International Seed Federation
Seed is Life

Plant Sciences and Risk Assessment
Department of Agriculture and Water Resources
GPO Box 858
Canberra ACT 2601

Nyon, 5 Oct 2018

Ref: Submission on the draft report for the pest risk analysis for Pepino mosaic virus and pospiviroids associated with tomato seed

Dear Sirs,

Thank you for the opportunity to make a submission on the PRA for Pepino mosaic virus (PepMV) and pospiviroids associated with tomato seed.

ISF's general comments have been integrated into the submission made by the Australian Seed Federation. This submission is specific to the proposed testing requirement for Pepino mosaic virus in tomato seed lots that weigh more than 300 g.

The ELISA test

The ELISA assay with a sample size of 3,000 seed is a widely recognised standard within and outside of the seed industry for more than a decade.

When Australia introduced emergency measures on imports of tomato and wild tomato seed for PepMV, it considered the use of the ELISA test sufficient for additional declarations on the seed lot being free of the virus. The draft PRA acknowledges that in the years since the emergency measures came into force no PepMV infected lots have been detected by Australian laboratories.

» ISHI-Veg/ISF would like to propose the ELISA test in ISHI-Veg's Manual of Seed Health Testing Methods be maintained as a department-approved test, the results of which can be used to issue an additional declaration that the lot is free of the virus.

PCR tests for detecting target pathogens

ELISA and PCR assays used for detecting a pathogen are in industry parlance "indirect" tests, as they detect proteins or nucleic acids that are specific to the target pathogen. Due to the combination of available sequence data and simple low-cost assay design, PCR tests can be specific to the target pathogen and sensitive.

However, indirect tests do not yield any information about pathogen viability and are not sufficient to prove pathogenicity. But as they can identify non-infected seed lots rapidly they can be used to screen seed lots before a "direct" test, such as a bioassay. This is particularly important in seed health testing as nucleic acids from dead or inactivated pathogens may remain detectable in seed samples following seed processing and/or seed treatments.

The ELISA test is, as a matter of fact, used in 2 ways: to confirm the presence of infectious virus in the leaves of tobacco plants that have been inoculated with an extract of ground tomato seed being tested for the presence of PepMV, or to screen seed lots. In the latter case, a negative result indicates the lot is

free from the virus. ELISA positive samples are considered 'suspect' till they are confirmed positive or negative using the bioassay.

As the aim of a pre-screen assay is to identify seed lots that are not infected with the target pathogen(s), false negatives must not occur. It is imperative, therefore, an indirect test like any other test be validated to show it is fit for its intended purpose (see http://www.worldseed.org/wp-content/uploads/2018/03/Real-time_PCR_pre-screens_2018.pdf).

» ISHI-Veg/ISF would respectfully like to know if the department has approved any PCR test and if the validation report is available.

ISHI-Veg's seed extract PCR (SE-PCR) test for PepMV

As technology progresses, the range of available techniques in seed health testing also grows and seed companies are actively engaged in developing and using them to improve the quality of their seed. ISHI-Veg has developed a PCR test on tomato seed extract.

The test does not replace the ELISA and so the sample and subsample sizes remain 3000 and 250 seeds, respectively. As it is based on a different biological principle to that of the ELISA, it can be used to confirm the results of the ELISA or as an alternative pre-screen to the ELISA.

The test has other important features, such as two TaqMan primers located on different regions of the PepMV genome so that in case of a PepMV strain with some rare mutations at least one TaqMan will always be positive. An Internal Amplification Control is included as a check of RNA extraction and the transcriptase step in the PCR reaction.

» ISF would be willing to share information on the ISHI-Veg SE-PCR test with the department lab(s) in the interest of harmonising the test used by seed companies and authorised labs.

ISHI-Veg and ISF thank you again for the opportunity to comment on the draft PRA and look forward to hearing from you on the remarks made and the offer to work collaboratively with your official labs. ISHI-Veg also remains at your disposal for any questions you may have.

Thanking you,

Yours sincerely

Radha Ranganathan
Director of Technical Affairs