International Rules for Seed Testing
2019

Chapter 7: Seed health testing

Including changes and editorial corrections adopted at the Ordinary General Meeting 2018, Sapporo, Japan

Effective from 1 January 2019
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.

Published by
The International Seed Testing Association (ISTA)
Zürichstr. 50, CH-8303 Bassersdorf, Switzerland

©2019 International Seed Testing Association (ISTA)

Online ISSN 2310-3655

All rights reserved. No part of this publication may be reproduced, stored in any retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior permission in writing from ISTA.
Contents

Preface to the 2019 Edition of the ISTA Rules ............... v

Chapter 7: Seed health testing ............................... 7-1
  7.1 Object ......................................................... 7-1
  7.2 Definitions .................................................... 7-1
    7.2.1 Seed health .............................................. 7-1
    7.2.2 Pretreatment ............................................ 7-1
    7.2.3 Seed treatment ......................................... 7-1
    7.2.4 ISTA Seed Health Method Validation
      Programme .................................................... 7-1
  7.3 General principles ......................................... 7-1
  7.4 Procedures .................................................. 7-1
    7.4.1 Working sample ......................................... 7-1
    7.4.2 Seed treatment .......................................... 7-2
    7.4.3 Sample storage .......................................... 7-2
    7.4.4 Specific directions ..................................... 7-2
  7.5 Calculation and expression of results ..................... 7-2
  7.6 Reporting results .......................................... 7-2

Table 7A. ISTA official seed health testing methods ..... 7-3
Preface to the 2019 Edition of the ISTA Rules

Since 2014, the International Rules for Seed Testing (ISTA Rules) are primarily available in electronic form only. The ISTA Rules can be downloaded as a complete PDF file or as individual chapters from:

http://www.ingentaconnect.com/content/ista/rules

If required, users of the ISTA Rules can print their own copies. For further information on the ISTA Rules, see:

http://www.seedtest.org/rules

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

Seed health testing methods

Previously, the seed health testing methods were published as a separate Annexe to Chapter 7 of the ISTA Rules. They are now available as separate method sheets from the ISTA web site at:

http://www.seedtest.org/seedhealthmethods

Details of changes

The 2019 changes are editorial corrections or Rules changes adopted at the Ordinary General Meeting held at Sapporo, Japan, in June 2018. Edits were made in Adobe InDesign by Vanessa Sutcliffe of HeartWood Editorial (www.heartwoodeditorial.co.uk).

The changes in the text content from the previous edition of the ISTA Rules are listed below. They can be displayed as yellow highlighted text as a ‘layer’ within the electronic copy with comments on what has changed.

For the previous history of amendments to the ISTA Rules, see the Prefaces for 2003 to 2018 on the ISTA website.

Ernest Allen, ISTA Rules Committee Chair
Susan Alvarez, ISTA Rules Committee Vice-Chair
ISTA Secretariat
Changes to the ISTA Rules for 2019

General editorial:
Renumbering of Chapter 9 to reflect other chapters and subsequent cross-referencing updated.

Chapter 1
1.4.5: Clarification of reporting on ISTA Certificates.
1.5.2: Details about the use of ‘N’ (test not done) amended to avoid any confusion in reporting results. Clarification of reporting on ISTA Certificates.
1.5.2.2: Clarification of reporting on ISTA Certificates. Points removed (‘The percentage by weight... are listed first.’ and ‘The kinds of inert matter... (to one decimal place.’).
1.5.2.4: Addition of other species of parasitic weeds with dust-like seed under ‘Determination of other seeds by number’.
1.5.2.13: Consequential change in ‘Reporting results’ due to revision of Chapter 10.

Chapter 2
2.2.12: Revision of the definition of treated seed to include film coating.
2.2.13: Clarification of the criteria to distinguish coated seed from treated seed.
2.5.4.5: Sentence moved (‘If the sample... single submitted sample.’) and sentence added (‘This also applies... exceptions listed below.’) to remove uncertainty over submitted samples.
Table 2A Part 3: New species added to ‘Lot sizes and sample sizes’ table (Eustoma exaltatum and Felicia heterophylla), based on thousand-seed weight determinations.
Table 2B Part 1: Consequential change in table due to revision of Chapter 10.

Chapter 3
3.7: Consequential changes in clarification of reporting on ISTA Certificates. Points removed (‘The percentage by weight... are listed first.’ and ‘The kinds of inert matter... (to one decimal place.’).
Table 3B Part 1: New PSD numbers included (for Eustoma and Felicia).

Chapter 4
4.5.3, 4.5.3.1, 4.5.3.2, 4.5.3.3, 4.5.3.4, 4.7: Addition of other species of parasitic weeds with dust-like seed under ‘Determination of other seeds by number’.
4.5.3.1: Sentence added (‘This test is not... pelleted seed.’).

Chapter 5
5.2.7.2: Addition of criterion on evaluation of the root system of Spinacia oleracea seedlings, supported by a validation study.
5.2.8.1: Changes in abnormal seedling evaluation – abnormality 21/01 revised (‘and/or’ added).
5.4.3.1: Addition of crepe cellulose paper as a medium for the TP germination method. Note: an abbreviation for crepe cellulose paper is not required as it is a type of paper used for the TP method.
5.6.2.1.1: Precision about TP method to indicate that germination boxes can be slanted/inclined during the germination test.
5.6.3.1: Change in wording from ‘non-imbibed’ to ‘dry’ for clarity about the preheating method applied to break seed dormancy.
Table 5A Part 1: Recommendation for the germination method for Raphanus sativus added, supported by a validation study.
Table 5A Part 3: Germination methods for Eustoma exaltatum and Felicia heterophylla included, supported by validation studies.
Table 5E Part 3: Values corrected so they are similar to those in Table 5B Part 1.

Chapter 7
7.2.4: Reference added to ISTA Technical Guidelines. Method 7-009, 7-010, 7-012, 7-014: Changes to scientific names of fungi, based on USDA database and Index Fungorum.
Method 7-019a: Method modified with addition of pre-screening methods and use of TaqMan assay as a third option for suspect screening.
Method 7-019b: Addition of reference to use of TaqMan assay (in 7-019a) as a third option for suspect screening.
Method 7-022: Editorial change to Methods step 5 – ‘/’ changed to ‘and/or’ and sentence added (‘As morphology can overlap... Microdochium spp.’).

Chapter 8
8.3.3: Sentence added (‘DNA can be extracted... equivalent to one seedling’).
8.9.4.3.2, 8.9.6.6.1.2, 8.9.6.6.2.2: Sentence added (‘Note that as an alternative... prior to pouring the gel.’) for consistency in gel polymerisation method.

Chapter 10
Change of title from ‘Weight determination’ to ‘Thousand-seed weight (TSW) determination’. Revision to whole chapter and addition of Table 10A.

Chapter 11
11.1.1: Cross reference updated under ‘Seed treatment’ to ‘2.2.12’.
11.2.5.4.1: Duplication of lot size definition deemed unnecessary so only the reference to Chapter 2 is kept.
Table 11A: Consequential change in table due to revision of Chapter 10.
Chapter 15
15.3, 15.8.1.2, Table 15A: Removal of ‘Kabuli type’ after *Cicer arietinum* for species to which the conductivity test can be applied, to indicate that Desi type chickpeas are also included.

Chapter 18
18.7, 18.8.4: Consequential changes in section titles due to revision of Chapter 10.

Chapter 19
Review and updates throughout whole chapter.
Chapter 7: Seed health testing

7.1 Object

The object of a seed health test is to determine the health status of a seed sample, and by inference that of the seed lot.

Health testing of seed is important for four reasons:

a. Seed-borne inoculum may give rise to progressive disease development in the field and reduce the commercial value of the crop.
b. Imported seed lots may introduce diseases into new regions. Tests to meet quarantine requirements may therefore be necessary.
c. Seed health testing may elucidate seedling evaluation and causes of poor germination or field establishment and thus supplement germination testing.
d. Seed health test results can/may indicate the necessity to carry out/perform seed lot treatment(s) in order to eradicate seed-borne pathogens or to reduce the risk of disease transmission.

7.2 Definitions

7.2.1 Seed health

Health of seed refers primarily to the presence or absence of disease-causing organisms, such as fungi, bacteria and viruses, and animal pests, including nematodes and insects, but physiological conditions such as trace element deficiency may be involved.

7.2.2 Pretreatment

Any physical or chemical laboratory treatment of the working sample preceding incubation, given solely to facilitate testing.

7.2.3 Seed treatment

See 2.2.12. For seed health testing, a seed lot may be treated for the purpose of controlling plant pathogens or insect pests, or correcting trace element deficiencies.

7.2.4 ISTA Seed Health Method Validation Programme

Before publication in the International Rules for Seed Testing, the ISTA seed health testing methods (new or equivalent) are validated. The principles and factors which should be considered in the validation of methods for the detection of seed-borne pathogens are described in the ISTA Technical Guidelines for Organising and Analysing Results of Proficiency Tests (PT) and Interlaboratory Tests for Validation of Methods (CT).

7.3 General principles

Seed health testing should be performed using methods and equipment which have been tested to ensure they are fit for purpose. Different methods of testing are available, varying in sensitivity and reproducibility and in the amount of training and equipment required. The method used will depend on the pathogen or condition to be investigated, the species of the seed, and the purpose of the test. Selection of the method and evaluation of the results requires knowledge and experience of the methods available. The presence or absence of disease organisms, pests and deleterious physiological conditions specified by the sender is estimated as accurately as the method used permits.

7.4 Procedures

7.4.1 Working sample

The entire submitted sample, or a proportion of it, depending on the test method, may be used as a working sample. The sample should be packaged and submitted in a manner which will not alter its seed health status.

Exceptionally, a submitted sample larger than that prescribed in 2.8 may be required and in such cases the sampler must be instructed accordingly.

When a portion of the submitted sample is required as a working sample, the reduction must be carried out in accordance with 2.5.2, taking appropriate precautions to avoid cross-contamination.

Normally the working sample must not be less than that specified in the method description.

Replicates containing a specified number of seeds, if required, must be taken at random from a subsample after thorough mixing.
Chapter 7: Seed health testing

7.4.2 Seed treatment

Test results may be influenced by treatment applied to the seed lot. Seed health tests on treated seeds will generally deliver unreliable test results caused by masking or inhibition of the growth of the target organism. Individual Method Sheets will determine whether the testing of treated seeds is acceptable.

7.4.3 Sample storage

The microflora of seed, in the lot or the sample, may change considerably during storage in conditions in which seed viability is satisfactorily maintained. The selection of the appropriate storage conditions must take into account the optimal storage temperature and container in order to maintain sample integrity.

Abundant development of saprophytic moulds including ‘storage fungi’ in tests can be an indication that the seed is not of good quality due to unfavourable harvesting, processing or storage conditions, or to ageing. Some fungi (such as Rhizopus spp.) spread rapidly over tests on blotters and may rot originally healthy seedlings or may interfere with outgrowth of the pathogen from the plated infected seeds. Pretreatment as described in the specific method may be advisable.

7.4.4 Specific directions

Specific seed health testing methods are published online on the ISTA web site at:

www.seedtest.org/seedhealthmethods

Seed health methods are normally based on one host, and one pathogen, but multi-pathogen methods may be included. Before publication, all seed health test methods must be validated through the ISTA Seed Health Method Validation Programme. Methods validated in this way at the time of printing are listed in Table 7A. Additions, updates and deletions to this list can be found on the ISTA web site (www.seedtest.org/seedhealthmethods). The definitive list is held by the ISTA Secretariat. It is the responsibility of the laboratory using the method to consult this list.

7.5 Calculation and expression of results

Results are expressed either qualitatively or quantitatively as specified in the individual prescribed methods.

7.6 Reporting results

The results of a test for seed health must be reported under ‘Other determinations’ as follows:
- either qualitative or quantitative results, as specified in the individual methods;
- negative and positive results, as specified in the individual methods;
- the scientific name of the pathogen detected;
- the percentage of infected seeds;
- the method used, including any pretreatment (7.2.2);
- the size of the sample or fraction examined;
- any additional permitted procedure used.

The absence of a statement concerning the health condition of the seed does not necessarily imply that the health condition is satisfactory.
### Table 7A. ISTA official seed health testing methods

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
<th>Pathogen(s)</th>
<th>Host</th>
<th>Date approved</th>
<th>Review due</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-003</td>
<td>Detection of <em>Botrytis cinerea</em> in <em>Helianthus annuus</em> (sunflower) seed</td>
<td><em>Botrytis cinerea</em> Pers. ex Pers. (Perfect state <em>Botryotinia fuckeliana</em> (de Bary) Whetzel, syn. <em>Sclerotinia fuckeliana</em> (de Bary) Fuckel.)</td>
<td><em>Helianthus annuus</em> L.</td>
<td>2011</td>
<td>2017</td>
</tr>
<tr>
<td>7-004</td>
<td>Detection of <em>Leptosphaeria macularis</em> and <em>Plenodomus biglobosus</em> in <em>Brassica</em> spp. seed</td>
<td><em>Leptosphaeria macularis</em> (Tode ex Fr.) Ces. &amp; de Not (previously <em>Phoma lingam</em>) or <em>Plenodomus biglobosus</em> (Shoemaker &amp; H. Brun) (previously <em>Leptosphaeria biglobosa</em>)</td>
<td><em>Brassica</em> spp.</td>
<td>2017</td>
<td>2016</td>
</tr>
<tr>
<td>7-007</td>
<td>Detection of <em>Alternaria linicola</em>, <em>Botrytis cinerea</em> and <em>Colletotrichum lini</em> in <em>Linum usitatissimum</em> (flax, linseed) seed</td>
<td><em>Alternaria linicola</em>, <em>Botrytis cinerea</em> and <em>Colletotrichum lini</em> in <em>Linum usitatissimum</em> (flax, linseed) seed</td>
<td><em>Linum usitatissimum</em> L.</td>
<td>2012</td>
<td>2017</td>
</tr>
<tr>
<td>7-008</td>
<td>Detection of <em>Caloscypha fulgens</em> in <em>Picea engelmannii</em> and <em>P. glauca</em> (spruce) seed</td>
<td><em>Caloscypha fulgens</em> (Pers.) Boud. (imperfect state <em>Geniculodendron pyriforme</em> Salt)</td>
<td><em>Picea engelmannii</em> Pary ex Engelm.; <em>Picea glauca</em> (Moench) Voss</td>
<td>2011</td>
<td>2016</td>
</tr>
</tbody>
</table>

**Effective 1 January 2019**
### Table 7A. ISTA official seed health testing methods (cont.)

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Host</th>
<th>Pathogen(s)</th>
<th>Date approved</th>
<th>Review due</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-010</td>
<td>Detection of <em>Bipolaris oryzae</em> in <em>Oryza sativa</em> (rice) seed</td>
<td><em>Oryza sativa</em> L.</td>
<td><em>Bipolaris oryzae</em> (Breda de Haan) Shoem., syn. Drechslera oryzae, syn. Helminthosporium oryzae Breda de Haan (Perfect state <em>Cochliobolus miyabeanus</em> (Ito &amp; Kurib.) Drechsler ex Dastur, syn. <em>Ophiobolus miyabeanus</em> Ito &amp; Kuribayashi)</td>
<td>2018</td>
<td>2023</td>
</tr>
<tr>
<td>7-011</td>
<td>Detection of <em>Pyricularia oryzae</em> in <em>Oryza sativa</em> (rice) seed</td>
<td><em>Oryza sativa</em> L.</td>
<td><em>Magnaporthe grisea</em> (Hebert) Barr (Imperfect state <em>Pyricularia oryzae</em> Cavara, syn. <em>P. grisea</em>)</td>
<td>2011</td>
<td>2016</td>
</tr>
<tr>
<td>7-012</td>
<td>Detection of <em>Trichoconiella padwickii</em> in <em>Oryza sativa</em> (rice) seed</td>
<td><em>Oryza sativa</em> L.</td>
<td><em>Trichoconiella padwickii</em> Ganguly, syn. <em>Alternaria padwickii</em> (Ganguly) Jain</td>
<td>2018</td>
<td>2023</td>
</tr>
<tr>
<td>7-013a</td>
<td>Detection of <em>Ustilago nuda</em> in <em>Hordeum vulgare</em> (barley) seed by embryo extraction</td>
<td><em>Hordeum vulgare</em> L.</td>
<td><em>Ustilago nuda</em> (Jens.) Rostr.</td>
<td>2011</td>
<td>2016</td>
</tr>
<tr>
<td>7-013b</td>
<td>Detection of <em>Ustilago nuda</em> in <em>Hordeum vulgare</em> (barley) seed by dehulling and embryo extraction</td>
<td><em>Hordeum vulgare</em> L.</td>
<td><em>Ustilago nuda</em> (Jens.) Rostr.</td>
<td>2011</td>
<td>2016</td>
</tr>
<tr>
<td>7-017</td>
<td>(Replaced by 7-007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-018</td>
<td>(Replaced by 7-007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-019a</td>
<td>Detection of <em>Xanthomonas campestris pv. campestris</em> in <em>Brassica</em> spp. seed</td>
<td><em>Brassica</em> spp.</td>
<td><em>Xanthomonas campestris pv. campestris</em> (Pammel) Dowson</td>
<td>2018</td>
<td>2023</td>
</tr>
<tr>
<td>7-019b</td>
<td>Detection of <em>Xanthomonas campestris pv. campestris</em> in disinfested/disinfected <em>Brassica</em> spp. seed</td>
<td><em>Brassica</em> spp.</td>
<td><em>Xanthomonas campestris pv. campestris</em> (Pammel) Dowson</td>
<td>2018</td>
<td>2023</td>
</tr>
</tbody>
</table>
Table 7A. ISTA official seed health testing methods (cont.)

7-020: Detection of Xanthomonas hortorum pv. carotae in Daucus carota (carrot) seed
Host: Daucus carota L.
Pathogen(s): Xanthomonas hortorum pv. carotae
(Kendrick) Vauterin, Hoste, Kersters & Swings, syn. X. campestris pv. carotae (Kend) Dye
Date approved: 2010
Review due: 2015

7-021: Detection of Xanthomonas axonopodis pv. phaseoli and X. axonopodis pv. phaseoli var. fuscans in Phaseolus vulgaris (bean) seed
Host: Phaseolus vulgaris L.
Pathogen(s): Xanthomonas axonopodis pv. phaseoli
(Smith) Vauterin, Hoste, Kersters & Swings, syn. X. campestris pv. phaseoli (Smith) Dye; Xanthomonas axonopodis pv. phaseoli var. fuscans Vauterin, Hoste, Kersters & Swings, syn. X. campestris pv. phaseoli var. fuscans (Burkholder) Starr & Burkholder
Date approved: 2011
Review due: 2016

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
Date approved: 2018
Review due: 2023

7-023: Detection of Pseudomonas savastanoi pv. phaseolicola in Phaseolus vulgaris (bean) seed
Host: Phaseolus vulgaris L.
Pathogen(s): Pseudomonas savastanoi pv. phaseolicola (Burk.) Gardan, Bollet, Abu, Ghorrhah, Grimont & Grimont, syn. P. syringae pv. phaseolicola (Burk.) Young, Dye & Wilkie
Date approved: 2012
Review due: 2017

7-024: Detection of pea early browning virus and pea seed-borne mosaic virus in Pisum sativum (pea) seed
Host: Pismum sativum L.s.l.
Pathogen(s): Pea early browning virus (PEBV) and pea seed-borne mosaic virus (PSbMV)
Date approved: 2012
Review due: 2017

7-025: Detection of Aphelenchoides besseyi in Oryza sativa (rice) seed
Host: Oryza sativa L.
Pathogen(s): Aphelenchoides besseyi Christie
Date approved: 2013
Review due: 2018

7-026: Detection of squash mosaic virus, cucumber green mottle mosaic virus and melon necrotic spot virus in curcubit seed
Host: Cucurbita
Pathogen(s): Squash mosaic virus (SqMV); cucumber green mottle mosaic virus (CGMMV); melon necrotic spot virus (MNSV)
Date approved: 2014
Review due: 2019

7-027: Detection of Pyrenophora teres and P. graminea in Hordeum vulgare (barley) seed
Host: Hordeum vulgare L.
Pathogen(s): Pyrenophora teres Drechsler (Imperfect state Drechslera teres) (Sacc.) Shoem.; Pyrenophora graminea Itô & Kurib. (Imperfect state D. graminea (Rabenh. Ex Schlecht.) Shoem.)
Date approved: 2011
Review due: 2016

7-028: Detection of infectious tobacco mosaic virus and tomato mosaic virus in Solanum lycopersicum (tomato) seed by the local lesion assay (indexing) in Nicotiana tabacum plants
Host: Solanum lycopersicum L.
Pathogen(s): Tobacco mosaic virus (TMV); tomato mosaic virus (ToMV)
Date approved: 2012
Review due: 2017

7-029: Detection of Pseudomonas syringae pv. pisi in Pismum sativum (pea) seed
Host: Pismum sativum L.s.l.
Pathogen(s): Pseudomonas syringae pv. pisi (Sack.) Young, Dye & Wilkie
Date approved: 2012
Review due: 2017

7-030: Detection of Acidovorax valerianellae in Valerianella locusta (corn salad) seed
Host: Valerianella locusta (L.) Later.
Pathogen(s): Acidovorax valerianellae sp. nov.
Date approved: 2014
Review due: 2019
7-031: Filtration method for detection of Ditylenchus dipsaci in Medicago sativa; D. dipsaci and D. gigas in Vicia faba

Host: Medicago sativa L. and Vicia faba L.

Pathogen(s): Ditylenchus dipsaci Kuhn, 1857; Ditylenchus gigas n. sp.

Date approved: 2017

Review due: 2022

7-032: Detection of Verticillium dahliae in Spinacia oleracea (spinach) seed

Host: Spinacia oleracea L.

Pathogen(s): Verticillium dahliae Kleb.

Date approved: 2017

Review due: 2022