

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

SN/T 4877.14-2019

**DNA barcoding screening method - Part 13: Quarantine
Sobemoviruses**

**基因条形码筛查方法 第14部分：检疫性南方菜豆花叶病毒属病毒
(English Translation)**

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Foreword

This section is drafted in accordance with the rules given in GB/T 1.1-2009.

Please note that some elements of this document may involve patents. Publication of this document the agency is not responsible for identifying these patents.

This section is proposed and managed by the General Administration of Customs of the People's Republic of China.

This section was drafted. Beijing Customs of the People's Republic of China, Xiamen Customs of the People's Republic of China.

1 Scope

This section specifies the RNA extraction, gene amplification, sequencing, and sequencing of quarantine southern bean mosaic virus virus gene barcode screening methods.

Column comparison analysis and result determination.

This section applies to the screening and detection of quarantine southern kidney mosaic virus in plant propagation materials and plant products.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this document.

file. For undated references, the latest version (including all amendments) applies to this document.

SN/T 3438 Southern bean mosaic virus quarantine and identification method.

SN/T 3436 Quarantine mosaic virus quarantine and identification method.

3 Terms and definitions

The following terms and definitions apply to this document.

DNA barcode refers to a standard, sufficiently mutated, easily amplified, and relatively short DNA that can represent the species in the organism.

Fragment.

4 Basic Information on Southern Bean Mosaic Virus

Chinese name. Southern kidney bean mosaic virus this genus virus can infect monocotyledonous and dicotyledonous plants, but the natural host range of each virus is relatively narrow, and the disease symptoms are mainly flowers for additional information on the genus, see Appendix A.

5 Principle

That is the bar code characteristic sequence of this genus. Based on this conserved region, degenerate primers were designed, RT-PCR amplification and PCR product sequencing were performed.

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6.1 Instruments and facilities

Enzyme-linked detector, electronic balance (sensitivity. 0.0001g), qualitative PCR instrument, electrophoresis instrument, electrophoresis tank, gel imaging system, constant temperature water bath, low Refrigerators, ordinary refrigerators, centrifuges, etc .; micropipettes (2.5 µL, 10 µL, 20 µL, 100 µL, .200 µL, 1000 µL); enzyme-linked plates, mortars, etc .; isolation greenhouse.

6.2 Reagents

See Appendix B for reagents.

7 Preparation

Pick out malformed, immature seeds and sown in sterilized soil. After growing 3-4 true leaves, number the plants that show symptoms, but no symptoms the plants are grouped (10 plants in 1 group) and numbered.

8 DNA barcode screening

The total RNA of the sample and the control were extracted separately, and cDNA was synthesized by reverse transcription, followed by PCR amplification. Healthy plant tissue negative as a result, the plant tissue infected with SBMV was used as a positive control, and sterile water was used as a blank control.

See Appendix B for specific operations.

9 Result judgment

9.1

If the general RT-PCR test result is negative, it is determined that no southern bean mosaic virus is detected.

9.2

If the general RT-PCR method detects a positive result, perform sequence determination and analysis.

a) If the translated amino acid sequence is more than 95% homologous to a known viral sequence of the southern bean mosaic virus (see Appendix C), it is determined that Southern bean mosaic virus is detected (specific virus types need to be further identified by other methods).

b) If the translated amino acid sequence is less than 95% homologous to a known virus sequence of the southern bean mosaic virus genus.

--- If phylogenetic analysis indicates clustering with southern bean mosaic virus, it is determined that southern bean mosaic leaves are detected Virus is a virus (specific virus types need to be further identified by other methods);

Note: The detected virus may belong to a known virus whose genome sequence has not been determined or a new virus species that has not been reported.

--- If the phylogenetic analysis indicates that they are not clustered with the southern bean mosaic virus, it is determined that southern bean is not detected Mosaic virus.

c) If the determined genomic sequence has the highest homology to the SBMV and SoMV viruses in the southern bean mosaic virus genus, determine Screening for quarantine plant viruses requires final identification according to standard methods (SN/T 3438 and Sn/T 3436).

10.1 Results Recording and Data Preservation

Record sample information and test data, including sample source, plant species, sampling time and place, test time, method and results, and SN/T 478.14--2019 ?????? ?????, ?????,?

Signed by personnel. For RT-PCR detection methods, electrophoretic photographs should be saved, and sequencing reports should be saved for sequencing results.

10.2 Sample preservation

Southern bean mosaic virus virus samples should be properly stored in an ultra-low temperature refrigerator, or stored at low temperature after lyophilization, and registered and Identification for review.

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Appendix A

(Informative appendix) Southern bean mosaic virus A. Morphology of virion Southern bean mosaic virus virus virions are equiaxed icosahedron ($T = 3$), without envelope, about 30 nm in diameter and 180 Protein subunit, each protein subunit has two regions, one forming an icosahedral shell approximately 3.5 nm thick, and the other forming within the virus Partial sequence of "arms".

A. 2 Genomic characteristics Leadframe (ORF) composition. ORF1, ORF2a, and ORF2b are all produced by translation of the genomic RNA, of which ORF2a is expressed through the leakage scanning mechanism; ORF2b forms a fusion protein with ORF2 through the -1 ribosome frameshift mechanism; ORF3 it is produced by subgenomic RNA translation. The motif encoding the replicase is located in ORF2b.

A. 3 types of viruses SN/T 478.14--2019 ?????? ?????, ?????,?

A. 4 Host range

After the virus, mosaic or mottled symptoms usually appear on the leaves.

A. 5 Ways of transmission Spread by aphids, villous mottle virus (VTMoV) spreads through blind maggots. Some viruses can also be transmitted through seeds, such as southern vegetables Bean Mosaic Virus (SBMV), Southern Cowpea Mosaic Virus (SCPMV), and Quinoa Mosaic Virus (SoMV).

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