International Rules for Seed Testing 2023

Validated Seed Health Testing Methods

7-022: Detection of *Microdochium nivale* and *Microdochium majus* in *Triticum* spp. (wheat) seed

Including changes and editorial corrections adopted at the Ordinary General Meeting 2022, Cairo, Egypt

Effective from 1 January 2023

Validation reports

See References. Copies are available by e-mail from the ISTA Secretariat at ista.office@ista.ch.

Please send comments, suggestions or reports of problems relating to this method to the ISTA Seed Health Committee, c/o ISTA Secretariat.

Disclaimer

Whilst ISTA has taken care to ensure the accuracy of the methods and information described in this method description, ISTA shall not be liable for any loss or damage, etc. resulting from the use of this method.

Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during weighing out of ingredients. It is assumed that persons carrying out this test are in a laboratory suitable for carrying out microbiological procedures and familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic techniques. Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.

Note on the use of the translations

The electronic version of the International Rules for Seed Testing includes the English, French, German and Spanish versions. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.

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7-022: Detection of *Microdochium nivale* and *M. majus* in *Triticum* spp. (wheat) seed

Host: *Triticum* spp.

Pathogen(s): *Microdochium nivale* Samuels & Hallett, syn. *Fusarium nivale* (Fr.) Rabenh. (Perfect state *Monographella nivalis* (Schaff.) Müller); *M. majus* (Wollenw.) Glynn & S.G.Edwards, syn. *M. nivale* var. *majus* (Wollenw.) Samuels & I.C.Hallett

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Revision history

Version 1.0, 2007-02-01
Version 1.1, 2011-01-01: *Microdochium majus* added as species
Version 1.2, 2012-01-01: 70 % ethanol removed as solvent for streptomycin
Version 1.3, 2014-01-01: Addition of positive control
Version 1.4, 2016-01-01: Revision of method, §5. Examination; replacement of Figures 1–5
Version 1.5, 2017-01-01: Reporting results revised
Version 1.6, 2018-01-01: Editorial change to Methods step 5
Version 1.7, 2021-01-01: Sample size revised; Media and solutions revised
Version 1.8, 2022-01-01: Methods (pretreatment) revised

Background

A method for *Microdochium nivale* (*Fusarium nivale*) was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 33 (Anon.,1964), and was never revised for inclusion in the second edition of the Handbook. The agar plate test proposed is based on the comparative test organised by the ISTA Seed Health Committee and experience of routine testing in a number of laboratories. Summary of changes to original working sheet: Either potato dextrose agar (PDA) or malt agar (MA) can be used; incubation temperature reduced to 20 °C; incubation in dark followed by 3–4 h in daylight or under near-ultraviolet (NUV); changes to format and layout. Within *Microdochium nivale*, two varieties were recognised: *M. nivale* var. *nivale* and *M. nivale* var. *majus*. In 2005, Glynn *et al.* provided molecular-based phylogenetic evidence of two species when taken together with biological differences already reported, thus elevating the two varieties to species status, *M. nivale* and *M. majus*. Both species are detected on PDA and MA; however, visually it is not possible to distinguish between the two, and there is an overlap in spore morphology, making it difficult to separate the two species on agar. Both species cause seedling blight in wheat, although symptoms may be more severe with *M. majus*.

Treated seed

This method has not been validated for the determination of *Microdochium nivale* or *M. majus* on treated seed. Seed treatments may affect the performance of the method. (Definition of treatment: any process, physical, biological or chemical, to which a seed lot is subjected, including seed coatings. See 7.2.3.)

Sample size

The sample (total number of seeds tested) size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum sample size should be 400 seeds.

Materials

Reference material: reference cultures or other appropriate material
Media: potato dextrose agar or malt agar plates with streptomycin sulphate
Petri dishes: 90 mm Petri dishes (one per five seeds)
Incubator: capable of operating at 20 ±2 °C.
1 % NaOCl (sodium hypochlorite)

Methods

Critical control points are indicated by CCP.

1. Pretreatment: Immerse seeds in a solution of sodium hypochlorite (NaOCl) (1 % available chlorine) for 10 min, then drain, rinse well in sterile water and drain again.

2.1 Plating: Aseptically place a maximum of 5 seeds, evenly spaced around the perimeter of the plate, onto the agar surface of each PDA or MA plate (CCP).

2.2 Positive control (reference material): Aseptically place seeds pretreated as in 1., evenly spaced (CCP), onto the agar surface of an appropriate number of malt agar plates to obtain the reference culture, or plate a reference culture on one malt agar plate or PDA plate. The number of plates required will depend on the level of contamination of the positive control seed lot.

3. Incubation: Incubate plates for 7 d at 20 °C in the dark.

4. Reference culture: Subculture a reference culture to a PDA or MA plate at the same time the seeds are plated and incubate with the test plates. Alternatively, a sample of seed known to be infected may be surface sterilised, plated on PDA or MA and incubated under the same conditions as the test samples.

5. Examination: Examine the plates after 7 d incubation. *M. nivale* and *M. majus* colonies from seed are relatively fast growing; felted aerial mycelium is white to very pale pink, the undersurface is colourless turning to light orange (Nelson *et al.*, 1983) or salmon pink (CMI mycological colour chart, sheet 1, no. 41, Rayner, 1970). After incubation in the dark, the colonies are usually white (Figs. 1, 2). If the suspect colonies are left in daylight or under near-ultraviolet light for 24 h, the salmon pink/orange colour should develop if *M. nivale* or *M. majus* (Figs. 3, 4). Further confirmation can be made by examining conidia if present. Conidial spores may be present, forming on sporodochia and seen as a pale orange mass. Microconidia are absent. Macroconidia are small, 1–3 septate, curved with blunt terminal cells that are indistinguishable from one another. The basal cell may be notched but is never foot-shaped (Nelson *et al.*, 1983). Size: 10–30 µm × 2.5–5 µm (Booth, 1971). Conidial morphology is variable with overlap between the two species *M. nivale* and *M. majus* (Glynn *et al.*, 2005). Compare with positive control. Record the number of seeds infected with *Microdochium nivale*/*M. majus*. As morphology can overlap between the two species, results can be expressed as *Microdochium* spp. (*Microdochium nivale* and/or *M. majus*).

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in results within or between tests are sufficiently wide as to raise doubts about the accuracy of the results. Suitable tolerances, which can be applied to most direct seed health tests, can be found in Table 5B Part 1 of Chapter 5 of the ISTA Rules, or Table G1 in Miles (1963).

Reporting results: The result of a seed health test should indicate the scientific name of the pathogen detected and the test method used. When reported on an ISTA Certificate, results are entered under 'Other Determinations'.

The report must indicate the number of seeds tested.

In the case of a negative result (pathogen not detected), the results must be reported as 'not detected'.

In the case of a positive result, the report must indicate the percentage of infected seeds.

Quality assurance

Specific training

This test should only be performed by persons who have been trained in fungal identification or under the direct supervision of someone who has.

Critical control points (CCP)

- Method section 2: *M. nivale, M. majus*, some *Fusarium* spp and many saprophytic fungi present on *Triticum* spp. are comparatively fast growing; with more than 5 seeds per plate, there is a high risk of the plates being unreadable.
- Preparation of PDA or MA plates: The source of agar may influence the results. The level of available nutrients may vary from manufacturer to manufacturer. Both PDA and MA can be bought as a powdered medium, or MA can be made up as per recipe (see below). Suitable products used in the comparative test include PDA (Oxoid, Basingstoke, UK), Cristomalt (DIFAL, Seysses, France); agar-agar (VWR, West Chester, Pa., USA) and streptomycin (Sigma-Aldrich, Mo., USA). Any equivalent products should be suitable. Whenever a new batch of agar is used, a check on the quality should be made, using a reference lot with a known infection level, or a reference isolate and sustainability of isolate measured. Pay particular attention to the growth characteristics of reference isolates.

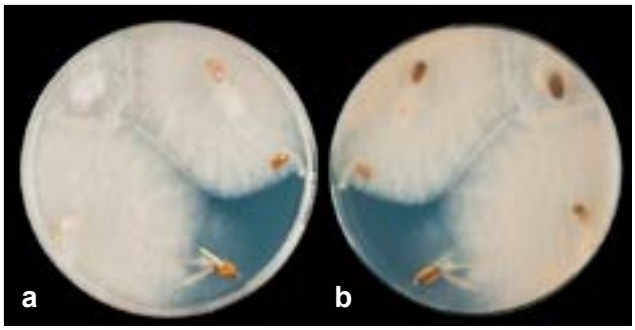

Figure 1. Colonies of *Microdochium nivale* and/or *majus* on PDA after 7 days incubation in the dark. **a** Top surface of plate. **b** Bottom surface of plate.

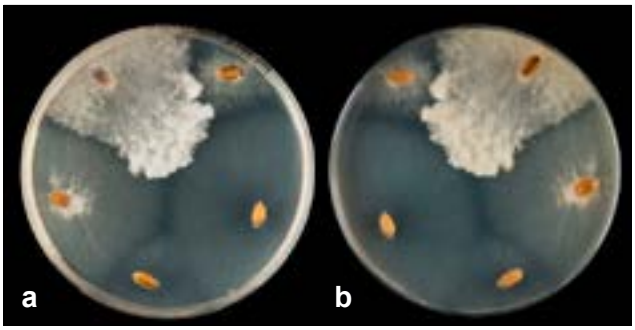

Figure 2. Colonies of *Microdochium nivale* and/or *majus* on MA after 7 days incubation in the dark. **a** Top surface of plate. **b** Bottom surface of plate.

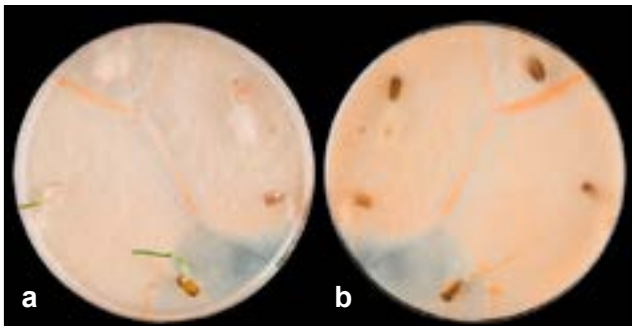

Figure 3. Colonies of *Microdochium nivale* and/or *majus* on PDA after 7 days incubation in the dark and 24 h under NUV, showing salmon-pink coloration. **a** Top surface of plate. **b** Bottom surface of plate.

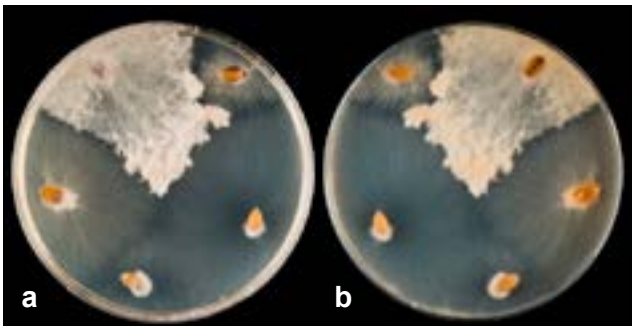

Figure 4. Colonies of *Microdochium nivale* and/or *majus* on MA after 7 days incubation in the dark and 24 h under NUV, showing salmon-pink coloration. **a** Top surface of plate. **b** Bottom surface of plate.

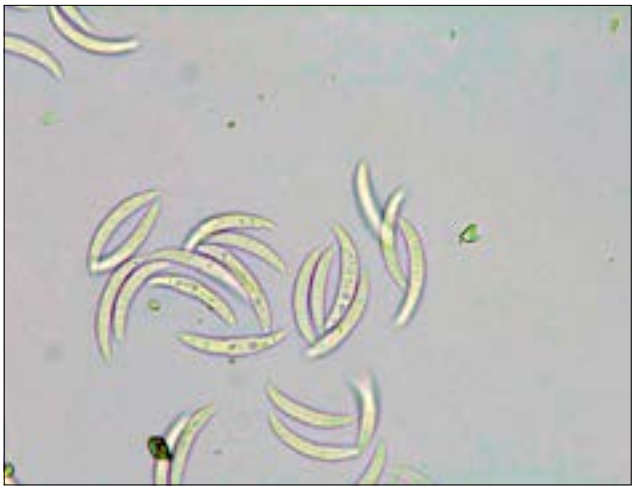

Figure 5. *Microdochium* conidia on CLA. ×400.

Media and solutions

Potato dextrose agar + streptomycin

Potato dextrose agar (CCP): make according to specification of supplier
Distilled/deionised water: 1000 ml
Streptomycin sulphate*: may be used between 50 mg and 130 mg, depending on the level of saprophytic bacterial contamination commonly encountered

* Added after autoclaving. Streptomycin sulphate can be dissolved in water. Filter sterilisation is required.

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 ml of distilled/deionised water.
3. Dissolve powdered PDA in the water by stirring.
4. Autoclave at 121 °C and 15 p.s.i. for 15 min.
5. Adjust to pH 5.6 ±0.2 pH units.
6. Allow agar to cool to approximately 50 °C and add streptomycin sulphate dissolved in water.
7. Pour 20–22 ml of molten agar into 90 mm Petri dishes and allow to solidify before use.

Storage

Prepared plates may be stored at 4 °C for up to 6 weeks.

Malt agar + streptomycin

Agar: 20 g
Malt: 10 g
Distilled/deionised water: 1000 ml
Streptomycin sulphate*: may be used between 50 mg and 130 mg, depending on the level of saprophytic bacterial contamination commonly encountered.

* Added after autoclaving. Streptomycin sulphate can be dissolved in water. Filter sterilisation is required.

Preparation

1. Weigh out the malt into a suitable container. Add two volumes of the distilled/deionised water per volume of malt, and bring to boiling point.
2. Add the agar and the remainder of the water, and dissolve completely before autoclaving.
3. Autoclave at 121 °C and 15 p.s.i. for 15 min.
4. Adjust to pH 7 ±0.2 pH units.
5. Allow agar to cool to approximately 50 °C and add streptomycin sulphate dissolved in water.
6. Pour 20–22 ml of the molten agar into 90 mm Petri dishes and allow to solidify before use.

If using ready-to-use 1 % malt agar, prepare according to specifications of supplier.

Storage

Prepared plates may be stored at 4 °C for up to 6 weeks.

Sodium hypochlorite solution

Sodium hypochlorite for pretreatment of seed can be prepared from commercial bleach diluted to 1 % available chlorine. The concentration of chlorine in commercial bleach varies considerably and declines with storage. Use the formula:

$$V_{stock} = V_{final} \times C_{final} / C_{stock}$$

(where V = volume and C = % available chlorine) to calculate the volume of commercial bleach stock solution required to prepare sodium hypochlorite solutions for use in seed pretreatment.

To prepare a 1 l solution of sodium hypochlorite containing 1 % chlorine from a stock of commercial bleach containing 12 % available chlorine:

$$V_{stock} = 1 \times 1/12 = 0.083$$

Thus add 83 ml of the 12 % stock to 917 ml water.

The percentage of active chlorine decreases rapidly in solution so, NaClO 1 % solution must be stored in the dark and used within 3 days of preparation. It is possible to check chlorine concentration with chlorine strip tests.

References

Anonymous (1964). Working Sheet S3 No. 3. In *International Seed Testing Association Handbook on Seed Health Testing*, International Seed Testing Association, Basserdorf, Switzerland.

Anonymous (2007). Chapter 7: Seed Health Testing. In *International Rules for Seed Testing*, International Seed Testing Association, Basserdorf, Switzerland.

Booth, C. (1971). *The Genus* Fusarium*, section Arachnites,* pp. 42–43, CMI, England.

Glynn, N. C., Hare, M. C., Parry D. W. & Edwards, S. G. (2005), Phylogenetic analysis of EF-1 alpha gene sequences from isolates of *Microdochium nivale* leads to elevation of varieties *majus* and *nivale* to species status. *Mycological Research*, **109:8,** 872–880.

Miles, S. R. (1963). Handbook of tolerances and of measures of precision for seed testing. *Proceedings of the International Seed Testing Association*, **28** (3), 525–686.

Nelson, P. E., Toussoun, T. A. & Marasas, W. F. O. (1983). Fusarium *species: An illustrated manual for identification*, pp. 62–63, Pennsylvania State University Press, USA.

Rayner, R. W. (1970). *CMI mycological chart, sheet 1 No. 41.* Commonwealth Mycological Institute, CABI, England.

Roberts, S. J., Phelps, K., Taylor, J. D. & Ridout, M. S. (1993). Design and interpretation of seed health assays. In *Proceedings of the First ISTA Plant Disease Committee Symposium on Seed Health Testing, Ottawa, Canada* (ed. J. W. Sheppard), pp. 115–125, Agriculture Canada, Ottawa, Canada.

Validation references

ISTA (2007). Method for the detection of *Microdochium nivale* on *Triticum* spp. *Method Validation Reports*. International Seed Testing Association, Bassersdorf, Switzerland.