International Rules for Seed Testing
2021

Validated Seed Health Testing Methods

7-006: Detection of Colletotrichum lindemuthianum in Phaseolus vulgaris (bean) seed

Including changes and editorial corrections adopted at the online Ordinary General Meeting 2020

Effective from 1 January 2021
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.
7-006: Detection of *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* (bean) seed

**Host:** *Phaseolus vulgaris* L.
**Pathogen(s):** *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav.

**Submitted by:** ISTA-PDC Method Validation Sub-committee
**Authors:** ISTA-PDC Method Validation Sub-committee

**Revision history**

Version 1.0, November 19, 2001
Revised 19.11.2001 J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.1, 2008-01-01: Treated seed revised;
Reporting results revised
Version 1.2, 2014-01-01: Addition to Background and caption of Figure 1
Version 1.3, 2017-01-01: Reporting results revised
Version 1.4, 2021-01-01: Sample preparation changed to Sample size and paragraph revised; Media and solutions revised

**Background**

This method was originally published in the *ISTA Handbook of Seed Health Testing* in 1981 as Working Sheet No. 45 prepared by C. Anselme & R. Champion, La Minière, France. 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. Lesions on severely infected seeds may be either brown with whitish centres surrounded by a pale brown to dark brown area or reddish lesions of variable size (Fig. 1). Direct inspection is not considered as a dependable method, as not all infected seeds bear symptoms and on dark-skinned varieties symptoms are more difficult to see.

**Treated seed**

This method has not been validated for the determination of *Colletotrichum lindemuthianum* 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 (total number of seeds tested) size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum sample size should be 400 seeds.

**Materials**

**Reference material:** reference cultures or other appropriate material
**Media:** paper towelling
**Sodium hypochlorite solution** (1 % available chlorine): for seed disinfection
**Incubator:** capable of operating in the range 20 ±2 °C

**Methods**

1. Pretreatment: Seeds are submerged in a solution of 1 % (available chlorine) sodium hypochlorite for 10 min and allowed to drain.
2. Between paper (BP): Spread the seeds in replicates of 50 on double sheets of paper towelling 350 × 450 mm which have been soaked in water. Cover seeds with one sheet of paper towelling soaked in water. Fold the paper twice lengthways and cover it with a sheet of polythene to maintain the moisture during incubation.
3. Incubation: 7 days at 20 °C in darkness
4. Examination: After 7 days remove the seed coats and examine the cotyledons by naked eye for black depressed areas with well delimited outlines (Fig. 2). Check each spot for the presence of acervuli with or without dark brown setae using ×25 magnification (Fig. 3). The septate setae measure approx. 6 µm × 100 µm. The pale orange acervuli contain cylindric, hyaline conidia with rounded ends containing one or two guttulae. Conidia measure 2.5–5.5 µm × 11–20 µm (Mordue, 1971; Kulshrestha, Mathur, & Neergaard, 1976). The use of a high-power microscope (magnification ×200) is sometimes necessary.
(Fig. 3). Small spots may require longer incubation for development of acervuli.

**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.

**Media and solutions**

**Sodium hypochlorite solution**

Sodium hypochlorite for pretreatment of seed can be prepared from commercial bleach diluted to 1 % available chlorine. The concentration of chlorine in commercial bleach varies considerably and declines with storage. Use the formula:

\[
V_{stock} = V_{final} \times C_{final} / C_{stock}
\]

(\text{where } V = \text{volume} \text{ and } C = \% \text{ available chlorine}) to calculate the volume of commercial bleach stock solution required to prepare sodium hypochlorite solutions for use in seed pretreatment.

To prepare a 1 l solution of sodium hypochlorite containing 1 % chlorine from a stock of commercial bleach containing 12 % available chlorine:

\[
V_{stock} = 1 \times 1/12 = 0.083
\]

Thus add 83 ml of the 12 % stock to 917 ml water.

The percentage of active chlorine decreases rapidly in solution so, NaClO 1 % solution must be stored in the dark and used within 3 days of preparation. It is possible to check chlorine concentration with chlorine strip tests.

**References**

The following references are extracted from the *ISTA Handbook of Seed Health Testing*, Working Sheet No. 45, C. Anselme & R. Champion, 1981.


**Validation references**

**Studied in ISTA comparative tests**: 1962
Figure 1. Dry, white bean seeds showing symptoms. Lesions on severely infected seeds may be either brown with whitish centres surrounded by a pale brown to dark brown area, or reddish lesions of variable size. Direct inspection is not considered as a dependable method, as not all infected seeds bear symptoms, and on dark-skinned varieties, symptoms are more difficult to see.

Figure 2. Cotyledons of seedlings after 7 days incubation with seed coats removed, showing black areas with well defined outline.
Figure 3. Acervuli with cylindric, hyaline conidia and dark brown septate setae. ×150.