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Replacing GB/T 18085-2000

Detection and identification of Tilletia controversa J.G. Kühn

小麦矮腥黑穗病菌检疫鉴定方法

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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. This document supersedes GB/T 18085-2000 "Plant Quarantine Identification Method for Wheat Dwarf Smut Disease", and is consistent with GB/T 18085- Compared to.2000, aside from structural adjustments and editorial changes, the main technical changes are as follows:

a) A quarantine flowchart has been added (see Chapter 6);

b) On-site quarantine was removed (see Chapter 6 of the.2000 edition);

c) A scanning electron microscope identification method has been added (see 10.1.3). 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 and is under the jurisdiction of the National Technical Committee on Standardization of Plant Quarantine (SAC/TC271). This document was drafted by: Institute of Plant Protection, Chinese Academy of Agricultural Sciences; National Agricultural Technology Extension Service Center; and Shenzhen Customs Animal and

Plant Protection Bureau. Inspection and Quarantine Technology Center. The main drafters of this document are. Gao Li, Feng Xiaodong, Chen Ranran, Chen Wanquan, Ma Chen, Wang Xiaoliang, Jiang Pei, Liu Taiguo, and Gao Ruifang. This document was first published in.2000, and this is its first revision. Quarantine identification method for wheat dwarf smut fungus

1.Scope This document describes the quarantine identification method for wheat dwarf smut fungus (Tilletia controversa J.G. Kühn). This document applies to the quarantine and identification of wheat dwarf smut fungus.

1.5

h) or moist heat (121°C, 20 min). Add 100mL of distilled water to the container, and then add 1-2 drops of the surfactant Tween-20 reagent.

9.3.1.2 Sealing Seal the Erlenmeyer flask with aluminum foil or sealing film.

9.3.1.3 Vibration Washing Place the Erlenmeyer flask on a reciprocating shaker and shake and wash for 5 minutes.

9.3.1.4 Centrifugation Immediately remove the Erlenmeyer flask and transfer the washing suspension into a 10mL-20mL graduated centrifuge tube that has been sterilized by dry heat. Centrifuge at 1000 rpm. Heart 3min.

9.3.1.5 Mixing and centrifugation Remove the centrifuge tube, discard the supernatant, add the remaining wash suspension, mix and centrifuge until all wash suspension has been centrifuged, and retain the sediment. sediment.

9.3.2 Fixed volume Add Schirr flotation agent to the precipitate to suspend it, and adjust the volume to 1mL~3mL depending on the amount of precipitate.

9.3.3 Production Use an adjustable micropipette to pipette 5 μL to 20 μL of the precipitate suspension onto a glass slide, then cover with a coverslip. (Precipitate suspension for slide preparation) The volume should be such that the suspension does not overflow after the cover glass is placed.

9.3.4 Microscopic examination For each test sample, the precipitate suspension should be examined on at least 5 slides, with each slide examined sequentially according to the field of view.

9.3.5 Measurement If suspected teliospores of wheat dwarf smut are found, randomly measure the ridge height of 30 mature teliospores. Measure 5 slides. If there are still fewer than 30 teliospores, increase the number of slides to be examined until all the precipitate suspension is used up.

2 Normative references

This document has no normative references.

3.Terms and Definitions This document does not contain any terms or definitions that need to be defined.

4.Basic Information Chinese name. Wheat dwarf smut fungus. Scientific name. Tilletiacontroversa J.G.Kühn. Taxonomic classification. Kingdom Fungi, Phylum Basidiomycota, Subphylum Ustilaginomycotina, Class Exobasidiomycetes (Exobasidiomycetes), Tiletiales, Tiletiaceae, Tilletia. Information on hosts, distribution, etc. can be found in Appendix A.

5 Identification Principles

5.1 Judgment Criteria The morphological characteristics, autofluorescence microscopic features, and germination physiology of the teliospores of this pathogen differ from those of other smut fungi, making it a key differentiator. The criteria for identifying the pathogen.

5.2 Morphological characteristics of teliospores The teliospores are stellate, elliptical, or irregular in shape, with reticulate ornamentation and a colorless to pale-colored gelatinous sheath, with a diameter (including the gelatinous sheath) of The micrometer diameter of teliospores ranges from 16.80 μm to 32.19 μm, typically from 18 μm to 24 μm; the optimal temperature for teliospore germination is 2°C to 8°C. During germination, promycelium is formed. (Basidiospores) produce primary microspores (basidiospores) at their apex. After "H"-shaped mating, the primary microspores produce crescent-shaped secondary microspores. The net ridge height value is the teliospore of *Tilletiacaries*, a close species of wheat dwarf smut. The most important distinguishing feature is that the former has a ridge height value (mean ± standard deviation) of 1.43 μm ± 0.14 μm, while the latter has 0.53 μm ± 0.19 μm. When the ridge height value of a certain teliospore is greater than the critical ridge height index value of 0.95 μm, it is considered to be a teliospore of wheat dwarf smut. Spores smaller than or equal to 0.95 μm are considered to be teliospores of the wheat net smut fungus.

5.3 Microscopic characteristics of autofluorescence in teliospores Under excitation by an excitation filter at 485 nm and a barrier filter at 520 nm, teliospores of wheat dwarf smut can be produced within 3 minutes. Self-fluorescence, a characteristic not found in its similar species, wheat smut.

5.4 Physiological characteristics of teliospore germination The teliospores of *Triticum aestivum*, the causal agent of wheat dwarf smut, germinate at 5°C but not at 17°C, while the teliospores of *Triticum aestivum*, the causal agent of wheat netted smut, germinate at both 5°C and 17°C. All of them can germinate.

6.Quarantine and Identification Process The quarantine and identification process is shown in Figure 1.

7 Instruments and tools

7.1 Microscope (equipped with an epi-mercury lamp, camera converter, and monitor).

7.2 Scanning electron microscope.

7.3 Filter group (450nm~490nm excitation, 520nm barrier).

7.4 Eyepiece and stage micrometer.

7.5 Reciprocating oscillator.

7.6 Low-speed centrifuge.

7.7 Dry heat sterilizer.

7.9 Ordinary balance (sensitivity 0.001g).

7.10 Clean bench.

7.11 Biological incubator (with light, adjustable low temperature up to 5°C).

7.12 Adjustable micropipette (5μL~200μL).

7.13 Erlenmeyer flask (250mL).

7.14 Graduated centrifuge tubes (1.5mL, 10mL, 20mL and 100mL).

7.15 Petri dish (9cm in diameter).

9 Experimental Procedure

9.1 Weighing the sample Weigh out 50g of the mixed sample as the test sample.

9.2 Examination of bacterial galls If galls of the smut fungus are found, scrape off a small amount of teliospore powder, add an appropriate amount of Shear's solution, and examine under a microscope for preliminary examination. If wheat dwarfism is suspected... The height of the reticulate ridges of 30 mature teliospores of the smut fungus was randomly measured.

9.3.1 Washing and centrifugation

9.3.1.1 Add water Pour the test sample into a 250 mL Erlenmeyer flask that has been sterilized by dry heat (165°C,

10.1.1 Or 10.1.2)

10.1.1 Microscopic identification method The morphology of teliospores is shown in Appendix A. Each teliospore was visualized by aligning it vertically and horizontally on the microscope monitor screen (or using an eyepiece micrometer). The height of four ridges