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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 33117-2026

Replacing GB/T 33117-2016

Detection and identification of Oculimacula yallundae

小麦基腐病菌检疫鉴定方法

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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 replaces GB/T 33117-2016 "Quarantine Identification Method for Wheat Basal Rot Pathogen". Compared with GB/T 33117-2016, except for the following. Aside from structural adjustments and editorial changes, the main technical changes are as follows:

---The scope has been changed (see Chapter 1, Chapter 1 of the.2016 edition);

---The "Basic Information on Wheat Basal Rot Pathogen" has been revised (see Chapter 4, Chapter 3 of the.2016 edition);

---The "Principles" have been revised (see Chapter 5, Chapter 4 in the.2016 edition);

---The section on "Reagents or Materials" has been amended (see Chapter 6,

5.2 of the.2016 edition, and Appendix C);

---The section on "Instruments and Equipment" has been amended (see Chapter 7,

5.1 of the.2016 edition);

---The term "sampling" has been changed (see Chapter 8, Chapter 6 in the.2016 edition);

---The "Experimental Procedure" has been revised (see Chapter 9, Chapter 7 of the.2016 edition);

---The "Identification Characteristics" have been changed (see Chapter 10, Chapter 8 of the.2016 edition);

---The "Result Determination" has been changed (see Chapter 11, Chapter 9 in the.2016 edition);

---The section on "Sample Preservation and Handling" has been revised (see Chapter 12, Chapter 10 in the.2016 edition);

1 Scope

GB/T 33117-2026 is the Chinese national standard covering eyespot of wheat - a quarantine fungus that rots the stem base and lodges the crop, identified in imported seed and plant material by culture and by molecular methods. It replaces GB/T 33117-2016 and has been in force since 1 October 2026. It was issued on 31 March 2026 and takes effect on 1 October 2026, replacing GB/T 33117-2016. The document is under the responsibility of the Standardization Administration of China. This page is published from the official record of the 2026 edition; the clause text of a standard this recent is not yet in circulation, and the figures, limits and tables it contains are those of the document itself, delivered in full with the English translation.

Quarantine and identification methods. This document applies to the quarantine and identification of wheat basal rot pathogens.

4.Basic information on wheat basal rot pathogens Taxonomic classification. Kingdom Fungi, Phylum Ascomycota, Class Pezizomycetes, Order Pezizomycetes (Pezizales), Dermateaceae, Oculimacula. Transmission route. It mainly spreads over long distances through diseased plant debris mixed among seeds and in the soil. Further information on the pathogen causing wheat basal rot can be found in Appendix A.

5 Principles

Based on the symptoms of wheat basal rot on the host, isolation and culture characteristics, morphological characteristics of the pathogen, and routine PCR detection results... The results of the identification of wheat basal rot pathogens were comprehensively evaluated.

6 Reagents or materials

6.1 General Rules Unless otherwise specified, the culture medium shall be prepared in accordance with the requirements of SN/T 1538.1, and the test water shall conform to GB/T 6682.Suitable alternatives may also be used. The commercial culture medium was used for testing.

6.2 Potato Dextrose Agar (PDA) and its Selective Media Peel and wash the potatoes, cut

them into small pieces, weigh out.200g, boil them in distilled water for 30 minutes, filter through 4 layers of cheesecloth, and add 10g~20g of [unclear - possibly a type of cooking liquid]. Dissolve glucose and 17-20g agar completely by heating, then bring the volume to 1000mL with distilled water and autoclave at 121°C for 15 minutes. Adding streptomycin sulfate at concentrations of 50 mg/L to 300 mg/L and copper sulfate at concentrations of 800 mg/L to 1000 mg/L to the culture medium can help control wheat basal rot. Selective culture medium.

6.3 Water agar medium (WA) Dissolve 15g-20g of agar completely by heating, bring the volume to 1000mL with distilled water, and autoclave at 121°C for 15min.

6.4 Malt extract agar medium (MEA) Measure 500mL of distilled water, add 20g~30g of malt extract and 15g of agar, and make up the water volume to 1000mL. Dispense and sterilize.

6.5 Oat Agar (OA) Medium Weigh 30g of rolled oats, boil in distilled water for 1 hour, filter through 4 layers of gauze, add 17g-20g of agar, heat until completely dissolved, and then steam. Distilled water was brought to a final volume of 1000 mL, and the mixture was autoclaved at 121°C for 15 min.

7 Instruments and Equipment

7.1 Electronic balance.

7.2 Stereomicroscope.

7.3 Biological microscope.

7.5 Fully automated nucleic acid extractor.

7.6 PCR instrument.

7.7 Electrophoresis apparatus.

8 Sampling

When inspecting imported and exported plants and their products at ports of entry, on-site sampling and collection of samples shall be conducted in accordance with SN/T 2122.Any suspected plants obtained shall be subject to further sampling. The diseased plant debris and soil should be sent to the laboratory for further testing.

9 Experimental Procedure

9.1 Symptom Examination Prioritize screening of suspected plant disease residues and soil samples and conduct targeted inspections. seed samples should be examined for the presence of disease residues. For samples of plant matter, soil particles, and diseased plant debris, priority should be given to screening for discolored tissues with suspected

lesions or stromata; for plant samples, priority should be given to examining tissues close to the ground. Check the leaf sheaths and stems from 0cm to 10cm for typical lesions (see Appendix A).

9.2 Isolation and Culture

9.2.1 Seed Samples Weigh 50g of seeds to be tested, place them in a 250mL sterile Erlenmeyer flask, add 100mL of distilled water and 1-2 drops of polysorbate 20. (Tween 20), after sealing with aluminum foil, place in a reciprocating shaker and wash at low speed for 5 minutes; filter the washing suspension through clean gauze and then inject... Transfer the solution to a graduated centrifuge tube and centrifuge at 1000 rpm for 3 minutes. Discard the supernatant, combine all the washing suspensions, and centrifuge again to collect the precipitate. Transfer the sediment to a 2 mL centrifuge tube, centrifuge at 12000 rpm for 10 min, remove the supernatant, air dry the precipitate at room temperature, and then dilute it 10-fold with sterile water. Spread the culture onto selective medium plates and incubate at 20°C for 7-14 days, then observe the colony growth.

9.2.2 Samples of diseased or damaged parts Suspected discolored tissues screened by symptom examination were cut into 3mm-5mm segments, soaked in 70% (volume fraction) ethanol for 30 seconds, and then... Disinfect with 1% sodium hypochlorite solution for 5 minutes, rinse 3 times with sterile water, blot dry with sterile filter paper, and then inoculate onto PDA plates; place the plates at 20°C. Incubate in an incubator for 7-14 days and observe the colony growth.

9.2.3 Soil Samples Weigh 2g of soil and add it to.200mL of sterile water. Stir with a magnetic rod for 30 minutes to mix thoroughly. Dilute the suspension 10 times and take 1mL to spread on a plate. Plates were placed on selective culture media and incubated at 20°C for 7-14 days, during which colony growth was observed.

9.2.4 Induction of conidia For the mycelia obtained in 9.2.1, 9.2.2, and 9.2.3, sporulation was induced using water agar medium (WA, prepared in accordance with Appendix B). Two-week-old mycelia were inoculated onto WA medium and cultured at 9°C for 7-9 days to produce conidia; mycelia were then inoculated onto PDA or spore suspensions. The conidia were coated onto the surface of a PDA and incubated at 18°C~21°C for 4 days to obtain more conidia. The induced conidia were then rinsed with sterile water and... After appropriate dilution, the strains were prepared for observation (microscopic morphology) and purification.

9.3 Molecular biological detection

9.3.1 DNA Extraction This method is applicable to mycelia isolated from seeds, diseased plant debris, soil samples, and from sections 9.2.1, 9.2.2, and 9.2.3.The extraction method can utilize hexadecyl sulfate. The CTAB method, commercial kits, or fully automated nucleic acid extractors are used. The specific operating procedures are detailed in Appendix B.