Method for the Detection of *Phoma lingam* on *Brassica* spp. seed

Crop	***Brassica* spp. (broccoli, cabbage, calabrese, canola, cauliflower, oilseed rape)**
Pathogens	*Phoma lingam* (Tode ex Fr.) Desm. (Synonym: *Plenodomus lingam* (Tode ex Fr.) Hohn. Teleomorph*: Leptosphaeria maculans* (Tode ex Fr.) Ces. & de Not.) *Plenodomus biglobosus* (Shoemaker & H. Brun) (Synonym: *Leptosphaeria biglobosa* (Shoemaker & H. Brun)
Revision history	Version 3, July 2017

Sample and sub-sample size

The test is done on a minimum sample size of 1000 seeds and a maximum sub-sample size of 100 seeds.

Principle

- Detection of *P. lingam* by incubation of seeds on a blotter or on malt agar
- Identification of the fungus by pycnidia and spore morphology

Restrictions on Use

- This test method is suitable for untreated seed.
- This test method is suitable for seed that has been treated using physical (hot water) or chemical (chlorine) processes with the aim of disinfestation and disinfection, provided that any residue, if present, does not influence the assay. It is the responsibility of the user to check for such antagonism and/or inhibition by analysis, sample spiking, or experimental comparisons.
- The ability to detect *P. lingam* can be influenced by the presence of other fungi. This can influence the reliability of the test.
- This test method has not been validated for seed treated with protective chemicals or biological substances. If a user chooses to test treated seed using this method, it is the responsibility of the user to determine empirically (through analysis, sample spiking, or experimental comparisons) whether the protective chemicals or biological substances have an effect on the method results.

Validation

The reference method for the detection of *P. lingam* (ISTA Rule 7-004) uses 2,4-Dichlorophenoxyacetic acid (2,4-D) to prevent seed germination. As 2,4-D is toxic and its use is not recommended in routine tests, two alternatives were developed: deep freezing and the use of malt agar.

Results of a comparative test of these alternatives to 2,4-D were validated by ISTA, see www.seedtest.org >>Technical Committees >>Seed Health Committee >>Testing

Note: the section **Validation** has been modified. A new section **Method Execution** has been added. The method validated by ISTA in 2017 is version 2 of ISTA Rule 7-004.

Methods >>Method Validation. The revised method was adopted as an ISTA Rule (7-004 version 2) in June 2017.

Method Execution

To ensure process standardization and valid results, it is strongly recommended following the best practices developed by ISHI-Veg for *Dilution Plating Assays in Seed Health Tests* (see http://www.worldseed.org/our-work/phytosanitary-matters/seed-health/ishi-veg/).

Method description

See www.seedtest.org (>>Technical Committees >>Seed Health Committee >>Testing Methods).