Report from the Germination Committee

Sylvie Ducournau

Committee report 2013 - 2014
Agenda

• Committee membership

• Working programme 2013 – 2016 and progress in 2014
  • Method validations
  • Guidance / Handbook
  • Specific projects

• Rules changes proposals
<table>
<thead>
<tr>
<th>Position</th>
<th>Name</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chair</td>
<td>Sylvie Ducournau</td>
<td>France</td>
</tr>
<tr>
<td>Vice-Chair</td>
<td>Gillian McLaren</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>Members</td>
<td>Ignacio Aranciaga</td>
<td>Argentina</td>
</tr>
<tr>
<td></td>
<td>Sarah Dammen</td>
<td>United States</td>
</tr>
<tr>
<td></td>
<td>Ronald Don</td>
<td>United Kingdom</td>
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<tr>
<td></td>
<td>Fabio Gorian</td>
<td>Italy</td>
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<td></td>
<td>Christine Herzog</td>
<td>Switzerland</td>
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<td></td>
<td>David Johnston</td>
<td>United States</td>
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<td></td>
<td>Andrea Jonitz</td>
<td>Germany</td>
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<td>Jin Wook Kim</td>
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<td>Augusto Martinelli</td>
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<td></td>
<td>Lea Mazor</td>
<td>Israel</td>
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<td></td>
<td>Harry Nijenstein</td>
<td>The Netherlands</td>
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<td></td>
<td>Takayuki Okuda</td>
<td>Japan</td>
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<td></td>
<td>Rita Zecchinelli</td>
<td>Italy</td>
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</table>
Validation of methods

• Use of soaking treatment for *Momordica charantia* seed germination (Yu Sung)
  • 3 laboratories, 3 samples
  • Soaking of the seeds for 10 minutes in water at 60°C
  • Germination in BP, 30°C, 4 – 14 days

• 1 lab disinfected seeds
• very variable results for lot 2
• variable results for soaking method

◊ New experiment with new samples and change of one lab

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Validation of methods

• A germination method for *Carica papaya* (Maggie Lin)
  • 7 laboratories, 3 samples
  • Methods tested: S and BP; 20°C–30°C; no soaking – soaking in water for 16h – soaking in water + GA₃

• 1 lab has lower results
• Best methods: Sand GA3_Water soaking, BP_No soaking and Sand_Water soaking

 İn progress
Validation of methods

• A germination method for *Chenopodium quinoa* (Ignacio Aranciaga, Lesly Gonzales)
  • 6 laboratories, 3 samples
  • Methods tested: TP and BP; 20 and 20°C – 30°C; no pre-treatment – KNO₃ – prechilling
  • Results to be statistically analyzed

• Agar substrate for germination method (David Johnston)
  • Work in conjunction with the FTS COM
  • AOSA/SCST Study for validating phyto agar for corn and tomato germination has been completed (results presented in the open GERCOM meeting)
Revised method for germination of
*Dactylis glomerata* & *Festuca arundinaceae*

Harry Nijënstein
- Start situation
- Test plan
- Results
- Conclusions
### Start situation

<table>
<thead>
<tr>
<th>Species</th>
<th>ISTA-rules</th>
<th>temp.</th>
<th>first count</th>
<th>final count</th>
<th>Recom. Breaking dormancy</th>
<th>AOSA</th>
<th>temp.</th>
<th>first count</th>
<th>final count</th>
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</thead>
<tbody>
<tr>
<td>Dactylis glomerata</td>
<td>TP</td>
<td>20 &lt;=&gt; 30; 15 &lt;=&gt; 25</td>
<td>7</td>
<td>21</td>
<td>KNO3; Prechill</td>
<td>P; TS</td>
<td>15 &lt;=&gt; 25</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Festuca arundinacea</td>
<td>TP</td>
<td>20 &lt;=&gt; 30; 15 &lt;=&gt; 25</td>
<td>7</td>
<td>14</td>
<td>KNO3; Prechill</td>
<td>P</td>
<td>20 &lt;=&gt; 30; 15 &lt;=&gt; 25</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Festuca pratensis</td>
<td>TP</td>
<td>20 &lt;=&gt; 30; 15 &lt;=&gt; 25</td>
<td>7</td>
<td>14</td>
<td>KNO3; Prechill</td>
<td>P</td>
<td>20 &lt;=&gt; 30; 15 &lt;=&gt; 25</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>x Festulolium</td>
<td>TP</td>
<td>20 &lt;=&gt; 30; 15 &lt;=&gt; 25; 20</td>
<td>5</td>
<td>14</td>
<td>KNO3; Prechill</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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</table>
Test plan

<table>
<thead>
<tr>
<th>Species</th>
<th>substrate</th>
<th>°C</th>
<th>days first count</th>
<th>days second count</th>
<th>days final count</th>
<th>days/°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dactylis glomerata</td>
<td>TP</td>
<td>20 &lt;=&gt; 30; 15&lt;= 25</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>2d 7°C</td>
</tr>
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<td>Festuca arundinacea</td>
<td>TP</td>
<td>20 &lt;=&gt; 30; 15&lt;= 25</td>
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<td>10</td>
<td>14</td>
<td>2d 7°C</td>
</tr>
</tbody>
</table>

8 participating laboratories (7 countries, 3 continents)
4 seed lots per species
- *Dactylis glomerata*: 79-94%
- *Festuca arundinacea*: 83-97%
## Results

### a. F. arundinacea

<table>
<thead>
<tr>
<th>Lot</th>
<th>10 days</th>
<th>14 days</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>84.6%</td>
<td>90.4%</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Lot 2</td>
<td>71.3%</td>
<td>74.5%</td>
<td>0.1167</td>
</tr>
<tr>
<td>Lot 3</td>
<td>94.0%</td>
<td>95.1%</td>
<td>0.0933</td>
</tr>
<tr>
<td>Lot 4</td>
<td>52.9%</td>
<td>61.7%</td>
<td>0.0015</td>
</tr>
<tr>
<td>Overall mean</td>
<td>79.8%</td>
<td>84.4%</td>
<td>0.0138</td>
</tr>
</tbody>
</table>

### b. D. glomerata

<table>
<thead>
<tr>
<th>Lot</th>
<th>14 days</th>
<th>21 days</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>82.4%</td>
<td>86.7%</td>
<td>0.0023</td>
</tr>
<tr>
<td>Lot 2</td>
<td>88.5%</td>
<td>90.5%</td>
<td>0.0504</td>
</tr>
<tr>
<td>Lot 3</td>
<td>94.4%</td>
<td>94.5%</td>
<td>0.7528</td>
</tr>
<tr>
<td>Lot 4</td>
<td>89.0%</td>
<td>94.2%</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Overall mean</td>
<td>89.6%</td>
<td>92.0%</td>
<td>0.0098</td>
</tr>
</tbody>
</table>

Bigger differences for:
- Lower quality samples
- Lower temperature
Results

Differences in germination between 21 and 14 days in *D. glomerata* were, on average, 2.7% and 2.9% for low and high temperatures respectively.

In *F. arundinacea* these differences were 6.6% and 3.2% respectively. Individual samples differed up to 11.6% in *F. arundinacea* and up to 5.1% in *D. glomerata*.

Repeatability and reproducibility were similar for the last two counts in *D. glomerata*, and were at acceptable levels.

In *F. arundinacea* only reproducibility at the final count after 14 days was at an acceptable level; second count reproducibility was not at an acceptable level.

Repeatability for *F. arundinacea* was at an acceptable level.
Conclusion

Because of the sometimes big differences between the two last counts in both species it is suggested to keep the duration for the germination tests of *D. glomerata* and *F. arundinacea* at the present level in the ISTA Rules.
Thank you for your attention

www.seedtest.org
Guidance / Handbook

- Document on soybean seedling evaluation (Ignacio Aranciaga)
  - A complete guidance with photos
  - Questionnaire sent to the committee members to gather comments / proposals
  - Proposals to be finalized regarding
    - specific defects such as the development of secondary roots
    - defects affecting other species

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Guidance / Handbook

• **Amendments to the HSE**
  • A description of counting “by hand”
  • A description of methods for seed disinfection
  • In Section 18: evaluation of loops and spirals on hypocotyl
  • In Section 18: evaluation of root system of ornamental and flower species of *Lupinus*
  • **Water Retention**: correction of formulas, change of the duration of the draining (from 12 to “a minimum of 16 hours to a maximum of 24 hours”), importance of the water retention measurement for germination testing
  • **Cleanliness and Innocuity**: the germination of sensitive species has to be evaluated after the final count
Specific projects

- Storability of KNO₃ and GA₃ solutions
  - Question raised by an ISTA laboratory before an audit
  - Different practices exist in laboratories (storage temperature, darkness vs. light, storage duration)
  - Difficulty in conducting experiments
  - AOSA has proposed a guidance to be added in the Rules

<table>
<thead>
<tr>
<th>Solution</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA₃</td>
<td>Store in sealed containers and keep in the dark, at 2-5°C. Solutions usually store poorly, so it is best to use within 2-5 days from preparation. Alternatively, stock or final solutions can be stored frozen (-20°C) for 1-2 years.</td>
</tr>
<tr>
<td>KNO₃</td>
<td>Should be kept in tightly closed, dark containers, at room temperature. When properly stored, solutions have a shelf life up to 5 years</td>
</tr>
</tbody>
</table>

Same guidance for ISTA? Short duration for GA₃ and long for KNO₃ compared to some common practices in ISTA laboratories
Specific projects

• Species for media toxicity checks
  • During audits, it has appeared that laboratories had difficulties in finding species listed above in their country.
  • The Germination Committee has thus suggested to send a survey to the ISTA member laboratories in order to:
    - Get information on how these checks are carried out by laboratories
    - Extend the list of sensitive species that could be used for media toxicity checks.
  • The species that are known to be sensitive to toxic substances are: *Agrostis gigantea, Eragrostis curvula, Festuca rubra, Hordeum vulgare, Lepidium sativum, Petunia* sp. and *Phleum pratense*.
  • “When the media is to be used for the testing of a limited number of species, for example *Hordeum vulgare* or *Zea mays*, the phytotoxicity tests can be carried out using these species rather than two sensitive species.”

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Specific projects

• Species for media toxicity checks
  • Toxicity checks are done by laboratories
    • On one to 6 different species
    • On sometimes different species depending on the substrates
  • Most common species used:
Specific projects

• Species for media toxicity checks
  • The sensitive species are not available in all countries
    • Difficult to find them in tropical countries
    • Laboratories have to buy seeds (problems of cost, time and quality)

• Suggestions for other sensitive species
  • Species listed in AOSA Rules: Lactuca sativa, Apium graveolens, Sorghum bicolor, Sesamum indicum, Zea mays
  • Species listed in Brazilian Rules: Allium cepa, Apium graveolens, Cichorium intybus, Lactuca sativa, Solanum lycopersicum
  • Other suggestions: Helianthus annuus, Capsicum annuum, Trifolium spp., Brassica spp., Sinapis alba...
Specific projects

• Other projects
  • Image analysis in germination
    • Seedling evaluation of grass seeds in BP
  • Developing tests based on “usable seedlings” for Young Plant Production Sector

 назначенов

Presentations in the Open Meeting of the GERCOM

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Rules changes proposals
Chapter 5: The germination test

• Possibility of testing 200 seeds for germination in case of BIC only.

5.6 Procedure

5.6.1 Working sample

The ISTA germination test is based on 400 seeds...

At the sender’s request, germination test can be carried out on 200 seeds, for BIC only, even if the sample is not composed of expensive seeds.

In this case, the number of seeds tested is less than 400, and has to be reported under “other determinations” as mentioned in 5.9.
Rules changes proposals
Chapter 5: The germination test

• Clarifying procedures for counting errors during the germination test (5.6.1)

5.6 Procedure

5.6.1 Working sample

... When more than ± 5 seeds (i.e. ± 1.25% on a total of 400 seeds) are tested for germination, due to counting error, then the test must be repeated.
Rules changes proposals
Chapter 5: The germination test

• Retesting

• Proposal is made to allow the option of a different test method to be used for a retest when replicates exceed the allowable tolerance.
• Another proposal is made to clarify the reporting of the results of a germination test when a retest has been made. The proposal is to indicate that in circumstances a), b), c) and e) only the best result has to be reported and that the report of the results of the other tests are not necessary unless the applicant requires them.
Rules changes proposals

Chapter 5: The germination test

• Evaluation, calculation and reporting of pellets “without seedlings”

11.5.6.5 Evaluation

... 

Pure pellets may not produce any seedling at the end of the test period. These pellets “without seedlings” can be evaluated as:

- Hard seeds: when ungerminated pellets include hard seeds (see 5.2.10)
- Fresh seeds: when ungerminated pellets include fresh seeds (see 5.2.10)
- Dead seeds: 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.
Workshop

• Germination Workshop – Estonia – 8-10 June 2016
  • Seed Testing Laboratory of the ARC (Agricultural Research Center) in Saku
  • 20 participants
  • Draft programme
    • Principles of the germination test
    • Seedling evaluation (cereals, legumes, grasses)
    • Results calculation and reporting
    • Quality management in germination testing
Thanks to
- All the members of the committee
- The working group leaders
- Our liaison officer
- The Statistics committee
- The ISTA Secretariat

Thank you for your attention