Bio-assay infectivity PepMV

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Prepared by:

Name:	Signature	Date
[redacted]	……..	……..

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Approved by:

Name:	Signature	Date
[redacted]	……..	……..
[redacted]	……..	……..

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0. Modifications

No modifications. This is first version of SOP.

1. Subject

This standard operating procedure describes the method to test the infectivity of pepino mosaic virus (PepMV) in a plant extract sample.

2. Scope

To test samples whether they contain infectious PepMV, tomato seedlings are inoculated with an appropriate dilution of the sample. 14 Days (± 2 days) after inoculation, seedlings are tested separately by ELISA to determine the percentage of infected plantlets. This percentage is a measure for the infectivity of PepMV in the sample.

3. Abbreviations

PepMV: pepino mosaic virus
ELISA: Enzyme-linked immunosorbent assay

4. Materials

4.1 Plants:

Tomato seedlings of 18 days (± 4 days) old after sowing. With at least two true leaves of more than 1 cm^2 each. The tomato seedlings are grown on rock wool plugs with regular tomato nutrient solution (8.1). The temperature in the greenhouse where the tomato seedlings are grown is 20ºC (± 2ºC). The rock wool plugs with the seedlings are placed in tempex plug trays. The tomato seedling are standing in the plug tray, not touching each other (more than 7 cm separated).

4.2 Carborundum:

Silicon carbide (carborundum) from Saint-Gobain; particle size 17 µm (cat. nr. F400/17) is used as an abrasive to facilitate virus infection in the leaves by mechanical inoculation.

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4.3 PBS (pH 7.4 ± 0.1) phosphate buffered saline
8.0 ± 0.4 g sodium chloride (NaCl)
0.2 ± 0.01 g mono-basic potassium phosphate (KH_2PO_4)
1.2 ± 0.06 g di-basic sodium phosphate (Na_2HPO_4)
0.2 ± 0.01 g potassium chloride (KCl)
Dissolve in 900 mL H_2O, adjust pH to 7.4 ± 0.1 with 1 M NaOH or 1 M HCl and fill up to one litre.

PBS can be stored for 6 months at -4ºC (± 3ºC). Before usage, the buffer should be checked whether it is clear after shaking the bottle to exclude the presence of accumulated micro-organisms. When turbidity is observed, the PBS should be discared and fresh PBS should be prepared.

4.4 Tubes. 50 mL polypropylene tubes, or equivalent.

4.5 Petridishes. Polystyrene petridishes with a diameter of 90 mm diameter, or equivalent

4.6 Gloves. Disposable gloves are from nitrile or latex.

5. Assay procedure

5.1 Label 50 ml tubes (4.4) with a separate code for each sample to be tested. Dilute each sample with PBS (4.3) in a total volume of 10-50 mL (standard 50x dilution) in the tube (4.4) by pipetting 0.2- 1.0 mL of the sample and fill with PBS to the volume of 50-times the volume of the sample.

5.2 Pour 10-25 ml of each diluted virus sample into a petri-dish (4.5). Add 3 ± 2% (w/v) carborundum (4.2) to the virus suspension.

5.4 Put on new disposable gloves (4.6) for each different mixture.

5.5 Inoculate for each sample, 10 tomato seedlings (4.1). Take for each sample two additional seedlings (4.1) as negative controls without inoculation. Inoculate each seedling to be inoculated on 2 leaflets per plant by gentle rubbing. Rubbing is carried out by first dipping thumb and index finger in virus-carborundum suspension, stir gently and then rub in the leaflet by lightly moving the thumb and index finger on leaflet (two times in opposite direction, leaflet between finger and thumb). Do not rub with too much force preventing hole formation. The leaflets should change somewhat in colour by the rubbing. Dip for each following seedling again your thumb and index finger in the virus-carborundum suspension.

5.6 After inoculation of all virus samples, collect all residual material and put this in a garbage bin or waste container. The residual material can be processed as regular garbage.

5.7 Test after 14 (± 2) days the 12 tomato plantlets (10 virus-inoculated plants and 2 negative

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controls) whether they are PepMV-infected by ELISA (SPV A517). Take from each plantlet two or three leaflets which are not damaged by the inoculation. Put the leaflets of each plantlet in a small plastic bag. Collect the 10 small bags with leaf samples of the virus-inoculated plants in a larger plastic bag which is coded for each tested sample. Also collect the 2 small bags of the negative controls in a coded bag. The 12 sub-samples will be tested by ELISA.

6. Reporting

When the two negative controls are negative for PepMV, the results of the inoculated plants are valid and can be reported. The results will be represented as X/10 +, where X = the number of PepMV-positive plants among 10 tested sub samples.

7. Quality control

The negative controls should be negative. When one or two negative controls are PepMV-positive the results are not valid. In case of doubt Head Micro Laboratory is consulted if the data is valid or not. If not the measurement should be repeated.

8. Literature reference

8.1 de Kreij C, Voogt W, van den Bos AL, Baas R (1999) Bemestings Adviesbasis Substraten. Proefstation voor Bloemisterij en Glasgroente.

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