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

GB/T 29397-2012

**Detection and identification of *Fusarium oxysporum* f. sp.
cubense race 4**

香蕉枯萎病菌4号小种检疫检测与鉴定

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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. National Agricultural Technology Extension Service Center, Plant Quarantine Station in Guangdong Province, South China Agricultural University. The main drafters of this standard. Wang Yuxi, Wang Lin, WU Li-feng, Jiang Zaide, Limin Hui, Ren Xiaoping, Lixiao Ni, Wu Shihao. *Fusarium oxysporum* Race 4 quarantine detection and identification

1 Scope

China's national quarantine method for race 4 of *Fusarium oxysporum* f. sp. *cubense* - the soil fungus behind Panama disease of banana, and specifically the race that attacks the Cavendish cultivars on which the world banana trade depends. The organism enters through the roots, colonises the vascular system and kills the plant; it survives in soil as chlamydospores for decades, so that an infested plantation cannot be replanted to banana and the only defence is to keep it out. The standard specifies the technical requirements for the detection and the identification of race 4 of the pathogen, covering the field symptoms, the isolation and culture of the fungus, its morphological identification, and the molecular identification that distinguishes race 4 from the other races and from the non-pathogenic formae speciales. It applies to the quarantine detection and identification of the organism on banana planting material, on plants and on soil. The reason the document runs to a

molecular endpoint is that morphology alone cannot separate the races of *F. oxysporum*: they are indistinguishable under the microscope, and the difference that matters - whether the isolate will kill a Cavendish - is genetic. The standard therefore sets the field symptoms and the vascular discoloration that raise the suspicion, the media and conditions for isolation and single spore culture, the macroconidia, microconidia and chlamydospore characters that confirm the species, the primers and cycling parameters that identify the race, and the controls and criteria by which the result is read, together with the handling and preservation of the specimens. Issued on 31 December 2012 and in force since 1 June 2013, it is held by SAC/TC 271 and was drafted with the Guangdong plant quarantine station and South China Agricultural University.

This standard specifies the *Fusarium oxysporum* Race 4 [*Fusarium oxysporum* f.sp. *Cubense* (EFSmith) Snyder Hansen Race 4] site inspection and laboratory testing to identify *Fusarium oxysporum* 4 races Damage symptoms, pathogen morphology, pathogenicity Assay results colony traits and host range Komada improved medium on the basis of clear-site inspection, laboratory identification, strain Preservation methods. This standard applies to bananas (including bananas, plantains, plantain and other *Fusarium oxysporum* 4 planted susceptible varieties) during the growth of the banana wilt Germs Races Test No. 4.

Principle

2 According pathogen *Fusarium* Damage symptoms Race 4, the Ministry of Health to take the junction tissue disease pathogens isolated and cultured according to the pathogen morphology, caused by Disease resistance measurement results on the suspected pathogen colonies traits Komada improved medium were determined. 3 instruments, appliances and medicines

3.1 equipment, appliances Instrument. clean benches, incubators, optical microscopes, autoclaves, cameras. Appliances. shovel, sticks scissors, machetes, paper, oil pen, notebooks, labels, small blade, petri dishes, slides, cover slips, tweezers, a pick, wine Fine lights.

3.2 Drugs 0.1% mercuric chloride, 70% ethanol, ethanol, sterile water, deionized water, 40% glycerol, dipotassium hydrogen phosphate, potassium chloride, magnesium sulfate heptahydrate, B Diamine tetra-acetic acid iron sodium, L- asparagine, galactose, agar powder, 75% pentachlorophenol nifedipine, wettable powders, ox bile powder, borax, streptomycin sulfate, 10% phosphoric acid and the like. 4 on-site inspection

4.1 Scene Investigation

4.1.1 The scope of the investigation Banana plantations and banana plantlets two seedling production and sales venues.

4.1.2 Survey Methods On *Fusarium* Race 4 host plants Tacha, to observe the growth condition; to banana growers and managers ask, OK suspected *Fusarium oxysporum* Race

4 damage plots, plots of the suspected test site.

4.1.3 identification of symptoms On the ground portion of plants showing symptoms, cut the roots and stems of vascular prosthesis organizations, to observe whether a color change occurs.

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