Method for the Detection of *Xanthomonas campestris* pv. *campestris* in disinfested / disinfected seed of *Brassica* spp.

Crop:	*Brassica* spp. (broccoli, cabbage, calabrese, canola, cauliflower, oilseed rape)
Pathogen:	*Xanthomonas campestris* pv. *campestris* (*X. c.* pv. *campestris* or Xcc)
Date:	July 2017

Sample and sub-sample size

The recommended minimum sample size is 30,000 seeds with a maximum sub-sample size of 10,000 seeds.

Background

The test method for detecting *Xanthomonas campestris* pv. *campestris* (Xcc) in untreated *Brassica* spp. seed (published as method 7-019a in the ISTA Rules (1)) involves extraction of the bacteria by soaking seed. An adaptation of this method involving wet grinding of the seed has been found to strongly enhance the detection of Xcc in disinfested/disinfected seed. Wet grinding disinfested/disinfected Brassica spp. seed facilitates the detection of internally located propagules of *Xanthomonas campestris* pv. *campestris* (Xcc) that may have survived the disinfestation/disinfection treatment.

It is emphasized that the method for testing untreated seed remains unchanged. It can detect bacteria located on the surface and in the funiculus of untreated seed, as soaking seed readily releases them. However, seed that has been treated to disinfest or disinfect them must be tested using the protocol described in this method to detect internal infections.

Principle

- The test is based on grinding seeds in buffer and plating the liquid obtained on semi-selective media. Grinding allows access to and detection of bacteria in the seed that may have survived disinfestation/disinfection.
- Confirmation of suspect bacterial colonies is completed by a pathogenicity assay or a PCR method.

Sensitivity and Restrictions on Use

- This test method is suitable for seed that has been treated using physical (hot water) or chemical (chlorine) processes with the aim of disinfestation/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 recover Xcc on plates can be influenced by the presence of other micro-organisms. It is the responsibility of the user to check for such antagonism

Note: The method was reviewed recently and found to be fit for purpose. The section **Validation** has been updated. A new section **Method Execution** has been added.

and/or inhibition by analysis, sample spiking, or experimental comparisons.

- 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.
- Dry grinding the seeds followed by the addition of an extraction buffer has been found to be inadequate. Soaking followed by wet grinding is a prerequisite.
- Comparative tests have shown higher numbers of positive subsamples using a buffered extraction solution when compared to a non-buffered solution (saline). A drop in pH is consistently measured when Brassica seeds are ground and buffering compensates for this reduction in pH.

Validation

Results of an ISHI–Veg comparative test were validated by ISTA, see www.seedtest.org (>>Technical Committees >>Seed Health Committee >>Testing Methods >>Method Validation). The method was adopted as an ISTA Rule (7-019b) in June 2013 and came into force in January 2014.

The method has also been approved by the US National Seed Health System (NSHS) as a Standard A (see http://seedhealth.org/seed-health-testing-methods/).

Method Execution

To ensure process standardization and valid results, it is strongly recommended to follow the best practices developed by ISHI-Veg for *Dilution Plating Assays in Seed Health Tests* and *Molecular Techniques 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)

References

1. Roberts, S.J. and Koenraadt, H (2005). Detection of *Xanthomonas campestris* pv. *campestris* on *Brassica* spp. Method description 7-019 (2005). ISTA International Rules for Seed Testing, Annex to Chapter 7 Seed Health Testing Methods 2003.