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

SN/T 5206-2020

**Quarantine and identification methods of onion yellow dwarf
virus**

洋葱黄矮病毒的检疫鉴定方法
(English Translation)

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Foreword

This document was drafted in accordance with the rules given in GB/T 1.1-2020.

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

Drafting organizations of this document. Fuzhou Customs of the People's Republic of China, Xiamen Customs of the People's Republic of China, China Inspection and Quarantine Research Institute Research Institute, Institute of Biotechnology, Chinese Academy of Agricultural Sciences, Fujian Agriculture and Forestry University.

1 Scope

This document specifies the procedures and methods for onion yellow dwarf virus detection.

This document is applicable to the detection of onion yellow dwarf virus in imported and exported plants and their products.

2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated references Documents, only the version corresponding to the date is applicable to this document; undated reference documents, the latest version (including all amendments) Applies to this document.

GB/T 6682 Analytical laboratory water specifications and test methods SN/T 2122 Quarantine sampling of incoming and outgoing plants and plant products SN/T 2964 Plant Virus Testing Specification SN/T 3457 Specification for Molecular Biology Testing of Plant Viruses

3 Terms and definitions

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

4 Principle

The serological and genomic characteristics of onion yellow dwarf virus are the main basis for formulating this quarantine identification method.

5.1 Instrument and equipment

High-speed refrigerated centrifuge, electronic balance (1/1000 g), enzyme-linked instrument, PCR instrument, electrophoresis instrument, horizontal electrophoresis tank, -20°C low temperature ice Box and -80°C ultra-low temperature refrigerator, gel imaging analysis system, pH meter, microwave oven, magnetic stirrer, constant temperature water bath, autoclaving Pot, ultra-clean workbench, etc.

5.2 Utensils

Adjustable pipettes of various volumes (1000 µL, 200 µL, 100 µL, 20 µL, 10 µL, 2 µL), PCR reaction tubes, centrifuge tubes (1.5 mL), mortar, etc.

5.3 Reagents

Unless otherwise specified, all reagents are of analytical grade, and the experimental water should meet the relevant regulations in GB/T 6682. DAS-ELISA reagent See Appendix A, and RT-PCR reagents in Appendix B.

6.1 Sampling

The sampling method shall be implemented in accordance with the relevant provisions of SN/T 2122.

Plant materials with symptoms of virus damage are sampled separately. For the description of the symptoms of OYDV, please refer to Appendix C; for plants without symptoms of damage the material sampling method shall be implemented in accordance with the relevant regulations in SN/T 2122.

6.2 Sample preparation

The sample preparation is carried out according to the symptomatic and non-symptomatic, and the specific method is carried out in accordance with the provisions of SN/T 2964 and SN/T 3457.

7.1 DAS-ELISA

See Appendix A for the specific method of DAS-ELISA.

7.2 RT-PCR

Refer to Appendix B for the specific method of RT-PCR.

8.1 Judgment of results

During sample testing, the testing process and result judgment shall be carried out according to the following principles.

First, use DAS-ELISA for preliminary screening.

8.2 Record of results

The record includes sample source, sample type, testing time, testing location, testing method and results, and the signature of the testing personnel. DAS-ELISA test should have enzyme-linked reaction value; RT-PCR test should have electrophoresis picture.

9 Storage of samples

The samples that are judged to be positive by the test results are properly stored in the refrigerator at -20°C~80°C, and the registration and labeling work should be done.

For review.

Appendix A

(Normative)

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) A.1

Reagents A.1.1 Coated antibody Specific antibodies to onion yellow dwarf virus.

A.1.2 Enzyme-labeled antibody Onion yellow dwarf virus antibody labeled with alkaline phosphatase.

A.1.3 Substrate

Disodium p-nitrophenyl phosphate (pNPP).

A.1.4 1xPBST buffer (pH 7.4) A.1.5 Sample extraction buffer (pH 7.4) A.1.6 Coating buffer (pH 9.6) A.1.7 Enzyme-labeled antibody dilution buffer (pH 7.4) A.1.8 Substrate buffer (pH 9.8) A.2 Test method A.2.1 Sample preparation A.2.1.1 Sample to be tested the sample to be tested is added with extraction buffer at a ratio of 1:10 (weight: volume, W/V), ground into a slurry with a mortar, and centrifuged at 8 000 g for 10 min.

The supernatant is the prepared test sample.

A.2.1.2 Positive control sample the pure virus or tissue extract of onion yellow dwarf virus is provided by the antiserum supplier and prepared with extraction buffer.

A.2.1.3 Negative control sample Provided by the antiserum supplier, prepared with extraction buffer.

A.2.1.4 Blank control Use extraction buffer as a blank control A.2.2 Coated antibody Dilute the antibody with coating buffer according to the instructions, add 100 µL/well to the wells of the enzyme-linked plate, cover, and incubate at 37°C for 2 h or 4°C on ice Incubate overnight in an incubator, pour out the solution in the wells of the enzyme-linked plate, and wash with PBST in an amount of 100 µL per well for 4-6 times, 1 min each time.

A.2.3 Sample addition Add the prepared test sample, negative control, positive control, blank control, and at least one replicate, 100 µL/well, add Cover, incubate at 37°C for 2 hours or incubate overnight at 4°C in a refrigerator, pour out the solution in the wells of the enzyme-linked plate, and wash with PBST for 4 to 6 times in an amount of 100 µL per well. 1 min each time.

A.2.4 Add enzyme-labeled antibody Dilute the enzyme-labeled antibody to the working