



International Seed Testing Association

Secretariat, Zürichstrasse 50, CH-8303 Bassersdorf, Switzerland

Phone: +41 44 838 60 00 Fax: +41 44 838 60 01

Email: ista.office@ista.ch - <http://www.seedtest.org>

Document OGM 20-05

Rules Proposals for the International Rules for Seed Testing 2021 Edition

This document was prepared by the Technical Committees (TCOMs) and the Rules Committee of the Association and has been endorsed by the ISTA Executive Committee (ECOM). The proposals are submitted to the ISTA Ordinary General Meeting 2020 for voting by the nominated ISTA Designated Members on behalf of their respective Governments.

It is submitted to all ISTA Designated Authorities, ISTA Members and ISTA Observer Organizations for information two months prior to the ISTA Ordinary General Meeting 2020.

It contains proposed amendments and changes for the ISTA *International Rules for Seed Testing* and will be discussed and voted on at the Ordinary General Meeting 2020 to be held on Thursday, May 28, 2020. *Consideration and Adoption of the Proposed Rules Changes.*

Introduction to the ISTA Rules Proposals to become effective 1 January 2021

The current version of the ISTA International Rules for Seed Testing (ISTA Rules) is the 2020 edition.

The ISTA Rules are only available electronically as a printable pdf file and are available for free download by ISTA members from the Ingenta website:

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

The electronic version also includes the French and German versions of the ISTA Rules. There is also now a separate official Spanish version for the main chapters of the ISTA Rules. If there are any questions on interpretation of the ISTA Rules the English version is the definitive version.

For further information on the ISTA Rules, see: <http://www.seedtest.org/rules>

The effective dates are changed annually. The changes from the previous edition of the ISTA Rules can be displayed as yellow highlighted text as a 'layer' within the electronic copy with comments on what has changed. Previous Prefaces as a 'history of changes' are available on the ISTA website.

The ISTA Rules are the result of the work of the ISTA Technical Committees (TCOMs) with input from many different sources. Thanks go to all the Technical Committee members and the ISTA Secretariat for their help with the annual proposals.

The following Rules Proposals will be discussed at the ISTA Ordinary General Meeting on May 28, 2020 and may be amended during the meeting. If the proposals are accepted by the membership, amendments will be issued, and they will become the 2021 edition of the ISTA Rules.

Please let us know about any problems with these proposals.

Many thanks.

Ernest Allen and Sue Alvarez

Chair and Vice-Chair of ISTA Rules Committee

Contact details:

Ernest Allen

E-mail: ernest.allen@usda.gov

Sue Alvarez

E-mail: suersl@silcom.com

Key to text changes:

~~Deleted text~~

New text

New text in large blocks, not underlined for ease of reading

Any changes made after the proposals were published to the membership

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PART A. INTRODUCTION OF EDITORIAL CHANGES

A.1. Editorial corrections

Safety and Health Statement	
The ECOM decided to include a Health and Safety statement in the introduction to the ISTA Rules. This statement was reviewed and approved by the ECOM and the Rules committee.	
CURRENT VERSION	PROPOSED VERSION
I-1 General Information ...	I-1 General Information ... Users of the ISTA Rules are responsible to comply with the Health and Safety Requirements for the jurisdiction they operate in. ISTA does not audit or accept any responsibility for compliance to Health and Safety Regulations. Any statements relating to Health and Safety in the ISTA Rules are for guidance only.

Reporting Results	
Changes required as a result of changes approved in Chapter 2 during OGM16.	
CURRENT VERSION	PROPOSED VERSION
1.5.2.2 Purity ... C. When the submitted sample received for purity testing weighs less than the weight in Table 2C, column 4, report under other determinations and use the current statement, according to 2.5.4.5 : ‘The submitted sample weighed only ... g and is not in accordance with the <i>International Rules for Seed Testing</i> .’ 3.7 Purity ... C. When the submitted sample received for purity testing weighs less than the weight in Table 2C, column 4, report under other determinations and use the current statement, according to 2.5.4.5 : ‘The submitted sample weighed only ... g and is not in accordance with the <i>International Rules for Seed Testing</i> .’	1.5.2.2 Purity ... C. When the submitted sample received for purity testing weighs less than the weight in Table 2C, column 4, report under other determinations and use the statement: ‘The submitted sample weighed only ... g and is not in accordance with the <i>International Rules for Seed Testing</i> .’ 3.7 Purity ... C. When the submitted sample received for purity testing weighs less than the weight in Table 2C, column 4, report under other determinations and use the statement: ‘The submitted sample weighed only ... g and is not in accordance with the <i>International Rules for Seed Testing</i> .’

Reporting Results	
Clarifying that authorities are not required for reporting.	
CURRENT VERSION	PROPOSED VERSION
<p>1.5.2.2 Purity</p> <p>...</p> <ul style="list-style-type: none"> The scientific name of the species of pure seed, in accordance with Table 2C (e.g. <i>Triticum aestivum</i> L. subsp. <i>aestivum</i>. Where it is impossible to determine species... <p>...</p> <ul style="list-style-type: none"> The scientific name of every species of other seeds found, in accordance, where applicable, with the current <i>ISTA List of stabilised Plant Names</i>, available at www.seedtest.org/stablist (e.g. <i>Elymus repens</i> (L.) Gould) <p>3.7 Purity</p> <p>...</p> <ul style="list-style-type: none"> The scientific name of the species of pure seed, in accordance with Table 2C (e.g. <i>Triticum aestivum</i> L. subsp. <i>aestivum</i>. Where it is impossible to determine species... 	<p>1.5.2.3 Purity</p> <p>...</p> <ul style="list-style-type: none"> The scientific name of the species of pure seed, in accordance with Table 2C (e.g. <i>Triticum aestivum</i> subsp. <i>aestivum</i>. Where it is impossible to determine species... <p>...</p> <ul style="list-style-type: none"> The scientific name of every species of other seeds found, in accordance, where applicable, with the current <i>ISTA List of Stabilised Plant Names</i>, available at www.seedtest.org/stablist (e.g. <i>Elymus repens</i>) <p>3.7 Purity</p> <p>...</p> <ul style="list-style-type: none"> The scientific name of the species of pure seed, in accordance with Table 2C (e.g. <i>Triticum aestivum</i> subsp. <i>aestivum</i>. Where it is impossible to determine species...

1.5.2.21 Genetically modified organisms	
Changes required as a result of changes approved in Chapter 19 during OGM18.	
CURRENT VERSION	PROPOSED VERSION
<p>1.5.2.21 Genetically modified organisms</p> <p>The result of a GMO test must be reported under ‘Other determinations’ as follows:</p> <ul style="list-style-type: none"> the request of the applicant; 	<p>1.5.2.21 Genetically modified organisms</p> <p>The result of a GMO test must be reported under ‘Other determinations’ as follows:</p> <ul style="list-style-type: none"> the request of the applicant;

<p>...</p> <ul style="list-style-type: none"> • the limit of detection of the method (when testing seed groups or seed bulk); • the limit of quantification of the method (when testing seed bulk with a quantitative method). <p>1.5.2.21.2 Quantitative results obtained by multiple qualitative tests of individuals or groups of seeds or seedlings</p> <p>...</p> <p>If the results do not show evidence that the seed lot meets a given specification with some confidence, then the applicant will report the point estimate with the 95 % confidence interval.</p> <p>1.5.2.21.3 Quantitative measurements of GMO in bulk samples</p> <p>...</p> <p>If the results do not show evidence that the seed lot meets a given specification with some confidence, then the applicant will report the point estimate with the 95 % confidence interval.</p>	<p>...</p> <ul style="list-style-type: none"> • the limit of detection of the method (when testing seed groups or seed bulk) according to the value verified by the laboratory; • the limit of quantification of the method (when testing seed bulk with a quantitative method) according to the value verified by the laboratory. <p>1.5.2.21.2 Quantitative results obtained by multiple qualitative tests of individuals or groups of seeds or seedlings</p> <p>...</p> <p>If the results do not show evidence that the seed lot meets a given specification at the desired confidence, then the estimated percentage of seed with the 95 % confidence interval will be reported.</p> <p>1.5.2.21.3 Quantitative measurements of GMO in bulk samples</p> <p>...</p> <p>If the results do not show evidence that the seed lot meets a given specification at the desired confidence, then the estimated percentage by mass or number of copies with the 95 % confidence interval will be reported.</p>
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Table 2C Part 1. Changes required as a result of approved nomenclature changes to the ISTA Stabilised List that have been incorporated into Table 2C.

CURRENT VERSION: Table 2C Part 1. Lot sizes and sample sizes: agricultural and vegetable seeds

Species	Maximum weight of lot (kg) (except see 2.8 Note 2)	Minimum submitted sample (g)	Minimum working sample for purity analysis (3.5.1) (g)	
			Purity Analysis (3.5.1)	Other Seeds by number (4.5.1)
1	2	3	4	5
<i>Hordeum vulgare</i> L.	30 000	1 000	120	1 000

PROPOSED VERSION: Table 2C Part 1. Lot sizes and sample sizes: agricultural and vegetable seeds

Species	Maximum weight of lot (kg) (except see 2.8 Note 2)	Minimum submitted sample (g)	Minimum working sample for purity analysis (3.5.1) (g)	
			Purity Analysis (3.5.1)	Other Seeds by number (4.5.1)
1	2	3	4	5
<i>Hordeum vulgare</i> L. subsp. <i>vulgare</i>	30 000	1 000	120	1 000

2.8 Changes required as a result of nomenclature changes to the ISTA Stabilised List that have been incorporated into Table 2C. Previous cross references were not removed prior to the printing of the 2020 rules. Previous cross references will be removed in the 2021 rules and the last sentence of the current version will be replaced.

CURRENT VERSION	PROPOSED VERSION
<p>2.8 Tables for lot size and sample sizes</p> <p>Note 1: ... Changes in the stabilised list agreed at the 2013 ISTA Congress are included in this version of Table 2C. Where plant names have been changed, the old name is included with a cross reference to the new name. This applies only to 2013 Congress changes; previous cross references have been removed.</p>	<p>2.8 Tables for lot size and sample sizes</p> <p>Note 1: ... Changes in the stabilised list agreed at the 2019 ISTA Congress are included in this version of table 2C. Where plant names have been changed, the old name is included with a cross reference to the new name. This applies only to 2019 Congress changes.</p>

4.2.1 Definitions

Changes required as a result of changes approved in Chapter 4 during OGM18.

CURRENT VERSION	PROPOSED VERSION
<p>4.2.1 Other Seeds</p> <p>Other seeds refer to species other than those under test as defined in Rule 3.2.2. In determining the numbers of other seeds, the definitions prescribed in 3.2 must be observed. The extent of the determination of other seeds by</p>	<p>4.2.1 Other Seeds</p> <p>Other seeds refer to species other than those under test as defined in Rule 3.2.2. In determining the numbers of other seeds, the definitions prescribed in 3.2 must be observed. The extent of the determination of other seeds by</p>

<p>number is for either all species or a selection of species in a working sample (see 4.5.1). Determinations of numbers of dust-like seeds of <i>Orobanchaceae</i> species, such as <i>Orobanche</i> or <i>Striga</i>, is only completed upon request of the applicant (see 4.5.3).</p>	<p>number is for either all species or a selection of species in a working sample (see 4.5.1). Determinations of numbers of dust-like seeds (see 4.5.3.1), such as <i>Orobanche</i> and <i>Striga</i>, is only completed upon request of the applicant (see 4.5.3).</p>
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5.8.2 Rounding Results A reference for 5.8.2 will replace the current erroneous reference of 5.8.1 in sections 1.5.2.17.2 and 15.8.2.8.	
CURRENT VERSION	PROPOSED VERSION
<p>1.5.2.17.2 Accelerated ageing test</p> <p>The result of a seed vigour test using the accelerated aging (AA) method must be reported under ‘Other determinations’ as follows:</p> <ul style="list-style-type: none"> • Results are expressed as a percentage, calculated to the nearest whole number (5.8.1) of normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds. If the result for any of these categories is found to be zero, it must be reported as ‘0’. <p>15.8.2.8 Reporting results</p> <p>The result of a seed vigour test using the AA method must be reported under ‘Other determinations’ as follows:</p> <ul style="list-style-type: none"> • Results are expressed as a percentage, calculated to the nearest whole number (5.8.1) of normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds. If the result for any of these categories is found to be zero, it must be reported as ‘0’. 	<p>1.5.2.17.2 Accelerated ageing test</p> <p>The result of a seed vigour test using the accelerated aging (AA) method must be reported under ‘Other determinations’ as follows:</p> <ul style="list-style-type: none"> • Results are expressed as a percentage, calculated to the nearest whole number (5.8.2) of normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds. If the result for any of these categories is found to be zero, it must be reported as ‘0’. <p>15.8.2.8 Reporting results</p> <p>The result of a seed vigour test using the AA method must be reported under ‘Other determinations’ as follows:</p> <ul style="list-style-type: none"> • Results are expressed as a percentage, calculated to the nearest whole number (5.8.2) of normal seedlings, abnormal seedlings, hard seeds, fresh seeds and dead seeds. If the result for any of these categories is found to be zero, it must be reported as ‘0’.

C.7 Clarifying instructions for preparing hypochlorite solutions for pre-treatment as prescribed in the methods. Adding the use of hypochlorite solutions for methods similar to those already approved in the rules.
 This proposal is supported by the Seed Health Committee.

CURRENT VERSION	PROPOSED VERSION

<p>7-008</p> <p>Media and solutions</p>	<p>The % 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.</p> <p>7-008</p> <p>Media and solutions</p> <p>Sodium hypochlorite solution</p> <p>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. Use the formula:</p> $V_{stock} = V_{final} \times C_{final} / C_{stock}$ <p>(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.</p> <p>To prepare a 1 L solution of sodium hypochlorite containing 1 % chlorine from a stock of commercial bleach containing 12 % available chlorine:</p> $V_{stock} = V_{final} \times C_{final} / C_{stock}$ $V_{stock} = 1 \times 1/12 = 0.083$ <p>Thus add 83 mL of the 12 % stock to 917 mL water</p> <p>The % 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</p>
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<p>1.5 % water agar</p> <p>7-016, 7-022</p> <p>Alternatively, sodium hypochlorite solutions can be prepared by using sodium dichloroisocyanurate tablets (e.g. Presept, Johnson & Johnson Medical Products) according to the manufacturer's instructions.</p> <p>7-032</p> <p>Sodium hypochlorite solution</p> <p>A sodium hypochlorite solution for pretreatment of seed can be prepared from commercial bleach diluted to 1.2% active ingredient (sodium hypochlorite). The concentration of chlorine in commercial bleach varies considerably. Use the formula:</p> $V_{stock} = (V_{final} \times C_{final}) / C_{stock}$ <p>(where V = volume and C = % active chlorine) to calculate the volume of commercial bleach stock solution required to prepare the sodium hypochlorite solution for seed treatment. For example, to prepare a 1 litre solution of sodium hypochlorite containing 1.2% sodium hypochlorite from a stock of commercial bleach containing 12% active chlorine:</p> $V_{stock} = (V_{final} \times C_{final}) / C_{stock}$	<p>is possible to check chlorine concentration with chlorine strip tests.</p> <p>1.5 % water agar</p> <p>7-016, 7-022</p> <p>7-032</p> <p>Sodium hypochlorite solution</p> <p>A sodium hypochlorite solution for pretreatment of seed can be prepared from commercial bleach diluted to 1.2% active ingredient (sodium hypochlorite). The concentration of chlorine in commercial bleach varies considerably. Use the formula:</p> $V_{stock} = (V_{final} \times C_{final}) / C_{stock}$ <p>(where V = volume and C = % active chlorine) to calculate the volume of commercial bleach stock solution required to prepare the sodium hypochlorite solution for seed treatment. For example, to prepare a 1 litre solution of sodium hypochlorite containing 1.2% sodium hypochlorite from a stock of commercial bleach containing 12% active chlorine:</p> $V_{stock} = (V_{final} \times C_{final}) / C_{stock}$
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<p>$= (1 \text{ L} \times 1.2\%) / 12\% = 0.1 \text{ L (or 100 mL)}$</p> <p>Thus, add 100 mL of the 12% stock to 900 mL water</p>	<p>$= (1 \text{ L} \times 1.2\%) / 12\% = 0.1 \text{ L (or 100 mL)}$</p> <p>Thus, add 100 mL of the 12% stock to 900 mL water.</p> <p>The % 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.</p>
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8.5 Procedures	
This editorial change is required due an incorrect cross reference to performance approved methods. The corrections were approved by the Variety Committee.	
CURRENT VERSION	PROPOSED VERSION
<p>8.5.3 Examination of seeds</p> <p>...For the application of performance approved methods see 8.2.3.</p> <p>8.5.4 Examination of seedlings</p> <p>...For the application of performance approved methods see 8.2.3.</p> <p>8.5.5 Examination of plants in glasshouse or growth chamber</p> <p>...For the application of performance approved methods see 8.2.3.</p> <p>8.5.6 Examination of plants in field plots</p> <p>...For the application of performance approved methods see 8.2.3.</p>	<p>8.5.3 Examination of seeds</p> <p>...For the application of performance approved methods see 8.3.3.</p> <p>8.5.4 Examination of seedlings</p> <p>...For the application of performance approved methods see 8.3.3.</p> <p>8.5.5 Examination of plants in glasshouse or growth chamber</p> <p>...For the application of performance approved methods see 8.3.3.</p> <p>8.5.6 Examination of plants in field plots</p> <p>...For the application of performance approved methods see 8.3.3.</p>

ACCEPTED BY VOTE	RESULT
Yes	47/48

PART B. NEW SPECIES AND CHANGES TO SPECIES NAMES

B.1.1 Addition of new species to Table 2C

None this year

B.1.2 Changes to the ISTA Stabilised List

None this year.

The next revision of the ISTA Stabilised List will be considered at the 2025 ISTA Meeting.

PART C. RULES CHANGES AND NEW METHODS REQUIRING A VOTE

Chapter 3: The purity analysis

C.3.1 Revision of Table 3A

The Purity committee met with the owner of several varieties that are no longer being traded. As a result of these discussions, the committee decided to delete several names from Table 3A.

The proposal was approved by majority vote in the Purity committee.

CURRENT VERSION	PROPOSED VERSION																																																								
<p>Table 3A. List of varieties of <i>Poa pratensis</i> with an average thousand-seed weight of less than 0.35 g.</p> <table border="1"> <thead> <tr> <th>Variety</th> <th>Thousand-seed weight (g)</th> </tr> </thead> <tbody> <tr> <td>Balin</td> <td>0.34</td> </tr> <tr> <td>Compact</td> <td>0.34</td> </tr> <tr> <td>Julia</td> <td>0.33</td> </tr> <tr> <td>Limousine</td> <td>0.33</td> </tr> <tr> <td>Enprima</td> <td>0.32</td> </tr> <tr> <td>Oxford</td> <td>0.32</td> </tr> <tr> <td>Ikone</td> <td>0.31</td> </tr> <tr> <td>Sobra</td> <td>0.31</td> </tr> <tr> <td>Pegasus</td> <td>0.29</td> </tr> <tr> <td>Platini</td> <td>0.29</td> </tr> <tr> <td>Slezanka</td> <td>0.28</td> </tr> <tr> <td>Mardona</td> <td>0.27</td> </tr> <tr> <td>Tommy</td> <td>0.26</td> </tr> <tr> <td>Lato</td> <td>0.24</td> </tr> <tr> <td>Harmony</td> <td>0.23</td> </tr> </tbody> </table>	Variety	Thousand-seed weight (g)	Balin	0.34	Compact	0.34	Julia	0.33	Limousine	0.33	Enprima	0.32	Oxford	0.32	Ikone	0.31	Sobra	0.31	Pegasus	0.29	Platini	0.29	Slezanka	0.28	Mardona	0.27	Tommy	0.26	Lato	0.24	Harmony	0.23	<p>Table 3A. List of varieties of <i>Poa pratensis</i> with an average thousand-seed weight of less than 0.35 g.</p> <table border="1"> <thead> <tr> <th>Variety</th> <th>Thousand-seed weight (g)</th> </tr> </thead> <tbody> <tr> <td>Julia</td> <td>0.33</td> </tr> <tr> <td>Limousine</td> <td>0.33</td> </tr> <tr> <td>Enprima</td> <td>0.32</td> </tr> <tr> <td>Oxford</td> <td>0.32</td> </tr> <tr> <td>Ikone</td> <td>0.31</td> </tr> <tr> <td>Platini</td> <td>0.29</td> </tr> <tr> <td>Slezanka</td> <td>0.28</td> </tr> <tr> <td>Mardona</td> <td>0.27</td> </tr> <tr> <td>Tommy</td> <td>0.26</td> </tr> <tr> <td>Lato</td> <td>0.24</td> </tr> <tr> <td>Harmony</td> <td>0.23</td> </tr> </tbody> </table>	Variety	Thousand-seed weight (g)	Julia	0.33	Limousine	0.33	Enprima	0.32	Oxford	0.32	Ikone	0.31	Platini	0.29	Slezanka	0.28	Mardona	0.27	Tommy	0.26	Lato	0.24	Harmony	0.23
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.3.1	48	0	YES

Chapter 4: Determination of other seeds by number

C.4.1 Complete Test

4.2.2 The dust-like seeds are in more species than Orobanchaceae, which harmonized with other changes related to “dust-like” seeds as an editorial change. However, adding “indistinguishable species” is a revision. Indistinguishable species are costly to or not reliably retrieved in the complete test quantity. A reduced test is allowed in the Rules for similar species searches.

The proposal was approved by majority vote in the Purity committee.

CURRENT VERSION	PROPOSED VERSION
<p>4.2.2. Complete test</p> <p>In a complete test, the whole working sample weight is examined for all other seeds present except for Orobanchaceae-species. Testing for Orobanchaceae-species is only completed upon request of the applicant.</p>	<p>4.2.2 Complete test</p> <p>In a complete test, the whole working sample weight is examined for all other seeds present except for species with dust-like (see 4.5.3.1) seeds and indistinguishable species (see 3.5.2.4). Testing for dust-like seeds is only completed upon request of the applicant (see 4.5.3).</p>

Vote to accept item	Yes votes	No votes	Result
C.4.1	48	0	YES

C.4.2 Reduced Test

4.2.4 This proposal harmonizes with recent updates regarding “dust-like” seeds and “small seed lots” in other sections.

The proposal was agreed to by majority vote in the purity committee.

CURRENT VERSION	PROPOSED VERSION
<p>4.2.4 Reduced test</p> <p>In a reduced test, less than the whole working sample seed weight is examined for all other seeds present except for Orobanchaceae-species.</p> <p>In the case of very expensive seed (see 2.5.4.5), a reduced test can be performed.</p>	<p>4.2.4 Reduced test</p> <p>A reduced test means that less than the whole working sample seed weight is examined for all other seeds present except for dust-like seeds (see 4.5.3.1).</p> <p>In the case of small seed lots (see 2.2.14), a reduced test can be performed.</p>

Vote to accept item	Yes votes	No votes	Result
C.4.2	48	0	YES

Chapter 5: The germination test

C.5.1 Germination method for *Brassica napus*

A validation study was conducted on *Brassica napus* to determine which temperature requirements for germination promoted higher normal seedling development and enhanced testing reproducibility among labs.

This proposal is supported by the Germination Committee.

PROPOSED: Table 5A Part 1. Detailed methods for germination tests: agricultural and vegetable Seeds

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
<i>Brassica napus</i>	BP; TP	20 ↔ 30 ; 20 15↔25	5	7	KNO ₃ ; Prechill	-	-

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.5.1.	33	15	YES

C.5.2. Precision of light for germination tests

This proposal is to make clear in the Germination Chapter that LED lights can be used for germination.

This proposal is supported by the Germination Committee.

CURRENT VERSION	PROPOSED VERSION
<p>5.6.3.1. Procedures for breaking physiological dormancy</p> <p>....</p> <p>Light: The quality and intensity of light may be important. The light intensity should be between 750 and 1250 lux from cool white lamps. Illumination is recommended especially for certain tropical and subtropical grasses</p>	<p>5.6.3.1. Procedures for breaking physiological dormancy</p> <p>....</p> <p>Light: The quality and intensity of light may be important. The light should be generated by lamps or LED equivalents between 3000K (neutral white) to 4000K (cool white). Illumination is recommended especially for certain tropical and subtropical grasses...</p> <p>5.6.2.4. Light</p> <p>....</p> <p>In certain cases (e.g. some tropical and subtropical</p>

<p>5.6.2.4. Light</p> <p>....</p> <p>In certain cases (e.g. some tropical and subtropical grasses), light may promote germination of dormant samples (5.6.3.1). In such cases, the light intensity should be between 750 and 1250 lux from cool white lamps.</p>	<p>grasses), light may promote germination of dormant samples (5.6.3.1). The light should be generated by lamps or LED equivalents between 3000K (neutral white) to 4000K (cool white).</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.5.2	46	1	YES

C.5.3. Reporting results

This proposal was submitted to the Germination Committee by the ISTA Accreditation Department. This proposal is to make clear that only the percentage germination is reported when the germination test is terminated due to a pre-determined germination level ~~or number of days.~~

If approved, a consequential change is required in 1.5.2.6.

The proposal is supported by the Germination Committee.

CURRENT VERSION	PROPOSED VERSION
<p>5.9 Reporting results</p> <p>The result of a germination test must be reported in the spaces provided as follows:</p> <p>.....</p> <p>– If an applicant requests that the test be terminated</p> <p>when the sample reaches a predetermined germination</p> <p>percentage, before the final count, then only the percentage of normal seedlings is reported. The results of the other categories (abnormal seedlings, hard seeds, fresh seeds and dead seeds) must be reported as ‘N’, because they have not been determined.</p> <p>....</p> <p>The following additional information must be reported under ‘other determinations’:</p> <p>.....</p>	<p>5.9 Reporting results</p> <p>The result of a germination test must be reported in the spaces provided as follows:</p> <p>.....</p> <p>– If an applicant requests that the test be terminated when the sample reaches a predetermined germination percentage or after a specific number of days, before the final count, then only the percentage of normal seedlings is reported. The results of the other categories (abnormal seedlings, hard seeds, fresh seeds and dead seeds) must be reported as ‘N’.</p> <p>....</p> <p>The following additional information must be reported under ‘other determinations’:</p> <p>.....</p>

<p>If an applicant requests that the germination test be terminated when the sample reaches a predetermined germination percentage, the following statement: ‘Upon request of the applicant, the germination test was terminated after ... days. The prescribed test period is ...days.’</p>	<p>If an applicant requests that the germination test be terminated when the sample reaches a predetermined germination percentage or after a specific number of days, the following statement: ‘Upon request of the applicant, the germination test was terminated after ... days. The prescribed test period is ...days.’</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.5.3	47	0	YES

C.5.4. Change in the germination evaluation of roots for *Helianthus annuus* to allow secondary roots

A validation study was carried out in the Germination Committee to change the evaluation of roots for *Helianthus annuus* to allow secondary roots when the primary root is defective.

This proposal is supported by a validation study and is approved the Germination Committee.

CURRENT VERSION	PROPOSED VERSION
<p>5.2.7.2 Slight defects</p> <p>The following defects are considered slight and therefore seedlings are classified as normal:</p> <p>.....</p> <p>– at least three secondary roots, each of which is greater than or equal to half the length of the hypocotyl, in <i>Glycine max</i>, when the primary root is defective;</p>	<p>5.2.7.2 Slight defects</p> <p>The following defects are considered slight and therefore seedlings are classified as normal:</p> <p>.....</p> <p>– at least three secondary roots, each of which is greater than or equal to half the length of the hypocotyl, in <i>Glycine max</i> and <i>Helianthus annuus</i>, when the primary root is defective;</p>

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.5.4.	45	1	YES

Chapter 7: Seed health testing

C.7.1 Sample and subsample size

This proposed change will increase harmonization for sample and subsample size throughout the chapter. It will also harmonize sample size for 7-004 with other methods to detect fungi.

This proposal was approved and supported by the Seed Health Committee.

CURRENT VERSION	PROPOSED VERSION
<p>7-001 a and b; 7-002 a and b; 7-016</p> <p>Sample preparation</p> <p>It is vital to exclude any possibility of cross-contamination between seed samples. This can be achieved by swabbing/ spraying equipment and gloved hands with 70 % ethanol.</p> <p>The test is carried out on a working sample of 400 seeds as described in Section 7.4.1 of the ISTA Rules.</p>	<p>7-001 a and b; 7-002 a and b; 7-016</p> <p>Sample Size</p> <p>It is vital to exclude any possibility of cross-contamination between seed samples. This can be achieved by swabbing/ spraying equipment and gloved hands with 70 % ethanol.</p> <p>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.</p>
<p>7-003</p> <p>Sample preparation</p> <p>The test is carried out on a working sample of 400 seeds as described in Section 7.4.1 of the ISTA Rules.</p>	<p>7-003</p> <p>Sample Size</p> <p>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.</p>
<p>7-004</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and</p>	<p>7-004</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and</p>

<p>subsample size to be tested depend on the desired tolerance standard (maximum acceptable number of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum recommended sample size is 1000 seeds.</p>	<p>subsample size to be tested depend on the desired tolerance standard (maximum acceptable number of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum sample size is 400 seeds.</p>
<p>7-005, 7-014</p>	<p>7-005, 7-014</p>
<p>Sample preparation</p>	<p>Sample Size</p>
<p>The test is carried out on a working sample of 400 seeds as described in Section 7.4.1 of the ISTA Rules as appropriate.</p>	<p>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.</p>
<p>7-006</p>	<p>7-006</p>
<p>Sample preparation</p>	<p>Sample Size</p>
<p>The test is carried out on a working sample of 400 seeds as described in Section 7.4.1 of the ISTA Rules.</p>	<p>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.</p>
<p>7-007</p>	<p>7-007</p>
<p>Sample size</p>	<p>Sample Size</p>
<p>The sample (total number of seeds tested) or subsample 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). In any case, the minimum sample size should be of 400 seeds.</p>	<p>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.</p>
<p>7-008, 7-010, 7-011and 7-012</p>	<p>7-008, 7-010, 7-011and 7-012</p>
<p>Sample preparation</p>	<p>Sample Size</p>
<p>The test is carried out on a working sample of 400</p>	<p>The sample (total number of seeds tested) size to</p>

<p>seeds as described in Section 7.4.1 of the ISTA Rules.</p> <p>7-013a</p> <p>Sample preparation</p> <p>The test is carried out on a working sample as described in Section 7.4.1 of the ISTA Rules.</p> <p>The method requires 2000–4000 seeds, and 1000 embryos are examined. Seed can be prepared either by weight or by counting. This test can be completed on both treated and untreated seed.</p> <p>Methods</p> <p>1. Working sample of embryo method</p> <p>1.1 Two replicates of 100–120 g containing, depending on 1000 seed weight, 2000–4000 seeds.</p>	<p>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.</p> <p>7-013a</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample 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 working sample consists of 100-120g containing 2000-4000 seeds depending on TSW. A minimum of 1000 embryos is examined.</p> <p>This test can be carried out on both treated and untreated seed. Further sample replicates can be tested if required.</p> <p>Methods</p> <ol style="list-style-type: none"> 1. Extraction and clearing of embryos <ol style="list-style-type: none"> 1.1 Place the seeds in ...
<p>7-013b</p> <p>Sample preparation</p> <p>The test is carried out on a working sample as described in section 7.4.1 of the ISTA Rules.</p> <p>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.</p>	<p>7-013b</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample 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 working sample consists of 100-120g containing 2000-4000 seeds depending on TSW. A minimum of 1000 embryos are examined.</p>
<p>7-015</p> <p>Sample preparation</p>	<p>7-015</p>

<p>The test is carried out on a working sample as described in Section 7.4.1 of the ISTA Rules.</p> <p>7-019a; 7-019b</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample 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 recommended sample size is 30 000 seeds. In any case the maximum subsample size should be 10 000 seeds. A full discussion of these aspects can be found in Geng et al. (1987); Roberts et al. (1993) and Roberts (1999).</p> <p>7-020</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample 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 recommended sample size is 10 000 seeds. In any case the maximum subsample size should be 10 000 seeds. A full discussion of these aspects can be found in Geng et al. (1987); Roberts et al. (1993) and Roberts (1999).</p> <p>7-021; 7-023;7-029</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample 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 recommended sample size is 5000</p>	<p>Sample Size</p> <p>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 100 seeds.</p> <p>7-019a; 7-019b</p> <p>Sample Size</p> <p>The sample (total number of seeds tested) and subsample 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 30000 seeds and the maximum subsample size should be 10000 seeds.</p> <p>7-020</p> <p>Sample Size</p> <p>The sample (total number of seeds tested) and subsample 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 10000 seeds and the maximum subsample size should be 10000 seeds.</p> <p>7-021; 7-023;7-029</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample 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</p>
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<p>seeds. In any case the maximum subsample size should be 1000 seeds.</p> <p>7-022</p> <p>Sample size</p> <p>The total number of seeds to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infected).</p> <p>7-024</p> <p>Sample size</p> <p>The sample (total number of seeds tested) or subsample 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 recommended sample size is 2000 seeds. In any case, the subsample size should not exceed 100 seeds.</p> <p>7-025</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample 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 recommended sample size is 1000 seeds. In any case, the maximum subsample size is 250 seeds.</p> <p>7-026</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and</p>	<p>minimum recommended sample size is 5000 seeds and the maximum subsample size should be 1000 seeds.</p> <p>7-022</p> <p>Sample Size</p> <p>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.</p> <p>7-024</p> <p>Sample size</p> <p>The sample (total number of seeds tested) or subsample 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 recommended sample size is 2000 seeds and the maximum subsample size should be 100 seeds.</p> <p>7-025</p> <p>Sample size</p> <p>The sample (total number of seeds tested) or subsample 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 recommended sample size is 1000 seeds and the maximum subsample size should be 250 seeds.</p> <p>7-026</p> <p>Sample size</p>
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<p>subsample size to be tested depend on the desired tolerance standard (maximum acceptable number of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum recommended sample size is 2000 seeds. In any case the subsample size should not exceed 100 seeds.</p>	<p>The sample (total number of seeds tested) or subsample 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 recommended sample size is 2000 seeds and the maximum subsample size should be 100 seeds.</p>
<p>7-027</p> <p>Sample size</p> <p>The total number of seeds to be tested depends on the de- sired tolerance standard (maximum acceptable percentage of seeds infested). In any case, the minimum sample size should be 400 seeds.</p>	<p>7-027</p> <p>Sample size</p> <p>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.</p>
<p>7-028</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample size to be tested depends on the desired tolerance standard (maximum acceptable number of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). In any case the maximum recommended subsample size is 250 seeds. The minimum recommended sample size is 3000 seeds.</p>	<p>7-028</p> <p>Sample size</p> <p>The sample (total number of seeds tested) or subsample 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 recommended sample size is 3000 seeds and the maximum subsample size should be 250 seeds.</p>
<p>7-030</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample 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 mini- mum recommended sample size is 10 000 seeds with a maximum subsample size of 5000 seeds.</p>	<p>7-030</p> <p>Sample size</p> <p>The sample (total number of seeds tested) or subsample 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 recommended sample size is 10000 seeds and the maximum subsample size should be 5000 seeds.</p>

<p>7-031</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample size to be tested depend on the desired tolerance standard (maximum acceptable number of seeds infested) and detection limit (theoretical minimum number of nematode per sample which can be detected). An example of a recommended minimum sample size for faba bean with a detection limit of 1.5 nematodes per 100 g in seed lot, and a zero tolerance is 900 g, using ISTA sampling methodology calculation adapted to sieving method (Macarthur et al., TESTA Deliverable 2.4). In any case, the minimum sample size is 100 g of seeds for alfalfa and 300 g for faba bean (TESTA WP2-Sampling), and the maximum subsample size is 100 g of seeds for alfalfa and 300 g for faba bean. The whole sample is tested.</p> <p>7-032</p> <p>Sample size</p> <p>The sample (i.e., total number of seeds tested) or subsample size to be tested depends on the desired tolerance standard (i.e., maximum acceptable percentage of seeds infested) and detection limit (i.e., theoretical minimum number of pathogen propagules per seed which can be detected). In either case, the minimum recommended sample size is 400 seeds with a maximum subsample size of 100 seeds.</p>	<p>7-031</p> <p>Sample size</p> <p>The sample (total number of seeds tested) and subsample size to be tested depend on the desired tolerance standard (maximum acceptable number of seeds infested) and detection limit (theoretical minimum number of nematode per sample which can be detected). An example of a recommended minimum sample size for faba bean with a detection limit of 1.5 nematodes per 100 g in seed lot, and a zero tolerance is 900 g, using ISTA sampling methodology calculation adapted to sieving method (Macarthur et al., TESTA Deliverable 2.4). The minimum sample size should be 100 g of seeds for alfalfa and 300 g for faba bean (TESTA WP2-Sampling), and the maximum subsample size should be 100 g of seeds for alfalfa and 300 g for faba bean. The whole sample is tested.</p> <p>7-032</p> <p>Sample size</p> <p>The sample (i.e., total number of seeds tested) or subsample size to be tested depends on the desired tolerance standard (i.e., maximum acceptable percentage of seeds infested) and detection limit (i.e., theoretical minimum number of pathogen propagules per seed which can be detected). The minimum sample size should be 400 seeds.</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.7.1.	44	1	YES

C.7.2 Identification Criteria

7-007 Detection of *Alternaria linicola*, *Boytrytis cinerea*, and *Colletotrichum lini* in *Linum usitatissimum*

This revision is requested by the SHC to make the description of *Alternaria linicola* more informative.

The proposal was discussed by the SHC.

CURRENT VERSION	PROPOSED VERSION
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7-007	7-007
<p>7.1 <i>Alternaria linicola</i> : Examine plates for dense olive grey colonies, 1.5–3 cm diameter. Some colonies of saprophytic <i>Alternaria</i> spp. can resemble those of <i>A. linicola</i> but the conidia of <i>A. linicola</i> are diagnostic (Fig. 1). Colonies should therefore be examined under $\times 50$–100 magnification. Conidiophores are simple, occurring singly or in bundles, pale olive-brown, septate, and variable in length 5–8 μm. Conidia form singly, are smooth walled, olive-brown, obclavate with long, tapering occasionally branched beaks muriform 4–16 μm with transverse septa and occasionally 1–4 longitudinal septa, sometimes slightly constricted at the septa (Fig. 2)(Corlett & Corlett 1999; David 1991; Malone & Muskett 1997). Short red streaks and water soaked areas may be visible on the hypocotyls and cotyledons of some infected seedlings (Fig. 3).</p> <p>7.2 <i>Botrytis cinerea</i> : Examine for roots showing a soft rot and covered by abundant grey mycelium (Fig. 4) or just mycelium very flat, diffuse and not aerial, possibility of sclerotia producing (Fig. 5). Colonies on agar measure up to 5 cm in diameter after 5 days. Identification can be checked by high-power microscope (magnification $\times 200$). Mycelium of tape-like hyphae producing bunches of branching conidiophores with ovoid-hyaline one-celled conidia 8–11 \times 6–19 μm (Fig. 6). When analysts are familiar with the fungus, naked eye examination is sufficient for identification (Muskett & Malone 1941; Tempe 1963; Malone & Muskett 1997; Ellis & Waller 1974).</p> <p>7.3 <i>Colletotrichum lini</i> : <i>C. lini</i> is easily recognised by visual examination. Examine the plates for shell pink to salmon coloured colonies (Fig. 7). Colonies of <i>C. lini</i> are a fine woolly-grey</p>	<p>7.1 <i>Alternaria linicola</i> : Examine plates for dense olive grey colonies, 1.5–3 cm diameter. Some colonies of saprophytic <i>Alternaria</i> spp. can resemble those of <i>A. linicola</i> but the conidia of <i>A. linicola</i> are diagnostic (Fig. 1). Colonies should therefore be examined under $\times 50$–100 magnification. Conidiophores are simple, occurring singly or in bundles, unbranched, erect, often geniculate, with 1-2 or more scars, pale olive-brown, and septate. Conidia form singly, are smooth walled, olive-brown, elongated conical to ellipsoid or obclavate, gradually tapering towards the beak. The beak is long, occasionally branched muriform 4–16 μm with transverse septa and occasionally 1–4 longitudinal septa,, sometimes slightly constricted at the septa (Fig. 2), 16–230 \times 3–4.5 μm (Corlett & Corlett 1999; David 1991; Malone & Muskett 1997). The size of conidia (body and beak) is given as an indication but should not be a compulsory identification criterion. Size of conidia depends on the growth media, isolate, growth conditions: depending on references they have been described as (13–)15–18(–22.5) \times (3–) 3.5–4(–4.5) μm (Damm et al., 2014) or 20–130 \times (7–)17–24(–30) μm (anonymous, 1991). Short red streaks and water soaked areas may be visible on the hypocotyls and cotyledons of some infected seedlings (Fig. 3).</p> <p>7.2 <i>Botrytis cinerea</i> : Examine for roots showing a soft rot and covered by abundant grey mycelium (Fig. 4) or just mycelium very flat, diffuse and not aerial, possibility of sclerotia producing (Fig. 5). Colonies on agar measure up to 5 cm in diameter after 5 days. Identification can be checked by high-power microscope (magnification $\times 200$). Mycelium of tape-like hyphae producing bunches of branching conidiophores with ovoid-hyaline one-celled conidia 8–11 \times 6–19 μm (Fig. 6). When analysts are familiar with the fungus, naked eye examination is sufficient for identification (Muskett & Malone 1941; Tempe 1963; Malone & Muskett 1997; Ellis & Waller 1974).</p> <p>7.3 <i>Colletotrichum lini</i> : <i>C. lini</i> is easily recognised by visual examination. Examine the plates for shell pink to salmon coloured colonies (Fig. 7). Colonies of <i>C. lini</i> are a fine woolly-grey</p>

<p>at the centre to salmon pink at the outer edge. Dark globose fruiting bodies (acervuli) may be scattered throughout the agar adjacent to the seed (Fig. 8). Characteristic long, black tapering hairs or setae 2–5 septate, 60–120 x 2–4 µm arise from the base of each acervulus. Bright orange conidial masses appear on the seed and agar adjacent to the seed. Conidia are hyaline; oblong to dumbbell shaped, one celled, straight ends 9–15 x 3–4 µm (Malone & Muskett 1997; Kulshrestha et al., 1976). Record the number of infected seeds in each plate.</p>	<p>Dark globose fruiting bodies (acervuli) may be scattered throughout the agar adjacent to the seed (Fig. 8). Characteristic long, black tapering hairs or setae 2–5 septate, 60–120 x 2–4 µm arise from the base of each acervulus. Bright orange conidial masses appear on the seed and agar adjacent to the seed. Conidia are hyaline; oblong to dumbbell shaped, one celled, straight ends 9–15 x 3–4 µm (Malone & Muskett 1997; Kulshrestha et al., 1976). Record the number of infected seeds in each plate.</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.7.2.	44	0	YES

C.7.3 Safety precautions

This proposal makes clear that alternative chemicals other than Ethidium bromide may be used for this method. It also provides additional safety considerations when using Ethidium bromide.

This proposal is supported by the Seed Health Committee.

CURRENT VERSION	PROPOSED VERSION
<p>7-019a; 7-019b; 7-020 Safety precautions</p> <p>... Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.</p>	<p>7-019a; 7-019b; 7-020 Safety precautions</p> <p>... Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.</p> <p>Ethidium bromide</p> <p>Ethidium bromide is carcinogenic. If possible, use an alternative chemical e.g. Gel Red™ (Biotium). Use ethidium bromide according to safety instructions. It is recommended to work with solution instead of powder. Some considerations are mentioned below.</p> <ul style="list-style-type: none"> – Consult the Material Safety Data Sheet on ethidium bromide before using the chemical. – Always wear personal protective equipment when handling ethidium bromide. This includes wearing a lab coat, nitrile gloves and closed toe shoes.

<p>7-019a (PCR option 1, PCR option 2, or PCR option 3)</p> <p>Fractionate 10 µL of the PCR products and water (negative PCR control) by gel electrophoresis in 1× tris acetate EDTA (TAE buffer) (CCP). Include a 100 bp ladder. Stain with ethidium bromide in a bath and rinse in water.</p> <p>7-020</p> <p>8.6 Fractionate 10 µL of the PCR products by gel electrophoresis during 1.5 h at 150V on a 1.5 % agarose gel in 0.5× Tris Borate EDTA (TBE buffer) stained with ethidium bromide. Include a 100 bp ladder.</p> <p>7-021; 7-030</p> <p>Ethidium bromide is carcinogenic. Use ethidium bromide according to safety instructions. It is recommended to manipulate solution instead of powder. Some considerations are mentioned below.</p>	<p>– Leave lab coats, gloves, and other personal protective equipment in the lab once work is complete to prevent the spread of ethidium bromide or other chemicals outside the lab.</p> <p>– All work with ethidium bromide is to be done in an “ethidium bromide” designated area in order to keep ethidium bromide contamination to a minimum.</p> <p>Ultraviolet light</p> <p>Ultraviolet UV light must not be used without appropriate precautions. Ensure that UV protective eyewear is utilised when working with ethidium bromide.</p> <p>7-019a (PCR option 1, PCR option 2, or PCR option 3)</p> <p>Fractionate 10 µL of the PCR products, the negative process control and sterile water (negative PCR control) by gel electrophoresis in 1x Tris acetate EDTA buffer (TAE buffer) (CCP). Include 100bp ladder. Stain with ethidium bromide and rinse in water.</p> <p>7-020</p> <p>8.6 Fractionate 10 µL of the PCR products, the negative process control and sterile water (negative PCR control), by gel electrophoresis during 1.5 h at 150V on a 1.5 % agarose gel in 0.5× Tris Borate EDTA (TBE buffer) (CCP). stained with ethidium bromide. Include a 100 bp ladder.</p> <p>7-021; 7-030</p> <p>Ethidium bromide is carcinogenic. If possible, use an alternative chemical e.g. Gel Red™ (Biotium). Use ethidium bromide according to safety instructions. It is recommended to work with solution instead of powder. Some considerations are mentioned below.</p>
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<p>8.5 Fractionate 10 µL of the PCR products, the negative process control and water (negative PCR control) by gel electrophoresis in a 1.5 % agarose gel in 1× Tris-acetate EDTA (TAE buffer)(CCP). Include a 100 bp ladder. Stain with ethidium bromide in a bath and rinse in water.</p> <p>7-031</p> <p>3.6 Fractionate 10 uL of the PCR products containing a loading buffer on an agarose gel of 1.5% with 100 bp ladder (migration conditions 180 V for 45 min) for example</p>	<p>8.5 Fractionate 10 µL of the PCR products, the negative process control and sterile water (negative PCR control) by gel electrophoresis in a 1.5 % agarose gel in 1× Tris-acetate EDTA (TAE buffer)(CCP). Include a 100 bp ladder. Stain with ethidium bromide in a bath and rinse in water.</p> <p>7-031</p> <p>3.6 Fractionate 10 µL of the PCR products containing a loading buffer on an agarose gel of 1.5% with 100 bp ladder (migration conditions 180 V for 45 min) for example. Stain with ethidium bromide in a bath and rinse in water.</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.7.3	44	0	YES

Chapter 9: Determination of moisture content

C.9.1 Changes to the use of “must”, “should”, and “may”.

At the ISTA Congress in India in 2019, the Moisture Committee discussed the usage of the words “must,” “should” and “may” in the ISTA Rules, Chapter 9. The MOI committee has reviewed this chapter and is recommending that some of the wording be changed to improve clarity on when a laboratory must perform an action and where it is suggested that the laboratory should perform the action (but would not be found in violation during an ISTA audit if they used a different but acceptable alternative process).

This proposal is approved by the Moisture Committee.

CURRENT VERSION	PROPOSED VERSION
<p>9.2.4.2</p> <p>...</p> <p>For individual samples, the difference in moisture content must not differ by more than 0.15 %. This check must be performed using a species that requires high temperature and a drying time less than or equal to 2 h. The same species can be used for 130 °C or 103 °C. Weighing should be performed in accordance with 9.2.5.3.</p>	<p>9.2.4.2</p> <p>...</p> <p>For individual samples, the difference in moisture content must not differ by more than 0.15 %. This check must be performed using a species that requires high temperature and a drying time less than or equal to 2 h. The same species can be used for 130 °C or 103 °C. Weighing must be performed in accordance with 9.2.5.3.</p>
<p>9.2.5.4</p> <p>...</p> <p>The grinding mill should be adjusted so that particles of the required dimensions are obtained. For those species requiring fine grinding (Table 9A Part 1), at least 50 % of the ground material should pass through a wire sieve with meshes of 0.50 mm, and not more than 10 % should remain on a wire sieve with meshes of 1.00 mm. For those species requiring coarse grinding (Table 9A Parts 1 and 2), at least 50 % of the ground material should pass through a sieve with meshes of 4.00 mm, and not more than 55 % should pass through a wire sieve with meshes of 2.00 mm.</p>	<p>9.2.5.4</p> <p>...</p> <p>The grinding mill should be adjusted so that particles of the required dimensions are obtained. For those species requiring fine grinding (Table 9A Part 1), at least 50 % of the ground material must pass through a wire sieve with meshes of 0.50 mm, and not more than 10 % must remain on a wire sieve with meshes of 1.00 mm. For those species requiring coarse grinding (Table 9A Parts 1 and 2), at least 50 % of the ground material must pass through a sieve with meshes of 4.00 mm, and not more than 55 % must pass through a wire sieve with meshes of 2.00 mm.</p>

<p>9.2.5.6</p> <p>...</p> <p>After predrying, the subsamples are reweighed in their containers to determine the loss in weight. Immediately thereafter the two partly dried subsamples are separately ground. One working sample is drawn from each subsample. Drawing of the working sample should be in accordance with 9.2.5.2. The moisture is determined as prescribed in 9.2.5.3.</p>	<p>9.2.5.6</p> <p>...</p> <p>After predrying, the subsamples are reweighed in their containers to determine the loss in weight. Immediately thereafter the two partly dried subsamples are separately ground. One working sample is drawn from each subsample. Drawing of the working sample must be in accordance with 9.2.5.2. The moisture is determined as prescribed in 9.2.5.3.</p>
<p>9.2.5.7</p> <p>The working sample, drawn according to 9.2.5.2, must be evenly distributed over the surface of the container.</p> <p>...</p>	<p>9.2.5.7</p> <p>The working sample, drawn according to 9.2.5.2, must be evenly distributed over the surface of the container.</p> <p>...</p>
<p>9.3.1.4</p> <p>...</p> <p>The housing of the moisture meters must be robust and so constructed that the main components of the instrument are inaccessible and protected from dust and moisture.</p> <p>...</p>	<p>9.3.1.4</p> <p>...</p> <p>The housing of the moisture meters should be robust and so constructed that the main components of the instrument are inaccessible and protected from dust and moisture.</p> <p>...</p>
<p>Grinder: Where the operating manual of the electronic moisture meter specifies grinding, a subsample from the submitted sample must be ground. The fineness of the grinding must be according to the specific moisture meter manual. If it is not specified in the manual it should be according to 9.2.5.4.</p>	<p>Grinder: Where the operating manual of the electronic moisture meter specifies grinding, a subsample from the submitted sample must be ground. The fineness of the grinding must be according to the specific moisture meter manual. If it is not specified in the manual it should be according to 9.2.5.4.</p>
<p>9.3.1.5.1</p> <p>...</p> <p>The moisture meter and the samples should be equilibrated to the same temperature before the assessments are made. During the determination, exposure</p>	<p>9.3.1.5.1</p> <p>...</p> <p>The moisture meter and the samples should be equilibrated to the same temperature before the assessments are made. During the determination, exposure</p>

<p>of the sample to the atmosphere of the laboratory should be reduced to the absolute minimum.</p> <p>9.3.1.5.2</p> <p>Five samples should be obtained from each of a minimum of two varieties of the species for which the moisture meter is being calibrated. The samples from each variety should have a range of moisture contents evenly covering the required measurement range of the moisture meter being checked. If the full range is not available from natural samples, sample may be conditioned.</p> <p>...</p> <p>Calibration sample containers should be moisture proof and filled to at least two-thirds of their capacity. If the container is too full, the sample cannot be mixed thoroughly. If the container is not filled sufficiently there can be hygrometric exchanges between the seeds and the air that is present in the container, and this can result in a modification of the moisture content of the sample in the period prior to testing. The containers should be sealed and stored at 5 ± 2 °C. The sealed containers must be moved to the room containing the moisture meter at least 24 h prior to use to ensure that the temperature of the seed has equilibrated with the temperature of the meter.</p> <p>9.3.1.5.3</p> <p>Working samples should be drawn after thoroughly mixing them using one of the following methods:</p> <p>either stir the sample in its container with a spoon,</p> <p>or place the opening of the original container against the opening of a similar container and pour the seed back and forth between the two containers.</p> <p>...</p>	<p>of the sample to the atmosphere of the laboratory must be reduced to the absolute minimum.</p> <p>9.3.1.5.2</p> <p>At least five samples should be obtained from each of a minimum of two varieties of the species for which the moisture meter is being calibrated. The samples from each variety should have a range of moisture contents evenly covering the required measurement range of the moisture meter being checked. If the full range is not available from natural samples, samples may be conditioned.</p> <p>...</p> <p>Calibration sample containers must be moisture proof and must be filled to at least two-thirds of their capacity. If the container is too full, the sample cannot be mixed thoroughly. If the container is not filled sufficiently there can be hygrometric exchanges between the seeds and the air that is present in the container, and this can result in a modification of the moisture content of the sample in the period prior to testing. The containers should be sealed and stored at 5 ± 2 °C. The sealed containers should be moved to the room containing the moisture meter at least 24 h prior to use to ensure that the temperature of the seed has equilibrated with the temperature of the meter.</p> <p>9.3.1.5.3</p> <p>Working samples must be drawn after thoroughly mixing them using one of the following methods:</p> <p>either stir the sample in its container with a spoon,</p> <p>or place the opening of the original container against the opening of a similar container and pour the seed back and forth between the two containers.</p> <p>...</p>
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<p>9.3.1.5.4</p> <p>Weighing, when required, should be in accordance with 3.5.1.</p>	<p>9.3.1.5.4</p> <p>Weighing, when required, must be in accordance with 3.5.1.</p>
<p>9.3.1.5.5</p> <p>...</p> <p>The calibration sample is then thoroughly mixed prior to drawing the next working sample (see 9.3.1.5.3). Where the determination is destructive, the measurements should be carried out on three independent working samples.</p> <p>The moisture content of the calibration samples should be rechecked after the measurement, using the reference oven method (see 9.2).</p> <p>...</p>	<p>9.3.1.5.5</p> <p>...</p> <p>The calibration sample is then thoroughly mixed prior to drawing the next working sample (see 9.3.1.5.3). Where the determination is destructive, the measurements must be carried out on three independent working samples.</p> <p>The moisture content of the calibration samples must be rechecked after the measurement, using the reference oven method (see 9.2).</p> <p>...</p>
<p>9.3.2.4.3</p> <p>Weighing, when required, should be in accordance with 3.5.1.</p> <p>...</p>	<p>9.3.2.4.3</p> <p>Weighing, when required, must be in accordance with 3.5.1.</p> <p>...</p>
<p>9.3.2.8</p> <p>Table 9D should be used when checking moisture meters against oven results.</p> <p>...</p>	<p>9.3.2.8</p> <p>Table 9D must be used when checking moisture meters against oven results.</p> <p>...</p>
<p>9.3.2.9</p> <p>Table 9E should be used when checking two moisture meters against each other.</p> <p>...</p>	<p>9.3.2.9</p> <p>Table 9E must be used when checking two moisture meters against each other.</p> <p>...</p>

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.9.1	47	1	YES

C.9.2 Containers for Moisture Testing in Chapter 9

The Moisture Technical Committee has been reviewing the wording in Chapter 9 of the ISTA Rules. The committee would like to clarify the definition of appropriate containers for use in oven moisture testing.

CURRENT VERSION	PROPOSED VERSION
<p>9.2.4.3 Containers</p> <p>Containers must be metal dishes, non-corrodible under the test conditions, or, failing this, glass dishes, with lids and an effective surface area enabling the test sample to be distributed so as to give a mass per unit area of not more than 0.3 g/cm².</p>	<p>9.2.4.3 Containers</p> <p>Containers must be non-corrodible and non-moisture absorbent under the test conditions (e.g. metal or glass), with lids and an effective surface area enabling the test sample to be distributed so as to give a mass per unit area of not more than 0.3 g/cm².</p>

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.9.2	47	0	YES

C.9.3 Use of mesh instead of meshes

The committee feels that in the instances below the use of “meshes” is incorrect as it implies multiples of mesh, and would prefer to use the singular mesh to describe sieves.

CURRENT VERSION	PROPOSED VERSION
<p>9.2.4.6 Sieves</p> <p>Wire sieves with meshes of 0.50, 1.00, 2.00 and 4.00 mm are required.</p> <p>...</p> <p>9.2.5.4 Grinding</p> <p>...</p> <p>The grinding mill should be adjusted so that particles of the required dimensions are obtained. For those species requiring fine grinding (Table 9A Part 1), at least 50 %, of the ground material should pass through a wire sieve with meshes of 0.50 mm, and not more than 10 % should remain on a wire sieve with meshes of 1.00 mm. For those species requiring coarse grinding (Table 9A Parts 1 and 2), at least 50 %</p>	<p>9.2.4.6 Sieves</p> <p>Wire sieves with 0.50, 1.00, 2.00 and 4.00 mm mesh are required.</p> <p>...</p> <p>9.2.5.4 Grinding</p> <p>...</p> <p>The grinding mill should be adjusted so that particles of the required dimensions are obtained. For those species requiring fine grinding (Table 9A Part 1), at least 50 %, of the ground material should pass through a wire sieve with 0.5mm mesh, and not more than 10 % should remain on a wire sieve with 1.00mm mesh. For those species requiring coarse grinding (Table 9A Parts 1 and 2), at least 50 % of the ground material should pass through a</p>

of the ground material should pass through a sieve with meshes of 4.00 mm, and not more than 55 % should pass through a wire sieve with meshes of 2.00 mm.	sieve with 4.00mm mesh , and not more than 55 % should pass through a wire sieve with 2.00mm mesh .
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.9.3	47	0	YES

C.9.4 Clarification of working sample

Section 9.2.5.2 of the Rules causes confusion, as there are differences in how many working samples are drawn depending on whether seeds are whole or ground. This section has been re-ordered and changed to improve clarity.

CURRENT VERSION	PROPOSED VERSION
<p>9.2.5.2. Working sample The determination must be carried out in duplicate on two independently drawn working samples, each of the following weight, depending on the diameter of the containers used: Diameter >5 cm and <8 cm: 4.5 ± 0.5 g Diameter ≥ 8 cm: 10.0 ± 1.0 g For large-seeded tree and shrub seeds that have to be cut (see Table 9A, Part 2), a different working sample size may be required. For cut seed, the working sample must be sufficient to draw two replicates of approximately 5 g each by cutting at least ten intact seeds (see 9.2.5.5). Before the working sample is drawn, the submitted sample must be thoroughly mixed by one of the following methods: either stir the sample in its container with a spoon, or place the opening of the original container against the opening of a similar container and pour the seed back and forth between the two containers. The mixing process must not take more than one minute. Take at minimum three subsamples with a spoon from different positions and combine them to form the subsample of the required size. The seed (whole seed,</p>	<p>9.2.5.2. Working sample Before obtaining the working sample(s), the submitted sample must be thoroughly mixed by one of the following methods: either stir the sample in its container with a spoon, or place the opening of the original container against the opening of a similar container and pour the seed back and forth between the two containers. The mixing process must not take more than one minute. Take at minimum three subsamples with a spoon from different positions and combine them to form the working sample of the required size. For whole seeds, the determination must be carried out in duplicate on two independently drawn working samples. In the case of cutting or grinding, one working sample must be drawn for cutting or grinding and from the cut/ground material two replicates must be obtained. For large-seeded tree and shrub seeds that have to be cut (see Table 9A, Part 2), a different working sample size may be required. For cut seed, the working sample must be sufficient to draw two replicates of approximately 5 g each by cutting at least ten intact seeds (see 9.2.5.5). The seed (whole seed, cut seed or ground material) must not be exposed to the air during sample reduction for more</p>

<p>cut seed or ground material) must not be exposed to the air during sample reduction for more than 30 s per replicate.</p> <p>In the case of cutting or grinding, one working sample must be drawn for cutting or grinding and from the cut/ground material two replicates must be obtained.</p> <p>The seed (whole seed, cut seed or ground material) must not be exposed to the air during sample reduction for more than 30 s per replicate.</p>	<p>than 30 s per replicate.</p> <p>The weight of the working sample (whole seeds) or replicate (cut or ground seed) is dependent on the diameter of the containers used:</p> <p style="padding-left: 40px;">Diameter > 5 cm and < 8 cm: 4.5 ± 0.5 g</p> <p style="padding-left: 40px;">Diameter ≥ 8 cm: 10.0 ± 1.0 g</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.9.4	48	0	YES

C.9.5 Moisture method for *Carica papaya*

A moisture method for *Carica papaya* is proposed for inclusion into the Rules.

The proposal is made following validation studies carried out within the Moisture Committee.

New entry: Table 9A Part 2

Species	Grinding/cutting (9.2.5.4, 9.2.5.5)	Remarks
<i>Carica papaya</i>	No	High oil content

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.9.5	47	0	YES

Chapter 15: Seed vigour Testing

C.15.1 Clarification of the assessment of radicle emergence for *Zea mays* and *Triticum aestivum* subsp. *aestivum* in the radicle emergence test

This proposal has three aims: 1) to clarify the assessment of a 2mm radicle 2) to be consistent in the description of the germination media for the radicle emergence test for *Zea mays* and *Triticum aestivum* subsp. *aestivum* 3) to express all Latin names according to the stabilised list.

The Vigour Committee has received queries regarding whether the assessment of a 2mm radicle for *Zea mays* and *Triticum aestivum* subsp. *aestivum* should or should not include any part of the radicle enclosed by the coleorhiza, and whether assessments should be made by eye or using magnification. The additional text clarifies the measurement of 2mm in the radicle emergence test for the above species and specifies that assessments should be made by eye.

All the validation for RE has been done without any magnification and achieved repeatability and reproducibility. Thus, the committee does not see the need for magnification. To allow magnification, the level of magnification that should be used would have to be specified; this would require further investigation to determine whether the results remain repeatable and reproducible.

Table 15B. Specific conditions for the radicle emergence test procedure.

CURRENT

Species	Germination medium	Replication	Germination temperature	Criterion of radicle emergence	Timing of radicle emergence count
<i>Brassica napus</i> (oil-seed rape, Argentine canola)	Pleated papers	2 replicates of 100 seeds	20 ± 1°C	Appearance of a radicle after breaking through the seed coat. Seeds in which the seed coat has split, but no radicle has emerged, must not be included.	30 h ± 15 min
<i>Raphanus sativus</i>	Top of paper	4 replicates of 50 seeds	20 ± 1°C	Production of 2 mm radicle	48 h ± 15 min
<i>Triticum aestivum</i> L. subsp. <i>aestivum</i> (excluding dormant seed lots)	<u>Between paper</u>	<u>4 replicates of 50 seeds</u>	<u>15 ± 1°C</u>	<u>Production of 2 mm radicle</u>	<u>48 h ± 15 min</u>
<i>Zea mays</i>	Paper towels	8 replicates of 25 seeds	20 ± 1°C or 13 ± 1°C	Production of 2mm radicle	66 h ± 15 min at 20 ± 1°C 144 h ± 1 h at 13 ± 1°C

PROPOSED

Table 15B. Specific conditions for the radicle emergence test procedure. All assessments of radicle emergence should be made by eye and without magnification.

Species	Germination medium	Replication	Germination temperature	Criterion of radicle emergence	Timing of radicle emergence count
<i>Brassica napus</i> (oil-seed rape, Argentine canola)	Pleated paper	2 replicates of 100 seeds	20 ± 1°C	Appearance of a radicle after breaking through the seed coat. Seeds in which the seed coat has split, but no radicle has emerged, must not be included.	30 h ± 15 min
<i>Raphanus sativus</i>	Top of paper	4 replicates of 50 seeds	20 ± 1°C	Production of 2 mm radicle.	48 h ± 15 min
<i>Triticum aestivum</i> subsp. <i>aestivum</i> (excluding dormant seed lots)	Between paper	4 replicates of 50 seeds	15 ± 1°C	Production of 2 mm radicle. The radicle includes the parts that are within the coleorhiza, as well as those that have emerged through it.	48 h ± 15 min
<i>Zea mays</i>	Between paper	8 replicates of 25 seeds	20 ± 1°C or 13 ± 1°C	Production of 2 mm radicle. The radicle includes the parts that are within the coleorhiza, as well as those that have emerged through it.	66 h ± 15 min at 20 ± 1°C 144 h ± 1 h at 13 ± 1°C

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.15.1.	46	1	YES