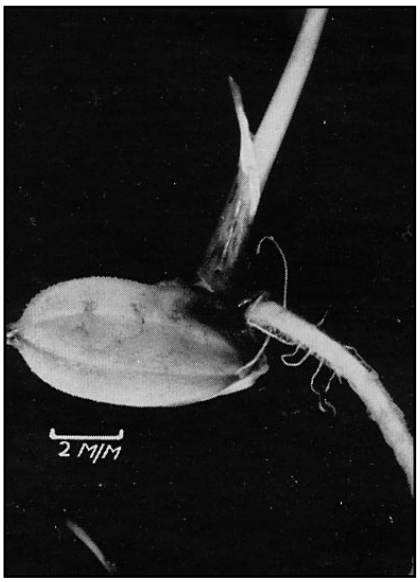

Fig. 2. Seedling in blotter test, lesion on coleoptile