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Document OGM19-11

OGM19-11 ISTA rules proposals method 7-019a

This document was prepared by the ISTA Seed Health Committee and has been endorsed by the ISTA Executive Committee (ECOM). The proposal is submitted to the ISTA Ordinary General Meeting 2019 for voting by the nominated ISTA Designated Members on behalf of their respective Governments.
It is submitted to all ISTA Designated Authorities, ISTA Members and ISTA Observer Organizations for information two months prior to the ISTA Ordinary General Meeting 2019.
It contains proposed changes for ed. 7 of the ISTA List of Stabilised Plant Names and will be discussed and voted on at the Ordinary General Meeting 2019 to be held on Tuesday, July 02, 2019 in Hyderabad, India under Agenda point 10. Consideration and Adoption of the Proposed Rules Changes.

Revised introduction

7-019a: Detection of *Xanthomonas campestris* pv. *campestris* and *Xanthomonas campestris pv. raphani* in *Brassica* spp. seed

Host: *Brassica* spp.

Pathogen(s): *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson and *Xanthomonas campestris pv. raphani*

Prepared by: International Seed Health Initiative for Vegetable Crops, ISF (ISHI-Veg)

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Revision history

Version 1.0, 2003-05-13

Version 2.0, 2004-08-06 (Koenraadt *et al.* 2004)

Version 3.0, 2006-07-05

Version 3.1, 2010-01-01: Editorial change: correction of autoclaving pressures

Version 4.0, 2013-01-01: Addition of PCR test; definition of sample size (Grimault *et al.* 2012)

Version 4.1, 2014-01-01: Renumbered 7-019a ~~(Sato *et al.* 2013)~~

Version 5.0, 2015-01-01 (Sato *et al.* 2013)

Version 5.1, 2017-01-01: Materials: numbers of Petri dishes for media deleted

Version 6.0, 2018-01-01: Addition of pre-screening methods and addition of a TaqMan assay ~~as a third option for suspect screening~~ for identification of suspect colonies (Bruinsma *et al.* 2018)

Version 6.1, 2020-01-01: Revision of the title, improved explanation of the method in the background and editorial changes to the method description.

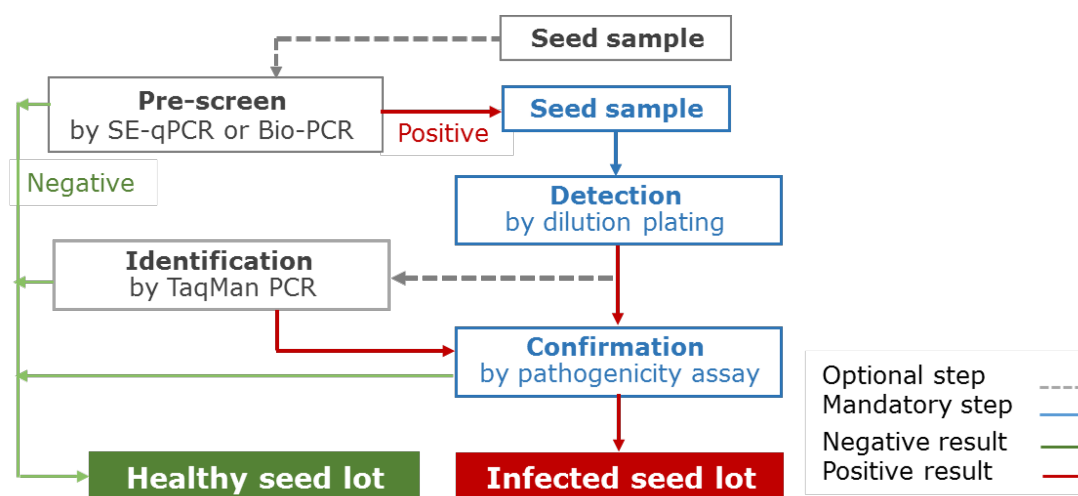
Background

This method includes a pre-screen for and assays to detect, identify suspect colonies and confirm the viability and pathogenicity of *Xanthomonas campestris* pv. *campestris* (Xcc) and *Xanthomonas campestris* pv. *raphani* (Xcr) in untreated Brassica seed. Dilution plating for detection and the bioassay for confirmation are mandatory steps in the method as together they show the presence, viability and pathogenicity of the target pathogens.

The original method was based on assays published by Franken *et al.* (1991) and in the 2nd edition of Working Sheet No. 50 in the ISTA Handbook of Seed Health Testing (Schaad & Franken, 1996). The ISTA Rules was published ISTA Rule in 2004 based on the method for the detection of Xcc, Xcr and *X. c.* pv. *armoraciae* on untreated cabbage seeds developed and validated by the International Seed Health Initiative for Vegetables (ISF/ISHI-Veg). Following a publication by Fargier and Manceau (2007) that their study did not "support the existence of another leaf spot disease caused by *X. c.* pv. *armoraciae*" the method was restricted to detecting Xcc and Xcr.

Version 4 of the method, published in 2013, included the option of identifying suspect colonies using either one of two gel-based PCRs that distinguished between Xcc and Xcr. In version 5.0, approved in 2015, the recipes for the semi-selective media mCS20ABN and FS were modified to improve performance and safety.

The latest version of the method includes a choice of two assays (a seed-extract PCR and a bio-PCR) as an optional "pre-screen". The aim of the pre-screen is to identify seed lots that are not infected with the target pathogens. A negative pre-screen result indicates the seed lot is negative and therefore, the end of the test. A positive pre-screen result is indicative of a "suspect" lot that needs to be investigated further using the dilution plating assay. It also replaces the gel-based PCR assays used for colony identification by a TaqMan qPCR assay. See process flow chart below:



Other consequential and editorial changes to the method description

1. In section 7.7 of the ISTA Rule, citing Fargier and Manceau (2007) following the assertion that leaf spot was caused by Xanthomonas – classified sometimes as Xca or Xcr.
2. Adding Fargier and Manceau (2007) to the list of references:
   Fargier, E. & Manceau, C. (2007). Pathogenicity assays restrict the species *Xanthomonas campestris* into three pathovars and reveal nine races within *X. campestris* pv. *campestris*. Plant Pathology. 56. 805 - 818. 10.1111/j.1365-3059.2007.01648.x.
3. Replacing all references to Xcc in the plating assay by Xcc/Xcr as these pathovars are undistinguishable on the semi-selective media used. It is only when the PCR assay is used in colony confirmation that one is able to distinguish them.
4. Taking out reference to gel based PCR in the method description.