ISTA Germination Committee

Presentation to the Ordinary Meeting

16 June 2009
Germination Committee Presentation

1. Welcome and Introduction

2. Summary of the work of the committee over the last year

3. Programme of work for the next year

4. Questions from the audience
Committee Membership

Chair: 1 Ronald Don United Kingdom
Vice-chair: 2 Sylvie Ducournau France
Members: 3 Ignacio Aranciaga Argentina
4 Kari Fiedler United States
5 Krystyna Kolasinska Poland
6 Joël Léchappé France
7 Lea Mazor Israel
8 Gillian McLaren United Kingdom
9 Günter Müller Germany
10 Enrico Noli Italy
11 Takayuki Okuda Japan
12 Anny van Pijlen Netherlands
13 Zdenka Procházková Czech Republic
14 Hakon Tangeras Norway
15 Grethe Tarp Denmark
Validation Studies and Rule Changes

Completed Work

✓ Crepe paper/sand method for beans, cotton, maize and sunflower.

✓ The use of Organic Growing Media for *Vicia faba* germination tests

✓ BP method for *Brassica* and *Sinapis*

✓ Germination method for *Brachiaria brizantha*
Validation Studies and Rule Changes

Work in Progress

- Dormancy breaking procedures for *Eruca sativa*
- Germination method for *Solanum nigrum*
- Germination methods for other tropical species, e.g., herbage species and *Jatropha curcas*
- Rules for testing Seed Mixtures
- Reduction in the time for final germination counts in temperate grasses
- Proposals for major revisions in Germination Chapter of the ISTA Rules
Completed Validation Studies

Method Validation Reports for Rules Proposals 2010

Contents

ISTA validation study on germination testing of Brachiaria brizantha (A. Rich.) Stapf [Rules Proposal 2010 B.1.] .................................................. 2
Validation of a cargo sampler and sampling stick without compartments for seed sampling in small seeded species [Rules Proposal 2010 C.2.2] .......... 22
Evaluation of Crepe Cellulose Paper Covered with Seed as an ISTA Medium for Glume Grass, Holcus lanatus, Phalaris arundinacea, Phalaris tuberosa and Zea mays [Rules Proposal 2010 C.5.1.] .................................................. 40
Between-paper method for the germination test of Brachiaria sp. and Stipa alba [Rules Proposal 2010 C.5.3.] .................................................. 46
Use of Organic Growing Media as primary substrate for the germination of Festuca glauca L. seeds [Rules Proposal 2010 C.5.3.5] .................................................. 58
Proposal for a new method for the detection of Cucumber Green Mottle mosaic Virus (CgMMV), Melon Necrotic Spot Virus (MNSV) and Squash Mosaic Virus (SqMV) in Cucurbita using DAS-ELISA [Rules Proposal 2010 C.7.1.] .................................................. 94
Proposal for the addition of Phasoleus vulgaris as a species to which the conductivity test for seed viability can be applied [Rules Proposal 2010 C.15.3.] ........ 92
Evaluation of the controlled desiccation test as a replaceable and reproducible vigour test for Brachiaria species [Rules Proposal 2010 C.15.3.5] .......... 92

1. Plant material

Three seed samples of Brachiaria brizantha of commercially traded quality were obtained from the Argentinean seed trade for this study.

The samples obtained were mechanically divided into subsamples by use of a soil divider. An in-house study was performed for the homogeneity of the seed samples. About 25 g of each sample were sent to each of the participating laboratories on 28 November 2008 with instructions to have the tests completed by 3 February 2009. The seeds were packed as blind samples (at lots 1–3), the numbering of the three samples was different for each laboratory.

2. Participating laboratories

Six laboratories from four countries participated in this validation study.

<table>
<thead>
<tr>
<th>Laboratory Name</th>
<th>Coordinator</th>
<th>ISTA Accessory Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTA LA CONSULTA (Argentina)</td>
<td>Marilis Makash, Jorge Valdés</td>
<td>ISTA Member Lab</td>
</tr>
<tr>
<td>Metalo Semillas (Brazil)</td>
<td>Paul Henrique</td>
<td>ISTA Accessory Lab</td>
</tr>
<tr>
<td>National Seed Institute of Uruguay</td>
<td>Teresa Fass, Debid Marfis</td>
<td>ISTA Accessory Lab</td>
</tr>
<tr>
<td>Queensland Seed Technology Lab (Australia)</td>
<td>Mrs. Karen A. Hill</td>
<td>ISTA Accessory Lab</td>
</tr>
<tr>
<td>SGS Mid-West Seed Services, Inc. (USA)</td>
<td>Karli Fiedler</td>
<td>ISTA Accessory Lab</td>
</tr>
<tr>
<td>INASE – National Seed Institute (Argentina)</td>
<td>Ignacio Aranciaga</td>
<td>ISTA Accessory Lab</td>
</tr>
</tbody>
</table>

In this report the laboratories are anonymously numbered as Labs 1–6, the sequence of these numbers is not identical to the alphabetical lot given here.
Use of Crepe Paper covered with Sand as a germination media for *Glycine max*, *Helianthus annuus*, *Phaseolus vulgaris* and *Zea mays*

- Last year Top crepe Paper Sand (TPS) was introduced as an approved media for the germination of *Pisum sativum* following a multi-laboratory validation study.
- A peer validation study has now been completed with the aim of having the media approved for the germination of *Glycine max*, *Helianthus annuus*, *Phaseolus vulgaris* and *Zea mays*. 
Top Crepe Paper Sand Methodology

- A standard volume of water is applied to crepe cellulose paper.
- Seeds are planted
- Dry sand is applied over the seeds and levelled
- Moisture moves by capillary action throughout the sand

The TPS medium utilizes a “Lean Manufacturing” approach to seed testing through sprayer tables, food service trays and carts and has the potential to save time and increase uniformity of results among seed testing laboratories.
For the four species TPS method gives statistically the same or better germination than the current ISTA approved methods
Top crepe Paper Sand Validation Study

Conclusion

The data generated in this validation study supports the inclusion of TPS as a new medium for ISTA laboratories for *Glycine max*, *Helianthus annuus*, *Phaseolus vulgaris*, and *Zea mays*.

A key advantage of this medium is moisture uniformity in sand based tests allowed by use of dry sand and calibrated water application through sprayer tables.
The use of Organic Growing Media for Vicia faba germination tests

A multi-laboratory was carried out to evaluate the germination of *Vicia faba* L. in organic growing media compared to sand and between paper substrates.
The use of Organic Growing Media for Vicia faba germination tests

Study design

- **Samples**
  Three samples of *Vicia Faba* L. seeds with various levels of germination quality (between 80% and 95% germination) were selected for this study.

- **Participants**
  Samples were sent to seven accredited laboratories in France, Netherlands, Scotland, Germany, USA, Norway and Israël.
Results of Vicia faba Organic Growing Media Validation Study

Repeatability and Reproducibility

Repeatability is higher with the use of Organic Growing Media compared to the two other media sand and between paper.

Reproducibility is higher with the use of Organic Growing Media compared with sand and between paper.
Results of *Vicia faba* Organic Growing Media Validation Study

Overall germination

<table>
<thead>
<tr>
<th>SUBSTRATE</th>
<th>Normal seedling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic</td>
<td>92</td>
</tr>
<tr>
<td>Paper</td>
<td>89</td>
</tr>
<tr>
<td>Sand</td>
<td>88</td>
</tr>
</tbody>
</table>

The level of normal seedlings also increased with the use of organic growing media compared to sand and between paper substrates.
Results of Vicia faba Organic Growing Media Validation Study

Conclusion

The results of this comparative test show that in germination tests of *Vicia faba* repeatability and reproducibility is higher with organic growing media than with sand and between paper substrates. The level of normal seedlings also increased with the use of organic growing media compared to sand and between paper substrates.

Organic growing media should be included as an additional media for the germination of *Vicia faba* L. seeds in the ISTA Rules.
The use of Between Paper Media for Brassica and Sinapis germination tests

To test whether BP could be included in the ISTA Rules as a prescribed substrate for *Brassica* *spp.* and *Sinapis alba* germinations a peer validation study was carried out.
The use of Between Paper Media for Brassica and Sinapis germination tests

Study design

This study involved the comparative testing of three different germination capacity seed samples of both Brassica spp. and Sinapis alba by three different accredited ISTA laboratories. The laboratories tested the samples using both BP and TP methods at the two alternative temperatures prescribed in the ISTA Rules, i.e. constant 20°C and alternating 20-30 °C.
The use of Between Paper Media for Brassica and Sinapis germination tests

Results

The statistical evaluation of the germination test results of this peer validation study show for both Brassica spp. and Sinapis alba, there is no significant difference between TP and BP germination methods.

The repeatability of the test method is purely associated with sampling variation and the interaction with method variance components is relatively small indicating that the two methods of TP and BP will provide similar results.
The use of Between Paper Media for Brassica and Sinapis germination tests

Conclusion

TP and BP provide similar results for the germination of Brassica spp and Sinapis alba.

Between paper should be included as an additional media for the germination of Brassica spp and Sinapis alba seeds in the ISTA Rules.
Development of a germination method for *Brachiaria brizantha*

To develop a germination procedure for *Brachiaria brizantha* that could be added to the ISTA Rules a multi-laboratory validation study was undertaken.
## Development of a germination method for Brachiaria brizantha

### Experimental Protocol

<table>
<thead>
<tr>
<th>Germination Media</th>
<th>Dormancy breaking pre-treatment</th>
<th>Temperature Regime</th>
<th>Light</th>
<th>Time for intermediate count</th>
<th>Time Final Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (Experiment 1)</td>
<td>KNO₃ (0.2%) on germination media</td>
<td>20 - 35 ºC</td>
<td>8 hrs. (During the high temp.)</td>
<td>7 days</td>
<td>21 days</td>
</tr>
<tr>
<td>TP (Experiment 2)</td>
<td>Dry in oven with forced air for 5 days (35-40ºC). KNO₃ (0.2%) on germination media</td>
<td>20 - 35 ºC</td>
<td>8 hrs. (During the high temp.)</td>
<td>7 days</td>
<td>21 days</td>
</tr>
<tr>
<td>TP (Experiment 3)</td>
<td>H₂SO₄ (96%, 36N) for 15 min, after acid draining, seed soaked in water for 1 hour, followed by one minute of washing in tap water and surface drying the seed over blotter paper. KNO₃ (0.2%) on germination media</td>
<td>20 - 35 ºC</td>
<td>8 hrs. (During the high temp.)</td>
<td>7 days</td>
<td>21 days</td>
</tr>
<tr>
<td>TP (Experiment 4)</td>
<td>KNO₃ (0.2%) on germination media</td>
<td>15 - 35 ºC</td>
<td>8 hrs. (During the high temp.)</td>
<td>7 days</td>
<td>21 days</td>
</tr>
<tr>
<td>TP (Experiment 5)</td>
<td>Dry in oven with forced air for 5 days (35-40ºC) KNO₃ (0.2%) on germination media</td>
<td>15 - 35 ºC</td>
<td>8 hrs. (During the high temp.)</td>
<td>7 days</td>
<td>21 days</td>
</tr>
<tr>
<td>TP (Experiment 6)</td>
<td>H₂SO₄ (96%, 36N) for 15 min, after acid draining, seed soaked in water for 1 hour, followed by one minute of washing in tap water and surface drying the seed over blotter paper. KNO₃ (0.2%) on germination media</td>
<td>15 - 35 ºC</td>
<td>8 hrs. (During the high temp.)</td>
<td>7 days</td>
<td>21 days</td>
</tr>
</tbody>
</table>

### Seed

Three seed samples of *Brachiaria brizantha* of commercially traded quality were obtained from Matzuda Semillas.

### Participants

Six ISTA laboratories from Australia, Argentina(2), Brazil, Uruguay and USA.

### Germination conditions

Six different germination procedures were employed based on experience of the species and methods used for other *Brachiaria* spp. presently in the ISTA Rules.
Development of a germination method for Brachiaria brizantha

Analysis of Results

Results were analysed using:

- Generalised linear mixed effect model to assess significance of effects and the repeatability and reproducibility
- The experimental error of replicate results from individual participating laboratories was quantified by calculating the f-ratio \( f \) between the observed standard deviation and the expected standard deviation based on the binomial distribution
- Tolerated ranges for comparing germinations from different laboratories were computed using the method of Miles’s tolerance tables

\[
\begin{align*}
\text{Normal}_\text{seedlings_counts}_i & \sim \text{Binomial}(400, \pi_i) \\
\logit(\pi_i) &= \log \left( \frac{\pi_i}{1 - \pi_i} \right) = \mu + \alpha_i + b_i \\
\text{Normal}_\text{seedlings_counts}_{ijklm} & \sim \text{Binomial}(100, \pi_{ijklm}) \\
\logit(\pi_{ijklm}) &= \log \left( \frac{\pi_{ijklm}}{1 - \pi_{ijklm}} \right) \\
&= \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_j + (\alpha\delta)_k + (\beta\delta)_jk + (\alpha\beta\delta)_jyk + c_l + (\alpha c)_ji + (\beta c)_jl + (\delta c)_kl \\
f &= \frac{\text{SD}_{\text{obs.}}}{\text{SD}_{\text{exp.}}} \\
f &= \frac{\text{SD}_{\text{obs.}}}{\text{SD}_{\text{exp.}}} = 2.38 - 0.008321p \\
S &= \left( \sqrt{(p \times q)/n} \right)(2.38 - 0.008321p)F \\
\text{SD}_{\text{exp.}} &= \sqrt{(p \times q)/n}
\end{align*}
\]
Development of a germination method for Brachiaria brizantha

Results

A summary of the results is given in the chart below:

It would appear that four methods gave equivalent results and could be recommended for inclusion in the ISTA Rules.
Development of a germination method for Brachiaria brizantha

Statistical analysis of results

Dormancy breaking treatment $\text{H}_2\text{SO}_4$ plus $\text{KNO}_3$ gives significantly lower germinations for all seed lots in both temperature regimes.

$\checkmark$ $\text{H}_2\text{SO}_4$ plus $\text{KNO}_3$ is not recommended as a dormancy breaking treatment for *Brachiaria brizantha*
Development of a germination method for Brachiaria brizantha

f-test and Maximum Tolerated Ranges

The f-factors indicate an acceptable experimental error among the 4 replicates within the tests for all dormancy breaking pretreatment and temperature regime combinations apart from seed given H₂SO₄ plus KNO₃ and tested at 20°C - 35°C.

Maximum tolerated ranges

- For two seed lots the range obtained experimentally was greater than the tolerated range for H₂SO₄ plus KNO₃ at both 15°C - 35°C and 20°C - 35°C.
- For temperature regime 15°C - 35°C the experimental range for dormancy breaking treatments KNO₃ and Heat plus KNO₃ was greater than the tolerated range for one and two seed lots respectively.
- For temperature regime 20°C - 35°C the results for KNO₃ and Heat plus KNO₃ were all within the tolerated range.
Development of a germination method for Brachiaria brizantha

Generalised linear mixed effect model

When excluding the H$_2$SO$_4$ plus KNO$_3$ results:

- There is no difference in the means over the 6 laboratories between the different temperature regimes and the different dormancy breaking treatments and their combinations.

- There is a significant seed lot x temperature regime interaction but no cross-over between effects.

- Repeatability standard-deviations are quantitatively higher for temperature regime 15 - 35°C and reproducibility standard-deviation higher for the combination of temperature regime 15 - 35°C and dormancy breaking treatment KNO$_3$. 
Development of a germination method for Brachiaria brizantha

Conclusion

- The range of results using temperature regime 20 - 35°C and dormancy breaking treatments KNO₃ and Heat plus KNO₃ are all within the theoretical tolerated range and there is no significant difference between replicate results obtained in participating laboratories.

- The use of temperature regime 20 – 35°C with dormancy breaking treatments KNO₃ and Heat plus KNO₃ gives acceptable levels of repeatability and reproducibility.

- For the germination of *Brachiaria brizantha* the use of the temperature regime 15 – 35°C, with dormancy breaking treatments KNO₃ and Heat plus KNO₃, is recommended for inclusion in the ISTA Rules.

<table>
<thead>
<tr>
<th>Species</th>
<th>Prescription for:</th>
<th>Additional directions recommendations for Breaking dormancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Substrate</td>
<td>Temperature (ºC)</td>
</tr>
<tr>
<td><em>Brachiaria brizantha</em></td>
<td>TP</td>
<td>20 - 35</td>
</tr>
</tbody>
</table>
Validation Studies and Rule Changes
Work in Progress

- Dormancy breaking procedures for *Eruca sativa*
- Germination method for *Solanum nigrum*
- Germination methods for other tropical species, e.g. Herbage species and *Jatropha curcas*
- Rules for testing Seed Mixtures
- Reduction in the time for final germination counts in temperate grasses
- Proposals for major revisions in Germination Chapter of the ISTA Rules
Work in progress - Validation studies
Dormancy breaking procedures for Eruca sativa

Eruca sativa seeds are very dormant just after harvest.

However there is no recommendation for dormancy breaking in the ISTA Rules.

Investigations in France indicate that the addition of GA₃ to the germination media was most successful in breaking dormancy.

A peer validation study was organised in 2007 but unfortunately dormancy in the study samples had been overcome naturally prior to the initiation of comparative tests. The study will be repeated when suitably dormant seed samples are available.
Work in progress - Validation studies
Germination procedure for *Solanum nigrum*
(and other tropical/sub-tropical species)

- A test plan has been approved and it is anticipated that a validation report and Rules proposal will be presented to the 2010 Congress.

The proposal of a germination method for *Brachiaria brizantha* this year and a method for *Solanum nigrum* next year are hopefully the first of many proposals for important tropical and Subtropical species. Validation studies for more tropical herbage species are in the pipeline as is a study on *Jatropha curcas*. The main obstacle to progress is the availability of sufficient seed of suitable quality for such studies.
The ISTA Bulking and Sampling, Purity and Germination Committees in conjunction with the Statistics Committee have initiated an experiment to collect information that may be used to determine the reliability of seed mixture test results and establish tolerances.

Details of the experiment are given on the ISTA website. So far thirteen laboratories have registered participation in the experiment. Although data from germination and other seed determination tests on mixtures is not required for analysis, procedures for these tests are given in the protocol for the guidance of those who test mixtures for these quality attributes.
The Germination Committee in conjunction with the ISTA Accreditation Department and Chairs of the Flower Seed and Forest Tree and Shrub Seed Committees are holding a workshop in September where a major revision of the Germination Chapter of the Rules will be considered.

The two main tasks for the workshop are:
1. To go through the germination rules chapter line by line and remove inconsistencies and add clarity where required; and
2. To fundamentally review the contents and see if changes are required to accommodate changes in the needs of customers.
Work in progress – Rules Proposals
Major Revision of the Germination Chapter of
the ISTA Rules

The Germination Committee has many ideas and there have been many suggestions from ISTA members for change.

Simple changes include denoting alternating temperatures as, e.g. 20°C⇌ 30°C rather than 20°C-30°C, and ensuring the alignment of abnormal seedlings in the Rules and Seedling Evaluation Handbook.

Fundamental changes to be discussed include the possibility of reporting germinated and non-germinated seeds only (not distinguishing between fresh and dead seeds) and reporting germination results as soon as a predetermined germination is achieved.
Questions to the Committee

The committee has been very active in answering questions with more than 50 enquiries since the last ISTA meeting. Topics attracting the most attention have been:

- Seedling Evaluation Guidance;
- Clarification of the Rules;
- Equipment supplies;
- Media Specification Checks; and
- QA requirements for germination testing.

<table>
<thead>
<tr>
<th>Subject of Question</th>
<th>Date</th>
<th>From (Country)</th>
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<tbody>
<tr>
<td>Programme for calculation of water retention of Germination media</td>
<td>Jun-08</td>
<td>USA</td>
</tr>
<tr>
<td>Storage conditions for maintenance of Petroselinum crispum</td>
<td>Oct-08</td>
<td>South Africa</td>
</tr>
<tr>
<td>Optimal conditions for germinating Lathyrus linifolius</td>
<td>Jun-08</td>
<td>UK</td>
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<tr>
<td>Calculation of water retention of Helianthus, Verbena, Catharanthus</td>
<td>Jul-08</td>
<td>France</td>
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<tr>
<td>Lolium</td>
<td>Jun-08</td>
<td>Canada</td>
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<tr>
<td>Evaluation of Lolium</td>
<td>Jun-08</td>
<td>India</td>
</tr>
<tr>
<td>Standards for climate room and germination boards</td>
<td>Jun-08</td>
<td>Japan</td>
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<tr>
<td>Lolium multiflorum proficiency test evaluation issues</td>
<td>Jun-08</td>
<td>Switzerland</td>
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<td>Evaluation of Glycine max Seedlings</td>
<td>Jun-08</td>
<td>Argentina</td>
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<tr>
<td>Lolium multiflorum proficiency test evaluation issues</td>
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<td>Switzerland</td>
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<td>Calculation of water retention of germination media</td>
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<td>Australia</td>
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<td>Standards for climate room and germination boards</td>
<td>Jul-08</td>
<td>Turkey</td>
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<tr>
<td>Introduction of new clover species to the Rules</td>
<td>Jul-08</td>
<td>Switzerland</td>
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<tr>
<td>Validity/longevity of ISTA certificate</td>
<td>Jul-08</td>
<td>Germany</td>
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<tr>
<td>Evaluation of Raphanus sativus seedlings</td>
<td>Jul-08</td>
<td>Germany</td>
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<tr>
<td>Germination test tolerance questions</td>
<td>Jul-08</td>
<td>Germany</td>
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<tr>
<td>Equipment required for an accredited laboratory?</td>
<td>Aug-08</td>
<td>Canada</td>
</tr>
<tr>
<td>Soybean seedling evaluation</td>
<td>Aug-08</td>
<td>Italy</td>
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<td>Specifications for testing maize samples to confirm International Certificate results</td>
<td>Sep-08</td>
<td>Greece</td>
</tr>
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<td>Testing of maize samples to confirm International Certificate results</td>
<td>Sep-08</td>
<td>Albania</td>
</tr>
<tr>
<td>Germination test of Helianthus, Verbena, Catharanthus</td>
<td>Sep-08</td>
<td>Switzerland</td>
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<tr>
<td>RH measurements in germination chambers</td>
<td>Sep-08</td>
<td>Netherlands</td>
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<td>Temperature control of testing germination of Sorghum spp</td>
<td>Sep-08</td>
<td>Germany</td>
</tr>
<tr>
<td>Reporting hard seed</td>
<td>Sep-08</td>
<td>Germany</td>
</tr>
<tr>
<td>Use of crepe paper for testing germination of Sorghum spp</td>
<td>Sep-08</td>
<td>Germany</td>
</tr>
<tr>
<td>Calculation of germination media water retention</td>
<td>Sep-08</td>
<td>United Kingdom</td>
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<tr>
<td>Use of binocular microscope for assessment of terminal buds of seedlings</td>
<td>Aug-09</td>
<td>Argentina</td>
</tr>
<tr>
<td>Number of seed to be tested – expensive hybrid seed lots</td>
<td>Aug-09</td>
<td>Turkey</td>
</tr>
<tr>
<td>Tolerance limits on germination 80% and 90%</td>
<td>Aug-09</td>
<td>Columbia</td>
</tr>
</tbody>
</table>
Problems in Proficiency tests

The committee is still often asked questions regarding problems laboratories have had with proficiency tests.

The most common problem is related to the evaluation of infected seedlings. If there are any doubts as to whether the infection is primary or secondary the seed should be retested using sand or organic growing media where paper was used in the first test.
Update of the Handbook on Seedling Evaluation

We have produced enhanced guidelines on:
- The calculation of growing media Water Retention;
- The evaluation of ungerminated seed; and
- The 50% Rule for the evaluation of foliated cotyledons.

It is anticipated that these will be published and sent out to members by the autumn.
Water Retention: Calculation

Details of the calculation are in the Handbook

The amount of water present in the substrate before it is saturated = ($H_2O$)$_s$

$\text{(H}_2\text{O)}_s = W_s \times \text{MC}$

The dry weight of the substrate used = (DW)$_s$

$\text{(DW)}_s = W_s - H_2O_s$

The amount of water present when the substrate is at Field Capacity = (H$2$O)$_{FC}$

$\text{(H}_2\text{O)}_{FC} = W_{FC} - W_s + (H_2O)_s$

The maximum amount of water held a growing media as percentage of its dry weight = (H$_2$O)$_{MAX}$

$\text{(H}_2\text{O)}_{\text{MAX}} = (H_2O_{FC} / DW_S) \times 100$

Some find this calculation very complex and have asked for some simple instructions.
We have produced a Water retention flow chart for the Handbook that gives step by step instructions.

and

We have also produced a spreadsheet (available of the ISTA website) that should take the strain out of the calculation.
Update of the Handbook on Seedling Evaluation Guidance for the evaluation of Fresh Seed

THE ASSESSMENT OF FRESH SEED AT THE END OF A GERMINATION TEST

- **Are there any ungerminated seed?**
  - **YES**: Perform quality checks and report the result of the test if appropriate.
  - **NO**: Continue with the following steps.

**Perform quality checks and report the result of the test if appropriate.**

- **Examine the ungerminated seed and make a visual/physical assessment of whether they are hard, fresh or dead.**
  - **HARD SEED**: seeds that have not imbibed water and remain firm and hard.
  - **FRESH SEED**: seeds that have imbibed water; they are clean and turgid and have the potential to germinate once dormancy is broken.
  - **DEAD SEED**: seeds that have imbibed water; they are soft and often discoloured and mouldy.

**Visual/Physical attributes of categories of ungerminated seed**

- **HARD SEED** - seeds that have not imbibed water and remain firm and hard
- **FRESH SEED** - seeds that have imbibed water; they are clean and turgid and have the potential to germinate once dormancy is broken
- **DEAD SEED** - seeds that have imbibed water; they are soft and often discoloured and mouldy

**NOTE**: Even with levels of fresh seed less than 5%, the customer can request that the viability of these seeds is verified even although this is not a requirement of the test.

- **Are levels of Fresh seed as determined by visual/physical assessment less than 5%?**
  - **YES**: Have dormancy breaking treatments been applied?
  - **NO**: Retest sample using dormancy breaking treatment.

**Perform quality checks and report the result of the test if appropriate.**

- **Evaluate the viability of the visually/physically assessed fresh seed by dissection, tetrazolium or excised embryo.**
- **Perform quality checks. Only seed assessed as viable by dissection, tetrazolium or excised embryo can be reported as fresh.**

The Committee have produced a flow chart that gives step by step instructions on how ungerminated seed should be evaluated at the end of a germination test. This will be included as an Annex to the Handbook on Seedling Evaluation.

Radical changes to how ungerminated seed are reported are being considered by the Committee at their workshop in September.
Update of the Handbook on Seedling Evaluation

50% rule for the evaluation of foliated cotyledons

The committee, under the leadership of Takayuki Okuda, have produced a major new Annex to the Handbook relating to the 50% rule for the evaluation of foliated cotyledons that gives illustrated guidelines relating to damaged, necrotic, decayed and discoloured tissue.

(a) (b)
Damaged areas are smaller than half of total cotyledon and more than half of the total cotyledon tissue is functional. (a) diagrammatic representation of defect, and (b) digital image of cotyledons of Lactuca sativa seedling with defect. (Normal Seedling)
The section on the evaluation of Necrosis should be particularly helpful

Intact cotyledons

Some few small necrotic spots can be observed but the conductive tissue is not damaged.

Necrotic spots can be observed clearly. The conductive tissue is not damaged seriously and more than half of the estimated tissue is functional.

Necrotic spots invade the conductive tissue. Cotyledons do not develop nor expand normally and less than half of the estimated tissue is functional.

Necrotic spots invade the conductive tissue completely. Cotyledons do not develop nor expand normally and deformed.

Necrotic spots invade the conductive tissue completely. Cotyledons do not develop nor expand normally and deformed severely.
In 2008 Workshops in conjunction with Moisture, Purity and Tetrazolium Committees have been held in Cordoba, Nakuru and Taichung.

The seed testing stations of Augustenberg and Jena will be organising a joint Germination and Tetrazolium workshop prior to the 2010 ISTA Congress in Cologne.
Any Questions
A few words of thanks

The Germination Committee

The Secretariat