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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

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Detection and identification of bean pod mottle virus

菜豆荚斑驳病毒检疫鉴定方法

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Standardization Administration of the PRC

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Foreword

This standard was drafted in accordance with GB/T 1.1-2009 given rules. This standard by the National Standardization Technical Committee of Plant Quarantine (SAC/TC271) and focal points. This standard was drafted. People's Republic of China, Xiamen Entry-Exit Inspection and Quarantine Fujian People's Republic of China, People's Republic of China, Shenzhen CIQ. The main drafters of this standard. Liao Furong, Shen Jianguo, Zheng Yun, Chen Qing, Lin Shiming, Chen Yun, Huang Peng Ying, Wu Yuan. Bean pod mottle virus, quarantine and identification methods

1 Scope

China's national method for the quarantine detection and identification of bean pod mottle virus. It specifies the serological and molecular biological methods for detecting and identifying the virus, and it applies to the detection and identification of the virus in legume seeds, seedlings and other plant propagation material, and in the insects that transmit it. Bean pod mottle virus is a comovirus of soybean and other legumes, transmitted by leaf-feeding beetles and, at a low rate, by seed. On its own it reduces yield modestly; in combination with soybean mosaic virus it causes a synergistic infection that is far more damaging, and it produces the seed coat mottling that downgrades a soybean crop commercially even where the yield is unaffected. For a country that imports soybean seed and germplasm in quantity, keeping it out is worth a standard. What makes detection a quarantine problem rather than a laboratory exercise is the material. A seed lot is dormant and symptomless, the seed transmission rate is very low, and the virus is therefore present in a small fraction of a large consignment - so the method has to be sensitive enough to find a positive among many negatives and specific enough not to report one that is not there. The standard specifies two approaches used together: double antibody sandwich ELISA, which is fast, cheap and suited to screening large numbers of samples, and reverse transcription PCR, which is more sensitive and more specific and confirms what the serology suggests. It also gives the basic information on the virus - the accepted and

synonym names, the abbreviation, and its position in the family Comoviridae and the genus Comovirus - which is what allows a positive result to be matched against a quarantine list. Issued on 30 December 2011 and in force since 1 June 2012.

This standard specifies the method for detection and identification of bean pod mottle virus serology and molecular biology. This standard applies may carry the virus detection and identification of legume seeds, seedlings and other plant propagation material, but also to spread The mediator virus detection and identification of insects.

2 Basic information of bean pod mottle virus

Chinese name. bean pod mottle virus. English name. beanpodmottlevirus. Synonyms. beanpodmottlecomovirus (bean pod mottle virus), podmottlevirus (pod mottle virus), desmodium virus (loosestrife virus). English abbreviation. BPMV. Genus cowpea mosaic virus family Comoviridae, cowpea mosaic virus genus Comovirus. Bean clip Mottle Virus For additional information, see Appendix A.

3 PRINCIPLE OF THE METHOD

The use of double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) based on antigen-antibody reaction, reverse transcription and in vitro DNA amplification technology Surgery reverse transcription polymerase chain reaction (RT-PCR) detection and identification.

4 Main equipment

The standard detection methods identified mainly with the following equipment. Trace juicer, microplate reader, washer, microbalance (sense of volume. 0.001g), PCR, fluorescence PCR, electrophoresis, horizontal electrophoresis Groove, gel imager, desktop high-speed refrigerated centrifuge, water bath, pH meter range and a variety of adjustable pipette (1000μL, 200μL, 100μL, 20μL, 2μL). Preparation of the test sample

5 Preparation of

5.1 DAS-ELISA and IC-RT-PCR for detecting samples Inspection of each batch of samples symptom checker, prefers the seeds have brown or black mottle symptoms (from the hilum start), or choose Having a blade symptoms mottled, wrinkled, chlorosis and so on. Seed samples. Weigh 0.5g ~ 1.0g seed coat as the test sample, is thoroughly ground in liquid nitrogen and warmed at

1.10 after added Sample extraction buffer and grinding continued. Leaf samples. Weigh 0.5g ~ 1.0g leaves as a test sample, grind in a mortar or grinding in liquid nitrogen,

1.10 Sample extraction buffer was added and grinding continued. The ground sample was

transferred to 5mL centrifuge tube, 8000r/min centrifugal 5min, supernatant as DAS-ELISA (see Annex Record B) and IC-RT-PCR (see Appendix C) detection extract.

Note. The extract used in 3h, otherwise the stored at 4 °C.