



International Rules for Seed Testing
Annexe to Chapter 7: Seed Health Testing Methods



7-013b: Detection of *Ustilago nuda* on *Hordeum vulgare* (Barley) by dehulling method and floating embryo extraction

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 damage etc., resulting from the use of this method.

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

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7-013b: *Ustilago nuda* on *Hordeum vulgare***Crop:** *Hordeum vulgare* (Barley)**Pathogen:** *Ustilago nuda* (Jens.) Rost r.**Prepared by:** Karin Sperlingsson

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Sponsored by: ISTA Seed Health Committee, *Ustilago nuda* Working Group and Nordic *Ustilago* Working Group**Revision history:** Version 1.0, 2010-10-01.**Submitted by:** Nordic Seed Pathology Working Group and ISTA Seed Health Committee

Background

Since the 1970s, the Nordic laboratories have used a modification of ISTA method 7-013a described by Joelson (1968). The method described here differs from 7-013a in the embryo extraction technique and the procedure used to clear embryos for examination of the *Ustilago* mycelium. A validation study comparing the two methods was carried out. Three seed lots with infection levels between 1% and 4% were tested by three laboratories using both the current method 7-013a and the 'Nordic' Method (7-013b). The validation study shows that the two methods produce equivalent results (Sperlingsson, 2011). The Nordic method offers an alternative method for laboratories that do not have access to plentiful warm water, nor a fume hood. The alternative embryo-clearing process adds a day to the duration of the test, so may not be suitable where a quicker turnaround is required. It does, however, offer an alternative clearing procedure which could be used in combination with the existing method to provide flexibility of resources within laboratories during busy periods.

Validation studies

Sperlingsson, K. (2011).

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Safety precautions

Ensure that you are familiar with hazard data and take appropriate safety precautions. It is assumed that this procedure is being carried out in a laboratory by persons familiar with the principles of Good Laboratory Practice. Great care must be taken when working with sulphuric acid and sodium hydroxide; the analyst should wear full protective clothing. Dispose of all waste materials in an appropriate way and in accordance with local health, environmental and safety regulations.

Treated seed

This method has not been validated for the determination of *Ustilago nuda* 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)

Materials

Reference material: seed known to be infected or other appropriate material.

Oven: capable of operating at 75 ± 5 °C.

Sulphuric acid (H₂SO₄): concentration 25–37% by weight.

Electric hand mixer: at low speed.

Sodium hydroxide + sodium chloride: 10–15% NaOH plus 110–175 g salt per litre of solution.

Brass sieves: 1 mm mesh, with one additional sieve of larger mesh (approx. 2.4 mm) and an additional fine sieve with mesh smaller than 1 mm.

Glycerol-ethanol solution: one part glycerol to two parts ethanol.

Lactic acid: more than 70%.

95% ethanol

Glycerol (glycerine)

Microscope: with substage illumination.

Sample preparation

The test is carried out on a working sample as described in section 7.4.1 of the International Rules for Seed Testing.

The method was validated on a maximum sample size of 120 g, and 1000 embryos were examined. Seed can be prepared either by weight or by counting.

7-013b: *Ustilago nuda* on *Hordeum vulgare***Method****Dehulling**

1. Place the working sample in a glass beaker with 25–37% H₂SO₄ until the seeds are covered.
2. Incubate in an oven at 75 °C for 50 minutes or until the seeds turn a medium-brown colour.
3. Carefully pour off the H₂SO₄ solution. Rinse seeds by pouring water into the beaker, gently mix and pour out the water. Add new water and remove the loosened hulls by stirring robustly with a rod. Remove hulls by carefully removing the water. If hulls remain, add new water, and either use an electric hand mixer at low speed (maximum 3 minutes) or continue stirring. Repeat procedure until all hulls are removed. Be careful not to lose any kernels (seed without hulls).
4. Place drained kernels in a container with the NaOH-NaCl solution.
5. Incubate overnight (approximately 15 hours) in 22 ± 3 °C.
6. Stir mixture gently to loosen the embryos from the kernels. Pour the loosened embryos which float to the top of the liquid into a beaker.
7. Repeat the procedure until all embryos are released.
8. To ensure that there are no remaining embryos, place the dissolved kernels on top of a coarse sieve combined with a fine sieve. The coarse sieve shall have a mesh of approximately 2.4 mm, enough to let the embryos pass but retain the remains of the kernels. The fine sieve should have a mesh of 1 mm. If there are any embryos in the bottom sieve, add these to the beaker.
9. Using a fine sieve, drain the NaOH-NaCl solution from the embryos and rinse in running water for approximately 10 s.
10. If there is a large amount of chaff with the embryos, add water and remove the floating chaff.
11. Drain the embryos, place in a beaker and cover with lactic acid.
12. Incubate overnight in an oven at 75 ± 5 °C
13. Using a fine sieve, drain the lactic acid from the embryos. The embryos can be made more transparent by being washed in ethanol or covered for a few minutes in 95% ethanol. Cover the embryos with glycerine-ethanol (95%) (1:3) solution or pure glycerine.
14. Examine the embryos under the microscope according to ISTA method 7-013a.

General methods (common to many test procedures)**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.

7-013b: *Ustilago nuda* on *Hordeum vulgare***Quality assurance****Critical control points**

None listed.

Preparation of chemicals**Preparation of sulphuric acid**

For safety reasons, ready-made sulphuric acid 25–37% (by weight) is preferable.

If ready-made 25–37% sulphuric acid is not available, add concentrated sulphuric acid to water. **Not the reverse, never add water to acid!** The density of H₂SO₄ is 1.8356 g/mL. The quantity is calculated depending on the required concentration. Weight/density = volume.

Preparation of sodium hydroxide

For safety reasons, ready-made NaOH solution 10–15% or ready-made NaOH-NaCl is preferable.

If these are not available, dissolve 130–175 g sodium hydroxide pellets and 110–150 g sodium chloride in 1 L of cold tap water.

References

- Joelson, G. (1968). Laboratoriemetod för bestämning av naket sot hos korn. Årsredogörelse 1967–1968, Lokala Frökontrollen I Skara.
- Joelson, G. (no date). Analys av naket sot enligt metod utarbetad I Skara av G. Joelson (available from ISTA Secretariat).
- Sperlingsson, K. (2011). Alternative embryo extraction procedure to 7-013b *Ustilago nuda*/ *Hordeum vulgare*. ISTA Method Validation Report 2011. International Seed Testing Association, Bassersdorf, Switzerland.

