PROPOSED RULES CHANGES 2004

For Consideration and Decision at the Ordinary Meeting 2004

[Logos: International Seed Testing Association and ISTA 2004]
Introduction by the Chairman of the ISTA Rules Committee

The 2003 loose-leaf edition of the rules has had its first set of amendments issued. For ease of tracing updates the amended version of the 2003 rules now becomes the 2004 edition.

The following proposals will be discussed at the voting part of the ISTA Congress in Budapest, Hungary. If the proposals are accepted by the membership amendments will be issued and they will become the 2005 edition of the Rules. Any subsequent editorial changes remain the responsibility of the Chair of the Rules Committee.

Please let me know of any problems with these proposals.

Many thanks.

Steve

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<thead>
<tr>
<th>Item</th>
<th>Title</th>
<th>Rules Chapter</th>
<th>Pages</th>
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</thead>
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<td>General editing issues</td>
<td>Chapter 5, 7, 8 &amp; 13</td>
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<td>Chapter 17</td>
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</table>
ITEM 1  GENERAL EDITING ISSUES

Item 1  Corrections

The following are editorial changes/errors that have been noticed in the 2004 edition of the Rules.

Item 1a List of edits

Table 5A Part 2
Pinus densiflora – there is a missing temperature for prechilling:
Add ‘at 3-5 °C’; so it will read ‘Prechill 14 days at 3-5°C’

Pinus strobus: missing words ‘Double test’ as both no prechill and prechill are recommended.
Add ‘Double test’; so it will read “1. No prechill and prechill 28 days at 3-5°C. Double test.”

In Table 5A Part 2 make sure that the singular term ‘double test’ is used throughout and not
the plural term ‘double tests’ for the following species: Nothofagus oblique, Picea glauca, P.
glehnii; P. jezoensis, P. sitchensis, Pinus contorta, P. elliottii, P. palustris, P. ponderosa, P.
taeda, Pseudotsuga menziesii, Syringa reflexa, and Zelkova serrata.

Page 13A-1 & 2
The change to ‘double test’ also applies to the header text for Table 13A and entries in Table
13A.
For header text for Table 13A delete the phrase ‘The less desirable methods are placed in
brackets in the Table.’ As there are no methods in brackets.

Page 7-4
7.4.3 Specific directions
Additions and/or deletions to these lists can be found in the Seed Testing International ISTA
News Bulletin or on… (Note: alter any other similar instances)

Page 8A-14
8.6.A.5 Figures 1-4
Fig 2: …; other patterns arise from self-pollination (same patterna patterns as female) or …

Pages 7-017-5 and 7-018-6 in Annexe to Chapter 7: Seed Health Methods
Should read ‘Bacto Malt Agar (Cat No 0024-01-1 Difco USA) 30 g  15 g’ (not 45 g  15 g)
**ITEM 2 PROPOSALS FOR CHAPTER 2 AND ANNEXE, SAMPLING**

**Item 2a New definition for primary sample**

Since there are sampling methods that take samples from non-defined positions in a seed lot (i.e. automatic seed samplers) or triers with several chambers and openings at different positions, the BSC is proposing a new definition of primary sample.

**Current:**

2.2.2 Primary sample

A *primary sample* is a small portion taken from one point in the lot.

**Proposed:**

2.2.2 Primary sample

A *primary sample* is a small portion taken from the seed lot during one sampling action.

**Item 2b Changes in submitted and working sample weights for *Oryza sativa***

The following Rules change proposal was submitted to the BSC Chair by K. Kant and K. Janaiah Division of Seed Science & Technology, Indian Agricultural Research Institute, New Delhi, India. Supporting data/evidence is presented in the document *Supporting Data and Evidence for the Proposed Rules Changes 2004– ITEM 2b* which is available on the ISTA website [www.seedtest.org](http://www.seedtest.org) or on request from the ISTA Secretariat.

<table>
<thead>
<tr>
<th>Species</th>
<th>Minimum sample weights</th>
<th>Species</th>
<th>Minimum sample weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum weight of lot</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Submitted sample</td>
<td>Working sample for purity analysis</td>
<td>Working sample for count of other species</td>
</tr>
<tr>
<td></td>
<td>Chapter 2</td>
<td>Chapter 2</td>
<td>Chapter 3</td>
</tr>
<tr>
<td>1 kg</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

**Current:**

*Oryza sativa L.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Minimum sample weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 000 400 40 400</td>
</tr>
</tbody>
</table>

**Proposed:**

*Oryza sativa L.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Minimum sample weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 000 700 70 700</td>
</tr>
</tbody>
</table>
ITEM 3 PROPOSALS FOR CHAPTER 3, PURITY

Item 3a Change to the uniform blowing method for *Poa pratensis* varieties

Changes to the uniform blowing method for small seeded varieties of *Poa pratensis*. Detailed supporting evidence is provided in the *Supporting Data and Evidence for the Proposed Rules Changes 2004 – ITEM 3a* which is available on the ISTA website [www.seedtest.org](http://www.seedtest.org) or on request from the ISTA Secretariat. The proposal includes a list of varieties classified as small seeded.

3.5.2.A.5. Uniform blowing method
This method is obligatory for *Poa pratensis*, *Poa trivialis* and *Dactylis glomerata*.

The working sample size is 1g for *Poa pratensis* and *Poa trivialis* and 3g for *Dactylis glomerata*. The blowing pressure is determined for *Poa pratensis* and *Dactylis glomerata* by means of a calibration samples issued under the authority of the International Seed Testing Association. The blowing pressure for the varieties of *Poa pratensis* listed below with an average weight of 1000 seeds <0.35g is obtained by multiplying the blower setting for *Poa pratensis* by 0.82 (applies only for General Seed Blowers). Seed identified as ‘variety not stated’ is excluded from the list.

The blowing pressure for *Poa trivialis* is obtained by multiplying the blower setting for *Poa pratensis* by 0.82 (applies only for General Seed Blowers).

Prior to calibration both the calibration and working samples must be exposed to room conditions. For those not having a General Seed Blower, please contact the International Seed Testing Association Secretariat.

**Table 3.5.2.A.5**
List of varieties of *Poa pratensis* with an average weight of 1000 seeds <0.35g.

<table>
<thead>
<tr>
<th>Variety</th>
<th>1000 seed weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balin</td>
<td>0.34g</td>
</tr>
<tr>
<td>Compact</td>
<td>0.34g</td>
</tr>
<tr>
<td>Julia</td>
<td>0.33g</td>
</tr>
<tr>
<td>Limousine</td>
<td>0.33g</td>
</tr>
<tr>
<td>Enprima</td>
<td>0.32g</td>
</tr>
<tr>
<td>Oxford</td>
<td>0.32g</td>
</tr>
<tr>
<td>Ikone</td>
<td>0.31g</td>
</tr>
<tr>
<td>Sobra</td>
<td>0.31g</td>
</tr>
<tr>
<td>Pegasus</td>
<td>0.29g</td>
</tr>
<tr>
<td>Platini</td>
<td>0.29g</td>
</tr>
<tr>
<td>Slezanka</td>
<td>0.28g</td>
</tr>
<tr>
<td>Mardona</td>
<td>0.27g</td>
</tr>
<tr>
<td>Tommy</td>
<td>0.26g</td>
</tr>
<tr>
<td>Lato</td>
<td>0.24g</td>
</tr>
<tr>
<td>Harmony</td>
<td>0.23g</td>
</tr>
</tbody>
</table>
Item 3b Amendment to Chapter 1 section 1.2

If the proposal on small seeded varieties of *Poa pratensis* is accepted section 1.2 of Chapter 1 will need amending so new varieties can be added to the list in Table 3.5.2.A.5.

1.2 Guidelines for the introduction of new species and methods into the Rules

*Small seeded varieties of Poa pratensis:*

Before a small seeded variety is included in 3.5.2.A.5, a determination of the 1000 seed weight must be performed on at least 20 samples from different seed lots, representing seeds grown either in two different harvest years or in two different countries.

The determination of the 1000 seed weight shall be carried out on pure seeds, obtained by blowing a 1g sample of *Poa pratensis* using the standard blower setting (factor 1.00). Only seed remaining in the heavy fraction shall be used for the 1000 seed weight. See Chapter 10 for the weight determination procedure.

Seed identified as ‘variety not stated’ is excluded from the list.

Results shall be submitted to the ISTA Purity Committee with a request to change the ISTA Rules.
**Item 4 Proposals for Chapter 5, Germination, Annex**

**Item 4a Amendment to entry for Chloris gayana**

The weighed replicate method was approved for *Chloris gayana* in 2002 and detailed in Table 13B but Table 5 was not amended to reflect this and make the weighed replicate test the mandatory germination test method. If laboratories want to be able to use either the TP or weighed replicate method then this needs to be made clear in the Rules and this proposal needs to be voted against.

<table>
<thead>
<tr>
<th>Species</th>
<th>Prescriptions for:</th>
<th>Additional directions including</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Substrate °C</td>
<td>Temperature</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<table>
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<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chloris gayana</em></td>
<td>TP 20-35; 20-30</td>
<td>7</td>
<td>14</td>
<td>KNO₃; Light; Prechill</td>
<td></td>
</tr>
</tbody>
</table>

| **Proposed:** | | | | |
| *Chloris gayana* | - | - | - | Test by the weighed replicates method (see Chapter 13, Table 13B) |

**Item 4b Amendment to entry for Eucalyptus spp. in Table 5**

Remove the word should for Eucalyptus spp. to be consistent with the entry for *Betula* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Prescriptions for:</th>
<th>Additional directions including</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Substrate °C</td>
<td>Temperature</td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eucalyptus</em> spp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>All <em>Eucalyptus</em> spp. should be tested by weighed replicates method (see Chapter 13, Table 13A)</td>
</tr>
</tbody>
</table>

| **Proposed:** | | | | |
| *Eucalyptus* spp. | - | - | - | - | All *Eucalyptus* spp. test by weighed replicates method (see Chapter 13, Table 13A) |
**Item 4c Addition of ‘Pinus brutia (Ten.)’ to ISTA Rules**

At the recent ISTA Forest Tree and Shrub Seed Workshop in the Czech Republic it was noted that the species list in ‘EU Directive 1999/105/EC on marketing forest reproductive material’ includes ‘Pinus brutia (Ten.)’ - hence the seeds must be tested according to ‘internationally accepted techniques’. Unfortunately the latest ISTA Rules (2003) do not contain any prescriptions for this ‘species’.

*Pinus brutia* (Ten.) is already included in the list of ISTA Stabilised Names so it would seem reasonable to add the species to the ISTA Rules. *Pinus brutia* is often thought of as a variety of *Pinus halepensis*. It is the opinion of the Forest Tree Seed Committee that the seeds of each are likely to be extremely similar, so existing methods for *Pinus halepensis* would be suitable. In our opinion, that it is very important to add this species at the next congress, and therefore provide the EU with a suitable testing method.

1. Add ‘*Pinus brutia* (Ten.)’ to Table 2A Part 2 - Tree and shrub seeds, and repeat the current figures for ‘*Pinus halepensis* (Mill.)’ in columns 2, 3 and 4.

2. Use the same pure seed definition (PSD 47) for ‘*Pinus brutia* (Ten.)’ as for ‘Pines II’ from Table 3.2.1.A.1 Part 1

3. Add ‘*Pinus brutia* (Ten.)’ to Table 5A Part 2 - Tree and shrub seeds, and repeat the current prescriptions for ‘*Pinus halepensis* (Mill.)’ in columns 2, 3, 4, 5 and 6.

4. Apply the existing Tetrazolium testing procedures to ‘*Pinus brutia* (Ten.)’ as currently appear for ‘*Pinus spp.*’ in Table 6A Part 2.

5. In common with moisture content testing for ‘All tree species’ – Apply Table 9B.

**Item 4d Clarification of the term ‘Double test’**

In Chapter 5 germination, Table 5A Part 2 there is a statement: "For certain species in column 6, duplicate tests (with and without prechilling) are necessary." From this statement it is not clear if both methods (with and without prechill) are mandatory and also if the stated temperatures and days of prechilling are mandatory. This situation is different from agricultural species where in most cases no time (days) has been specified.

Text following heading to Chapter 5 Germination, Table 5A Part 2 Tree and shrub seeds.

**Current:** For certain species indicated in column 6, duplicate tests (with and without prechilling) are necessary. The less desirable methods are placed in brackets in the table.

**Proposed:** For certain species in column 6, a double test (with and without prechilling) is mandatory. Alternative methods are placed in brackets in the table.
NOTE: Given the above changes in Item 4d, Table 13A will also need amending:

**Proposed:** For certain species in column 7, a double test (with and without prechilling) is mandatory.

**Item 4e Clarification of how to report ungerminated seeds**

Proposal from Ronnie Don of the Executive Committee.

If more than 5% ungerminated seeds are found at the end of the germination test a method is required to check whether they are fresh or dead. It needs to be made clear in the rules that whatever method is used the subsequent classification should be into fresh or dead. Also x-ray tests will only determine if seeds are full or empty, they will not determine if the seed is viable, hence this term is deleted as an appropriate method.

5.6.5.A.3. Ungerminated seeds:

............

2. Fresh seeds: measures as described in Annexe 5.6.3.A. must be taken to induce germination, especially if large numbers are found. If fresh seeds are to be reported at a rate of 5% or more, it must be verified that these seeds have the potential to produce a normal seedling. This may be done with a tetrazolium test (Chapter 6) or other appropriate method (such as dissection, or embryo excision or x-ray). If there is any doubt as to whether the seed is fresh or dead, then it must be classified as dead. The ungerminated fresh seed are then reclassified as fresh and any ungerminated dead seed added to the numbers of any dead seed already found.

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Item 5 Proposals for Annexe to Chapter 7, Seed Health testing methods
Item 5a: Modifications and additions to the validated seed health testing methods.

The following methods have been validated by the SHC method validation sub-committee, and are submitted for acceptance by the membership to be included in the Annexe to Chapter 7 of the International Rules for Seed Testing as Methods 7-019 and 7-020.

Any problems or errors should be directed to the Chairpersons of the Seed Health and Rules Committees. Details of the validation study are published in ISTA Method Validation Reports.

7-019 Detection of *Xanthomonas campestris pv. campestris* (Black rot) on *Brassica* spp. (with centrifugation step)

**Crop:** Brassica spp  
**Pathogen:** *Xanthomonas campestris pv. campestris*

**Background:**
This method is based on methods originally published by Franken et al. (1991) and in the 2nd edition of Working Sheet No. 50 in the ISTA Handbook of Seed Health Testing (Schaad and Franken, 1996). Compared to the 2nd edition of Working Sheet No. 50, this version incorporates a number of modifications resulting from comparative tests in 13 laboratories (Koenraadt et al., 2004), a study done in a single laboratory (Roberts et al., 2004), and experience of routine testing in a number of laboratories. Summary of modifications: no fungicides used in extraction buffer; NSCAA medium replaced by mCS20ABN; centrifugation after 5 min done in micro-centrifuge tubes; continuous shaking instead of static incubation; only one plate of each medium per dilution; removal of a check for antagonistic bacteria; minor changes to media preparation; simplified pathogenicity test method; removal of IF and direct plating assays; changes to format and layout. This method differs from method 7-020 by the inclusion of a centrifugation step, which may theoretically give an improved analytical sensitivity. Users of this method should be aware that the values quoted for analytical sensitivity (detection limits) are theoretical; in practice the actual level of sensitivity achieved will vary with the background level of saprophytes.

**Method Abstract**

Seeds are suspended in saline plus Tween 20 in a conical flask, which is then shaken for 5 min. Two 1 ml samples are removed and centrifuged. The flask is then shaken for a further 2.5 h and the extract diluted. Both centrifuged and diluted extracts are plated on FS and mCS20ABN media. Plates are incubated at 28-30°C for 3-4 d and then examined for the presence of suspected colonies of *Xanthomonas campestris pv. campestris*. Suspected colonies are sub-cultured to plates of YDC medium and their identity confirmed by a pathogenicity test on susceptible Brassica seedlings.

7-020 Detection of *Xanthomonas campestris pv. campestris* (Black rot) on *Brassica* spp. (without centrifugation step)

**Crop:** Brassica spp  
**Pathogen:** *Xanthomonas campestris pv. campestris*

**Background:**
This method is based on methods originally published by Franken et al. (1991) and in the 2nd edition of Working Sheet No. 50 in the ISTA Handbook of Seed Health Testing (Schaad and Franken, 1996). Compared to the 2nd edition of Working Sheet No. 50, this version incorporates a number of modifications resulting from comparative tests in 13 laboratories (Koenraadt et al., 2004), a study done in a single laboratory (Roberts et al., 2004), and experience of routine testing in a number of laboratories. Summary of modifications: no fungicides used in extraction buffer; NSCAA medium replaced by mCS20ABN; no centrifugation step after 5 min; continuous shaking instead of static incubation; only one plate of each medium per dilution; removal of check for antagonistic bacteria; minor changes to media preparation; simplified pathogenicity test method; removal of IF and direct plating assays; changes to format and layout. This method differs from method 7-019 by the omission of a centrifugation step, which may theoretically give a reduced analytical sensitivity. Users of this method should be aware that the values quoted for analytical sensitivity (detection limits) are theoretical; in practice the actual level of sensitivity achieved will vary with the background level of saprophytes.

**Method Abstract**

Seeds are suspended in saline plus Tween 20 in a conical flask, which is then shaken for 2.5 h. The extract is then diluted and plated on FS and mCS20ABN media. Plates are incubated at 28-30°C for 3-4 d and then examined for the presence of suspected colonies of *Xanthomonas campestris pv. campestris*. Suspected colonies are sub-cultured to plates of YDC medium and their identity confirmed by a pathogenicity test on susceptible Brassica seedlings.
ITEM 6 PROPOSALS FOR CHAPTER 10, WEIGHT DETERMINATION

Items 6a Allowing pure seed for the 1000 seed weight determination to be taken from a fraction of the submitted sample

Proposal from Ronnie Don of the Executive Committee. Pure seed are allowed to be taken from a representative fraction of the submitted sample for use in germination testing; the same should be applied to the determination of the weight of a 1000 seeds.

10.4. Procedure

Either the whole working sample (10.4.2.) or replicates of pure seed from it or from a representative fraction of the submitted sample (10.4.3.) shall be used.

10.4.1 Working sample
The working sample shall be the entire pure seed fraction of a purity analysis carried out in accordance with Chapter 3 of these Rules, or pure seed taken from a representative fraction of the submitted sample.
**ITEM 7 PROPOSALS FOR CHAPTER 17, CERTIFICATES**

**Item 7a Changes to the certificate statements**

The paragraph (17.4.2, 17.4.3j and 17.4.4f), as it is presented in the Rules 2003, gives the impression that the signatory’s confirmation needs to be retyped on the Certificate. To avoid confusion it shall be made clear that the confirmation is already written on the back of the Certificate and thus does not need to be retyped.

17.4.2 *Orange International Seed Lot Certificate*

(j) The following statement signed by Signature of the director of the issuing laboratory, or his assignee: With his signature the signatory confirms the statement written on the back of the Certificate which reads as follows: …

17.4.3 *Green International Seed Lot Certificate*

(j) The following statement signed by Signature of the director of the issuing laboratory, or his assignee: With his signature the signatory confirms the statement written on the back of the Certificate which reads as follows: …

17.4.4 *Blue International Seed Sample Certificate*

(f) The following statement signed by Signature of the director of the issuing laboratory, or his assignee: With his signature the signatory confirms the statement written on the back of the Certificate which reads as follows: …

**Item 7b Changes to the Green International Certificate statements**

A change is required to the text for the Green Certificate to reflect what appears on the recently amended Green Certificate

17.4.3 *Green International Seed Lot Certificate*

(j) …:

“I certify that sampling, sealing and testing have been carried out in accordance with the International Rules for Seed Testing of the ISTA and that the tests have been made at the Official Station authorised a laboratory accredited by the International Seed Testing Association to issue International Seed Analysis Certificates.”
Item 7c Changes to the Orange International Certificate statements

It is suggested to omit the requirement to test the sample in the country the lot is located in. Reason: In some countries there are no authorised seed testing laboratories but a laboratory from another country may send a sampler to or license samplers in that country to draw the sample.

17.4.2 Orange International Seed Lot Certificate

For an Orange International Seed Lot Certificate, the submitted sample must be tested by an authorised seed testing laboratory of the country in which the lot is located. The testing laboratory shall be responsible for sampling, sealing, labelling, testing and issuance of the Certificate.