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**Technical code of practice for the identification and evaluation
of eggplant resistance to verticillium wilt**

茄子抗黄萎病鉴定与评价技术规程

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1. Structure and Drafting Rules of Standardization Documents". Drafting. Please note that some content in this document may involve patents. The issuing organization of this document assumes no responsibility for identifying patents. This document was proposed by the Ministry of Agriculture and Rural Affairs of the People's Republic of China. This document is under the jurisdiction of the National Technical Committee on Standardization of Vegetables (SAC/TC467). This document was drafted by: Zhejiang Academy of Agricultural Sciences, Zhejiang Provincial Seed Management Station, Hangzhou Fuyang District Agricultural Technology Extension Center, and Cangnan County. County Agricultural Technology Extension Station. The main drafters of this document are. Wang Hanrong, Wu Jun, Chen Xiaoyang, Wu Zaogui, Wang Mingyu, Zhu Wei, Hu Minjun, Ke Jingjing, Jin Lixin, and Shen Nianqiao. Fang Li. Technical Specifications for Identification and Evaluation of Eggplant Resistance to Verticillium Wilt

1. Scope This document defines the relevant terms and definitions for the identification of eggplant resistance to Verticillium wilt, and specifies the identification process and result evaluation. This document applies to the identification and evaluation of resistance to Verticillium wilt in eggplant (*Solanum melongena* L.).

4. Identification Process The identification process for eggplant resistance to Verticillium wilt includes three stages. material preparation, resistance identification, and result evaluation. The identification process is shown in Figure 1. Figure

5.1 Vaccination Preparation

5.1.1 Basic Equipment Sterile room, constant temperature incubator, constant temperature shaking incubator, ultra-clean workbench, autoclave, refrigerator, greenhouse (temperature maintained at 20°C~) Items include. plastic basins (30°C), aluminum pots, electric stoves, petri dishes, test tubes, scissors, tweezers, wide-mouth bottles, alcohol lamps, filter paper, Erlenmeyer flasks, and various culture media.

5.1.2 Preparation of inoculum Diseased tissue was obtained from the stems at the boundary between diseased and healthy cells of eggplant plants infected with *Verticillium* wilt from various regions; it was inoculated onto water agar (WA) plates. Culture medium was used for routine tissue isolation; single-spore purification and isolation were performed on potato dextrose agar (PDA) plates. The organism was identified as **V. dahlia** Kleb., the causal agent of *Verticillium* wilt in eggplant, through morphological and molecular biological identification and pathogenicity testing. Selected species were found in relatively densely distributed areas. The dominant strain of *Verticillium* wilt, the causal agent of eggplant wilt, was used as an inoculum for artificial inoculation and identification, and was amplified on PDA agar plates. For future reference, please save this file.

5.1.3 Preparation of inoculum suspension Under aseptic conditions, preserved mycelial blocks of *Verticillium* wilt of eggplant were picked and placed onto PDA plates for incubation at 23°C~26°C. Activation. An activated culture of *Verticillium* wilt pathogen (2 mm in diameter) was aseptically inoculated into potato liquid culture medium, and then placed... Incubate the mycelia in a shaking incubator at 25°C±1°C for 10-15 days. Filter the mycelia through sterile gauze, retain the filtrate, and prepare a solution with a concentration of [missing value] per milliliter. A spore suspension of *Verticillium* wilt of eggplant containing 1.0×10^6 to 5.0×10^6 spores. Preparation of potato liquid culture medium..200g potato, 20g glucose, 1000mL distilled water, pH 7.0, autoclaved at 121°C. bacteria for 20 minutes.

5.2 Seedling Cultivation for Identification

5.2.1 Seed quality It should comply with the requirements of GB 16715.3.

5.2.2 Seed soaking and germination Eggplant seeds for identification should first be soaked in 55°C warm water for 15 minutes, or in a 10% sodium hypochlorite solution for 5-10 minutes, then rinsed with clean water. After rinsing, soak the seeds in clean water at 28°C~30°C for 8h~10h, and then germinate them at varying temperatures of 20°C for 16h and 30°C for 8h for 5d~6d.

5.2.3 Sowing After germination, the seeds are sown in a greenhouse and raised using a nutrient pot method. The seedling substrate should meet the requirements of NY/T 2118. Fill new plastic seedling pots (11-13cm in diameter, 10-13cm in height) to two-thirds full, arranging them neatly. Before sowing, use a... After thoroughly watering the seedling substrate with tap water, sow the pre-germinated seeds of the eggplant variety (line) to be

identified into the aforementioned plastic seedling pots, two seeds per pot. Then cover the seeds with peat moss seedling substrate, just enough to cover them. When the seedlings have two leaves and one heart, thin them out to one seedling per pot and set aside for use.

5.2.4 Management of eggplant seedlings before inoculation After sowing, the seedlings are cultivated in a greenhouse. Before emergence, the greenhouse temperature is maintained at 25°C~30°C, and the substrate in the seedling pots is kept at a suitable moisture level. After emergence, maintain the greenhouse temperature at 23°C~28°C and the soil relative humidity at 60%~80%, and carry out normal cultivation and management without applying pesticides. Bacterial agent.

6 Resistance Identification

6.1 Control Variety Setup A susceptible control variety was included in the identification process. The susceptible control variety was "Solanum acinosa," or a control variety with a concentration of 1.0×10^6 spores/mL to 5.0×10^6 spores/mL. Locally prevalent susceptible varieties with a disease index greater than

50.0 at an inoculum concentration of 1 spore/mL, and susceptible control varieties should not be omitted. During identification, [the following criteria can be used]. Add a local routine antibiotic standard with a disease index of less than

15.0 at an inoculum dosage of 1.0×10^6 to 5.0×10^6 spores per milliliter. Disease control varieties.

6.2 Identification Methods

6.2.1 Design Evaluation Each variety (line) was randomly arranged, with 3 replicates, each with 60 plants, for a total of 180 plants. A control variety was also placed around the perimeter of the identification nursery. Escort.

6.2.2 Identification Time The optimal time for identifying and evaluating eggplant resistance to Verticillium wilt is from April to July and from September to November each year.

6.2.3 Vaccination period The optimal inoculation period is from the 3-leaf-1-heart stage to the 5-leaf-1-heart stage of eggplant seedlings.

6.2.4 Preparation of inoculum Dilute the inoculum suspension with sterile water to prepare an inoculum solution with a concentration of 1.0×10^6 spores to 5.0×10^6 spores per milliliter.

6.2.5 Inoculation Method Remove the eggplant seedlings, wash away the substrate from the roots, cut the roots 1cm from the root tip with scissors, and then soak the seedling roots in water. The seed was placed in a suspension and transplanted into a nutrient pot after 20 minutes.

6.2.6 Post-inoculation management of eggplant seedlings After inoculating the eggplant seedlings, maintain the greenhouse temperature at 23°C~28°C and the relative humidity at 85%~100% for 24h~48h. Provide sufficient light daily. Water every 12-14 hours, water as needed, maintain normal cultivation management, and do not apply fungicides.

6.3 Disease Investigation

6.3.1 Survey period A survey was conducted 20-25 days post-inoculation. Alternatively, the time it took for the disease severity of the susceptible control variety to be extended to the highest disease severity level could be considered. Adjustment.

6.3.2 Disease classification The disease severity levels and corresponding symptom descriptions are shown in Table 1.

6.3.3 Survey Methodology Based on the description of disease symptoms, the disease incidence of each identified variety (line) was investigated. Individual plants were examined separately for each variety (line), and the disease status was recorded. Situation classification.

6.4 Results Statistics

6.4.1 Incidence rate The incidence rate of each identified variety (line) was calculated based on the survey results, and the corresponding data were recorded. The incidence rate is expressed in "Ri" and the value is expressed in "%".

6.4.2 Disease Index The disease index of each identified variety (line) was calculated based on the survey results, and the corresponding data were recorded. The disease index was expressed as "DI" and calculated according to formula (2).

7 Results Evaluation

7.1 Validity determination When the disease index DI of the susceptible control variety is ≥ 50.0 , the disease resistance test of that batch is considered valid.

7.2 Resistance Evaluation The resistance type is determined based on the average disease index of the identified varieties (lines). The resistance assessment criteria are shown in Table 2.

7.3 Results Recording The results of the identification and evaluation of resistance to Verticillium wilt in eggplant are recorded in Table 3.