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

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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 blotter method appears in Annexe 7.4.3.A.7 of the ISTA Rules (1999). It has been incorporated into the new Annexe to Chapter 7, Seed Health Testing Methods as method 7-010 and is subject to review before 2006.

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 may be influenced by treatment applied to the seed lot. Seed health tests on chemically treated seeds will generally deliver unreliable test results caused by masking or inhibition of the growth of the target organism.

Materials

Reference Material	-	The use of reference cultures or other appropriate material is recommended when ever possible.
Media	-	Blotters
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 22ºC ± 2ºC. To stimulate sporulation, alternating 12-hour 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 22ºC in Near Ultra Violet light in 12 hr. light/12 hr. 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 x200 magnification. Conidia are crescent-shaped 35-107µ x 11-17µ (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. A tolerance table, which can be applied to most direct seed health tests, can be found in Table 5.1 of Annex 15 of the ISTA Rules or in Table G1 of 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, pp 644).

2. Reporting Results

   The result of a seed health test should indicate the scientific name of the pathogen detected and the percentage of infected seeds. When reported on an ISTA Certificate results are entered under Other Determinations. he results should be accompanied by information on the test method used, including any pretreatment.

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* oryzae 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.

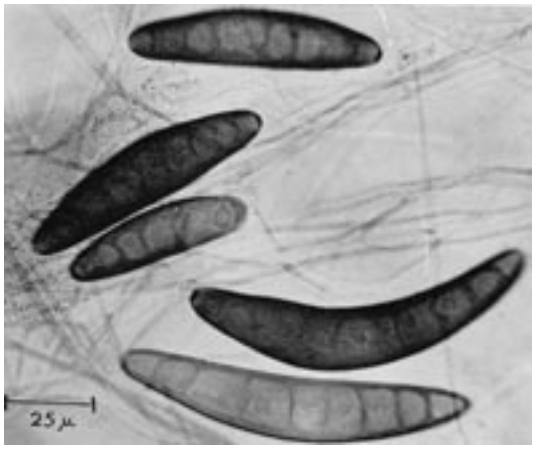

Fig. 1. Conidia

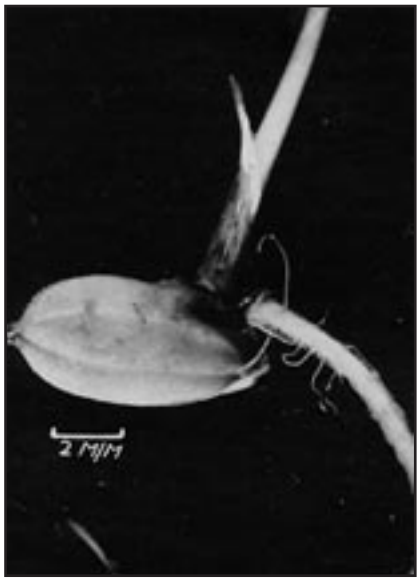

Fig. 2. Seedling in blotter test, lesion on coleoptile