Germination Committee
Chair: Sylvie Ducournau
Committee report 2010 – 2013
Agenda

• Germination committee members
• Rules development
  – New species
  – New methods
  – Special project
  – Proposals for rules changes
• Publications
• Workshops / Seminar
• Future work of the committee
## Committee membership 2010-2013

<table>
<thead>
<tr>
<th></th>
<th>Name</th>
<th>Nationality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sylvie Ducournau</td>
<td>France</td>
</tr>
<tr>
<td>2</td>
<td>Anny van Pijlen</td>
<td>The Netherlands</td>
</tr>
<tr>
<td>3</td>
<td>Ignacio Aranciaga</td>
<td>Argentina</td>
</tr>
<tr>
<td>4</td>
<td>Ronald Don</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>5</td>
<td>Kari Fiedler</td>
<td>United States</td>
</tr>
<tr>
<td>6</td>
<td>Fabio Gorian</td>
<td>Italy</td>
</tr>
<tr>
<td>7</td>
<td>Andrea Jonitz</td>
<td>Germany</td>
</tr>
<tr>
<td>8</td>
<td>Krystyna Kolasinska</td>
<td>Poland</td>
</tr>
<tr>
<td>9</td>
<td>Augusto Martinelli</td>
<td>Argentina</td>
</tr>
<tr>
<td>10</td>
<td>Lea Mazor</td>
<td>Israel</td>
</tr>
<tr>
<td>11</td>
<td>Gillian McLaren</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>12</td>
<td>Harry Nijenstein</td>
<td>The Netherlands</td>
</tr>
<tr>
<td>13</td>
<td>Takayuki Okuda</td>
<td>Japan</td>
</tr>
<tr>
<td>14</td>
<td>Hakon Tangeras</td>
<td>Norway</td>
</tr>
<tr>
<td>15</td>
<td>Rita Zecchinelli</td>
<td>Italy</td>
</tr>
</tbody>
</table>

**New members 2013-2016**

<table>
<thead>
<tr>
<th></th>
<th>Name</th>
<th>Nationality</th>
</tr>
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<tbody>
<tr>
<td>16</td>
<td>Sarah Dammen</td>
<td>United States</td>
</tr>
<tr>
<td>17</td>
<td>Christine Herzog</td>
<td>Switzerland</td>
</tr>
<tr>
<td>18</td>
<td>Jin Wook Kim</td>
<td>South Korea</td>
</tr>
</tbody>
</table>

*15 June 2013 – Sylvie DUCOURNAU*
Rules development – New species

• Introduction of germination methods for new species was planned in 2010 for six species

<table>
<thead>
<tr>
<th>Species</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum nigrum</em></td>
<td>Δ A germination method has been included into the Rules in 2011</td>
</tr>
<tr>
<td><em>Chenopodium quinoa</em></td>
<td>Δ The validation study has started at the end of 2012</td>
</tr>
<tr>
<td><em>Cleome gynandra</em></td>
<td>Δ A validation study is planned in 2013-2014</td>
</tr>
<tr>
<td><em>Bambusa bambos</em></td>
<td>Δ Information has been given from Indian laboratory on germination method but no contact since July 2012</td>
</tr>
<tr>
<td><em>Bambusa vulgaris</em></td>
<td></td>
</tr>
<tr>
<td><em>Eustoma sp.</em></td>
<td>Δ No work in the committee for that species proposed by a member</td>
</tr>
<tr>
<td><em>Brassica carinata</em></td>
<td>Δ No work in the committee for that species proposed by a member</td>
</tr>
</tbody>
</table>
Chenopodium quinoa

- **Test organizers:** Ignacio Aranciaga and Lesly González

- Amendments have been proposed and the test plan has been validated again in August 2012
  - 6 participating laboratories
  - 3 seed lots with different levels of germination
  - Substrates: TP and BP
  - Temperatures: 20°C and 20°C–30°C
  - Dormancy breaking treatments: None, Prechilling, KNO₃
  - 2 counts after 4 and 7 days
New requests

• *Carica papaya*
  – Two laboratories from Separate Customs Territory expressed the need to introduce germination method for this species
    • Seeds are available
    • Test plan is in progress and is being reviewed by the committee
      Sand, 20°C–30°C, 21-28 days, soaking seeds
    • 2 or 3 more laboratories are needed

• *Panax ginseng*
  – Request from a Korean laboratory
Difficulties with new species

• The committee receives a lot of requests for germination on new species

• These new species are usually produced in one area of the world
  – Difficulties in finding laboratories to test the species
  – Difficulties in sending seeds to laboratories

• Seeds from these species often exhibit seed coat or/and embryo dormancy

• In order to be able to issue Certificates, sample weight and PSD shall be known
  – Difficulties in finding time to interact with other committees
Rules development – New methods

• New germination methods for existing species were planned in 2010

<table>
<thead>
<tr>
<th>Method Description</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add the temperature of 20°C for the germination of <em>Spinacia oleracea</em></td>
<td>No work on this</td>
</tr>
<tr>
<td>Use of KNO3 for lettuce seed germination test</td>
<td>Another test leader had to be found but the study has not started yet</td>
</tr>
<tr>
<td>Use of GA3 for <em>Panicum maximum</em> and <em>Panicum coloratum</em> seed germination test</td>
<td>The study has not yet started</td>
</tr>
<tr>
<td>Use of BP for <em>Lolium perenne</em> and <em>Festuca rubra</em></td>
<td>Due to the results of repeatability the committee decided not to validate the use of BP for the germination method of these species</td>
</tr>
<tr>
<td>Shortening the time of the final count for certain grass species</td>
<td>The study is completed. A rules change has been proposed</td>
</tr>
<tr>
<td>Shortening the time of the final count for <em>Trifolium</em> and <em>Medicago</em></td>
<td>The test plan has been started</td>
</tr>
</tbody>
</table>
Germination Committee

Harry Nijënstein

Shortening the duration of the germination test for Lolium, Festuca and Poa species
Shortening the duration of the germination test for *Lolium*, *Festuca* and *Poa* species

- Start situation
- Test plan
- Results
- Conclusions
Present situation

- Duration of germination period of most important temperate grasses:
  - Lp: 14 days + prechill
  - Fr: 21 days + prechill
  - Pp: 28 days + prechill

- This duration may be shortened, because
  - Hardly any if any seeds germinate in last week.

- Positive effects of shortening duration:
  - This will save space in germination cabinet.
  - This will improve logistics in grasses
    (especially because often two tests have to be done: after cleaning and after blending).
  - This will save money (in lab and in logistics).
Test plan comparative test - labs and lots

8 participating laboratories (7 countries, 3 continents)

Species tested
- *Lolium perenne*
- *Festuca rubra*
- *Poa pratensis*

4 seed lots per species
- 1 fast germinating variety
  - 2 levels of germination quality
- 1 slow germinating variety
  - 2 levels of germination quality
Test plan comparative test
- tests

• On pure seeds
• With all temperatures available for each species
• Counts after:
  – 7, 10 and 14 days for *Lolium perenne*
  – 7, 14 and 21 days for *Festuca rubra*
  – 7, 14, 21 and 28 days for *Poa pratensis*
• Several statistical studies have been carried out
  – Repeatability and reproducibility
  – Statistical comparison between temperature and test duration for each species
Results
- general

• A draft validation report has been circulated between the participants and within GER

• The final validation report was approved by the members of GER
Results
- statistical analysis

• In Lolium at 15-25°C the repeatability and reproducibility criteria are all met after excluding lab 2. These criteria are almost met for temp 20-30°C at 10 and 14 days.

• For Festuca repeatability and reproducibility criteria are almost met at 14 and 21 days for the two temperatures.

• In Poa repeatability and reproducibility at 15-25°C these criteria are almost met at 14, 21 and 28 days.

• This means that there is more variation within lab (repeatability variance) and across labs (reproducibility variance) than expected.

• At this moment no statistical tests exist for comparing observed repeatabilities/reproducibilities with expected values.

• However, as the observed results are only slightly different from the expected ones, it can be concluded that the results are acceptable.
Results
- Differences between counting dates and temperatures compared

<table>
<thead>
<tr>
<th></th>
<th>between last two counts</th>
<th>differences between temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15-25</td>
<td>20</td>
</tr>
<tr>
<td>Lp</td>
<td>14-10</td>
<td>14-10</td>
</tr>
<tr>
<td>Lp3</td>
<td>fast high</td>
<td>1.3</td>
</tr>
<tr>
<td>Lp1</td>
<td>fast low</td>
<td>1.3</td>
</tr>
<tr>
<td>Lp2</td>
<td>slow high</td>
<td>0.4</td>
</tr>
<tr>
<td>Lp4</td>
<td>slow low</td>
<td>1.9</td>
</tr>
<tr>
<td>avg</td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>Fr1</td>
<td>fast high</td>
<td>1.5</td>
</tr>
<tr>
<td>Fr3</td>
<td>fast low</td>
<td>2.4</td>
</tr>
<tr>
<td>Fr4</td>
<td>slow high</td>
<td>3.0</td>
</tr>
<tr>
<td>Fr2</td>
<td>slow low</td>
<td>2.0</td>
</tr>
<tr>
<td>avg</td>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td>Pp1</td>
<td>fast high</td>
<td>1.8</td>
</tr>
<tr>
<td>Pp2</td>
<td>fast low</td>
<td>1.7</td>
</tr>
<tr>
<td>Pp3</td>
<td>slow high</td>
<td>3.6</td>
</tr>
<tr>
<td>Pp4</td>
<td>slow low</td>
<td>2.2</td>
</tr>
<tr>
<td>avg</td>
<td></td>
<td>2.3</td>
</tr>
</tbody>
</table>
Conclusions
- general

• Repeatability and reproducibility were similar for the last two counts in all species, and were at acceptable levels.

• Different temperature regimes and shortening the duration of the germination test resulted in statistically significant differences of less than 3%.

• The variation caused by shortening the duration of the germination test is of the same magnitude as the variation caused by different temperature regimes.
Conclusions
- rules change proposal

• Part of the bias is probably caused by postponing the final evaluation of questionable seedlings.
• Therefore it is suggested to change the duration of the germination test as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Old (days)</th>
<th>New (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lolium spp</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Festuca spp</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Poa sp</td>
<td>28</td>
<td>21</td>
</tr>
</tbody>
</table>
Conclusions
- prolonging the test

In order for allowing sufficient time in case of deep dormancy being present, the extended periods will stay as they are (and not be reduced to 50% of the duration).

<table>
<thead>
<tr>
<th>CURRENT VERSION</th>
<th>PROPOSED VERSION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>5.6.4 Duration of the test</strong></td>
<td><strong>5.6.4 Duration of the test</strong></td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
| If it seems advisable, when for example some seeds have just started to germinate, the prescribed test period may be extended by 7 days or up to half the prescribed period for the longer tests. | If it seems advisable, when for example some seeds have just started to germinate, the prescribed test period may be extended by:

a) 7 days;
b) up to half the prescribed period;
c) up to 21 days for *Lolium* spp.;
d) up to 32 days for *Festuca* spp. (except *F. arundinacea* and *pratensis*);
e) up to 42 days for *Poa* spp. (except *P. bulbosa*);
f) up to 54 days for *Poa bulbosa*. |
Conclusions
- extrapolation

• The results can be extrapolated to other species of the same genus that have similar germination patterns (have already the same test conditions and test durations in the present ISTA Rules):

• *Lolium perenne*
  – *L. xbochaneum (hybridum)*, *L. multiflorum*

• *Festuca rubra*
  – *F. filiformis*, *F. heterophylla*, *F. ovina*.

• *Poa pratensis*
  – *P. nemoralis*, *P. palustris*,

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Rules changes proposals

• Harmonisation between ISTA Rules and ISTA Handbook on Seedling Evaluation regarding the evaluation of the cotyledons (50% rule)

5.2.6 The 50% rule

The 50% rule is used in the evaluation of cotyledons and primary leaves.

Cotyledon tissue:
– Seedlings are considered normal as long as half or more of the total cotyledon tissue is functional
– Seedlings are abnormal when more than half of the cotyledon tissue is missing, necrotic, decayed or discoloured.

The 50% rule does not apply if the tissue around the terminal bud or the two points of attachment of the cotyledons to the seedling axis or the terminal bud itself is necrotic or decayed; such seedlings are abnormal irrespective of the condition of the cotyledons or primary leaves. It does not apply also if one point of attachment of one cotyledon is necrotic or decayed and if the other cotyledon is not intact; such seedlings are also considered as abnormal.

5.2.8.1. Seedling abnormalities

3 Abnormalities of the cotyledons and primary leaves

Note: damage or decay of the cotyledons at the two points of attachment of the cotyledons to the seedling axis or near the terminal bud renders a seedling abnormal, irrespective of the 50% rule. The 50% rule also does not apply if one point of attachment of one cotyledon is necrotic or decayed and the other cotyledon is not intact; such seedlings are also considered as abnormal.
Rules changes proposals

• Growing media for germination test
  – It is suggested to delete a sentence referring to the use of “moistened porous paper or absorbent cotton” in order to avoid confusion regarding the need to determine the water content and the water retention capacity of the substrates.

5.6.2.1.1. Methods using paper

Top of paper (TP): the seeds are germinated on top of one or more layers of paper which are placed:
- ...
- ...
- directly on trays in germination incubators. The relative humidity in the incubators must then be maintained at a level that prevents tests drying out. Moistened porous paper or absorbent cotton can be used as a base for substrates.
Rules changes proposals

• List of seedling abnormalities

5.2.8.1. Seedling abnormalities

Abnormalities of the coleoptiles and the primary leaf

41  The coleoptile
...

41/12  is trapped under the lemma or the seed coat
Rules changes proposals

• Change in Germination Chapter following the proposal from the Purity Committee to move the genus *Arachis* from PSD 11 to PSD 21.

5.6 Procedure
5.6.1 Working sample

... Multigerm seed units, except for *Arachis*, are not broken up for the germination test but are tested as though they were single seeds.

For *Arachis*, although a pod is a pure seed unit, seed must be removed from the pod before use in a germination test.
Rules changes proposals

- Duration of germination test for certain grass species
  
  Table 5A Part 1 Agricultural and vegetable seeds (PROPOSED VERSION)

<table>
<thead>
<tr>
<th>Species</th>
<th>Substrate</th>
<th>Temperature (°C)</th>
<th>First count (d)</th>
<th>Final count (d)</th>
<th>Recommendations for breaking dormancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Festuca filiformis</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25</td>
<td>5</td>
<td>2+ 14</td>
<td>KNO₃; prechill</td>
</tr>
<tr>
<td>Festuca heterophylla</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25</td>
<td>5</td>
<td>2+ 14</td>
<td>KNO₃; prechill</td>
</tr>
<tr>
<td>Festuca ovina</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25</td>
<td>5</td>
<td>2+ 14</td>
<td>KNO₃; prechill</td>
</tr>
<tr>
<td>Festuca rubra</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25</td>
<td>5</td>
<td>2+ 14</td>
<td>KNO₃; prechill</td>
</tr>
<tr>
<td>Lolium × hybridum</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25; 20</td>
<td>5</td>
<td>14-10</td>
<td>KNO₃; prechill</td>
</tr>
<tr>
<td>Lolium multiflorum</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25; 20</td>
<td>5</td>
<td>14-10</td>
<td>KNO₃; prechill</td>
</tr>
<tr>
<td>Lolium perenne</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25; 20</td>
<td>5</td>
<td>14-10</td>
<td>KNO₃; prechill</td>
</tr>
<tr>
<td>Poa nemoralis</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25; 10 &lt;==&gt;30</td>
<td>10</td>
<td>28 21</td>
<td>KNO₃; prechill</td>
</tr>
<tr>
<td>Poa palustris</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25; 10 &lt;==&gt;30</td>
<td>10</td>
<td>28 21</td>
<td>KNO₃; prechill</td>
</tr>
<tr>
<td>Poa pratensis</td>
<td>TP</td>
<td>20&lt;==&gt;30; 15&lt;==&gt;25; 10 &lt;==&gt;30</td>
<td>10</td>
<td>28 21</td>
<td>KNO₃; prechill</td>
</tr>
</tbody>
</table>
Rules development – Some questions after the 2010 update

• Philosophical issues
  – Some major philosophical issues have been raised to the committee in 2010:
    • Finishing germination tests once a pre-determined germination level is achieved
      ∆ This question has led to a change into the Rules in 2012 that allows the possibility of reporting results before the end of the germination, at the request of an applicant
    • Not differentiating ungerminated seed
      ∆ It has been decided in the committee not to go on with this change proposal for the following reasons:
        – Reporting hard and fresh seeds is needed for EU certification purposes in some species.
        – Not differentiating ungerminated seeds could also be a problem as fresh seeds could not be identified anymore and so the retest when 5% or more fresh seeds are present could not be applied anymore.
Rules development – Some questions after the 2010 update

• Philosophical issues
  – Some major philosophical issues have been raised to the committee in 2010:
    • Introducing performance based germination procedures.

  Proposal has been made by the Germination Committee in 2011 in order to use in-house germination methods or variations of ISTA germination methods as alternative germination methods

  The Executive Committee decided that the Rules committee organizes a debate on this item during the Venlo Meeting involving the Germination and Statistics Committee and the ISTA Accreditation Department
Most of the germination committee members are in favour of increasing flexibility of germination methods, in particular on substrates, temperatures, durations and dormancy breaking treatments.

Whereas few members are against any changes due to concern about the resulting lack of uniformity in seed testing that they feel the changes would generate.

In-house methods should be validated. Peer validation methods could be used, including tests on repeatability, reproducibility, and also tests on accuracy. Simple tools on Excel would be necessary for in-house method validation.

Introducing in-house methods would increase the complexity of the accreditation supervision. A list of available in-house methods and a list of laboratories using these methods should be provided to auditors prior to audits.
Rules development – Some questions after the 2010 update

• Technical issues
  – After the update of the Chapter 5 in 2010, some items remained for discussion in order to provide changes and/or clarifications into the Rules
    • Light used for germination testing
    • Particle size of sand
    • Seed disinfection prior to germination test
  – In September 2012, Ronnie Don created questionnaires sent to ISTA laboratories in order to gather information on what it is done and what is required on these items
  – A report has been produced and some work has to be done in order to transform answers into rules proposals or guidance in the Handbook
Germination Committee

Ronald Don

Responses to Questionnaires on seed treatment, light, sand and organic growing media
Last July the 122 ISTA Laboratories accredited for germination testing were sent three questionnaires:

1. On disinfection of seed prior to germination testing
2. On light used in germination testing
3. On particle size of sand and the particle size and composition of organic growing media used in germination testing

These questionnaires were designed to collect information on current laboratory practices in ISTA Accredited Laboratories in order to make proposals for improving the ISTA Rules or ISTA Handbook for Seedling Evaluation.

Responses were received from 91 laboratories, which represents a response rate of 75%.
Questionnaire on the disinfection of seed prior to germination testing
The ISTA Rules allows laboratories to apply a fungicidal seed treatment before planting the seed of *Arachis hypogaea* and *Beta vulgaris* when the seed is known not to have received such a treatment.

When the laboratory applies such treatment germination is reported in the normal manner but the name of the chemical applied, the percentage of the active ingredient and the method of treatment must be reported on the ISTA International Seed Analysis Certificate.
For other species ISTA does not allow the laboratory to report the germination of seed to which a fungicide is applied by the laboratory in the germination section of the ISTA International Seed Analysis Certificate.

They can only report such germination results in the other determination part of the certificate provided:

- The germination of untreated seed is reported in the germination part of the certificate; and
- The result of the germination of the fungicidal treated seed is followed by details of the treatment (the name of the chemical applied, the percentage of the active ingredient and the method of treatment) and the statement: “This method is not covered by the International Rules for Seed Testing”. 
Questionnaire Responses
Laboratory Treatment prior to germination testing

Disinfection of seed prior to germination testing by ISTA Accredited Laboratories
Species disinfected as a matter of routine prior to germination testing

Other species/crop groups that were routinely disinfected prior to germination testing include: species of the Cucurbitaceae, Pulses, Temperate Cereals, Tree seed, *Abelmoschus esculentus*, *Allium* spp., *Canna* spp., *Capsicum* spp., *Coriandrum sativum*, *Foeniculum vulgare*, *Glycine max*, *Onobrychis sativa* and *Zea mays*. 
Views of accredited laboratories on the reporting arrangements for seed that are treated with fungicide prior to germination testing

- The present reporting arrangements should not be changed: 40%
- The present reporting arrangements for Beta and Arachis should be applied to all species: 20%
- The present reporting arrangements applied to species other than Beta and Arachis should be applied to all species: 10%
- Did not express a view regarding reporting: 50%
Seed should be tested as received since the certificate should state the quality of the seed lot as it is presented and sealed under supervision of the sampler.

There are many products on the market and treatments applied by laboratories could be different from those applied prior to the sowing of seed. There could also be issues with the efficacy of seed treatments selected by the laboratory and problems with rates of treatment and methods of application.

There is a need for treatment guidance and standardised of procedures.
No Consensus of Opinion

For

The object of the germination test is to determine the germination potential of seed lot.

Without application of fungicide it can be impossible to determine the potential of seed particularly when the artificial environment of germination tests encourages the proliferation of microorganisms that can suppress germination and prevent an assessment of its true potential.

“Truth in testing” should be the ultimate aim. Provided it is reported that the seed was treated for the germination test then it would be clear to the client and report user that the germination result is based on seed that was treated in the lab.
Other factors to be considered

Whereas some species need to be treated for better field performance, some only need the seed treatment in the lab situation.

Labs also suggested that the application of fungicides to the germination media could be an alternative to laboratories disinfecting seed.

Results both with and without treatment would be most useful to the customer, especially since labs may not be able to provide the equivalent commercial application of the treatments, but customers would know the potential benefit, or not, of applying a treatment.
Views of Laboratories

If disinfection can be applied for *Arachis* and *Beta*, then disinfection should be applied to all species and results should be reported in the same way for all species.

If labs are applying treatment prior to germination testing then that fact should be clearly stated on the certificate.

There is no need for a change in reporting arrangements since there is already the possibility to treat seed and report the result under Other Determinations.
Questionnaire on light used in germination testing
The ISTA Rules recommend the use of light as one of the procedures for breaking physiological dormancy for a number of species:

ISTA Rules
5.6.3.1 Procedures for breaking physiological dormancy

Prechilling: ..............................

Light: The tests should be illuminated during at least 8 hours in every 24 hour cycle and during the high temperature period when the seeds are germinated at alternating temperatures. The quality and intensity of light may be important. The light intensity should be between 750 and 1250 lux from cool white lamps. Illumination is recommended especially for certain tropical and subtropical grasses (e.g. Chloris gayana, Cynodon dactylon).

Sealed Polyethylene envelopes: ..................
Even when not being used to break dormancy light is generally recommended for germination tests as its use gives better developed seedlings that are easier to assess:

ISTA Rules

5.10 Germination methods
Table 5A indicates.............
Light: Illumination of tests is generally recommended for better development of seedlings. .........................

Where light is used to promote the development of seedlings there are no recommendation in the ISTA Rules regarding the intensity or quality of light that should be used.

The questionnaire was designed to obtain information on the use of light in the germination laboratory with the aim of improving guidance given to laboratories.
Use of Light

Whereas only 29% of laboratories use light as a dormancy breaking treatment, 92% of laboratories used light to improve the development of seedlings.
Only 2% of laboratories conducted germination tests in the dark.
Of the 98% of labs who used light either to break dormancy or improve the development of seedlings only 36% measured the intensity of the light they used with Lux meters being the most commonly used instrument.
Only 38% of laboratories using light meters had them calibrated.
Apart from one lab that made measurements in micromoles of photons per square meter second all labs made measurements in LUX with one of the labs also measuring in PAR (photosynthetically active radiation) as well as LUX.
Of those who measure light intensity, 49% of labs did this annually. Twelve percent of labs made measurements more frequently than this, and 9% of labs conducted measurements every 3 years. For 30% of labs, measurement was sporadic with measurement only being undertaken once or with no schedule.
There is no consistency on the number of hours of light provided when seed are germinated in a constant temperature environment. Whereas 64% of laboratories provided 8 hours of light, 34% provided more than 8 hours with 7% providing constant light. Only 2% of laboratories used natural daylight and this would vary throughout the year according to day length.
Three laboratories reported that they measure the quality of the light they use for germination testing. However, two equated quality with intensity and the other laboratory made only a visual assessment of quality. 

It is concluded therefore that no ISTA laboratory accredited for germination testing made a quantitative measurement of the quality of light they used in germination testing.
Light Systems

Laboratories provided detailed descriptions of the light systems they used. Cool white fluorescent lamps of various wattages and from various manufactures were the most common sources of light used. Neon tubes and Glow light were also mentioned by some laboratories.
Comments on Light

• Difficult to standardizing and getting even lighting.
• Measurements depend on the loading of germinators and the position of tests.
• Light is complex because the germination system and the intensity, quality and the duration of must be considered.
• Advice and directions from the Germination Committee would be helpful but any guidelines should not be too strict to avoid discouraging the use of light by laboratories.
• The germination of many species is unaffected by the intensity of light. Whilst light contributed to a better development of the seedlings and an easier and more straightforward evaluation it is not necessary to measure its quality and intensity.
Comments on Light

The problems of temperature control at seed bed level and the use of fluorescent lights with test near the source drying was highlighted by a number of laboratories. They and others would like information on the use and suitability of light emitting diode (LED), which would not have the same temperature effects, for germination tests.
Questionnaire on particle size of Sand and particle size and composition of Organic Growing Media used in germination testing
The ISTA Rules recommend particle sizes for sand and organic growing media that are used for germination testing and gives a recommendation for the composition of organic growing media:

**ISTA Rules**

5.4.3 Growing media characteristic
5.4.3.1 Paper growing media

5.4.3.2 Sand growing media
The sand should be ..........................................
It is recommended that 90% of the particles should pass through a sieve with wholes or meshes of 0.8mm width, and be retained on a sieve with holes or meshes of 0.05mm width.

5.4.3.3 Organic growing media
Organic growing media are defined .................
**Organic compounds**: fibres such as peat, coconut fibres or wood tables, with a size of less than 5mm.
**Mineral particles**: for example sand, perlite and vermiculite. The proportion should be around 20% in volume. It is recommended that 90% of particles should pass through a size with holes or meshes of 2mm width and be retained on a sieve with holes or meshes of 0.05mm width.

5.4.4 Water.................................
To improve the rules and guidance given in the Seedling Evaluation Handbook, the ISTA Germination Committee wanted to obtain information on the actual particle sizes of sand and organic growing media used by ISTA laboratories. In addition it wanted to obtain information on the composition of organic growing media used by ISTA laboratories.
Laboratories using sand as a germination medium

Sand was used as a germination medium by 80% of respondent laboratories, but only 9% of these laboratories re-used sand.

To sterilise sand that had been previously used for germination tests: all but one laboratory heated the sand at various temperatures for various durations of time; one laboratory mixed the sand with boiling water.
Of the laboratories that used sand as a germination medium, 87% measured the particle size of each batch with 60% doing the measurements in-house and 27% having the measurements conducted externally.
Specifications set by laboratories for the batches of sand they procure for germination tests

Sixty-one per cent of laboratories set the specification for the particle size of sand recommended by ISTA whereas 26% of laboratories set a specification that allows a larger particle size than recommended by ISTA.

Thirteen per cent of labs set no specification.
Only 34% of laboratories used organic growing media for germination tests and of these only 39% measured the particle size of the organic growing media they used.
Only 36% of laboratories set a specification for organic growing media and only 11% set the specification recommended by ISTA. Eleven per cent of laboratories applied the ISTA recommended specification for the mineral component but set no specification for the organic material component. Fourteen per cent of laboratories applied a specification that was different from the one recommended by ISTA.
From details of the compositions of organic growing media used by laboratories it is clear that the term organic growing media covers a wide range of material with no consistency between laboratories in terms the composition.

<table>
<thead>
<tr>
<th>Constituents of organic and mineral fractions of the Organic Growing Medias used by laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic fraction</strong></td>
</tr>
<tr>
<td>Examples of Constituents contained in fraction</td>
</tr>
<tr>
<td>Soil organic components, sphagnum peat moss, peat, weakly decomposed white and high grade frozen black sphagnum peat, peat moss, course sphagnum peat, aged fine bark, fine peat</td>
</tr>
</tbody>
</table>

Some laboratories use soil as organic growing media, others mix mineral and organic components and others purchase commercial mixes the composition of some of which are unknown.
Laboratories made a number of general comments on the questionnaire regarding the particle size of sand and the particle size and composition of organic growing medium.

One common theme of some was the feeling that the current recommendations for particle size of sand are too restrictive and that a larger particle sizes should be permitted with laboratories considering innocuity, pH, conductivity and water capacity to be more important than particle size.

Some laboratories consider that presently too much time is spent doing media checks and they would be reluctant to do more for no good reason.
Thanks:

• To all the accredited laboratories who responded to the questionnaires;

• To Nadine Ettel (TCord) who send out the questionnaires and collated the responses; and

• To you, for your attention

Any Questions

Thank You!
Publications

• Amendments to the ISTA Handbook for Seedling Evaluation
  – Addition of sections concerning counting equipment and germination apparatus in Section 4 Laboratory conditions for seedling evaluation
  – Proposal for a method for evaluating counting equipment

15 June 2013 – Sylvie DUCOURNAU
• Amendments to the ISTA Handbook for Seedling Evaluation

  – Revision of Appendix 2: Index of Seedling Groups, regarding the classification of seedling types for *Vigna subterraneae* and *Vigna angularis*

  – Revision of Appendix 3: Index of Seedling Abnormalities

  – Revision of Section 12: Seedling Type D and Section 13: Seedling Type D to include abnormal type “scutellum detached from the endosperm”
Workshops and Seminar

• Germination Seminar in Zürich in 2011
• Contribution to the ISTA Workshop on Flower Seed Testing in Roelofarendsveen in 2012
• ISTA Germination Workshop in Saskatoon in 2012
  – 21 participants from Canada, USA, Separate Customs Territory and France
  – All aspects of germination covered from testing to reporting the results and QA items
  – 11 species germinated and evaluated
Workshops and Seminars

• ISTA Workshop on Purity and Germination, in Ankara, in June 2013
  – 25 participants from 13 countries (Belgium, Brazil, Denmark, Finland, France, Macedonia, Norway, Serbia, Sweden, Separate Customs Territory, Turkey, USA)
  – Species studied in germination: cereals, grass seeds, sugar beet seeds, clover seeds and vegetable seeds
Future work of the committee

• Method validation
  – Introduction of new methods and new species
  – Continuation of the current working programme

• Publications
  – Handbook: improvement of tree and shrubs photos of seedlings
  – Handbook: guidance on assessment of Glycine max. seedlings
  – Rules / Handbook: clarifications on substrates, light and disinfection

• Special projects
  – Use of variation of germination methods or in-house methods
  – Use of control seed samples for germination testing

…” And more following members’ meeting
Thanks to all the members of the committee and Joël Léchappé, the Statistics committee and the ISTA Secretariat

Thank you for your attention