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**Document OGM23-05**

## Rules Proposals for the International Rules for Seed Testing 2024 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 2023 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 2023.

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 2023 to be held on 01 June, 2023, in Verona, Italy.

## **Introduction to the ISTA Rules Proposals to become effective 1 January 2024**

The current version of the ISTA International Rules for Seed Testing (ISTA Rules) is the 2023 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, 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.

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.

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 in Verona, Italy on 01 June, 2023, and may be amended without changing the intent of the proposal. If the proposals are accepted by the membership, amendments will be issued, and they will become the 2024 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

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### **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

The ISTA Secretariat moved its office in 2021. The 2022 rules were not updated to reflect this address change.

CURRENT VERSION	PROPOSED VERSION
<p><b>I-1 General Information</b></p> <p>.....</p> <p>For further information on the ISTA Rules and their use, please contact:</p> <p>ISTA Secretariat  <del>Zurichstrasse 50</del>  <del>CH-8303 Basersdorf</del>                      Switzerland                      Phone +41 44 838 6000                      FAX +41 44 838 6001</p> <p>or visit the ISTA website: <a href="http://www.seedtest.org">www.seedtest.org</a></p>	<p><b>I-1 General Information</b></p> <p>.....</p> <p>For further information on the ISTA Rules and their use, please contact:</p> <p>ISTA Secretariat  <a href="#">Richtiarkade 18</a>  <a href="#">CH-8304 Wallisellen</a>                      Switzerland                      Phone +41 44 838 6000                      FAX +41 44 838 6001</p> <p>or visit the ISTA website: <a href="http://www.seedtest.org">www.seedtest.org</a></p>

An inconsistency between instructions on how to report results of GMO testing given in 1.5.2.21 and in those given in 19.7 was identified. The reason is that Chapter 19 in ISTA Rules 2023 was amended but changes were not reflected in Chapter 1. Therefore, text in 1.5.2.21 should be edited according to 19.7.

CURRENT VERSION	PROPOSED VERSION
<p><b>1.5.2.21 Genetically modified organisms</b></p> <p>The result of a genetically modified organism test must be reported under 'Other determinations' as follows:</p> <ul style="list-style-type: none"> <li>• the request of the applicant;</li> <li>• <del>the name and scope (with reference to the target) of the method(s) used;</del></li> </ul>	<p><b>1.5.2.21 Genetically modified organisms</b></p> <p><a href="#">As for any other testing in the ISTA Rules, also for GMO testing results can be reported on an Orange International Certificate under the condition that both sampling and testing have been carried out by accredited laboratories and that the total number of seeds in the working sample is known. In the</a></p>

<ul style="list-style-type: none"> <li>• a description of the working sample (e.g. pure seed fraction, inert matter present, other seeds present, washed seed);</li> <li>• the number of seeds in the working sample;</li> <li>• a description and the source of the reference material used (e.g. certified reference material, provider);</li> <li>• the limit of detection of the method (when testing seed groups or seed bulk) according to the value verified by the laboratory;</li> <li>• the limit of quantification of the method (when testing seed bulks with a quantitative method) according to the value verified by the laboratory.</li> </ul>	<p>case the size of the working sample is unknown (bulk sample) then only a Blue International Certificate can be issued.</p> <p>The result of a genetically modified organism test must be reported under 'Other determinations' as follows:</p> <ul style="list-style-type: none"> <li>• the request of the applicant (testing objectives);</li> <li>• the testing approach;</li> <li>• the testing technology;</li> <li>• the assay target;</li> <li>• a description of the working sample (e.g. pure seed fraction, inert matter present, other seeds present, washed seed);</li> <li>• the number of seeds in the working sample (in this case the results can be reported on an Orange International Certificate) or alternatively, the weight of the working sample (in this case the result can be reported only on a Blue International Certificate);</li> <li>• a description and the source of the reference material used (e.g. certified reference material, provider);</li> <li>• the limit of detection of the method (when testing seed groups or seed bulks) according to the value verified by the laboratory;</li> <li>• the limit of quantification of the method (when testing seed groups or bulks with a quantitative method) according to the value verified by the laboratory.</li> </ul> <p>For the items indicated above, the terms to be used on an ISTA Certificate should be those reported in the List of Standardised Terms.</p>
<p><b>1.5.2.21.1 Qualitative test results</b></p> <p>Suggested phrases for reporting the detection of test targets depending upon the result are as follows:</p> <p>a) If the test target(s) was(were) not detected: 'The test target was not detected.'</p>	<p><b>1.5.2.21.1 Assessment of presence of GMO</b></p> <p>Suggested phrases for reporting the detection of test targets depending upon the result are as follows:</p> <p>a) If the test target(s) was (were) detected: 'The test target(s) was (were) detected.'</p>

<p><del>b) If the test target(s) was (were) detected: 'The test target was detected.'</del></p>	<p>b) If the test target(s) was (were) not detected: 'The test target(s) was (were) not detected.'</p> <p>A negative result obtained with a qualitative assay even on a single group can allow to declare that the seed lot meets the specification of ...% (maximum or minimum) with ...% confidence.</p>
<p><b>1.5.2.21.2 Quantitative results obtained by multiple qualitative tests of individuals or groups of seeds or seedlings</b></p>	<p><b>1.5.2.21.2 Estimation of the level of GMO by multiple qualitative tests of individuals or groups</b></p>
<p><b>1.5.2.21.3 Quantitative measurements of GMO in bulk samples</b></p> <p>Results should be reported relative to the percentage of the test target specified by the applicant by mass or number of DNA copies. <del>(e.g. number of replicate seed samples, number of replicate flour samples per seed sample, number of extracts per flour sample, number of replicate measurements per extract)</del> must be indicated. Required phrases for reporting depending upon the results are as follows:</p> <p>a) If the test target was not detected (no signal or below the limit of detection): 'The test target was not detected at a level above the limit of detection.'</p> <p>b) If the test target was detected at a level above the limit of detection and below the limit of quantification: 'The test target was detected at a level below the limit of quantification of the method used.'</p> <p>c) If seeds showing the test target were found at a level above the limit of quantification: 'The test target(s) percentage in the seed lot was determined to be ...% by mass or number of copies, with a 95 % confidence interval of [...%, ...%]'</p> <p><b>or</b></p>	<p><b>1.5.2.21.3 Estimation of the level of GMO by quantitative measurements on groups or bulks</b></p> <p>Results should be reported relative to the percentage of the test target specified by the applicant by mass or number of DNA copies. <b>If an Orange International Certificate is to be issued, the total number of seeds tested must be reported. If a Blue International Certificate is to be issued, either the number or the weight of the seeds tested must be reported.</b></p> <p><b>In any case, the number of groups or bulks, the number of replicate flour samples per group/bulk, and the number of replicate measurements per flour sample must be indicated.</b></p> <p>Required phrases for reporting depending upon the results are as follows:</p> <p>a) If the test target(s) was (were) not detected (no signal or below the limit of detection): 'The test target(s) was (were) not detected at a level above the limit of detection.'</p> <p>b) If the test target(s) was (were) detected at a level above the limit of detection and below the limit of quantification: 'The test target(s) was (were) detected at a level below the limit of quantification of the method used.'</p>

<p>‘For the test target(s) specified by the applicant, the seed lot meets the specification of ...% (maximum or minimum) by mass or number of copies with ...% confidence.’</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>	<p>c) If seeds showing the test target(s) were found at a level above the limit of quantification: ‘The test target(s) percentage in the seed <b>sample (in case of bulks)/in the seed lot (in case of groups)</b> was determined to be ...% by mass or number of copies, with a 95 % confidence interval of [...%, ...%]’</p> <p><i>or</i></p> <p>‘For the test target(s) specified by the applicant, the <b>sample (in case of bulks)/seed lot (in case of groups)</b> meets the specification of ...% (maximum or minimum) by mass or number of copies with ...% confidence.’</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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An inconsistency between 3.7 and 3.6.1.3 was identified by an ISTA member lab. ISTA Purity Committee addressed the gap by submitting an editorial correction to the rules. The correction provides consistent requirements between section 3.7 and section 3.6.1.3. Similarly, it was noticed that Section 1.5.2.2 also needed this correction.

**“3.6.1.3 Rounding procedure**

*Fraction percentages must be rounded to one decimal place. After rounding, add together the percentages of all fractions. Fractions that are to be reported as a ‘trace’ (see 3.7) are excluded from this calculation; the other fractions must then together total **100.0 %**.”*

The proposal was approved by voting of Purity Committee.

CURRENT VERSION	PROPOSED VERSION
<p><b>1.5.2.2 Purity</b></p> <p>The results of a purity test must be reported in the spaces provided as follows:</p> <ul style="list-style-type: none"> <li>• 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 the species with certainty on the basis of seed characteristics, reporting must be done to the most precise taxon possible.</li> <li>• ...</li> </ul> <p>The percentage by weight of pure seed, inert matter and other seeds, given to one decimal place. The percentage of all components must total 100 %. ...</p> <p><b>3.7 Reporting results</b></p> <p>The results of a purity test must be reported in the spaces provided as follows:</p> <ul style="list-style-type: none"> <li>• 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 the species with certainty on the basis of seed characteristics, reporting must be done to the most precise taxon possible.</li> <li>• ...</li> <li>• The percentage by weight of pure seed, inert matter and other seeds, given to one decimal place. The percentage of all components must total 100 %. ...</li> </ul>	<p><b>1.5.2.2 Purity</b></p> <p>The results of a purity test must be reported in the spaces provided as follows:</p> <ul style="list-style-type: none"> <li>• 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 the species with certainty on the basis of seed characteristics, reporting must be done to the most precise taxon possible.</li> <li>• ...</li> </ul> <p>The percentage by weight of pure seed, inert matter and other seeds, given to one decimal place. The percentage of all components must total 100.0 %. ...</p> <p><b>3.7 Reporting results</b></p> <p>The results of a purity test must be reported in the spaces provided as follows:</p> <ul style="list-style-type: none"> <li>• 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 the species with certainty on the basis of seed characteristics, reporting must be done to the most precise taxon possible.</li> <li>• ...</li> <li>• The percentage by weight of pure seed, inert matter and other seeds, given to one decimal place. The percentage of all components must total 100.0 %. ...</li> </ul>

Editorial change required 5.10 clarifying the column to be checked in in Table 5A Part 2 'double tests'.  
Column 7 should be examined and not column 6.

CURRENT VERSION	PROPOSED VERSION
<p><b>5.10 Germination methods</b></p> <p>...</p> <p>For certain species in Table 5A Part 2, 'double tests' (with and without prechilling) are mandatory, as indicated in column 6. Less desirable methods are placed in brackets, e.g. TTZ (or EET).</p> <p>...</p>	<p><b>5.10 Germination methods</b></p> <p>...</p> <p>For certain species in Table 5A Part 2, 'double tests' (with and without prechilling) are mandatory, as indicated in column 7. Less desirable methods are placed in brackets, e.g. TTZ (or EET).</p> <p>...</p>

ACCEPTED BY VOTE	RESULT

## PART B. NEW SPECIES AND CHANGES TO SPECIES NAMES

### Introduction to the ISTA Rules

#### I.1. Revision of the process to add new species to Table 2C

The proposal is to provide a standard method to determine the working weight of purity and other seed determination (OSD) for adding a new taxon (taxa) to Table 2C, including data rounding rules. With collaboration among BSC, STA, and Purity Committee, the working weight determinations were developed based on statistically recognised methods for estimating variables such as lots, variety, and testing laboratories, and removing data outliers. The **Calculator for adding working weights to Table 2C** is available on the ISTA website. The proposal is provided by BSC and Purity Committee, and the statistical methods applied and the experimental design recommended in the Calculator were developed by Statistics Committee.

The following proposal has been approved by a majority vote of the BSC and Purity Committees

CURRENT VERSION	PROPOSED VERSION
<p><b>I-2.2 Proposals for new species</b></p> <p><b>2. Maximum lot size and sample sizes.</b>                      Proposals for maximum lot size should take into account the general principles that have been applied to species already in the ISTA Rules and to the feasibility of achieving reasonably homogenous seed lots. Seed size is generally the significant factor in determining maximum lot size, but this is also influenced by whether the species is for agriculture or horticulture use, a tree or shrub species, or a flower, spice, herb or medicinal species. This, in turn, will determine whether the species should be placed in Part 1, 2 or 3, respectively, of Table 2C. Proposals for maximum lot size and submitted sample size should then be based on those already to be found in the corresponding part of Table 2C. For agricultural and horticultural species, the submitted sample is larger in relation to the purity working sample, based on the weight of 2500 seeds, than for the other species, to allow for determination of other species by number based on 10 times the purity weight.</p>	<p><b>I-2.2 Proposals for new species</b></p> <p><b>2. Maximum lot size and sample sizes.</b>                      Proposals for maximum lot size should take into account the general principles that have been applied to species already in the ISTA Rules and to the feasibility of achieving reasonably homogenous seed lots. Seed size is generally the significant factor in determining maximum lot size, but this is also influenced by whether the species is for agriculture or horticulture use, a tree or shrub species, or a flower, spice, herb or medicinal species. This, in turn, will determine whether the species should be placed in Part 1, 2 or 3, respectively, of Table 2C. Proposals for maximum lot size and submitted sample size should then be based on those already to be found in the corresponding part of Table 2C. For agricultural and horticultural species, the submitted sample is larger in relation to the purity working sample, based on the weight of 2500 seeds, than for the other species, to allow for determination of other species by number based on 10 times the purity weight.</p> <p style="color: blue; text-align: center;"><a href="#">To determine the weight of the purity and other seed determination (OSD) working samples for a new taxon (or a group of taxa) to be added to</a></p>

	<p>Table 2C, perform and analyse an experiment for assessing multiple sources of variation of 100 seed units weights. Guidelines for the experimental design and data analysis for deriving the minimum 2500 or 25 000 seeds weight are provided in the <b><i>Calculator for adding working weights to Table 2C</i></b> available on the ISTA website.</p> <p>...</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
I.1			

I.2. Revision of Form 1: Proposal for inclusion of new species in the ISTA Rules

C. revised bullet 2, 3, added a new bullet 4

Form 1 is updated with the newly developed *Calculators for adding working weight to Table 2C* under bullet 2. Bullet 3 was revised to clarify the purpose of providing morphological features for PSD (not for identification). The requirement to provide validated working weight for a new taxon or a group of taxa was clarified in a new step; bullet 4. This revision is to improve the procedures and guidance for proposing a new taxon to Table 2C.

The proposal was approved by the purity committee members with voting.

PROPOSED VERSION

**Form 1: Section 2 proposed changes:**

***2. Lot and sample weights***

(information as it should appear in Table 2 C)

Species	Maximum weight of lot (kg)	Minimum submitted sample (g)	Minimum working samples (g)
			<a href="#"><i>Use Calculator for adding working weights to Table 2C</i></a>
			Purity analysis (3.5.1)      Count of other species (4.5.1)

**Form 1: Section 3 proposed changes:**

**Form 1: Section 3 proposed changes:**

No existing definition covers this species:

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**Distinguishing Characteristics of this species to support the PSD proposal:**

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**Form 1: Sections 4, 5, and 6 proposed changes:**

4. *Validated working weight determinations* provided according to the guideline and experiment design of the *Calculator for adding working weights to Table 2C*

YES

5. *Validated germination test method(s)*

(Information as it should appear in Table 5A)

Species	Prescriptions for: Substrate-Temp-pret-tu-re-(c_C) --F-ir-st-c-ou_n_t(d)--F-in_al_c_o_u_n_t( d_)	Additional directions incl. recommendations for breaking dormancy
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6. *Validated tetrazolium test procedure*

(Information as it should appear in Table 6A)

Species	Pretreatment: type/minimum	Preparation before staining	Staining solu- tion(%)	Optimum staining time (h)	Preparation for evaluation	Permitted non- viable tissue	Remarks
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
I.2			

### B.1.1 Addition of new species to Table 2C

None this year.

### B.1.2 Changes to the ISTA List of Stabilised Plant Names

None this year.

The next revision of the ISTA List of Stabilised Plant Names will be discussed at the 2025 ISTA Congress and voted on during the 2025 OGM.

**PART C. RULES CHANGES AND NEW METHODS REQUIRING A VOTE**

**Chapter 1: ISTA Certificates**

**C.1.1. Revision of maximum lot size allowance for *Solanum lycopersicum* L.**

The following proposal has been developed by the ECOM-VSI working group and supported by experimental data, a summary report and analysis supplied by the Statistics Committee. The report of the Statistics Committee (see validation reports provided as Rules proposal supporting evidence) shows that the tomato seed lots in the experiment using separate primary spatial samples from the original lot were clean, with 100% pure seed and no other seeds, but with different % germination values. The seed lots were homogenous for % germination. The analysis used real data and a computer model to answer the question: what is the maximum number of sublots that can be traded commercially and still reflect the quality of the original seed lot? The answer was there is no limit to the number of sublots that could be made from the original seed lot, because the tomato seed lots were found to be homogenous.

This finding was discussed by ISTA members of the ECOM-VSI working group, Kirk Remund (Statistics Committee Chair), Jean-Louis Laffont (Statistics Committee Vice-Chair), Corinne Guimier (B&S TCOM Chair) and the Executive Committee of ISTA who recommended that although in theory there is no maximum number of sublots for homogenous seed lots of tomato, a maximum of 20 sublots be set. The reason to set a maximum of 20 is that a larger survey of tomato seed lots shows some can have a low level of other seeds content, and to ensure both buyers and sellers that the test results represent what is traded and therefore take a more cautious approach. In addition, the 20 subplot maximum is also known to be a practical solution for the companies producing tomato seed lots.

This proposal is approved by the Statistics Committee and a unanimous vote of the BSC TCOM and the ECOM-VSI working group.

CURRENT VERSION	PROPOSED VERSION
<p><b>1.3 Conditions for issuance of ISTA Certificates</b></p> <p>.....</p> <p>i. For an Orange International Seed Lot Certificate to be issued for a subplot, the subplot must represent a minimum size of 20 % of the weight of the original seed lot. A maximum of five Orange International Seed Lot Certificates may be issued for sublots of any one original seed lot.</p> <p>j.</p> <p>.....</p>	<p><b>1.3 Conditions for issuance of ISTA Certificates</b></p> <p>.....</p> <p>i. For an Orange International Seed Lot Certificate to be issued for a subplot, the subplot must represent a minimum size of 20 % of the weight of the original seed lot. A maximum of five Orange International Seed Lot Certificates may be issued for sublots of any one original seed lot, <i>except for <b>Solanum lycopersicum</b> L. where 20 sublots are allowed with a minimum size of 5 % of the weight of the original seed lot.</i></p> <p>j.</p> <p>.....</p>

<p>Each container within the lot or subplot must be identified in such a way that the containers can be readily recognised by the information provided on the certificate issued. Each container of a subplot must be marked with the identification of the original seed lot. A subplot-specific identification is not necessary.</p> <p><b>2.2.2 Sublot</b></p> <p>A subplot is a portion of not less than 20 % of the seed lot. Each container of a subplot must be marked with the identification of the seed lot.</p>	<p>Each container within the lot or subplot must be identified in such a way that the containers can be readily recognised by the information provided on the certificate issued. Each container of a subplot must be marked with the identification of the original seed lot. A subplot-specific identification is not necessary <a href="#">unless the seed owner requests this</a>.</p> <p><b>2.2.2 Sublot</b></p> <p>A subplot is a portion of not less than 20 % of the seed lot, <a href="#">except for <i>Solanum lycopersicum</i> L. seed lots which is not less than 5 % of the original seed lot</a>. Each container of a subplot must be marked with the identification of the seed lot.</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
<b>C.1.1</b>			

C.1.2. Reporting the subplot weight on an ISTA Certificate

The following proposal has been developed by the ECOM-VSI working group to allow the weight of the original seed lot and the weight of the subplot to be recorded in the same place on the ISTA certificate, i.e. in the area that records the information as supplied by the applicant. With this change the statement in 1.4.2.I was no longer needed and therefore the proposal is to delete 1.4.2.I and renumber the other sections that follow.

This proposal is approved by the Statistics Committee and a unanimous vote of the BSC TCOM and the ECOM-VSI working group.

CURRENT VERSION	PROPOSED VERSION
<p><b>1.4 Completing ISTA Certificates</b></p> <p><b>1.4.1 General</b></p> <p>.....</p> <p>d. The name and address of the applicant. Other information stated by the applicant, such as country of origin, species, cultivar, weight of lot <del>or</del> subplot, certification category and applicant's</p>	<p><b>1.4 Completing ISTA Certificates</b></p> <p><b>1.4.1 General</b></p> <p>.....</p> <p>d. The name and address of the applicant. Other information stated by the applicant, such as country of origin, species, cultivar, weight of lot <a href="#">(and subplot, if applicable)</a>, certification category</p>



<p>lot reference must be entered as stated by the applicant.</p> <p><b>Note:.....</b></p> <p><b>1.4.2 Orange International Seed Lot Certificate</b></p> <p>.....</p> <p>k. analysis results;</p> <p><del>l. In the case of certificates for sublots, under 'Other determinations': 'The results reported represent the sample drawn from the original seed lot of ... kg';</del></p> <p><u>m. country where the seed lot was sampled, when the seed lot is located in a different....</u></p> <p><u>n. the signature of the Head of the issuing laboratory...</u></p> <p><u>o. Under the signature it must state at least, the job position of the person signing or 'Authorised signatory'.</u></p>	<p>and applicant's lot reference must be entered as stated by the applicant.</p> <p><b>Note:.....</b></p> <p><b>1.4.2 Orange International Seed Lot Certificate</b></p> <p>.....</p> <p>k. analysis results;</p> <p><u>l. country where the seed lot was sampled, when the seed lot is located in a different...</u></p> <p><u>m. the signature of the Head of the issuing laboratory...</u></p> <p><u>n. Under the signature it must state at least, the job position of the person signing or 'Authorised signatory'.</u></p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.1.2			

### C.1.3. Reporting of uncertainty of measurement on ISTA certificates

The proposed change is necessary to be consistent with what stated in Chapter 19 since several years. The need to report uncertainty of measurement when testing genetically modified organisms is specifically indicated at 19.7.2 and 19.7.3 in current version, and also at 19.7.1 starting from 2023.

This proposal is approved by vote within the GMO TCOM.

#### Additional comments for Proposal C1.3

The current version of 1.5.3 states “Uncertainties of measurements associated with test results are accessible through the tolerance tables in the ISTA Rules and are not reported on ISTA Certificates.” This obviously contradicts what was established in Chapter 19, in particular at 19.7, where it is stated that uncertainty must be indicated when reporting estimates of GMO level (this concept is in the GMO Chapter at least since 2016, perhaps even since the first version in 2013).

In addition to this Rule amendment, in order to maintain consistency, we think an amendment is necessary also to the “ISTA Position Paper on Quantifying and Reporting Uncertainty of Measurement in Seed Testing” (<https://www.seedtest.org/api/rm/67T9WYK66H693XM/09-2007-omistapositionpaperonquantifyingandreporti.pdf>), adopted at the Ordinary Meeting in Iguassu in 2007, which also excluded this possibility.

Of course, other Committees (Variety, Seed Health, others) could think that uncertainty could be applied to their cases too, even though no tolerance tables are present, nor uncertainty is mentioned in the relevant Chapters up to now.

Considering that a comprehensive revision of this Position Paper would require a more thorough discussion, for the time being we would suggest that the following sentence would be applied to the front page of the Position Paper:

“Not valid for GMO testing described in Chapter 19 (Testing for seeds for genetically modified organisms) of the Rules” or “The provisions of this paper do not apply to GMO testing...”).

CURRENT VERSION	PROPOSED VERSION
<p><b>1.5.3 Reporting of uncertainty of measurement on ISTA certificates</b></p> <p>Uncertainties of measurements associated with test results are accessible through the tolerance</p>	<p><b>1.5.3 Reporting of uncertainty of measurement on ISTA certificates</b></p> <p>Except for GMO tests, uncertainties of measurements associated with test results are accessible through the tolerance tables in the</p>

tables in the ISTA Rules and are not reported on the ISTA Certificates.	ISTA Rules and are not reported on the ISTA Certificates.
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.1.3			

## Chapter 2: Sampling

### C.2.1. Revision of the method to add working and OSD weights to Table 2C

The proposal is to provide a standard method to determine the working weight of purity and other seed determination (OSD) for adding new weight to Table 2C, including data rounding rules. With collaboration among BSC, Statistics and Purity Committees, the working weight determinations were developed based on statistically recognised methods for estimating variables such as lots, variety, and testing laboratories, and removing data outliers. The **Calculator for adding working weights to Table 2C** will be available on the ISTA website. The proposal is provided by BSC and Purity Committee, and the statistical methods applied, and the experimental design recommended in the Calculator were developed by STA.

The following proposal has been approved by a majority vote of the BSC and PUR Committees.

CURRENT VERSION	PROPOSED VERSION
<p><b>2.5.2.1 Minimum size of working sample</b></p> <p>Minimum sizes of working samples are prescribed in the appropriate chapter for each test. The working sample weights for purity analyses given in Table 2C are calculated to contain at least 2500 seeds. These weights are recommended for normal use in purity tests, see 3.5.1.</p> <p>The sample weights in column 5 of Table 2C, Part 1, for <del>counts of other species</del> are 10 times the weights in column 4, subject to a maximum of 1000 g.</p>	<p><b>2.5.2.1 Minimum size of working sample</b></p> <p>Minimum sizes of working samples are prescribed in the appropriate chapter for each test. The working sample weights for purity analyses given in Table 2C are calculated to contain at least 2500 seeds. These weights are recommended for normal use in purity tests, see 3.5.1.</p> <p>The sample weights in column 5 of Table 2C, Part 1, for <b>other seed determination (OSD)</b> are 10 times the weights in column 4, subject to a maximum of 1000 g. <b>These weights are recommended for normal use in Other Seed Determination (OSD), see 4.5.1.</b></p> <p><b>Where the seed weight obviously deviates from the purity working sample weight listed in column 4 or other seed determination (OSD) working sample weight listed in column 5 for the taxon concerned, perform and analyse an experiment for assessing multiple sources of variation of 100 seed unit weights. Guidelines for the experiment design and data analysis for deriving the minimum 2500 or 25 000 seeds weight are</b></p>

<p>Working samples of all coated seeds except those defined as treated seed in 2.2.12 must contain at least the number of pellets, seeds or granules indicated in column 3 of Table 2D, Part 1 and Part 2. If a smaller sample is used, the actual number of pellets, seeds or granules in the sample must be reported.</p>	<p>provided in the <a href="#">Calculator for adding working weights to Table 2C</a> available from the <a href="#">ISTA website</a>.</p> <p>Working samples of all coated seeds except those defined as treated seed in 2.2.12 must contain at least the number of pellets, seeds or granules indicated in column 3 of Table 2D, Part 1 and Part 2. If a smaller sample is used, the actual number of pellets, seeds or granules in the sample must be reported.</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
<b>C.2.1</b>			

C.2.2. Revision of maximum lot size allowance for *Solanum lycopersicum* L.

The following proposal has been developed by the Vegetables Working Group to recognise that tomato seed lots are not usually produced or traded internationally in the same weights as equivalent sized agricultural crop seeds (i.e 10 000kg). A survey of ISF members producing tomato seed lots concluded that 200 kg is a maximum lot size for the international trade in tomato seed. The proposal is to reflect the usual seed lot size in commercial production by amending Table 2C. This proposal was discussed in depth within the BSC and only approved by a close majority vote of the BSC as it was thought unnecessary to revise the weight when the maximum is just that, a maximum, and seed lots can be traded below the maximum. Also, that if future amendments were required to reflect the commercial trade in other species in Table 2C that this would be a lot of work and effort that might be best spent elsewhere by BSC TCOM.

This proposal is approved by the Statistics Committee and a majority vote of the BSC and the ECOM-VSI working group.

CURRENT VERSION				
<b>Table 2C Part 1. Lot sizes and sample sizes: agriculture and vegetable seeds</b>				
Species	Maximum weight of lot (kg) (except see 2.8 Note 2)	Minimum submitted sample (g)	Minimum working samples (g)	
			Purity analysis (3.5.1)	Other seeds by number (4.5.1)
1	2	3	4	5
<i>Solanum lycopersicum</i> L.	10 000	15	7	-
PROPOSED VERSION				
<i>Solanum lycopersicum</i> L.	<del>10 000</del> 200	15	7	-

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
<b>C.2.2</b>			

### Chapter 3: The purity analysis

#### C.3.1 Table 3B part 2: Numbered pure seed definitions: 33

The discrepancy, between PSD 33, Table 3B Part 2 (Figure 3.1), and ISTA Handbook on Pure Seed Definitions (Figure 33.1) was identified from analyst training. The proposal was developed by the purity committee to harmonize the wording in PSD 33 with the example provided in Figures 3.1 and 33.1 to include the multi-seed unit that has both fertile and sterile florets.

The proposal was developed and approved by the Purity Committee.

CURRENT VERSION	PROPOSED VERSION
<b>PSD 33</b>	<b>PSD 33</b>

<p><b>Multiple seed units</b></p> <p>Seed units may consist of spikelets or parts of spikelets with more than one floret. Such structures with or without glumes are called multiple seed units (MSUs) when formed by the following structures:</p> <ul style="list-style-type: none"> <li>• one fertile floret with one attached fertile or sterile floret that extends to or beyond the tip of the fertile floret, excluding the awns (Fig. 3.1, 8–12).</li> <li>• one fertile floret with two or more attached fertile or sterile florets of any length (Fig. 3.1, 5–7).</li> <li>• ...</li> </ul>	<p><b>Multiple seed units</b></p> <p>Seed units may consist of spikelets or parts of spikelets with more than one floret. Such structures with or without glumes are called multiple seed units (MSUs) when formed by the following structures:</p> <ul style="list-style-type: none"> <li>• one fertile floret with one attached fertile or sterile floret that extends to or beyond the tip of the fertile floret, excluding the awns (Fig. 3.1, 8–12).</li> <li>• one fertile floret with two or more attached fertile <b>and/or</b> sterile florets of any length (Fig. 3.1, 5–7).</li> <li>• ...</li> </ul>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
<b>C.3.1</b>			

C.3.2 Table 3B part 2: Numbered pure seed definitions: 15

Testing laboratories identified that for some species the schizocarp could be broken and present in a sample. To make the definition more inclusive for those situations, a schizocarp more than one-half the original size is added to the 15.

The proposal was developed and approved by the Purity Committee.

CURRENT VERSION	PROPOSED VERSION
<p>...</p> <p>15. Schizocarp/mericarp, with or without pedicel (of any length), unless it is obvious that no seeds are present.</p> <p>Piece of mericarp larger than one-half the original size, unless it is obvious that no seed is present.</p> <p>...</p>	<p>...</p> <p>15. Schizocarp/mericarp, with or without pedicel (of any length), unless it is obvious that no seeds are present.</p> <p>Piece of <b>schizocarp</b>/mericarp larger than one-half the original size, unless it is obvious that no seed is present.</p> <p>...</p>

Vote to accept item	Yes votes	No votes	Result
C.3.2			

C.3.3. Revision to clarify C.3.5.2.4 regarding the identification of indistinguishable species.

The purity committee received many inquiries from auditors and accredited labs when a reported seed to species level for listed genus under 3.5.2.4. The amendment is to clarify C.3.5.2.4 procedures only apply when the seed is deemed by laboratories as “indistinguishable” with discretion. When a lab can identify those example genera, such as *Brassica rapa*, (not *Brassica* spp), it is outside of the scope of 3.5.2.4, and should be correct as long as proficient evidence is provided.

The proposal was supported by a majority vote of the Purity Committee.

CURRENT VERSION	PROPOSED VERSION
<p><b>3.5.2.4 Indistinguishable species</b></p> <p>When it is difficult or impossible to distinguish between species, one of the two following procedures may be followed:</p> <ol style="list-style-type: none"> <li>Only the genus name is reported on the analysis Certificate, all seeds of that genus (e.g. both awned and awnless seeds of <i>Lolium</i>) being classed as pure seed; additional information may be reported under ‘Other determinations’, or</li> <li>The similar seeds are separated from the other components and weighed together. From this mixture at least 400 seeds, and preferably about 1000, are taken at random; a final separation is made on this portion and the proportion of each species determined by weight. From this proportion the percentage of each species in the entire sample can be calculated (3.6).</li> </ol> <p>If this procedure is followed, the details must be reported including the number of seeds examined.</p>	<p><b>3.5.2.4 Indistinguishable species</b></p> <p>When it is difficult or impossible to distinguish between species, one of the two following procedures may be followed:</p> <ol style="list-style-type: none"> <li>Only the genus name is reported on the analysis Certificate, all seeds of that genus (e.g. both awned and awnless seeds of <i>Lolium</i>) being classed as pure seed; additional information may be reported under ‘Other determinations’, or</li> <li>The similar seeds are separated from the other components and weighed together. From this mixture at least 400 seeds, and preferably about 1000, are taken at random; a final separation is made on this portion and the proportion of each species determined by weight. From this proportion the percentage of each species in the entire sample can be calculated (3.6).</li> </ol> <p>If this procedure is followed, the details must be reported including the number of seeds examined.</p>

The procedures are applicable when the seed is described by the <b>sender</b> as a species of <i>Agrostis</i> , <i>Brassica</i> , <i>Lolium</i> , <i>Poa</i> , and <i>Festuca rubra</i> or <i>F. ovina</i> , <del>and in other cases at the discretion of the analyst.</del>	The procedures are applicable <b>at the discretion of the laboratory</b> when the taxon is considered indistinguishable. This may happen when the seed is described by the <b>applicant</b> as a species of, <b>for example</b> , <i>Agrostis</i> , <i>Brassica</i> , <i>Lolium</i> , <i>Poa</i> , and <i>Festuca rubra</i> or <i>F. ovina</i> .
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.3.3			

## Chapter 4: Determination of other seeds by number

### C.4.1 Reporting Results

The sample weight of the determination of other seeds must be reported with a fixed decimal place. To express it clearer, the misleading word "minimum" is erased. As well the table got a number and a heading, in consequence the already existing tables 4A and 4B are renamed.

The following proposal has been developed and approved by the Purity Committee.

CURRENT VERSION	PROPOSED VERSION																								
<p><b>4.7 Reporting results</b></p> <p>The sample weight examined must be reported according to the <b>minimum</b> number of decimals indicated in <del>the</del> <b>table below</b>.</p> <table border="1"> <thead> <tr> <th>Weight of sample (g)</th> <th>Number of decimal places for reporting</th> </tr> </thead> <tbody> <tr> <td>Lower than 1.000</td> <td>4</td> </tr> <tr> <td>1.000–9.999</td> <td>3</td> </tr> <tr> <td>10.00–99.99</td> <td>2</td> </tr> <tr> <td>100.0–999.9</td> <td>1</td> </tr> <tr> <td>1000 or greater</td> <td>0</td> </tr> </tbody> </table>	Weight of sample (g)	Number of decimal places for reporting	Lower than 1.000	4	1.000–9.999	3	10.00–99.99	2	100.0–999.9	1	1000 or greater	0	<p><b>4.7 Reporting results</b></p> <p>The sample weight examined must be reported according to the number of decimals indicated in <b>table 4A</b>.</p> <p><b>Table 4A. Number of decimals for reporting the sample weight for the determination of other seeds</b></p> <table border="1"> <thead> <tr> <th>Weight of sample (g)</th> <th>Number of decimal places for reporting</th> </tr> </thead> <tbody> <tr> <td>Lower than 1.000</td> <td>4</td> </tr> <tr> <td>1.000–9.999</td> <td>3</td> </tr> <tr> <td>10.00–99.99</td> <td>2</td> </tr> <tr> <td>100.0–999.9</td> <td>1</td> </tr> <tr> <td>1000 or greater</td> <td>0</td> </tr> </tbody> </table>	Weight of sample (g)	Number of decimal places for reporting	Lower than 1.000	4	1.000–9.999	3	10.00–99.99	2	100.0–999.9	1	1000 or greater	0
Weight of sample (g)	Number of decimal places for reporting																								
Lower than 1.000	4																								
1.000–9.999	3																								
10.00–99.99	2																								
100.0–999.9	1																								
1000 or greater	0																								
Weight of sample (g)	Number of decimal places for reporting																								
Lower than 1.000	4																								
1.000–9.999	3																								
10.00–99.99	2																								
100.0–999.9	1																								
1000 or greater	0																								



<p><b>4.8 Tolerance tables</b></p> <p><b>Table 4A</b> gives the maximum difference in the numbers of other seeds,...</p> <p>...</p> <p><b>Table 4A.</b> Tolerances for the determination of other seeds by number when tests are made on the same or a different submitted sample in the same or a different laboratory (two-way test at 5 % significance level)</p> <p>...</p> <p><b>Table 4B</b> gives the tolerances for counts of number of other seeds,...</p> <p><b>Table 4B.</b> Tolerances for the determination of other seeds by number when tests are made on different submitted samples, the second being made in the same or in a different laboratory (one-way test at 5 % significance level)</p>	<p><b>4.8 Tolerance tables</b></p> <p><b>Table 4B</b> gives the maximum difference in the numbers of other seeds,...</p> <p>...</p> <p><b>Table 4B.</b> Tolerances for the determination of other seeds by number when tests are made on the same or a different submitted sample in the same or a different laboratory (two-way test at 5 % significance level)</p> <p>...</p> <p><b>Table 4C</b> gives the tolerances for counts of number of other seeds,...</p> <p><b>Table 4C.</b> Tolerances for the determination of other seeds by number when tests are made on different submitted samples, the second being made in the same or in a different laboratory (one-way test at 5 % significance level)</p>
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Vote to accept item	Yes votes	No votes	Result
C.4.1			

## Chapter 5: The germination test

### C.5.1. Revision of retesting when fresh seed is present

The purpose of this proposal is to address the issue in ISTA Rules Section 5.7(a), considering test results of the initial germination test “unsatisfactory” and the instruction to not report these test results and to require additional testing (i.e., retesting), when dormancy is suspected in the initial germination test.

There should be an option provided to laboratories to either report the percentage of germination and the percentage of fresh seeds determined by the initial test, or to not report the results of the initial test and to conduct additional testing using dormancy breaking procedures listed in Table 5A. When a fuller assessment is requested by the customer or desired by the laboratory, a retest would be conducted.

The ISTA Rules should allow for the same testing and reporting option for “fresh seeds” as they do for “hard seeds” (ref. ISTA Rules 5.6.3.2). When, at the end of the test period, ungerminated seeds are determined to be “fresh seeds”, the percentage of “fresh seeds” is reported. When a fuller assessment is desired or requested, the test results are not reported, and additional testing is conducted.

This proposal originates from and is supported by the Germination Committee.



<p>the temperature indicated in Table 5A column 3...</p> <p>...</p> <p><b>5.6.5.3 Ungerminated seeds</b></p> <p><b>Hard seeds:</b> At the end of a germination test, hard seeds are counted and reported as such on the ISTA Certificate.</p> <p><b>Fresh seeds:</b> When 5 % or more of fresh seeds are believed to be present, their potential to germinate must be determined by dissection, tetrazolium or excised embryo. Those determined to have the potential to germinate are reported as fresh. Those determined not to have the potential to germinate are reported as dead. After this determination, if there is any doubt as to whether the seed is fresh or dead, it must be classified as dead. <del>If not already applied, measures described in 5.6.3 must be taken to break dormancy if 5% or more of fresh ungerminated seeds are found.</del></p> <p>....</p> <p><b>5.7 Retesting</b></p> <p>The result of a test must be considered unsatisfactory and must not be reported, and a second test must be made by the same or an alternative method, under the following circumstances:</p> <p><del>a. When dormancy is suspected (fresh ungerminated seeds), any procedure to break dormancy indicated in column 6 of Table 5A or in 5.6.3.1 may be applied in one or more additional tests. The best result achieved must be reported and the procedure must be indicated on the ISTA Certificate.</del></p> <p>b. When the result may not be reliable because of phytotoxicity or spread of fungi or bacteria, retests must be made using one or more alternative methods as indicated in Table 5A, or in sand, organic growing</p>	<p>temperature indicated in Table 5A column 3...</p> <p>...</p> <p><b>5.6.5.3 Ungerminated seeds</b></p> <p><b>Hard seeds:</b> At the end of a germination test, hard seeds are counted and reported as such on the ISTA Certificate.</p> <p><b>Fresh seeds:</b> When 5 % or more of fresh seeds are believed to be present, their potential to germinate must be determined by dissection, tetrazolium or excised embryo. Those determined to have the potential to germinate are reported as fresh. Those determined not to have the potential to germinate are reported as dead. After this determination, if there is any doubt as to whether the seed is fresh or dead, it must be classified as dead. <b>When a fuller germination assessment is required by the laboratory or upon the request of the customer, retesting utilising a procedure for removing dormancy described in 5.6.3 is essential.</b></p> <p>....</p> <p><b>5.7 Retesting</b></p> <p>The result of a test must be considered unsatisfactory and must not be reported, and a second test must be made by the same or an alternative method, under the following circumstances:</p> <p>a. When the result may not be reliable because of phytotoxicity or spread of fungi or bacteria, retests must be made using one or more alternative methods as indicated in Table 5A, or in sand, organic growing</p>
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<p>media, or soil. If necessary, the distance between the seeds must be increased. The best result achieved must be reported, and the method used must be indicated on the ISTA Certificate.</p> <p><b>e.</b> When there is difficulty in deciding the correct evaluation of a number of seedlings, retests must be made using one or more alternative methods as prescribed in Table 5A, or in sand, organic growing media, or soil. The best result achieved must be reported and the method used must be indicated on the ISTA Certificate.</p> <p><b>d.</b> When there is evidence of errors in test conditions, seedling evaluation or counting, a retest must be made using the same method or an alternative method as described in Table 5A, and the result of the retest must be reported on the ISTA Certificate.</p> <p><b>e.</b> If a sample does not respond satisfactorily to the method selected, it will be necessary to retest it by one or more of the alternative methods. When seedlings occur which cannot be easily evaluated or show phytotoxic symptoms, a retest should be made in sand, organic growing media, or soil at the temperature prescribed in Table 5A. Planting another sample of the same cultivar, known to germinate satisfactorily, alongside, may provide a useful guide to evaluation of this retest. The best result achieved must be reported and the method used must be indicated on the ISTA Certificate.</p> <p><b>f.</b> When the range for the replicates exceeds the maximum tolerated range in Table 5B, a retest must be carried out using the same test method or an alternative method. If the results of the retest using the same method are compatible with the first (i.e. the difference does not exceed the tolerance indicated in either Table 5C, 5D or 5E), the average of the test results must be reported on the ISTA Certificate (see 5.8.1 Tolerances). If an alternative method is used and if the results are better and within accepted tolerances, then these results must be reported on the ISTA Certificate</p>	<p>media, or soil. If necessary, the distance between the seeds must be increased. The best result achieved must be reported, and the method used must be indicated on the ISTA Certificate.</p> <p><b>b.</b> When there is difficulty in deciding the correct evaluation of a number of seedlings, retests must be made using one or more alternative methods as prescribed in Table 5A, or in sand, organic growing media, or soil. The best result achieved must be reported and the method used must be indicated on the ISTA Certificate.</p> <p><b>c.</b> When there is evidence of errors in test conditions, seedling evaluation or counting, a retest must be made using the same method or an alternative method as described in Table 5A, and the result of the retest must be reported on the ISTA Certificate.</p> <p><b>d.</b> If a sample does not respond satisfactorily to the method selected, it will be necessary to retest it by one or more of the alternative methods. When seedlings occur which cannot be easily evaluated or show phytotoxic symptoms, a retest should be made in sand, organic growing media, or soil at the temperature prescribed in Table 5A. Planting another sample of the same cultivar, known to germinate satisfactorily, alongside, may provide a useful guide to evaluation of this retest. The best result achieved must be reported and the method used must be indicated on the ISTA Certificate.</p> <p><b>e.</b> When the range for the replicates exceeds the maximum tolerated range in Table 5B, a retest must be carried out using the same test method or an alternative method. If the results of the retest using the same method are compatible with the first (i.e. the difference does not exceed the tolerance indicated in either Table 5C, 5D or 5E), the average of the test results must be reported on the ISTA Certificate (see 5.8.1 Tolerances). If an alternative method is used and if the results are better and within accepted tolerances, then these results must be reported on the ISTA Certificate (see 5.8.1 Tolerances) and</p>
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<p>(see 5.8.1 Tolerances) and must not be averaged with the previous test results. When retesting is carried out under the circumstances a., b., c. or e., the best results achieved must be indicated on the ISTA Certificate. The results of the other tests do not have to be reported on the ISTA Certificate, except on specific request by the applicant.</p> <p><del>g.</del> When due to counting errors more than 5 seeds are lost or found during a germination test (i.e. <math>\pm 1.25\%</math> for a total of 400 seeds), then the test must be repeated.</p>	<p>must not be averaged with the previous test results. When retesting is carried out under the circumstances a., b., c. or e., the best results achieved must be indicated on the ISTA Certificate. The results of the other tests do not have to be reported on the ISTA Certificate, except on specific request by the applicant.</p> <p>f. When due to counting errors more than 5 seeds are lost or found during a germination test (i.e. <math>\pm 1.25\%</math> for a total of 400 seeds), then the test must be repeated.</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
<b>C.5.1</b>			

C.6.1 Adding a tetrazolium test method for *Ulmus* spp. L.

This proposal introduces into Chapter 6 (Table 6A Part 2) of the ISTA Rules a method to test *Ulmus* spp. seeds with Tetrazolium salts. The method being introduced is already described in ISTA Working Sheets on Tetrazolium Testing Volume II, published by ISTA in 2003 (ISBN 978-3-906549-41-5). In addition, three other *Ulmus* species are already included in Table 5A Part 2 of the germination chapter.

The following proposal has been approved by the ISTA TEZ Committee and is supported by a method validation study.

**New method in Chapter 6**

**Table 6A Part 2.** Standard procedures for tetrazolium testing: tree and shrub seeds

Species	Pretreatment type/minimum time (h)	Preparation before staining	Staining solution (%)	Optimum staining time (h)	Preparation for evaluation	Permitted non- viable tissue	Remarks
<i>Ulmus</i> spp.	Soak 18 hours in water at 20°C	Cut transversely 1/3 from stalk base	1	18 hours, 30°C	Extract embryo	none	none

Vote to accept item	Yes votes	No votes	Result
C.6.1			

## Chapter 9: Determination of moisture content

### C.9.1 Specifying oven temperatures and tolerances

Current version of the paragraph “9.2.5.7” provides only tolerated ranges for drying temperatures. In this proposal, prescribed temperatures are explicitly given, as in paragraphs 9.1.2 and 9.1.3 (103°C resp. 130°C). The proposed tolerated range for the high temperature method (127-133°C) deviates from the current one (130-133°C), but it is the same range prescribed by AOSA (127-133°C). A comparison was conducted in 2 labs. The Statistics TCOM analysed the data and supports the proposed change, the report of the peer method validation is added.

This proposal originates from and is supported by the Moisture Committee.

CURRENT VERSION	PROPOSED VERSION
<p><b>9.2.5.7 Prescribed methods</b></p> <p>...</p> <p>e. <del>Tolerances for the temperatures and durations are:</del></p> <p><del>101–105 °C</del> (low temperature): 17 ±1 h;</p> <p><del>130–133 °C</del> (high temperature): 1 h ±3 min, 2 h ±6 min or 4 h ±12 min. ...</p>	<p><b>9.2.5.7 Prescribed methods</b></p> <p>...</p> <p>e. <b>Methods and their Tolerances for temperatures and durations:</b></p> <p>Low temperature <b>103°C (± 2°C)</b>; 17 h ± 1 h;</p> <p>High temperature <b>130°C (± 3°C)</b>; 1 h ±3 min, 2 h ±6 min or 4 h ±12 min....</p>

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

### C.9.2 Reporting moisture content in seed mixtures

In special cases it should be possible, to report the moisture content of seed mixtures.

This proposal originates from and is supported by the Moisture Committee. Note: If this proposal is accepted, the remaining sections in Chapter 18 will be renumbered (18.8 **Reporting Results** will be renumbered 18.9, etc.).

CURRENT VERSION	PROPOSED VERSION
<p><b>18.8 Reporting Result</b></p> <p>.....</p>	<p><b>18.8 Moisture content</b></p> <p>Moisture content can be determined on seed mixtures containing only species with the same moisture method prescribed (Table 9A). The result for moisture content must be entered in the appropriate field in the BIC. For all other mixtures, there is no ISTA moisture method and moisture content cannot be reported.</p> <p><b>18.9 Reporting Result</b></p> <p>.....</p>

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

### C.9.3 Moisture test for coated seeds

The moisture test on seeds is usually done with naked, treated or pelleted seeds. Per definition given in chapter 11 also seed mats and seed tapes are coated seeds. This proposal describes clearly now how to handle seed mats and seed tapes regarding the moisture test.

This proposal originates from and is supported by the Moisture Committee.

CURRENT VERSION	PROPOSED VERSION
<p><b>9.2.7 Reporting of results</b></p> <p>...</p> <ul style="list-style-type: none"> <li>In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted, and the moisture content reported is the average of seed and pelleting materials'.</li> </ul>	<p><b>9.2.7 Reporting of results</b></p> <p>...</p> <ul style="list-style-type: none"> <li>In the case of pelleted seeds (see Chapter 11), the following statement must be entered: 'The seeds of the submitted moisture sample were pelleted and the moisture content reported is <b>that of the combined unit comprising seed and pelleting material</b>'.</li> </ul>

<p><b>1.5.2.12 Moisture content</b></p> <p>...</p> <ul style="list-style-type: none"> <li>• In the case of pelleted seeds (see Chapter 11), the following statement must be entered: ‘The seeds of the submitted moisture sample were pelleted, and the moisture content reported is the average of seed and pelleting materials’.</li> </ul>	<p><b>1.5.2.12 Moisture content</b></p> <p>...</p> <ul style="list-style-type: none"> <li>• The moisture content of seed tapes and seed mats cannot be reported on an OIC or BIC since there is no ISTA method for this kind of sample.</li> <li>• In the case of pelleted seeds (see Chapter 11), the following statement must be entered: ‘The seeds of the submitted moisture sample were pelleted and the moisture content reported is <b>that of the combined unit comprising</b> seed and pelleting material’.</li> <li>• The moisture content of seed tapes and seed mats cannot be reported on an OIC or BIC since there is no ISTA method for this kind of sample.</li> <li>• For <i>Arachis</i>...</li> </ul>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
<b>C.9.3</b>			

### C.9.4 Merging Parts 1 and 2 of Table 9A

Part 1 and Part 2 of Table 9A are merged due to changes in the crop groups (e.g. Malva transferred from tree and shrub to flower crop group). This change would allow easier inclusions and modifications in future. Some species are renamed according to table 2C. Additional Information of species that are included according to new Taxonomy are given in the last column, this column won't be in the Rules.

As well the method, which has to be applied, can be found better and faster.

This proposal originates from and is supported by the Moisture Committee.

CURRENT VERSION



**Table 9A Part 1.** Details of methods for moisture determination: agricultural and vegetable seeds

The low-temperature method (low) or high temperature (high) method must be used as specified for the species in this Table.

9: Determination of moisture content

Species	Grinding/cutting (9.2.5.4, 9.2.5.5)	Method to be used	Drying at high temperature (h)	Predrying requirement (9.2.5.6)
1	2	3	4	5
<i>Agrostis</i> spp.	No	High	1	–
<i>Allium</i> spp.	No	Low	–	–
<i>Alopecurus pratensis</i>	No	High	1	–
<i>Anethum graveolens</i>	No	High	1	–
<i>Anthoxanthum odoratum</i>	No	High	1	–
<i>Anthriscus</i> spp.	No	High	1	–
<i>Apium graveolens</i>	No	High	1	–
<i>Arachis hypogaea</i>	Cut	Low	–	To 17 % moisture content or less
<i>Arrhenatherum</i> spp.	No	High	1	–
<i>Asparagus officinalis</i>	No	High	1	–
<i>Avena</i> spp.	Coarse	High	2	To 17 % moisture content or less
<i>Beta vulgaris</i>	No	High	1	–
<i>Brassica</i> spp.	No	Low	–	–
<i>Bromus</i> spp.	No	High	1	–
<i>Camelina sativa</i>	No	Low	–	–
<i>Cannabis sativa</i>	No	High	1	–

**Table 9A Part 2.** Details of methods for moisture determination: tree and shrub seeds

The low-temperature method must be used for all species in Table 9A Part 2.

9: Determination of moisture content

Species	Grinding/cutting (9.2.5.4, 9.2.5.5)	Remarks
<i>Abies</i> spp. (TSW ≤200 g)	No	–
<i>Abies</i> spp. (TSW >200 g)	Cut	High oil content
<i>Acacia</i> spp.	Coarse	–
<i>Acer</i> spp.	Coarse	Because of heterogeneity
<i>Aesculus hippocastanum</i>	Cut	–
<i>Ailanthus altissima</i>	Coarse	–
<i>Alnus</i> spp.	No	–
<i>Amorpha fruticosa</i>	Coarse	Moved from Table 9A Part 1
<i>Berberis aquifolium</i>	No	–
<i>Betula</i> spp.	No	–
<i>Calocedrus decurrens</i>	Coarse	–
<i>Caragana arborescens</i>	Coarse	–
<i>Carica papaya</i>	No	High oil content
<i>Carpinus betulus</i>	Coarse	–
<i>Castanea sativa</i>	Cut	–
<i>Catalpa</i> spp.	Coarse	–
<i>Cedrela</i> spp.	No	–
<i>Cedrus</i> spp.	Cut	High oil content
<i>Chamaecyparis</i> spp.	No	–
<i>Cornus</i> spp. (TSW ≤200 g)	Coarse	Hard integument
<i>Cornus</i> spp. (TSW >200 g)	Coarse	–

PROPOSED VERSION

Table 9A Details of methods for moisture determination

The oven method must be used as specified for the species in this Table.

Species	Grinding/cutting (9.2.5.4, 9.2.5.5)	Drying Temp. High: 130°C Low: 103°C	Drying time (h)	Tolerances of replicates (9.2.6.2)	Predrying require- ment (9.2.5.6) / remarks
1	2	3	4	5	6
<i>Abies</i> spp. (TSW >200 g)	Cut	Low	17	Table 9B	– / High oil content
<i>Abies</i> spp. (TSW ≤200 g)	No	Low	17	Table 9B	–
<i>Acacia</i> spp.	Coarse	Low	17	Table 9B	–
<i>Acer</i> spp.	Coarse	Low	17	Table 9B	– / Because of heterogeneity
<i>Aegilops</i> spp.	Fine	High	2	0.2%	To 17 % moisture content or less
<i>Aesculus hippocastanum</i>	Cut	Low	17	Table 9B	–
<i>Agrostis</i> spp.	No	High	1	0.2%	–
<i>Ailanthus altissima</i>	Coarse	Low	17	Table 9B	–
<i>Allium</i> spp.	No	Low	17	0.2%	–
<i>Alnus</i> spp.	No	Low	17	Table 9B	–
<i>Alopecurus pratensis</i>	No	High	1	0.2%	–
<i>Amorpha fruticosa</i>	Coarse	Low	17	Table 9B	– / Moved from Table 9A Part 1
<i>Anethum graveolens</i>	No	High	1	0.2%	–
<i>Anthoxanthum odoratum</i>	No	High	1	0.2%	–
<i>Anthriscus</i> spp.	No	High	1	0.2%	–
<i>Apium graveolens</i>	No	High	1	0.2%	–
<i>Arachis hypogaea</i>	Cut	Low	17	0.2%	To 17 % moisture content or less
<i>Arrhenatherum</i> spp.	No	High	1	0.2%	–
<i>Asparagus officinalis</i>	No	High	1	0.2%	–
<i>Avena</i> spp.	Coarse	High	2	0.2%	To 17 % moisture content or less
<i>Berberis aquifolium</i>	No	Low	17	Table 9B	–
<i>Beta vulgaris</i>	No	High	1	0.2%	–
<i>Betula</i> spp.	No	Low	17	Table 9B	–
<i>Brassica</i> spp.	No	Low	17	0.2%	–
<i>Bromus</i> spp.	No	High	1	0.2%	–
<i>Calocedrus decurrens</i>	Coarse	Low	17	Table 9B	–
<i>Camelina sativa</i>	No	Low	17	0.2%	–

one species from genus *Triticum* has been placed into genus *Aegilops*

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<i>Cannabis sativa</i>	No	High	1	0.2%	–
<i>Capsicum</i> spp.	No	Low	17	0.2%	–
<i>Caragana arborescens</i>	Coarse	Low	17	Table 9B	–
<i>Carpinus betulus</i>	Coarse	Low	17	Table 9B	–
<i>Carum carvi</i>	No	High	1	0.2%	–
<i>Castanea sativa</i>	Cut	Low	17	Table 9B	–
<i>Catalpa</i> spp.	Coarse	Low	17	Table 9B	–
<i>Cedrela</i> spp.	No	Low	17	Table 9B	–
<i>Cedrus</i> spp.	Cut	Low	17	Table 9B	– / High oil content
<i>Cenchrus</i> spp.	No	High	1	0.2%	–
<i>Chamaecyparis</i> spp.	No	Low	17	Table 9B	–
<i>Chloris gayana</i>	No	High	1	0.2%	–
<i>Cicer arietinum</i>	Coarse	High	1	0.2%	To 17 % moisture content or less
<i>Cichorium</i> spp.	No	High	1	0.2%	–
<i>Citrullus lanatus</i>	Coarse	High	1	0.2%	To 17 % moisture content or less
<i>Cornus</i> spp. (TSW >200 g)	Coarse	Low	17	Table 9B	–
<i>Cornus</i> spp. (TSW ≤200 g)	Coarse	Low	17	Table 9B	– / Hard integument
<i>Corylus avellana</i>	Cut	Low	17	Table 9B	–
<i>Corymbia</i> spp.	No	Low	17	Table 9B	–
<i>Cotoneaster</i> spp.	No	Low	17	Table 9B	–
<i>Crataegus monogyna</i>	Coarse	Low	17	Table 9B	–
<i>Cryptomeria japonica</i>	No	Low	17	Table 9B	–
<i>Cucumis</i> spp.	No	High	1	0.2%	–
<i>Cucurbita</i> spp.	No	High	1	0.2%	–
<i>Cuminum cyminum</i>	No	High	1	0.2%	–
<i>Cupressus</i> spp.	No	Low	17	Table 9B	–
<i>Cydonia oblonga</i>	No	Low	17	Table 9B	–
<i>Cynodon dactylon</i>	No	High	1	0.2%	–
<i>Cynosurus cristatus</i>	No	High	1	0.2%	–
<i>Cytisus scoparius</i>	Coarse	Low	17	Table 9B	–
<i>Dactylis glomerata</i>	No	High	1	0.2%	–
<i>Daucus carota</i>	No	High	1	0.2%	–
<i>Deschampsia</i> spp.	No	High	1	0.2%	–
<i>Elaeagnus angustifolia</i>	Coarse	Low	17	Table 9B	–
<i>Elymus</i> spp.	No	High	1	0.2%	–
<del><i>Elytrigia</i> spp.</del>	<del>No</del>	<del>High</del>	<del>1</del>	<del>0.2%</del>	<del>–</del>

from *Elytrigia* spp.

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<i>Eucalyptus</i> spp.	No	Low	17	Table 9B	–
<i>Euonymus europaeus</i>	Coarse	Low	17	Table 9B	–
<i>Fagopyrum esculentum</i>	Fine	High	2	0.2%	To 17 % moisture content or less
<i>Fagus sylvatica</i>	Cut	Low	17	Table 9B	–
<i>Festuca</i> spp.	No	High	1	0.2%	–
<i>Fraxinus</i> spp.	Coarse	Low	17	Table 9B	–
<i>Galega orientalis</i>	No	High	1	0.2%	–
<i>Ginkgo biloba</i>	Cut	Low	17	Table 9B	–
<i>Gleditsia triacanthos</i>	Coarse	Low	17	Table 9B	–
<i>Glycine max</i>	Coarse	Low	17	0.2%	To 12 % moisture content or less
<i>Gossypium</i> spp.	Fine	Low	17	0.2%	To 17 % moisture content or less
<i>Helianthus annuus</i>	No	Low	17	0.2%	–
<i>Holcus lanatus</i>	No	High	1	0.2%	–
<i>Hordeum vulgare subsp. vulgare</i>	Fine	High	2	0.2%	To 17 % moisture content or less
<i>Ilex aquifolium</i>	Coarse	Low	17	Table 9B	–
<i>Juniperus</i> spp.	Coarse	Low	17	Table 9B	–
<i>Koeleria paniculata</i>	Coarse	Low	17	Table 9B	–
<i>Laburnum</i> spp.	Coarse	Low	17	Table 9B	–
<i>Lactuca sativa</i>	No	High	1	0.2%	–
<i>Larix*marschlinsii</i>	No	Low	17	Table 9B	–
<i>Larix</i> spp.	No	Low	17	Table 9B	–
<i>Lathyrus</i> spp.	Coarse	High	1	0.2%	To 17 % moisture content or less
<i>Lepidium sativum</i>	No	High	1	0.2%	–
<i>Ligustrum vulgare</i>	Coarse	Low	17	Table 9B	–
<i>Linum usitatissimum</i>	No	Low	17	0.2%	–
<i>Liquidambar styraciflua</i>	No	Low	17	Table 9B	– / High oil content
<i>Liriodendron tulipifera</i>	Coarse	Low	17	Table 9B	–
<i>Lolium</i> spp.	No	High	2	0.2%	– /also 103°C 17h possible
<i>Lotus</i> spp.	No	High	1	0.2%	–
<i>Lupinus</i> spp.	Coarse	High	1	0.2%	To 17 % moisture content or less
<del><i>Macroptilium atropurpureum</i></del>	<del>Coarse</del>	<del>High</del>	<del>1</del>	<del>-</del>	<del>To 17 % moisture content or less</del>

name changed

to be replaced with *Macroptilium* spp. due to transfer of some species from genus *Phaseolus*

<i>Macroptilium spp.</i>	Coarse	High	1	0.2%	To 17 % moisture content or less	Macroptilium spp. due to transfer of some species from genus Phaseolus	
<i>Malus spp.</i> (except <i>M. sylvestris</i> )	No	Low	17	Table 9B	–		
<i>Malus sylvestris</i>	Coarse	Low	17	Table 9B	–		
<i>Malva sylvestris</i>	No	Low	17	Table 9B	–		
<i>Medicago spp.</i>	No	High	1	0.2%	–		
<i>Megathyrus maximus</i>	No	High	2	0.2%	–		
<i>Melilotus spp.</i>	No	High	1	0.2%	–		
<i>Morus spp.</i>	No	Low	17	Table 9B	–		
<i>Nicotiana tabacum</i>	No	High	1	0.2%	–		
<i>Nothofagus spp.</i>	No	Low	17	Table 9B	–		
<i>Onobrychis viciifolia</i>	No	High	1	0.2%	–		
<i>Ornithopus sativus</i>	No	High	1	0.2%	–		
<i>Oryza sativa</i>	Fine	High	2	0.2%	To 13 % moisture content or less		
<i>Panicum spp.</i>	No	High	2	0.2%	–		
<i>Papaver somniferum</i>	No	High	1	0.2%	–		
<i>Paspalidium spp.</i>	No	High	1	0.2%	–		one species from genus Setaria has been moved to this genus
<i>Paspalum spp.</i>	No	High	1	0.2%	–		
<i>Pastinaca sativa</i>	No	High	1	0.2%	–		
<i>Pennisetum spp.</i>	No	High	1	0.2%	–	species from genus Cenchrus	
<i>Petroselinum crispum</i>	No	High	1	0.2%	–		
<i>Phacelia tanacetifolia</i>	No	High	1	0.2%	–		
<i>Phalaris spp.</i>	No	High	1	0.2%	–		
<i>Phaseolus spp.</i>	Coarse	High	1	0.2%	To 17 % moisture content or less		
<i>Phleum spp.</i>	No	High	1	0.2%	–		
<i>Picea spp.</i>	No	Low	17	Table 9B	–		
<i>Pinus spp.</i> (TSW >200 g)	No	Low	17	Table 9B	–		
<del><i>Pinus spp.</i> (TSW ≤200 g)</del>	<del>No</del>	<del>Low</del>	<del>17</del>	<del>Table 9B</del>	<del>–</del>		
<i>Pisum sativum</i>	Coarse	High	1	0.2%	To 17 % moisture content or less		
<i>Platanus spp.</i>	No	Low	17	Table 9B	–		

<i>Platycladus spp.</i>	No	Low	17	Table 9B	–	from the genus Thuja	
<i>Poa spp.</i>	No	High	1	0.2%	–		
<i>Populus spp.</i>	No	Low	17	Table 9B	–		
<i>Prunus spp.</i>	Coarse	Low	17	Table 9B	–		
<i>Pseudotsuga menziesii</i>	No	Low	17	Table 9B	–		
<i>Pyrus spp.</i>	No	Low	17	Table 9B	–		
<i>Quercus spp.</i>	Cut	Low	17	Table 9B	–		
<i>Raphanus sativus</i>	No	Low	17	0.2%	–		
<i>Ricinus communis</i>	Cut	Low	17	0.2%	To 17 % moisture content or less		
<i>Robinia pseudoacacia</i>	Coarse	Low	17	Table 9B	–		
<i>Rosa spp.</i>	No	Low	17	Table 9B	–		
<i>Salix spp.</i>	No	Low	17	Table 9B	–		
<i>Scorzonera hispanica</i>	No	High	1	0.2%	–		
<i>Secale cereale</i>	Fine	High	2	0.2%	To 17 % moisture content or less		
<i>Senegalia spp.</i>	Coarse	Low	17	Table 9B	–	from the genus Acacia	
<i>Sequoia sempervirens</i>	No	Low	17	Table 9B	–		
<i>Sequoiadendron giganteum</i>	No	Low	17	Table 9B	–		
<i>Sesamum indicum</i>	No	Low	17	0.2%	–		
<i>Setaria spp.</i>	No	High	1	0.2%	–		
<i>Sinapis spp.</i>	No	Low	17	0.2%	–		
<i>Solanum lycopersicum</i>	No	High	1	0.2%	–		
<i>Solanum melongena</i>	No	Low	17	0.2%	–		
<i>Sorbus spp.</i>	No	Low	17	Table 9B	–		
<i>Sorghum spp.</i>	Fine	High	2	0.2%	To 17 % moisture content or less		
<i>Spartium junceum</i>	Coarse	Low	17	Table 9B	–		
<i>Spinacia oleracea</i>	No	High	1	0.2%	–		
<i>Styphnolobium japonicum</i>	Coarse	Low	17	Table 9B	–		
<i>Syringa spp.</i>	No	Low	17	Table 9B	–		
<i>Taxodium distichum</i>	Cut	Low	17	Table 9B	–		
<i>Taxus spp.</i>	Coarse	Low	17	Table 9B	–		
<i>Tectona grandis</i>	Cut	Low	17	Table 9B	–		
<i>Thinopyrum spp.</i>	No	High	1	0.2%	–		from Elytrigia spp.
<i>Thuja spp.</i>	No	Low	17	Table 9B	–		

<i>Tilia</i> spp. (TSW >200 g)	Coarse	Low	17	Table 9B	–
<i>Tilia</i> spp. (TSW ≤200 g)	No	Low	17	Table 9B	–
<i>Trifolium</i> spp.	No	High	1	0.2%	–
<i>Trisetum flavescens</i>	No	High	1	0.2%	–
<i>Triticum</i> spp.	Fine	High	2	0.2%	To 17 % moisture content or less
x <i>Triticosecale</i>	Fine	High	2	0.2%	To 17 % moisture content or less
<i>Tsuga</i> spp.	No	Low	17	Table 9B	–
<i>Ulmus</i> spp.	No	Low	17	Table 9B	–
<i>Urochloa</i> spp.	No	High	1	0.2%	–
<i>Vachellia</i> spp.	Coarse	Low	17	Table 9B	–
<i>Valerianella locusta</i>	No	High	1	0.2%	–
<i>Viburnum opulus</i>	Coarse	Low	17	Table 9B	–
<i>Vicia</i> spp.	Coarse	High	1	0.2%	To 17 % moisture content or less
<i>Vigna</i> spp.	Coarse	High	1	0.2%	To 17 % moisture content or less
<i>Zea mays</i>	Fine	High	4	0.2%	To 17 % moisture content or less; see also 9.2.5.6
<i>Zelkova serrata</i>	No	Low	17	Table 9B	–

from the genus *Acacia*

CURRENT VERSION	PROPOSED VERSION
<p><b>9.2.4.7 Cutting tool</b> Where cutting is required according to Table 9A <del>Parts 1 and 2</del>,...</p> <p><b>9.2.5.1 General directions and precautions</b> See Table 9A <del>Parts 1 and 2</del> for directions for individual species. ...</p> <p><b>9.2.5.2 Working sample</b> ... In the case of cutting or grinding, one working sample must be drawn for cutting or grinding and two replicates must be obtained from the cut/ground material. For large-seeded</p>	<p><b>9.2.4.7 Cutting tool</b> Where cutting is required according to Table 9A,...</p> <p><b>9.2.5.1 General directions and precautions</b> See Table 9A for directions for individual species. ...</p> <p><b>9.2.5.2 Working sample</b> ... In the case of cutting or grinding, one working sample must be drawn for cutting or grinding and two replicates must be obtained</p>

<p>tree and shrub seeds that have to be cut (see Table 9A, <a href="#">Part 2</a>), ...</p> <p><b>9.2.5.3 Weighing</b></p> <p>...</p> <p>Open containers and their lids are placed rapidly into an oven maintained at the required temperature for the species being tested (Table 9A <a href="#">Parts 1 and 2</a>). The ...</p> <p><b>9.2.5.4 Grinding</b></p> <p>...</p> <p>It is obligatory to grind seed of a particular species if this is indicated in Table 9A <a href="#">Part 1 or Part 2</a>.</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 <a href="#">Part 1</a>), at least 50 % of the ground material must pass through a wire sieve with 0.50 mm mesh, and not more than 10 % must remain on a wire sieve with 1.00 mm mesh. For those species requiring coarse grinding (Table 9A <a href="#">Parts 1 and 2</a>), ...</p> <p><b>9.2.5.5 Cutting</b></p> <p>Large tree seeds (thousand-seed weight &gt;200 g and if prescribed in Table 9A <a href="#">Part 2</a>) and ...</p> <p><b>9.2.5.6 Predrying</b></p> <p>If the species is one for which grinding is necessary and the moisture content is higher than indicated in Table 9A <a href="#">Part 1</a>, predrying is obligatory. Two subsamples, each weighing 25 ±1 g are placed in weighed containers. The two subsamples, in their containers, are then dried at 130 °C for 5 to 10 min, depending on the moisture content, to reduce the moisture content to below that required in Tables 9A <a href="#">Part 1</a>. ...</p> <p>Predrying is not obligatory for any seeds that are cut (Table 9A <a href="#">Part 2</a>).</p>	<p>from the cut/ground material. For large-seeded tree and shrub seeds that have to be cut (see Table 9A), ...</p> <p><b>9.2.5.3 Weighing</b></p> <p>...</p> <p>Open containers and their lids are placed rapidly into an oven maintained at the required temperature for the species being tested (Table 9A). The ...</p> <p><b>9.2.5.4 Grinding</b></p> <p>...</p> <p>It is obligatory to grind seed of a particular species if this is indicated in Table 9A.</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), at least 50 % of the ground material must pass through a wire sieve with 0.50 mm mesh, and not more than 10 % must remain on a wire sieve with 1.00 mm mesh. For those species requiring coarse grinding (Table 9A), ...</p> <p><b>9.2.5.5 Cutting</b></p> <p>Large tree seeds (thousand-seed weight &gt;200 g and if prescribed in Table 9A) and ...</p> <p><b>9.2.5.6 Predrying</b></p> <p>If the species is one for which grinding is necessary and the moisture content is higher than indicated in Table 9A, predrying is obligatory. Two subsamples, each weighing 25 ±1 g are placed in weighed containers. The two subsamples, in their containers, are then dried at 130 °C for 5 to 10 min, depending on the moisture content, to reduce the moisture content to below that required in Tables 9A. ...</p> <p>Predrying is not obligatory for any seeds that are cut (Table 9A).</p> <p><b>9.2.5.7 Prescribed methods</b></p>
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<p><b>9.2.5.7 Prescribed methods</b></p> <p>a. The working sample, drawn according to 9.2.5.2, must be evenly distributed over the surface of the container.</p> <p>b. Weigh the container and its cover before and after filling.</p> <p>c. Place the container rapidly, on top of its cover or next to its cover, in an oven.</p> <p>d. See Table 9A <del>Parts 1 and 2</del> for additional details regarding grinding, temperature and duration per species.</p> <p>e. Tolerances for the temperatures and durations are: 101–105 °C (low temperature): 17 ±1 h;</p> <p><b>9.2.6.2 Tolerances</b></p> <p>...</p> <p>For tree and shrub species (Table 9A <del>Part 2</del>) it</p> <p>...</p>	<p>a. The working sample, drawn according to 9.2.5.2, must be evenly distributed over the surface of the container.</p> <p>b. Weigh the container and its cover before and after filling.</p> <p>c. Place the container rapidly, on top of its cover or next to its cover, in an oven.</p> <p>d. See Table 9A for additional details regarding grinding, temperature and duration per species.</p> <p>e. Tolerances for the temperatures and durations are: 101–105 °C (low temperature): 17 ±1 h;</p> <p><b>9.2.6.2 Tolerances</b></p> <p>...</p> <p>For tree and shrub species (Table 9A) it ...</p>
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VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
<b>C.9.4</b>			

C.9.5 Clarifying the wording “initial moisture content”

The wording 'initial moisture content' is used in '9.2.6.2 Tolerances' in the sense of the moisture content measured by the lab, but it is not defined. To make it clear, the word 'initial' is deleted 2 times in the text and as well in the header of column 2-4 of table 9B.

This proposal originates from and is supported by the Moisture Committee.

CURRENT VERSION	PROPOSED VERSION
<p><b>9.2.6.2 Tolerances</b></p> <p>...</p> <p>These are related to seed size and <b>initial</b> moisture content (Table 9B).</p> <p>To use Table 9B, in column 1, select the relevant row depending on seed size. Then select the</p>	<p><b>9.2.6.2 Tolerances</b></p> <p>...</p> <p>These are related to seed size and moisture content (Table 9B).</p> <p>To use Table 9B, in column 1, select the relevant row depending on seed size. Then select the</p>

<p>correct column (2, 3 or 4) depending on the <del>initial</del> moisture content of the sample.</p> <p>...</p> <p><b>Table 9B.</b> Tolerance levels for differences between the two duplicate determinations of moisture content of tree and shrub seeds (significance level not defined)</p> <table border="1"> <thead> <tr> <th rowspan="2">Seed size</th> <th colspan="3">Average <del>initial</del> moisture content</th> </tr> <tr> <th>&lt;12 %</th> <th>12–25 %</th> <th>&gt;25 %</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>2</td> <td>3</td> <td>4</td> </tr> <tr> <td>Small: TSW &lt;200 g</td> <td>0.3 %</td> <td>0.5 %</td> <td>0.5 %</td> </tr> <tr> <td>Large: TSW ≥200 g</td> <td>0.4 %</td> <td>0.8 %</td> <td>2.5 %</td> </tr> </tbody> </table>	Seed size	Average <del>initial</del> moisture content			<12 %	12–25 %	>25 %	1	2	3	4	Small: TSW <200 g	0.3 %	0.5 %	0.5 %	Large: TSW ≥200 g	0.4 %	0.8 %	2.5 %	<p>correct column (2, 3 or 4) depending on the moisture content of the sample.</p> <p>...</p> <p><b>Table 9B.</b> Tolerance levels for differences between the two duplicate determinations of moisture content of tree and shrub seeds (significance level not defined)</p> <table border="1"> <thead> <tr> <th rowspan="2">Seed size</th> <th colspan="3">Average moisture content</th> </tr> <tr> <th>&lt;12 %</th> <th>12–25 %</th> <th>&gt;25 %</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>2</td> <td>3</td> <td>4</td> </tr> <tr> <td>Small: TSW &lt;200 g</td> <td>0.3 %</td> <td>0.5 %</td> <td>0.5 %</td> </tr> <tr> <td>Large: TSW ≥200 g</td> <td>0.4 %</td> <td>0.8 %</td> <td>2.5 %</td> </tr> </tbody> </table>	Seed size	Average moisture content			<12 %	12–25 %	>25 %	1	2	3	4	Small: TSW <200 g	0.3 %	0.5 %	0.5 %	Large: TSW ≥200 g	0.4 %	0.8 %	2.5 %
Seed size		Average <del>initial</del> moisture content																																					
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Large: TSW ≥200 g	0.4 %	0.8 %	2.5 %																																				

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
<b>C.9.5</b>			

## Chapter 11: Testing Coated Seeds

### C.11.1 Updates and clarifications

With more and more seed treatments available, Chapter 11 requires an update remain current with new advancements. The whole chapter was reviewed by the Purity Committee and updated to include recent changes to Chapter 10 TSW. There were also clarifications made on how to report and what to report.

The proposal was approved by a majority vote of the Purity Committee.

CURRENT VERSION	PROPOSED VERSION
<p><b>11.1.1 Definitions</b></p>	<p><b>11.1.1 Definitions</b></p> <p><b>11.1.1.1 Treated seed</b></p> <p>‘Seed treatment’ is a generic term which indicates that a seed lot has been subjected to:</p> <ol style="list-style-type: none"> <li>the application of a compound including film coatings, polymers, pesticides, fungicides, biologicals, identifying colourants, dyes and/or other additives;</li> <li>the application of a biological product including micro-organisms;</li> <li>a process including wetting and drying; or</li> </ol>

<p><b>Seed pellets</b> More or less spherical units developed for precision sowing, usually incorporating a single seed with the size and shape of the seed no longer readily evident. <del>The pellet, in addition to the pelleting material, may contain pesticides, dyes or other additives.</del></p> <p><b>Encrusted seed</b> Units more or less retaining the shape of the seed with the size and weight changed to a greater or lesser extent. <del>The encrusting material may contain pesticides, fungicides, dyes or other additives.</del></p> <p><b>Seed granules</b> Units more or less cylindrical, including types with more than one seed <del>joined together. The granule, in addition to the granulating material, may contain pesticides, dyes or other additives.</del></p> <p><b>Seed tapes</b> Narrow bands of material, such as paper or other degradable material, with seeds spaced randomly, in groups or in a single row.</p> <p><b>Seed mats</b> Broad sheets of material, such as paper or other degradable material, with seeds placed in rows, groups or at random throughout the sheets.</p> <p><b>Seed treatment</b> See 2.2.12. Seeds which have received seed treatment must still be tested according to the methods prescribed in other chapters.</p>	<p>d. an energy form including heat, radiation, electricity or magnetism; but does not specify the application method.</p> <p>Seed treatment does not significantly change the shape and the general size of the untreated seed with only a minimal weight gain. Treated seeds are usually tested without removing the treatment and according to the same rules as untreated seeds.</p> <p><b>11.1.1.2 Coated seeds</b></p> <p>Coated seeds are seeds covered with material in such a way that in most cases the seeds cannot be identified without removing the covering material. The material may contain pesticides, fungicides, biologicals, identifying colourants, dyes and/or other additives. The following types of coated seeds are defined:</p> <p><b>Seed pellets</b> More or less spherical units, usually incorporating a single seed with the size and shape of the seed no longer readily evident.</p> <p><b>Encrusted seed</b> Units more or less retaining the shape of the seed with the size and weight changed to a measurable extent.</p> <p><b>Seed granules</b> Units more or less cylindrical, including types with more than one seed <b>per granule</b>.</p> <p><b>Seed tapes</b> Narrow bands of material, such as paper or other degradable material, with seeds spaced randomly, in groups or in a single row.</p> <p><b>Seed mats</b> Broad sheets of material, such as paper or other degradable material, with seeds placed in rows, groups or at random throughout the sheets.</p> <p><b>Seed treatment</b> See 2.2.12. Seeds which have received seed treatment must still be tested according to the methods prescribed in other chapters.</p>
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<p><b>11.3.2.2 Unpelleted seed</b> Unpelleted seed must include:</p> <p>a Free seeds of any species,</p> <p>b Broken pellets containing a seed that is recognisably not of the species stated by the applicant,</p> <p>c Broken pellets containing seed recognisable as being of the species stated by the applicant but not included in the pure pellets fraction.</p> <p><b>11.3.7 Reporting results</b></p> <p>The result of a purity test on coated seeds must be reported as follows:</p> <ul style="list-style-type: none"> <li>Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered.</li> <li>The results must be reported to one decimal place, and the percentage of all components must total 100 %. Components amounting to less than 0.05 % must be reported as 'Trace' or 'TR' (for 'Trace').</li> <li>In the case of pelleted seeds only, the percentages of pure pelleted seeds, inert matter and unpelleted seeds must be reported in the spaces provided for 'Pure seeds', 'Inert matter', and 'Other seeds', respectively.</li> </ul> <p>The name and number of the seeds of each species found in the examination of the 100 seeds removed from the pellets or tapes must be reported under 'Other determinations'.</p>	<p><b>11.3.2.2 Unpelleted seed</b> Unpelleted seed must include:</p> <p>a. Free seeds of any species, <b>including species stated by the applicant,</b></p> <p>b. Broken pellets containing a seed that is recognisably not of the species stated by the applicant,</p> <p>c. Broken pellets containing seed recognisable as being of the species stated by the applicant but not included in the pure pellets fraction.</p> <p><b>11.3.7 Reporting results</b></p> <p>The result of a purity test on coated seeds must be reported as follows:</p> <ul style="list-style-type: none"> <li>Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered.</li> <li>The results must be reported to one decimal place, and the percentage of all components must total 100.0 %. Components amounting to less than 0.05 % must be reported as 'Trace' or 'TR' (for 'Trace').</li> <li>In the case of pelleted seeds only, the percentages of pure pelleted seeds, inert matter and unpelleted seeds must be reported in the spaces provided for 'Pure seeds', 'Inert matter', and 'Other seeds', respectively. <ul style="list-style-type: none"> <li><b>Kind of inert matter.</b></li> <li><b>The scientific name of every species of other seeds (unpelleted/pelleted) found, in accordance, where applicable, with the current ISTA List of Stabilised Plant Names (e.g. <i>Elymus repens</i>). If it is the species stated by the applicant, then report this name with 'not pelleted' or 'not encrusted'.</b></li> </ul> </li> <li>The name and number of the seeds of each species found in the examination of the 100 seeds removed from the pellets or tapes must be reported under 'Other determinations'.</li> </ul>

<p><b>11.10 Weight determination and size grading of pelleted seed</b>                  Because of the technical requirements of precision drilling, <b>weight</b> determination or size grading may be necessary.</p> <p><b>11.10.1 Object</b></p> <p>The object is to determine the weight per 1000 pellets and/ or size grading of the sample as submitted.</p> <p><b>11.10.2 Principles</b></p> <p>For a <b>weight</b> determination, the number of pellets in a weighed quantity of pure pellets is counted and the weight per 1000 calculated. For size determination, a sample of the size specified in Chapter 16 is screened as specified and the percentage of each screening fraction determined.</p> <p><b>11.10.2 Apparatus</b></p> <p>For <b>weight</b> determination, a suitable counting machine or counting equipment for germination tests may be used. For size determination, a suitable screening machine is used.</p> <p><b>11.10.4-6 Procedure</b></p> <p>For <b>weight</b> determination, follow the procedure prescribed in Chapter 10 sections 10.4 to 10.6. For size determination, follow the procedure prescribed in Chapter 16.</p>	<p><b>11.10 Thousand-seed weight (TSW) determination and size grading of pelleted seed</b>                  Because of the technical requirements of precision drilling, <b>TSW</b> determination or size grading may be necessary.</p> <p><b>11.10.1 Object</b></p> <p>The object is to determine the weight per 1000 pellets and/ or size grading of the sample as submitted.</p> <p><b>11.10.2 Principles</b></p> <p>For a <b>TSW</b> determination, the number of pellets in a weighed quantity of pure pellets is counted and the weight per 1000 calculated. For size determination, a sample of the size specified in Chapter 16 is screened as specified and the percentage of each screening fraction determined.</p> <p><b>11.10.3 Apparatus</b></p> <p>For <b>TSW</b> determination, a suitable counting machine or counting equipment for germination tests may be used. For size determination, a suitable screening machine is used.</p> <p><b>11.10.4-6 Procedure</b></p> <p>For <b>TSW</b> determination, follow the procedure prescribed in Chapter 10 sections 10.4 to 10.6. For size determination, follow the procedure prescribed in Chapter 16.</p>
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**Consequential changes in Chapter 1 resulting from changes in C.11.1**

CURRENT VERSION	PROPOSED VERSION
<p><b>1.5.2.3 Purity tests on coated seeds</b></p> <p>The result of a purity test on coated seeds must be reported as follows:</p> <ul style="list-style-type: none"> <li>Following the species name, the words ‘seed pellets’, ‘encrusted seeds’, ‘seed granules’, ‘seed tapes’ or ‘seed mats’, as applicable, must be clearly entered.</li> <li>The results must be reported to one decimal place, and the percentage of all components must total 100 %. Components amounting to less than 0.05 % must be reported as ‘Trace’ or ‘TR’ (for ‘Trace’). If no inert matter or other seeds are found, this must be reported as ‘0.0’.</li> <li>In the case of pelleted seeds only, the percentages of pure pelleted seeds, inert matter and unpelleted seeds must be reported in the spaces provided for ‘Pure seeds’, ‘Inert matter’, and ‘Other seeds’, respectively.</li> </ul> <ul style="list-style-type: none"> <li>The name and number of the seeds of each species found in the examination of the 100 seeds removed from the pellets or tapes must be reported under ‘Other determinations’.</li> </ul>	<p><b>1.5.2.3 Purity tests on coated seeds</b></p> <p>The result of a purity test on coated seeds must be reported as follows:</p> <ul style="list-style-type: none"> <li>Following the species name, the words ‘seed pellets’, ‘encrusted seeds’, ‘seed granules’, ‘seed tapes’ or ‘seed mats’, as applicable, must be clearly entered.</li> <li>The results must be reported to one decimal place, and the percentage of all components must total 100 %. Components amounting to less than 0.05 % must be reported as ‘Trace’ or ‘TR’ (for ‘Trace’). If no inert matter or other seeds are found, this must be reported as ‘0.0’.</li> <li>In the case of pelleted seeds only, the percentages of pure pelleted seeds, inert matter and unpelleted seeds must be reported in the spaces provided for ‘Pure seeds’, ‘Inert matter’, and ‘Other seeds’, respectively.                         <ul style="list-style-type: none"> <li>Kind of inert matter.</li> <li>The scientific name of every species of other seeds (unpelleted/pelleted) found, in accordance, where applicable, with the current ISTA list of Stabilised Plant Names (e.g. <i>Elymus repens</i>). If it is the species stated by the applicant, then report this name with ‘not pelleted’ or ‘not encrusted’.</li> </ul> </li> </ul> <ul style="list-style-type: none"> <li>The name and number of the seeds of each species found in the examination of the 100 seeds removed from the pellets or tapes must be reported under ‘Other determinations’.</li> </ul>

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.11.1			

**C.11.2 Revision of Chapter 11 germination related sections**

This proposal is to improve the germination area of Chapter 11: Testing coated seeds by rewording the general principles, evaluation and reporting results.

This proposal originates from and is supported by the Germination Committee.

CURRENT VERSION	PROPOSED VERSION
<p><b>11.5.3 General principles</b></p> <p><del>Germination tests on pelleted seeds must be made with pellets from the pure pellet fraction of a purity test.</del> The pellets must be placed on the substrate in the condition in which they are received (e.g. without rinsing or soaking). Germination tests on seed tapes are made on the tape without removing the seeds from the tape material or in any way pre-treating the tape.</p> <p><b>11.5.6.5 Evaluation</b></p> <p>...</p> <p>Pure pellets may not produce any seedlings at the end of a test period. These pellets 'without seedlings' <del>can</del> be evaluated as:</p> <p><b>Hard seeds:</b> when ungerminated pellets include hard seeds (see 5.2.10)</p> <p><b>Fresh seeds:</b> when ungerminated pellets include fresh seeds (see 5.2.10)</p> <p><b>Dead seeds:</b> when ungerminated pellets include inert matter, no seed or ungerminated other seeds, not detected as such prior the germination test. They can also include dead seeds for the species stated.</p> <p><b>11.5.8 Reporting results</b></p> <p>The result of a germination test on coated seeds must be reported as follows:</p> <ul style="list-style-type: none"> <li>• Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered in the space provided.</li> <li>• The percentage of pellets or seed in tapes with normal seedlings, with abnormal seedlings <del>and without seedlings.</del></li> <li>• The duration of the test.</li> </ul> <p>...</p> <p>Seedlings that are obviously not of the species stated by the applicant, even if otherwise</p>	<p><b>11.5.3 General principles</b></p> <p>Germination tests on pelleted seeds must be made with pellets from the pure pellet fraction of a purity test or pure pellets (see 11.3.2.1) from a representative fraction of the submitted sample. The pellets must be placed on the substrate in the condition in which they are received (e.g. without rinsing or soaking). Germination tests on seed tapes are made on the tape without removing the seeds from the tape material or in any way pre-treating the tape.</p> <p><b>11.5.6.5 Evaluation</b></p> <p>...</p> <p>Pure pellets may not produce any seedlings at the end of a test period. These pellets 'without seedlings' <b>must</b> be evaluated as:</p> <p><b>Hard seeds:</b> when ungerminated pellets include hard seeds (see 5.2.10)</p> <p><b>Fresh seeds:</b> when ungerminated pellets include fresh seeds (see 5.2.10)</p> <p><b>Dead seeds:</b> when ungerminated pellets include inert matter, no seed or ungerminated other seeds, not detected as such prior the germination test. They can also include dead seeds for the species stated.</p> <p><b>11.5.8 Reporting results</b></p> <p>The reporting of germination test results for coated seeds must be in accordance with Chapter 5 (see 5.9). Additionally, the result of a germination test on coated seeds must be reported as follows:</p> <ul style="list-style-type: none"> <li>• Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered in the space provided.</li> <li>• The percentage of pellets or seed in tapes with normal seedlings, with abnormal seedlings, <b>with hard seeds, fresh seeds and dead seeds.</b></li> <li>• The duration of the test.</li> </ul> <p>...</p>



<p>normal, must not be included in the germination result, but their number must be reported separately under 'Other determinations'.</p>	<p>Seedlings that are obviously not of the species stated by the applicant, even if otherwise normal, must not be included in the germination results, but their number must be reported separately under 'Other determinations'.</p> <p>If requested or at the discretion of the laboratory, the percentage of coated seeds that did not contain a seed must be reported under 'Other determinations'.</p>
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**Consequential changes in Chapter 1 resulting from changes in C.11.2**

CURRENT VERSION	PROPOSED VERSION
<p><b>1.5.2.7 Germination of coated seeds</b>                      The result of a germination test on coated seeds must be reported as follows:</p> <ul style="list-style-type: none"> <li>• Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered in the space provided.</li> <li>• The percentage of pellets or seed in tapes with normal seedlings, with abnormal seedlings <b>and without seedlings.</b></li> <li>• The duration of the test.</li> </ul> <p>...</p> <p>Seedlings that are obviously not of the species stated by the applicant, even if otherwise normal, must not be included in the germination result, but their number must be reported separately.</p>	<p><b>1.5.2.7 Germination of coated seeds</b>                      The reporting of germination test results for coated seeds must be in accordance with Chapter 5 (see 5.9). Additionally, the result of a germination test on coated seeds must be reported as follows:</p> <ul style="list-style-type: none"> <li>• Following the species name, the words 'seed pellets', 'encrusted seeds', 'seed granules', 'seed tapes' or 'seed mats', as applicable, must be clearly entered in the space provided.</li> <li>• The percentage of pellets or seed in tapes with normal seedlings, with abnormal seedlings, <b>with hard seeds, fresh seeds and dead seeds.</b></li> <li>• The duration of the test.</li> </ul> <p>...</p> <p>Seedlings that are obviously not of the species stated by the applicant, even if otherwise normal, must not be included in the germination results, but their number must be reported separately under 'Other determinations'.</p> <p>If requested or at the discretion of the laboratory, the percentage of coated seeds that did not contain a seed must be reported under 'Other determinations'.</p>

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.11.2			



## Chapter 13: Testing seeds by weighed replicates

### C.13.1 Revision of Chapter 13 germination related sections.

This proposal is to improve the germination area of Chapter 13: Testing seeds by weighed replicates rewording the calculation and expression of results and reporting results.

This proposal originates from and is supported by the Germination Committee with consultation with the Forest Tree and Shrub Committee.

CURRENT VERSION	PROPOSED VERSION
<p><b>13.6 Calculation and expression of results</b></p> <p>The result for the no prechill test is obtained by adding together the four individual replicate no prechill results. It is expressed as the number of normal seedlings in the total weight of seed tested.</p> <p>The prechill test results are calculated and expressed similarly.</p> <p>To check the reliability of a test result, the sum of the numbers of seeds germinated in the four replicates is calculated and compared with Table 13C.</p> <p><b>13.7 Reporting results</b></p> <p>The result of a weighed replicates test must be reported in the space provided as follows:</p> <ul style="list-style-type: none"> <li>• The result of the purity test (if requested), in the spaces provided for purity tests.</li> <li>• 'N' must be entered in all the spaces provided for reporting the percentages of the components of the germination tests.</li> </ul> <p>The following additional information must also be reported under 'Other determinations':</p> <ul style="list-style-type: none"> <li>• average weight of four replicates;</li> <li>• average number of normal seedlings in four replicates;</li> <li>• number of normal seedlings per kilogram;</li> <li>• other information as specified in 1.5.2.6 and 5.9.</li> </ul> <p>Upon request, other seeds found to be present in the weighed replicates may be reported, giving the scientific name(s) and number(s) of seeds found.</p>	<p><b>13.6 Calculation and expression of results</b></p> <p>The result for the no prechill test is obtained by adding together the four individual replicate no prechill results. It is expressed as the number of normal seedlings in the total weight of seed tested.</p> <p>The prechill test results are calculated and expressed similarly.</p> <p>To check the reliability of a test result, the sum of the numbers of seeds germinated in the four replicates is calculated and compared with Table 13C.</p> <p><b>13.7 Reporting results</b></p> <p>The result of a weighed replicates test must be reported in the space provided as follows:</p> <ul style="list-style-type: none"> <li>• The result of the purity test (if requested), in the spaces provided for purity tests.</li> <li>• 'N' must be entered in all the spaces provided for reporting the percentages of the components of the germination tests.</li> </ul> <p>The following additional information must also be reported under 'Other determinations':</p> <ul style="list-style-type: none"> <li>• <b>the number of normal seedlings in the total weight of seed tested.</b></li> <li>• average weight of four replicates;</li> <li>• average number of normal seedlings in four replicates;</li> <li>• number of normal seedlings per kilogram;</li> <li>• other information as specified in 1.5.2.6 and 5.9.</li> </ul> <p>Upon request, other seeds found to be present in the weighed replicates may be reported, giving</p>

	the scientific name(s) and number(s) of seeds found.
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**Consequential changes in Chapter 1 resulting from changes in C.13.1**

CURRENT VERSION	PROPOSED VERSION
<p><b>1.5.2.15 Weighed replicates</b></p> <p>The result of a weighed replicates test must be reported in the space provided as follows:</p> <ul style="list-style-type: none"> <li>• The result of the purity test (if requested), in the spaces provided for purity tests.</li> <li>• ‘N’ must be entered in all the spaces provided for reporting the percentages of the components of the germination tests.</li> </ul> <p>The following additional information must also be reported under ‘Other determinations’:</p> <ul style="list-style-type: none"> <li>• average weight of four replicates;</li> <li>• average number of normal seedlings in four replicates;</li> <li>• number of normal seedlings per kilogram;</li> <li>• other information as specified in 1.5.2.6 and 5.9.</li> </ul> <p>Upon request, other seeds found to be present in the weighed replicates may be reported, giving the scientific name(s) and number(s) of seeds found.</p>	<p><b>1.5.2.15 Weighed replicates</b></p> <p>The result of a weighed replicates test must be reported in the space provided as follows:</p> <ul style="list-style-type: none"> <li>• The result of the purity test (if requested), in the spaces provided for purity tests.</li> <li>• ‘N’ must be entered in all the spaces provided for reporting the percentages of the components of the germination tests.</li> </ul> <p>The following additional information must also be reported under ‘Other determinations’:</p> <ul style="list-style-type: none"> <li>• <b>the number of normal seedlings in the total weight of seed tested.</b></li> <li>• average weight of four replicates;</li> <li>• average number of normal seedlings in four replicates;</li> <li>• number of normal seedlings per kilogram;</li> <li>• other information as specified in 1.5.2.6 and 5.9.</li> </ul> <p>Upon request, other seeds found to be present in the weighed replicates may be reported, giving the scientific name(s) and number(s) of seeds found.</p>

Vote to accept item	Yes votes	No votes	Result
C.13.1			

**Chapter 14: X-ray test**

C.14.1 Revise and update the chapter to improve clarity

Background: Chapter 14 is obsolete due to new generations of x-ray machines and applications of digital technology. Technical committees of ATC, FTS, and Purity Committee organised the revision and developed the proposal. Significant revisions and updates are focused on equipment and its operations, as well as clarification on reporting.

The following proposal was developed and approved by the ISTA ATC, FTS, and Purity Committees.

CURRENT VERSION	PROPOSED VERSION
<p><b>14.1. Object</b></p> <p>The objects of X-radiography are:</p> <ul style="list-style-type: none"> <li>- to provide a quick, non-destructive method of differentiating between filled, empty, insect-damaged and physically damaged seed from the morphological characteristics <del>evident on an X-radiograph;</del></li> <li>- to <del>create a permanent photographic record of</del> the proportions of filled, empty, insect-damaged and physically damaged seeds in a sample.</li> </ul> <p>Further information on the X-ray test may be found in the <i>ISTA Tree and Shrub Seed Handbook</i>.</p> <p><b>14.1. Definitions</b></p> <p><b>14.1.1. Radiograph</b></p> <p><del>A radiograph is an image on photosensitive film or paper that is formed when an object is placed between the film or paper and an X-ray source. Photographic processing converts a latent image to one that is visible.</del></p> <p><del>A radiograph is also a digital image that is formed when an object is placed between an imaging device and an X-ray source. An imaging device can be an image intensifier (scintillator + photo multiplier) in combination with a digital camera, or a scintillator + photodiodes (flat panel detector, line scan camera, charge coupled device based receptor).</del></p> <p><b>14.1.2. X-rays</b></p> <p>X-rays are electromagnetic waves in the electromagnetic spectrum travelling at the speed of light, but with variable wavelengths (1/10 000 to 1/100 000 of that of light). High-energy (shorter wavelength) X-rays are more suitable for <del>large and/or</del> dense objects. Low-energy (longer wavelength) X-rays are suitable for</p>	<p><b>14.1. Object</b></p> <p>The objects of using an X-ray machine are:</p> <ul style="list-style-type: none"> <li>- to provide a quick, non-destructive method of differentiating between filled, empty, insect-damaged, and physically damaged seeds from the morphological characteristics;</li> <li>- to <b>obtain and report</b> the proportions of filled, empty, insect-damaged, and physically damaged seeds in a sample.</li> </ul> <p>Further information on the X-ray test may be found in the <i>ISTA Tree and Shrub Seed Handbook</i>.</p> <p><b>14.1. Definitions</b></p> <p><b>14.2.1. X rays</b></p> <p>X-rays are electromagnetic waves in the electromagnetic spectrum travelling at the speed of light, but with variable wavelengths (1/10 000 to 1/100 000 of that of light). High-energy (shorter wavelength) X-rays (<b>hard X-rays</b>) are more suitable for dense objects. Low-energy (longer wavelength) X-rays (<b>soft X-</b></p>

<p><del>small</del> objects such as seeds.</p> <p><b>14.1. General principles</b></p> <p><del>Seeds are placed between a low energy X-ray source and photosensitive film or paper. The various types of seed tissue absorb the X-rays to varying extents, depending on their thickness and/or density. The sensitive photographic emulsion is excited to varying degrees, depending on the amount of radiation it receives, thus creating a latent image. When the film or paper is processed, a visible image of varying shades of light and dark is formed.</del></p> <p><del>In a digital image the same principle applies, but in the reverse way. The radiation that is absorbed by the seed does not reach the detector, meaning that the more dense the material is, the less intensely the detector is excited, and thus the darker the image is. The result is the same, a visible image of varying shades of light and dark is formed.</del></p> <p>Several factors can affect the quality of the X-ray image:</p> <p>The voltage, measured in kilovolts (kV), is the measure of potential between the electrodes within the X-ray tube. An increase in voltage will</p>	<p>rays) are suitable for light or thin objects such as seeds. X-rays pass through the matter and are partially absorbed depending on their density and the energy of the radiation. This makes it possible to obtain information on the interior structures.</p> <p>14.2.2 X-ray image</p> <p>An X-ray image is obtained when an object is placed between a detector and an X-ray source. X-ray imaging provides contrast related to the variation of attenuation of X-ray photons. The image is a projection of all densities between the source and the detector for 2-D X-ray images. This could be used for profiles such as different shapes and internal structures of seeds. An X-ray image shows the different constituents according to the quantity of X-ray absorbed.</p> <p><b>14.3. General principles</b></p> <p>X-ray imaging of seeds follows a multi-step workflow (sample preparation, sample image acquisition, image collection and data interpretation).</p> <p><b>14.3.1. Sample preparation</b></p> <p>Seeds should not move during image acquisition. It is, therefore, necessary to pay special attention to how to hold the sample stable.</p> <p>The choice of material for the sample holder is crucial. For example, a sample tray can be used with a sticky side of tape to fix or stable the position of seeds. A large range of materials can be used as sample holders (floral foam, PMMA cylinders, polystyrene, etc.). Preferably, the seeds should not touch each other for a clear interpretation of the results. However, if this is not possible, this issue can sometimes be solved digitally during image processing.</p>
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~~produce shorter wave-length X-rays. The voltage affects the contrast of the image; a lower voltage improves the resolution, while a higher voltage reduces the density difference.~~

~~The electric current applied to the tube is measured in milliamperes (mA). Increasing the current increases the number of X-rays produced in a given time. The current influences the density, but not the contrast of the image. A high current will overexpose the image. A radiograph on photographic paper will be darkened and a digital radiograph will be lighter.~~

~~The exposure time is the time during which the specimen is exposed to X-rays for making the radiograph. There is an interaction between exposure time and current, so exposures should be expressed in milliamperere seconds (mAs) or milliamperere minutes (mA-min). Changing the exposure time alters the density of the image. To retain the same image quality, any increase in exposure time requires a proportional decrease in current. For example, an exposure of 100 mAs obtained with a tube current of 5 mA and an exposure time of 20 s produces the same image density as an exposure made at 10 mA for 10 s.~~

~~The distance between the focal spot (or target) and the film surface is the focus-film distance (FFD). An increase in the FFD decreases the intensity of the radiation according to the inverse square law. Thus, doubling the FFD requires four times the exposure to achieve the same degree of image density on the film or paper.~~

~~The distance between the object and the film surface (OFD) affects the image quality. The greater the distance, the poorer the image, as details will be less distinct. If fine detail is necessary, the seeds may be placed directly on the film surface, although in routine work the film is usually kept in a carrier or envelope to make handling easier.~~

~~In digital imaging, it is possible to have more distance between the object and the detector. When a microfocus tube is used, the distance can be~~

Sample preparation can also be facilitated, e.g., via a conveyor belt or a vacuum seed planting head.

It is also possible to use contrast agents that differentially permeate the subject, making some parts more radiographically dense than others in order to enhance certain characteristics of the image. In this case, an additional sample preparation step may be necessary.

Special attention should also be paid to the spatial positioning of the seeds, which can also influence the quality of the result.

#### 14.3.1. Image acquisition

Seeds are placed between a low-energy X-ray source and a detector. When this step is completed, the sample image is ready to be acquired. Currently, there are two main image acquisition configurations (2D radiography and 3D tomography) used. In 2D radiography, the image is obtained from a single projection of the X-ray through the sample. In 3D tomography, the sample is rotated along an axis and a projection is acquired for each angle.

Several parameters can be adjusted during the acquisition phase:

- The voltage, measured in kilovolts (kV), is the measure of potential between the electrodes within the X-ray tube. An increase in voltage will produce shorter wavelength X-rays. The voltage affects the contrast of the image; a lower voltage improves the contrast, while a higher voltage reduces the density difference.
- The electric current applied to the tube is measured in milliamperes (mA). Increasing the current raises the number of X-rays produced in a given time. The current influences the density, but not the contrast of the image. A high current will overexpose the image.

~~enlarged, resulting in a magnification on the object.~~

~~It is possible to use contrast agents that differentially permeate the subject, making some parts more radio-graphically dense than others in order to enhance certain characteristics of the image.~~

**Apparatus**

~~The following apparatus is necessary to make a radiographic film:~~

- ~~— X-ray machine;~~
- ~~— X-ray film or paper;~~
- ~~— developer for film or paper;~~
- ~~— holder for film;~~
- ~~— holder for seeds.~~

~~—The following apparatus is necessary to make a digital image.~~

- ~~- X-ray machine;~~
- ~~X-ray camera~~
- ~~- Holder for seeds~~

- The exposure time is the time during which the sample is exposed to X-rays. There is an interaction between exposure time and current, so exposures should be expressed in milliamperere-seconds (mAs) or milliamperere-minutes (mA · min). Changing the exposure time alters the density of the image. To retain the same image quality, any increase in exposure time requires a proportional decrease in current.
- The distance between the sample and the detector is very important to define a sufficient magnification or resolution to observe the morphological characteristics.

Incorrect settings can lead to “noisy” poor quality images, loss of detail, overexposure, and saturation. The choice of parameters could be automatically set or adapted by the analysts depending on the seed species and the purpose of the analysis.

**14.3.1. X-ray image collection and interpretation**

The X-ray images can be in film or digital format. Digital images are more common now with the newer generations of X-ray equipment. The X-ray images must be labeled and stored for traceability.

- In 2D radiography, the X-ray images can be directly viewed and analysed.
- In 3D radiography, different projections are used to reconstruct a 3D digital image of the sample. For this purpose, there are different methods (analytical methods or algebraic methods).

Once the X-ray images have been acquired, the interpretation of the images can be completed visually by an analyst or automatically by image analysis using verified

<p><b>14.2. Procedures</b></p> <p><b>14.1.1. Loading the film, preparing the seed and developing the image</b></p> <ol style="list-style-type: none"> <li><del>1. Load film/paper in cassettes or holder, or use prepack aged film/paper.</del></li> <li>2. The test is performed on four replicates of 100 seeds, each drawn at random from the pure seed fraction <del>(they may be the same seeds as those used for the germination test).</del></li> <li><del>3. Spread the seeds (with or without a holder) evenly on top of the film or paper.</del></li> <li><del>4. Place lead letters or other X-ray opaque marking devices on the film or paper to identify the sample.</del></li> <li><del>5. Make the exposure. Individual X-ray machines will require different exposure time and voltage settings to produce the best image. Settings will also vary for different species. For the best results, a time voltage exposure series should be made whenever new material or a different machine is used.</del></li> <li><del>6. Develop the film or paper. Paper is usually developed in instant processors, which produce a print within a few seconds. Film must be developed in a darkroom.</del></li> </ol> <p><b>14.1.1. Evaluating the image</b></p> <p>Seeds are classified according to the internal anatomy revealed by the <b>radiograph</b> as:</p>	<p>computing algorithms.</p> <p><b>14.4. Apparatus</b></p> <p>The following apparatus is necessary:</p> <ul style="list-style-type: none"> <li>- 2D or 3D X-ray machine</li> <li>- Seeds holder</li> <li>- Computer for image processing or data storage</li> </ul> <p><b>14.4. Procedures</b></p> <p><b>14.5.1 Equipment use</b></p> <ol style="list-style-type: none"> <li>a. Set up an X-ray machine following the manufacturer-provided operation procedures.</li> <li>b. Select or determine the best parameters for testing needs because each X-ray machine requires different exposure times and voltage settings to produce the best image.</li> <li>c. It is recommended to pre-determine the best parameters for a test of each species with the X-ray machine used in a laboratory.</li> </ol> <p><b>14.5.2 Digital image acquisition</b></p> <ol style="list-style-type: none"> <li>a. The test is performed on four replicates of 100 seeds, each drawn at random from the pure seed fraction.</li> <li>b. Distribute the seeds on a suitable seed holder, ensuring seeds are spaced out and are not touching each other, and cannot move during the imaging process.</li> <li>c. Perform the image acquisition step with the best parameters selected for each test and species. Note: the settings could vary with different species. For best results, a series of time/voltage exposures should be made each time a new or different machine is used.</li> </ol> <p><b>14.5.3 Evaluating the image</b></p>
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<p>filled: fruit or seed containing all <del>tissues essential</del> for germination;</p> <p>empty: fruit or seed containing less than 50 % of seed tissue;</p> <p>insect-damaged: fruit or seed containing insects, insect larvae or frass, or showing <del>other evidence of insect damage affecting the ability of the seed to germinate</del>;</p> <p>physically damaged: filled fruit or seed <del>with the coat outline cracked or broken</del>.</p> <p><b>14.6. Calculations and expression of results</b></p> <p>Results are expressed as percentages of filled, empty, insect-damaged, or physically damaged seeds.</p> <p><b>14.7. Reporting results</b></p> <p>The results of an X-ray test must be reported under 'Other determinations' as percentages of filled, empty, insect- damaged or physically damaged seeds, as follows:</p> <p>'X-ray test results:</p> <p>.. % filled;</p> <p>.. % empty;</p> <p>.. % insect-damaged;</p>	<p>The evaluation of the images can be completed either visually by trained analysts or automatically by image analysis using verified computing algorithms.</p> <p>Seeds are classified according to the internal anatomy revealed by the X-ray image as:</p> <ul style="list-style-type: none"> <li>filled: fruit or seed containing 50% or more of all essential tissues for germination;</li> <li>empty: fruit or seed containing less than 50% of seed tissue;</li> <li>insect-damaged: fruit or seed containing insects, insect larvae or frass, or showing evidence of insect damage.</li> <li>physically damaged: filled fruit or seed is mechanically damaged internally or externally, e.g. the seed coat outline is cracked or broken.</li> </ul> <p><b>14.6. Calculations and expression of results</b></p> <p>Results are expressed as percentages of filled, empty, insect-damaged, or physically damaged seeds.</p> <p><b>14.7. Reporting results</b></p> <p>The results of an X-ray test must be reported under 'Other determinations' as percentages of filled, empty, insect-damaged or physically damaged seeds in the pure seeds fraction, as follows:</p> <p>"X-ray test results:</p> <p>. % filled</p> <p>. % empty</p> <p>. % insect-damaged</p> <p>. % physically damaged</p>
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<p>.. % physically damaged'.</p>	<p>On request, testing results can be reported under 'Other determinations'. For example, % insect-damaged seeds for 100 seeds tested.</p>
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**Consequential changes in Chapter 1 resulting from changes in C.14.1**

CURRENT VERSION	PROPOSED VERSION
<p><b>1.5.2.16 X-ray test</b></p> <p>The results of an X-ray test must be reported under 'Other determinations' as percentages of filled, empty, insect-damaged or physically damaged seeds, as follows:</p> <p><b>X-ray test results</b></p> <p>.....% filled                  .....% empty                  .....% insect-damaged                  .....% physically damaged</p>	<p><b>1.5.2.16 X-ray test</b></p> <p>The results of an X-ray test must be reported under 'Other determinations' as percentages of filled, empty, insect-damaged or physically damaged seeds <b>in the pure seeds fraction</b>, as follows:</p> <p><b>X-ray test results</b></p> <p>.....% filled                  .....% empty                  .....% insect-damaged                  .....% physically damaged</p> <p>On request, testing results can be reported under 'Other determinations'. For example, % insect-damaged seeds on 100 seeds tested.</p>

VOTE TO ACCEPT ITEM	YES VOTES	NO VOTES	RESULT
C.14.1			