

## Validity of the ISHI-Veg method for Detection of CMM in tomato seed, Version 5.0.

### Introduction

Since the start of GSPP, GSPP only accepts *Clavibacter michiganensis* subsp *michiganensis* (Cmm) seed health tests based on ISHI-Veg published method as this is the industry standard. ISHI-Veg has published an addition (SE-PCR) to its dilution plating method and this affects Cmm testing in GSPP. This document describes the consequences of this change for seed health testing for Cmm in GSPP.

### The method

The 'method for the detection of *Clavibacter michiganensis* subsp *michiganensis* on tomato seed, version 4.3.1' has been the required method for testing for GSPP. The method consists of soaking seeds in buffer, grinding seeds, concentrating extract, plating on semi-selective media, selection of suspect colonies, followed by identification through Taqman PCR and pathogenicity testing. This method shows presence of viable and pathogenic Cmm, and results generated with this method and the method itself are and remain valid for use in GSPP.

Draw back of the 'method for detection of *Clavibacter michiganensis* subsp *michiganensis* on tomato seed, version 4.3.1', is its long duration, relatively low throughput, need for trained and skilled people and occasional invalid test results due to saprophytic overgrowth or inhibitors which require retesting of the seed lot before the seeds can be used.

To address the constraints above, ISHI-Veg has developed a prescreening method based on SE-PCR, to quickly select for seed lots that do not show any suspicion of Cmm, as target DNA is absent. Consequently, in case a signal is picked up, the test has to be continued with dilution plating to confirm presence of viable and pathogenic Cmm on the same seed extract or on a new sample as is described in the ISHI-Veg protocol: 'Method for detection of *Clavibacter michiganensis* subsp *michiganensis* on tomato seed, version 5.0'. The decision flow for GSPP participants is presented in figure 1.

### Conclusions

- SE\_PCR is an optional pre-screen step. A result generated with ISHI- veg: 'method for detection of *Clavibacter michiganensis* subsp *michiganensis* on tomato seed, version 4.3.1 or 5.0', without SE\_PCR prescreen is a valid result for GSPP.
- SE-PCR is very sensitive and is able to detect both viable, non-viable and potentially viable but non-pathogenic organisms and should be regarded as a pre-screen method.
- A Cmm SE-PCR negative result yields the conclusion that the seed lot meets the GSPP requirements and there is no need for additional testing.
- A Cmm SE-PCR positive result yields the conclusion that the seed lot is suspect and needs further investigation and there is need for additional dilution plating testing to show it meets the GSPP requirements.
- Seed lots that are Cmm suspect based on positive SE\_PCR, but that have been tested negative in dilution plating according to ISHI- veg: 'method for detection of *Clavibacter michiganensis* subsp *michiganensis* on tomato seed, version 4.3.1 or 5.0', are meeting the GSPP requirements and should be regarded as being free from Cmm.
- Seeds lots that are tested SE-PCR positive and dilution plating positive should be regarded Cmm infected.

