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INDUSTRY STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

SN/T 3446-2012

Detection and identification of Candidatus phytoplasma Australiense

澳大利亚植原体候选种检疫鉴定方法

(English Translation)

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Foreword

This standard was drafted in accordance with GB/T 1.1-2009 given rules.

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

This standard was drafted. China Inspection and Quarantine Science Research Institute, People's Republic of China Shandong Exit Inspection and Quarantine, the People's Republic of China Exit Inspection and Quarantine and the State of Liaoning.

1 Scope

This standard specifies the Australian quarantine and identification of phytoplasma candidate species.

This standard applies to Australian quarantine and identification of candidate species phytoplasma host seedlings in Australia phytoplasma candidate species.

Principle 2

2.1 Taxonomic status

Australia phytoplasma candidate species (candidatusphytoplasmaaustraliense), soft wall bacteria door (Tendericutes), flexible membrane bacteria Gang (Molicuteria), Mycoplasma mesh (Mycoplasmatales), non-alcoholic original body Division (Acholeplasmataceae), phytoplasma candidate belongs (Candidatusphytoplasma), 16SrXII group phytoplasma.

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2.2 Based detection

This standard mainly morphological characteristics and biological methods of molecular biology techniques for combining Australian phytoplasma candidate species were seized Test identification.

Symptoms of plant material was observed and found that arbuscular, yellow, leaves, curling and other symptoms are further detected; DAPI fluorescence Dyeing method can be used as a preliminary detection of phytoplasma methods; PCR technology combined with RFLP technology is currently phytoplasma international identification points the main method of the class, in this standard PCR technique and RFLP technology combined with an accurate method phytoplasma Australia as a candidate species identification;

Electron microscopy can clearly and directly observed phytoplasma particle morphology.

3.1 Instrument

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

3.2 Reagents

DAPI (4 ', 6- diamidino-2-phenylindole), the PCR buffer, MgCl2, dNTP, TaqDNA polymerase, primers and the like.

4.1 Observation of Symptoms

Carefully observe whether the performance of seedlings phytoplasma infection symptoms, the symptoms described in Appendix A.1.

4.2 DAPI staining

Select young tissues (new leaf stems, shoots, stems and roots phloem tissue), slices, 1μg/mLDAPI solution (4 ', 6-diamidino Indole-2-phenylindole) staining, 460nm excitation conditions, fluorescence microscopy, in phloem sieve portion obvious blue fluorescent signal

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