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**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 5205-2020

**Quarantine and identification method of Boping melon mosaic
virus**

柏平缕瓜花叶病毒检疫鉴定方法
(English Translation)

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Foreword

This document is drafted in accordance with GB/T 1.1-2020.

1 Scope

This document specifies the methods for quarantine and identification of Boping melon mosaic virus.

This document is applicable to the quarantine and identification of plant seeds, seedlings and products that may carry BoPinggua mosaic virus.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

There are no terms and definitions that need to be defined in this document.

4 Principle

Scientific name. Pepino mosaic virus Abbreviation. PepMV Taxonomic status. Alphaflexiviridae (Alphaflexiviridae), the antigenic characteristics of members of the Potato X virus genus (Potexvirus) and genomic characteristics are the main basis for quarantine identification.

See Appendix A for details.

5.1 Main equipment and utensils

Micro grinder, microplate reader, plate washer, micro balance (sensitivity. 0.001g), PCR machine, electrophoresis machine, horizontal electrophoresis tank, coagulation Gel imager, high-speed refrigerated benchtop centrifuge, ice maker, vortex shaker, water bath, pH meter, adjustable pipette (1000 μ L, 200 μ L, 20 μ L, 2 μ L), 96-well microtiter plate, mortar, etc.

5.2 Main reagents

The main reagents and buffers are shown in Appendix B, C, D.

6.1 Seed sample preparation

Pick out malformed and immature seeds and sown them in sterilized soil in the insect-proof net room. After 3 to 4 true leaves have grown, the plants that will show symptoms Number, the plants that did not show symptoms were grouped (10 plants as a group) and numbered. The collected true leaves were divided into 2 parts, and they were used separately for later use as needed.

Continued trials. Seeds can also be tested directly.

6.2 Seedlings and plant products

Seedlings and plant product numbers with symptoms (such as leaf deformities, mosaics, mottled, etc.) are tested separately. Group and number without symptoms the detection, grouping method and detection method are the same as 6.1.

7.1 Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)

Set up a negative control and a positive control (healthy plant tissue is used as a negative control, and PepMV infected plant tissue is used as a positive control), the sample extraction buffer is used as a blank control, and the type and material of the negative control (such as seeds or leaves) should be as consistent with the test sample as possible. Do not the same detection reagents or kits are operated according to the instructions of the reagents or kits. See Appendix B for specific operations.

7.2 One-step RT-PCR detection

Extract the total RNA of the sample and the control separately, and perform PCR amplification in one step by RT-PCR. Healthy plant tissue is used as a negative control, Plant tissue infected with PepMV was used as a positive control, and ddH₂O was used as a blank control. See Appendix C for specific operations.

7.3 One-step real-time fluorescent RT-PCR detection

The total RNA of the sample and the control were extracted separately, and the one-step real-time fluorescent RT-PCR was performed for detection. Healthy plant tissue is used as a negative control, Plant tissue infected with PepMV was used as a positive control, and ddH₂O was used as a blank control. See Appendix D for specific operations.

8 Result judgment

After the enzyme-linked test is positive, if the RT-PCR or real-time fluorescent RT-PCR test is positive, it can be judged that the sample carries the flowers and leaves virus.

9.1 Results recording

A complete experiment record includes. the source, type, time of the sample, the time, location, method and results of the experiment, etc., and must have the signature of the handler and the experimenter. The original data of the enzyme-linked plate reaction is required for the enzyme-linked assay; the photo of the electrophoresis result is required for the PCR Fluorescent RT-PCR must have an amplification curve.

9.2 Sample storage

After inspection, it is determined that the samples carrying PepMV should be stored under appropriate conditions. The seeds should be stored at 4°C, and the diseased leaves should be stored at -20°C or Do a good job of marking and registration in the refrigerator at -80°C.

Appendix A

(Informative)

Boping melon mosaic virus related information A.1 Antigen characteristics PepMV has strong antigenicity.

A.2 Chondrial morphology Boping cucurbita mosaic virus particles are linear, with a length of 500 nm and a diameter of 13 nm.

A.3 Genome

Single-stranded linear RNA, the genome length is 6.5kb, the genome is a single component, base ratio. G. A. C. U=25.26.19.30, it accounts for 6% of the particle weight. The genome does not have a Poly(A) tail, but has a tRNA-like structure, encoding 4 proteins, 5' of the viral genome and the 3'ends each contain a non-coding region.

A.4 Host range

Boping melon mosaic virus is mainly found in tomato (*Lycopersicon esculentum*) and phoenix (*Solanum muricatum*) in nature.

A.5 Disease symptoms Tomato. Leaf mosaic, twisted, bright yellow mosaic, shrunken, brown spots on old leaves, brown streaks on the stem, and red spots on the fruit.

A.6 Distribution

PepMV was first discovered in Peru in 1974. It has occurred in the Netherlands, many European countries, including Austria, Bulgaria, car Ministers, Italy, Finland, France, Germany, Hungary, Netherlands, Norway, Poland, Slovakia, Spain, Sweden, Switzerland, Turkey Ear it and the United Kingdom.

A.7 Transmission route PepMV can be spread through contact or through seeds.

Appendix B

(Normative)

Double antibody sandwich enzyme-linked immunosorbent assay B.1 Test materials B.1.1 ELISA plate B.1.2 Coated antibody Specific Boping Cucumber Mosaic Virus Antibody B.1.3 Enzyme-labeled antibody Alkaline Phosphatase-labeled Boping Wax Gourd Mosaic Virus Antibody B.1.4 Substrate Disodium p-nitrophenyl phosphate (rhoNPP) B.1.5 PBST buffer (washing buffer pH7.4) B.1.6 Sample extraction buffer (pH7.4) B.1.7 Coating buffer (pH 9.6) B.1.8 Enzyme-labeled antibody dilution buffer (pH7.4) B.1.9 Substrate (rhoNPP) buffer (pH 9.8) B.2 Procedure B.2.1 Coating antibody Dilute the antibody with the coating buffer according to the instructions, add it to the well of the enzyme-linked plate, 100 µL/well, incubate at 37°C for 2 h, and empty the well.

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PBST wash 3 times.

B.2.2 Sample preparation B.2.3 Sample addition B.2.4 Add enzyme-labeled antibody Use