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

SN/T 2398-2010

Identification of candidatus phytoplasma mali

苹果丛生植原体检疫鉴定方法

(English Translation)

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Foreword

This standard is part of EPPO phytosanitary standards (PM7/62 (1)), the main technical differences are as follows.

--- Remove ELISA detection method;

--- Remove indicator plant identification methods;

--- Added electron microscopy methods.

The Standard Appendix B, Appendix C, Appendix D is a normative appendix, Appendix A is an information appendix.

This standard is proposed and managed by the National Certification and Accreditation Administration Committee.

1 Scope

This standard specifies the overgrown apple quarantine and identification of phytoplasma.

This standard applies to entry and exit in apple seedlings and scion overgrown plant quarantine and identification of the original body.

Principle 2

2.1 Taxonomic status

Doors, flexible membrane mycetes without steroids Mycoplasma mesh, without steroids MLO Section, phytoplasma belong, 16SrX phytoplasma group.

2.2 Detection Methods

Its molecular biology and gene sequence information and other biological characteristics is based on the quarantine and identification methods.

DAPI staining method is simple, low cost, in this standard as a primary screening method phytoplasma; PCR technology Ling Sensitive and high specificity, RFLP technology is currently the main method of identification and classification of phytoplasma international, PCR technology in this standard and RFLP technique combined with an accurate method tufted apple phytoplasma as identification.

3 Equipment and reagents

PCR instrument, clean bench, sterilizer, ice maker, nucleic acid protein analyzer, high-speed refrigerated centrifuge, small desktop centrifuge, ultra-low temperature Refrigerator, conventional refrigerator, whirlpool oscillator, micro-injector, electrophoresis, gel imaging system.

DAPI, PCR buffer, magnesium chloride, dNTP, T Rao q DNA polymerase, primers and the like.

4.1 Observation of Symptoms

Careful observation apple seeds, fruits, leaves and other symptoms described in Appendix A.

4.2 DAPI staining

Select young tissues (new leaf stems, shoots, stems and roots phloem tissue), slices, 1µg/mL DAPI solution (4', 6-diamidino Indole-2-phenylindole) staining, 460nm excitation conditions, fluorescence microscopy, in phloem sieve portion obvious blue fluorescent signal it indicates a phytoplasma presence. Within the plant due to the uneven distribution of phytoplasma, so each sample required to select different parts of the tender material for testing.

4.3 Universal primer PCR and RFLP gel electrophoresis detection

Universal primer PCR and RFLP gel electrophoresis detected in Appendix B.

4.4-specific primers PCR gel electrophoresis Specific primers PCR gel electrophoresis in Appendix C.

4.5 Phytoplasma Morphology

Preparation of ultra-thin sections of plant samples collected by electron microscopy

Appendix phytoplasma form method D.

5 Results found

5.1

presenting symptoms and A. 1.1 Description of consistent and DAPI staining showed blue fluorescence can be initially determined to be overgrown apple phytoplasma Body, subject to any program by 5.2,5.3,5.4 further testing.

5.2 4.3 test, if the PCR product gel electrophoresis test result is positive, and RFLP analysis fragment size consistent with the description, can be Apple determined that the carryover overgrown plant phytoplasma original body or 16SrX group.