ISTA PURITY COMMITTEE
REPORT 2012

PROGRESS IN 2012 AND FUTURE GOALS

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Jane Taylor
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COMMITTEE MEMBERS

1 CHAIR: Adriel Garay, USA
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3 Myriam Alvisi, Brazil
4 Pernilla Andersson, Sweden
5 Gerarda de Boer, Netherlands
6 Maria Duter, New Zealand
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8 Charan Jeet Mehta, India
9 Andrea Jonitz, Germany
10 Augusto Martinelli, Argentina
11 Deborah Meyer, USA
12 Kepha Oganda, Kenya
13 Andreas Ratzenboeck, Austria
14 Zita Ripka, Hungary
15 Ruojing Wang, Canada
A1. Development of New Methods:

Uniform Blowing Procedures: There is interest of ISTA members to adopt the AOSA method.

Goals: Finish comparing AOSA and ISTA procedures and make rule proposal in 2013

Working Group Leader: Gerarda de Boer;
Collaborators: Ruojing Wang, Adriel Garay, Pernille Hvolbol Jensen, Dot Vittrup Pederson
Variability in Calibration Samples
Results with Master Calibration Samples

- Uniform separation of pure seed and inert matter of ten orchardgrass samples with different levels of inert matter content across seven blowers using the air velocity calibration method.
Features of AOSA Procedure

a) *Uses MCS to calibrate blower*
Positive Feature of AOSA procedure

\(b\) Uses air velocity for daily monitoring of correct point.
Basic Principle

- Use the optimum blowing point established during the blower calibration to set the air-gate opening.

- Pure seed (heavy fraction) is separated from the inert matter (light fraction) by air flowing through the blower tube.

Purity working sample of orchardgrass.

After UBP, pure seed (heavy fraction) on left and inert matter (light fraction) on right.
### Comparison of AOSA-ISTA Calibration Samples: Orchardgrass calibration samples

<table>
<thead>
<tr>
<th>System</th>
<th>Date cal. sample production</th>
<th>Wt. sample (g)</th>
<th>Air-gate opening</th>
<th>EAV (m/s)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISTA - SCS</td>
<td>2009</td>
<td>2.9413</td>
<td>13.45</td>
<td>2.5 0.1</td>
<td>ISTA calibration sample Ref. #4 6</td>
</tr>
<tr>
<td>AOSA - MCS</td>
<td>2010</td>
<td>3.1943</td>
<td>15.1</td>
<td>3.0 0.1</td>
<td>Received from AOSA June 2010 Ref. 14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>System</th>
<th>Date of cal. sample production</th>
<th>Wt. sample (g)</th>
<th>Air-gate opening</th>
<th>EAV (m/s)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISTA - SCS</td>
<td>1985</td>
<td>3.1120</td>
<td>14.1</td>
<td>2.1</td>
<td>Monthly calibration requirement using SCS takes time and not frequent enough</td>
</tr>
<tr>
<td>AOSA - MCS</td>
<td>2006</td>
<td>3.1955</td>
<td>16.1</td>
<td>2.7</td>
<td>Using EAV monitor the blower daily. The point has not changed in 5 years.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>System</th>
<th>Date of cal. sample production</th>
<th>Wt. sample (g)</th>
<th>Air-gate opening</th>
<th>EAV (m/s)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISTA - SCS</td>
<td>2007</td>
<td>3,044</td>
<td>15.4</td>
<td>2.3</td>
<td>Cal Sample #39</td>
</tr>
<tr>
<td>AOSA - MCS</td>
<td>2002</td>
<td>3,189</td>
<td>17.8</td>
<td>2.7</td>
<td>MCS #20</td>
</tr>
</tbody>
</table>
Difference AOSA-ISTA Calibration Samples on Inert Assessment of *Orchardgrass samples*
Germination of OG with AOSA and ISTA calibration samples
Current Actions

• New ISTA blower calibration samples are now available to service urgent needs.

• Additional data should be available during 2012 to make final decisions.

• The work is aimed is to harmonising the ISTA and AOSA calibration samples and to have a user friendly procedure for member labs.
Handbook on Tropical Species:

• Project Team: Myriam Alvisi in the Ministry of Agriculture in LASO-Minas Gerais in cooperation with Drs. Angela Tillman and Doris Groth of UFPel, Brasil and Augusto Martinelli in Argentina, Kepha Oganda, Kenya

• List will include approximately 75 species
• Images of tropical seed species
• Expected completion date is 2013 or 2014
Example of Image Quality

*Brachiaria brizantha*
**SPECIES DETAILS**

**Latin name:** *Sida cordifolia* L.

**Family:** Malvaceae

**Dispersal units:** mericarp (segment of fruit with one seed)

**Size:** 3,0 - 3,5 mm (except awns)

**Description:**
Mericarp trigones, 3,0-3,5 x 2,2-2,3 x 1,2-1,5mm, dull, faces and dorsum straw yellow with slightly darker sculptures. Long awns and apical cleft pale to redish-brown. Botanical seeds, trigone globosas, 2,0 - 2,2 x 1,5-1,7 x 1,0-1,1mm, redish brown to dark brown.

**Remarkable characteristics:**
Long awns (2.5 to 2.8 mm) and divergent, with the simple long, white-translucent.

**Importance:** weeds of pastures of different cultures, being too aggressive in fertile soils.

**Origin and Distribution:** native to tropical America, now distributed in many tropical and subtropical regions of the world. In the Americas occurs in the southern U.S. to Argentina. In Brazil it occurs in all states, with higher concentrations in the Amazon, Minas Gerais, São Paulo and northern Paraná.

**Synonyms:** *Sida rotundifolia* Lam; *Sida herbaceae* Dias, *Sida tomentosa* Vell.; *Sida multiflora* Cav.
Jane Taylor, UK
Rule proposals for voting 2012

1. Addition of *Solanum nigrum* to Table 2.A, Part 1
2. Moving *Arachis* from PSD 11 to PSD 21
3. Modification to PSDs 11 & PSD 20-24 re wording on broken / separated cotyledons contained within the testa.
4. Modification to PSD 36, new wording ‘with or without pedicel’.
5. Clarification of wording/cross referencing to Chapter 13, Table 3B Part 1 & Table 5.A, Part 1.
A2. Introduction of New Species

1: Addition of *Solanum nigrum* to Table 2.A, Part1

*Solanum nigrum*

added to germination Table 5.A, 01/01/2012 but not Table 2.A.  
*Solanum* = PSD 10, non chaffy.

<table>
<thead>
<tr>
<th>Species</th>
<th>Max. weight of lot (kg)</th>
<th>Min. submitted sample (g)</th>
<th>Min. Working samples (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum nigrum</em> L.</td>
<td>10 000</td>
<td>25</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Other seeds by number</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4.5.1)</td>
</tr>
</tbody>
</table>

Thanks to purity committee members (Kenya, India, UK) for carrying out the work to collect the 1000 seed weight data.
A2. Introduction of New Species

Other species previously put forward by a WG on tropical species but on which the purity committee has no seed or data:

*Cleome gynandra, Bupleurum rotundifolium, Carica papaya, Passiflora edulis.*

**Purity WG Leader:** Deborah Meyer

**Collaborators:** Kepha Oganda, Augusto Martinelli, Charan Jeet Mehta, Jane Taylor, need more helpers & liaise with GER & MOI

Germination committee now working on *Chenopodium quinoa*. Augusto Martinelli is collecting data on 1000 seed weight.
2: Moving *Arachis* from PSD 11 to PSD 21

*Why change?*

PSD 11 is:

• Time consuming.

• Not always reproducible among seed analyst.

• The amount of inert matter can be high e.g. more than 25%.

• Damage caused to seed when removing pod?
Purity Analysis

PSD 11
69.8% pure seed

PSD 21
97.9% pure seed
3: Modification to PSDs 11 & PSD 20-24

Why change the wording?

• Broken / separated cotyledons contained within the testa seem to be a particular problem for *Glycine max*, (PSD 11)

• Topic discussed in 1997 and raised again 2010 & 2011 by members seeking clarification on definition of how to assess broken/separated cotyledons contained within the testa.

NEW WORDING

*Fabaceae: cotyledons that are broken apart but held together within the seed coat are regarded as pure seed.*

Images from 1997 ISTA workshop, Hungary
3: Modification to PSDs 11 & 20-24

PSD 11

- Seed, provided a potion of the testa is attached.
- Piece of seed larger than one-half the original size, provided a portion of the testa is attached.
- *Fabaceae*: cotyledons that are broken apart but held together within the seed coat are regarded as pure seed.
- Seeds and pieces of seed entirely without testa are regarded as inert matter.
- *Fabaceae*: separated cotyledons are regarded as inert matter irrespective of whether the radicle-plumule axis and/or more than half of the testa is attached.
4: Modification to PSD 36, new wording ‘with or without pedicel’.

Why change the wording?

• In *Panicum maximum* PT 2006:
  • Only 10% of the laboratories detached the pedicel when it was attached to the spikelet
  • Slight change in percentage of inert matter:
    rachilla detached = 1.5% IM
    rachilla not detached = 1.4% IM
  • Purity workshop, Cordoba 2008 Suggestion raised by participants from laboratories experienced in *Panicum* analysis

NEW WORDING PSD36:
Spikelet, **with or without pedicel**, with glumes, lemma and palea enclosing a caryopsis, plus attached sterile lemma.
5: Clarification of wording/cross referencing to Chapter 13, Table 3B, Part 1 & Table 5.A, Part 1.

Why amend the wording & cross referencing?

In Table 3B part 1 – PSD numbers by genus, it is not obvious that under Chapter 13 testing weighted replicates is allowed.

This proposal will add cross referencing to Table3B Part1 and Table 5A Part 1 and clarify in Chapter 13 that a purity test is not normally performed unless requested by the applicant.
5: Clarification of wording/cross referencing to Chapter 13, Table 3B, Part 1 & Table 5A, Part 1.

Table 3B Part 1.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Family</th>
<th>PSD number</th>
<th>Chaffiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betula</td>
<td>Betulaceae</td>
<td>53 (seed also Chapter 13)</td>
<td>C</td>
</tr>
<tr>
<td>Chloris</td>
<td>Poaceae (Gramineae)</td>
<td>42 (seed also Chapter 13)</td>
<td>C</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>Myrtaceae</td>
<td>60 (seed also Chapter 13)</td>
<td>C</td>
</tr>
</tbody>
</table>

Table 5BA Part 1. Additional advice (column 8)
Testing by weighted replicates also allowed (Chapter 13 Table 13B)
Workshops and Seminars

• ISTA workshop on Purity analysis, OSD & seed ID, Hosted by CFIA, Saskatoon, Canada, September, 2011
• 27 participants from 6 countries
• Presenters: Jane Taylor and Deborah Meyer, Roujing Wang, Steve Jones
• Many thanks to local organizer Roujing Wang & Steve Jones and to all the staff who put in so much work in preparing the material.
• Programme covered *Orobanchaceae* testing methods, seed identification of large seeded *Fabaceae* species and also small seeded *Fabaceae* where retrieval and identification problems had been noted in proficiency tests.
• Future workshops? exploring potential hosts 2013/2014
Questions to purity committee 2011/2012

- Weighing of the purity components fractions - different numbers of decimal places, or all the same for all components?
- Clarification regarding 3.5.2.4 Indistinguishable species – how to decide when to use option (a) or (b),
- Reporting of the result of an examination for *Orobanche* seeds
- Which weight to use for *Cucurbita pepo* F1 hybrid
- Soybean grading why visual characteristics, split testa etc are mentioned but not Oil content, FFA, protein in grading requirements
- Blowing procedure for small seeded varieties *Poa Pratensis* <0.35 not listed in ISTA rules. Procedure how to add a new variety name to the list in ISTA Rules
Questions to purity committee 2011/2012

• Seed Identification help requested for differentiation between *Elymus trachycaulus* and *Elytrigia repens*
• Reply to clarify ISTA rules, PSD and assessment of Cotyledons that are broken apart but held together by the seed coat in *Glycine*
• Interpretation of an ISTA rounding procedures from 3rd and 4th place figures
• Query re ISTA stabilized list and reporting correct names re word ‘where applicable’
• Query re statements for OSD ‘complete tests’
Publications on specific seed testing topics

• Handbook on Purity testing, is in discussion

• Identification of Species of the Universal List 2013. WG leader: Andrea Jonitz; Collaborators: Rita Zipka, Augusto Martinelli and Ruojing Wang

• Handbook on Tropical Species: Seed Identification and Seed Technology.
  WG leader: Myriam Alvisi; Collaborators: Kepha Oganda, Augusto Martinelli
New Technologies for Purity Testing (purity separations and other seed determinations)

• Aim:
  a) To spread information of techniques or equipment used in some laboratories which might be of interest also for others.
  b) To get to know what kind of techniques/equipment the laboratories would like to use in the future.

• The survey was distributed by the ISTA secretariat on behalf of the Purity Committee.

• It was sent to all ISTA member laboratories, 210 labs.

• Answers were received from 57 laboratories.
Question 1:
Is your laboratory using equipment, a procedure or technique, which in your experience is a new technology, or which you have acquired in the last 10 years?
Would that new technology be helpful in other laboratories?
Answers to question 1

- Techniques for image analysis: Microscope connected to a digital camera, seed scanner for cereals et.c.
- Molecular and Biochemical methods: SSR markers, SDS-page, A-page
- Uniform Blowing Procedures
- X-ray analysis
- Diaphanoscope or similar equipment
- Adjustable oculars on binocular
- Use of acid to de-lint cotton
Digital microscope camera
Seed scanner

- Used for cereals
- Can be loaded with up to 30 samples
- The scanner sorts the seeds into *good seeds* and *questionable seeds*
- Around 10% of the sample goes into the fraction questionable seeds
Illuminator stand with LED transmitted light mounted to a microscope
Seeds of *Festuca rubra*

As seen in microscope with light from above

As seen in microscope with LED transmitted light from below
Uniform blowing procedure

• The most usual ones separate the sample into two fractions, light and heavy fraction.
• The following pictures show a more advanced continuous blower with a built-in anemometer.
• This blower can separate the sample into three fractions.
Continuously Flowing Blower:
Can calibrate air velocity
Works with any seed size
Can use large seed samples
Example of Separations in Clover seeds:

a) Light-weight immature, damaged seeds
b) Medium-weight seeds
c) Heavy weight- select seeds
Separation with Continuous Blower:

A: select seeds, high planting value
B: rejected seeds, no planting value
Purity Testing using Ergovision System

Advantages:
- Ergonomic work position
- High quality focus-resolution
- Fiber optic light
- High output
Digital X-ray to distinguish filled pure seeds from unfilled inert structures
2. Wish list for future purity testing: What critical technologies do you think we need to develop or incorporate into purity testing in the future?
Answers to question nr 2

- Seed scanner: 20 laboratories would like some kind of seed scanner. They want to use it for different species such as
  - Tree species
  - Maize and cereals
  - Peas and beans
  - Species of *Trifolium*, *Medicago* and *Vicia*
  - *Brassica* (to find *Galium aparine*, *Sinapis arvensis* and *Brassica rapa*)
Molecular and biochemical techniques:
• Microarrays to detect the presence of weeds
• SSR markers and other DNA markers
• DNA based fingerprinting for precise genetic identification of seeds
• Species identification based on shared DNA sequences (using a database)
• Biochemical markers, mono- and polyclonal antibodies et.c.
Answers to question 2 cont.

Answers connected to Seed blowing:

• Digital calibration of blowers so that it is not necessary to use calibration samples

• An anemometer

• Guidelines for blowing of Festuca rubra/ovina/lemanii

• A different method for examining Poaceae for the presence of caryposis without having to use the blower and calibration samples.
Answers to question 2 cont.

• A seed collection containing all species on "ISTA Universal List of Species"
• A large online seed collection published on ISTA website and available through password to all ISTA member labs
• Simple machines for de-linting cotton seed instead of using acid
• X-ray imaging to separate between seeds with caryopsis and empty seeds.