# Seed Testing

# ISTA News Bulletin No. 129 April 2005





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# Seed Testing



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### www.seedtest.org

# Editorial

By Michael Muschick, ISTA Secretary General

Dear Reader,

Only a few weeks before the ISTA Ordinary Meeting in Bangkok, Thailand, the preparations for this important event are going very well and all documents relevant to the meeting have been distributed to the membership and are also available on the ISTA Website:

- the Minutes of the Ordinary Meeting 2004,

- the Proposed Changes to the ISTA Constitution,

 the Proposed Rules Changes 2005 including the Editorial Merger of Chapter 2 as separate document, and Supporting Data and Evidence for the Proposed Rules Changes 2005 - Item 4B and ISTA Method Validation Reports, Volume 2005/1,

- the Activity Report 2004 of the International Seed Testing Association.

In Bangkok, as in the years before, we will have a large international participation. To date 97 delegates from 42 different countries have registered. However, not only the participants are very interesting, but also the programme. In this issue of Seed Testing International you will find the final programme of this meeting as well as the proposed constitution changes, which will be voted on during the Ordinary Meeting. I am looking forward to personally welcome you in Bangkok at this important meeting.

As in the issues before, GMO testing again takes a large portion. A summary of the results of the  $3^{rd}$  ISTA Proficiency Test on GMO testing on *Zea mays* is reported on as well as the results of the  $4^{th}$  ISTA Proficiency Test on GMO testing on *Glycine max* and the announcement for the upcoming  $5^{th}$  ISTA Proficiency Test on GMO testing. But also you will find workshop reports on the workshop on statistical aspects of GMO detection in the US and the ISTA/FAO workshops in Vietnam and Egypt.

Proficiency testing is an important part of



ISTA's activities, not only in GMO testing, but also in a number of other seed testing areas. In this issue you will find valuable information on the ISTA Tetrazolium and Moisture Proficiency Test.

Also seed health testing is a very important task, with major influence on the seed trade today.

In this issue you will find the programme of the 5<sup>th</sup> ISTA Seed Health Symposium to be held in Angers, France. Furthermore read the interesting article on the Distribution of Tilletia tritici and Microdochium nivale in wheat seed bulks, and significance for seed sampling strategies.

All these activities could not be performed without the active input from the ISTA Technical Committee Chairpersons and the Technical Committee Members. On page 25 you will find a short introduction on some of these persons, namely those who have newly taken over the chairmanship for an ISTA Technical Committee at the ISTA Congress 2004 in Budapest.

I certainly hope that you will find a lot of interesting and helpful information from the world of seed testing in this issue of Seed Testing International. Your comments and your proposals to continuously enhance our journal are most welcome.

And with the topic I started - the ISTA Ordinary Meeting 2005 - I would also like to close this editorial. Do not miss the fascination of Bangkok and of Thailand as well as the interesting and important discussions and decisions at our Ordinary Meeting.

I am looking forward to meeting you there.

### Yours sincerely, Michael Muschick

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The Final Programme for the ISTA Ordinary Meeting 2005 is now available

We are looking forward to a most interesting ISTA Ordinary Meeting 2005 in Bangkok, Thailand

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ISTA Proficiency Tests on GMO.

Read about the summary of results of the  $3^{rd}$  and  $4^{th}$  Proficiency Test on GMO

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Read about the validation of the controlled deterioration vigour test for small seeded vegetables.

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5<sup>th</sup> ISTA Seed Health Symposium in Angers, France

The final programme is available now

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# **President's Report**

### By Pieter Oosterveld, ISTA President

Within a few days we hopefully will meet in Bangkok. A historical meeting we could say, because it is the first time that ISTA holds an Ordinary Meeting in Asia. I myself remember Thailand as a very interesting and pleasant country with a rich history and a beautiful nature. In April 1999 the Executive Committee met in the northern town of Chang Mai. I remember that we had some interesting discussions on the experiment of accreditation of company laboratories. The discussions were quite heavy, but finally we agreed on the installation of the experiment. Last year in Budapest we discussed on the results of this experiment and made some very important decisions.

In my report of October 2004, I referred to more important discussions and decisions of the Budapest Congress. Directly after the Congress the newly elected Executive Committee started the work for the next triennial period. Also the Technical Committees prepared the working programme (terms of references), which has been approved by the Executive Committee. The Working Programme (Terms of references) is published twice this time. Once as a separate standalone document and additionally in the Activity Report of the year 2004. In Bangkok the Committee Chairs will report about this programme and on the progress they made over the past period.

Last January, the Executive Committee held a three days meeting at the ISTA Secretariat in Switzerland. Most members were able to attend this meeting. The Secretariat had prepared the meeting in a very professional way, this allowed us to work very efficiently.



Regarding the update on the ISTA Constitution, the Executive Committee considered it important to further update and enhance the ISTA Constitution. Therefore a working group for the preparation of new proposals, especially focusing on the membership categories has been installed. The proposal must result in categories that are more in line with the constitution being aligned with the current subscription structure. The working group will come up with proposals for further consideration by the Executive Committee at the next meeting in Bangkok.

Some more about the meeting of the Executive Committee. We discussed the possible installation of an ISTA seed analyst training programme. Also we worked on a better financial structure for Seed Science and Technology (SST). The Executive Committee decided on the introduction of a page charge of CHF 25 per published page with a maximum of CHF 150 per article. Members will not be charged. Together with the Chief Editor of SST, the Secretary General will decide whether we continue with three volumes per year or bring the number back to two.

The Executive Committee decided to change the voting system during the Ordinary Meeting. Until now we counted each time all the votes. A very time consuming procedure. From now on the President will only ask for the 'yes' votes and the 'no' votes. When a clear majority appears, no counting will be done. In case of doubt, the President or a member can ask for a count. The new procedure will only be followed for simple majority voting.

The Executive Committee discussed the structure of future Congresses, taking into consideration, that the programme for the Congress in Brazil, 2007, has already been set in headlines and therefore can not be changed dramatically. The Executive Committee prefers a one week programme, starting on Monday and ending on Saturday. This programme will include the meetings of the Technical Committees (2 days), the Seed Symposium (3 days) and the Ordinary Meeting. The Executive Committee agreed that the Ordinary Meeting should not take more than 1.5 days, so the meeting can be closed on Saturday at noon. The Executive Committee will make a decision in the beginning of 2006.

Of course, the Executive Committee discussed also on technical matters. The GMO Task Force has prepared a final proposal for the meeting in Bangkok. The accreditation of GMO laboratories was one of the items still to be solved. Final conclusions on this matter



were made in October 2004. The Executive Committee took note of the ISO initiative, installing a working group on biomolecular testing, including seeds. The Executive Committee expressed its surprise on this development, because the ISO initiative obviously is overlapping the work already done by ISTA. ISTA will contact ISO and explain its position.

The working group on tropical seeds has started with a questionnaire in order to get a clear picture on the problems and the questions arising. Together with the working group the Executive Committee was disappointed by the poor response. Only 5 laboratories reflected to the questionnaire. Nevertheless, the working group continues the work and hopes that after this disappointing start more laboratories will participate.

The Executive Committee agreed on the approach of the Bulking and Sampling Committee concerning the amalgamation of the rules and annexes and agreed that the work of the Bulking and Sampling Committee will be the model for the other chapters. Furthermore, the Executive Committee took proper decisions on a number of questions of Technical Committees.

This short summary indicates, that the Executive Committee is active and in good mood to do the things that are of benefit to the Association and its members. The Executive Committee is looking forward to meeting you in all at the Ordinary Meeting in Bangkok.

See you in Bangkok,

### Your President, Pieter Oosterveld

### **MEMBERSHIP MEETINGS**

ISTA Ordinary Meeting 2005

# **ISTA Ordinary** Meeting 2005

Bangkok, Thailand April 25 - 28, 2005

### **FINAL PROGRAMME**

### **SUNDAY** APRIL 24, 2005 REGISTRATION

16:00 - 18:00	Registration of Participants at Sofitel Central Plaza Hotel						
MONDAY	April 25, 2005 SESSIONS						
Presentation of W	orking Programmes, activities and special						
projects							
08:00 - 18:00	Registration of Participants at Sofitel Central						
	Plaza Hotel						
08:45 - 09:00	Welcome by ISTA						
09:00 - 09:30	Bulking and Sampling Committee Session						
09:30 - 10:00	Purity Committee Session						
10:00 - 10:30	Germination Committee Session						
10:30 - 10:45	Coffee break						
10:45 - 11:15	Tetrazolium Committee Session						
11:15 - 11:45	Vigour Committee Session						
11:45 - 12:15	Moisture Committee Session						
12:15 - 13:15	Lunch						
13:15 - 13:45	Editorial Board Session (Seed Science &						
	Technology)						
13:45 - 14:15	Statistics Committee Session						
14:15 - 14:45	Seed Health Committee Session						
14:45 - 15:15	Proficiency Test Committee Session						
15:15 - 15:30	Coffee break						
15:30 - 16:00	Variety Committee Session						
16:00 - 16:30	Flower Seed Committee Session						
16:30 - 17:00	Forest Tree and Shrub Seed Committee Session						
17:00 - 17:30	Nomenclature Committee Session						
17:30 - 18:00	Seed Storage Committee Session						
TUESDAY	APRIL 26, 2005 SESSIONS						
Discussion on curr	rent important issues						
09:00 - 10:30	GMO Testing issues:						
10.00	1 Deport from the Exchange of Information						

GMO Testing issues:	12:00
1. Report from the Exchange of Information	13:00
Working Group	
2. Proposal for a Rules Chapter for GMO testing	
Coffee break	
3. Results of the ISTA GMO Proficiency Tests	
4. Accreditation of GMO testing laboratories	
Lunch	
Amalgamation of Rules and Annexes	
Accreditation Session	
Coffee break	14.30
Rules Committee Session	15:00
	<ul> <li>GMO Testing issues:</li> <li>1. Report from the Exchange of Information Working Group</li> <li>2. Proposal for a Rules Chapter for GMO testing <i>Coffee break</i></li> <li>3. Results of the ISTA GMO Proficiency Tests</li> <li>4. Accreditation of GMO testing laboratories <i>Lunch</i></li> <li>Amalgamation of Rules and Annexes</li> <li>Accreditation Session</li> <li><i>Coffee break</i></li> <li>Rules Committee Session</li> </ul>



### WEDNESDAY APRIL 27, 2005 ORDINARY MEETING

09:00 - 10:30 10:30 - 11:00 11:00 - 12:30	Opening Ceremony <i>Coffee break</i> Ordinary Meeting (Block 1) 1. Call to order 2. President's address 3. Roll call of Designated Members entitled to vote 4. Reading and acceptance of Minutes
12:30 - 13:30	Lunch
13:30 - 15:00	Ordinary Meeting (Block 2) 5. Report of the Executive Committee 5.1 Report of ISTA's Activities on Tropical Seeds 6. Report of the Secretary Caneral (incl.
	Presentation of Multi-Annual Budget)
15:00 - 15:30	Coffee break
15:30 - 17:15	Ordinary Meeting (Block 3)
	<ul><li>7. Constitution changes</li><li>8. Consideration and Adoption of the proposed Rules Changes 2005</li></ul>
19:30	Official Dinner
THURSDAY	APRIL 28, 2005 ORDINARY MEETING
09:00 - 10:30	Ordinary Meeting (Block 4) 8. Consideration and Adoption of the proposed Rules Changes 2005 [cont.]
10:30 - 11:00	Ordinary Meeting (Block 5)
11.00 - 12.00	8. Consideration and Adoption of the proposed Rules Changes 2005 [cont.]
12:00 - 13:00	Lunch
13:00 - 14:30	Ordinary Meeting (Block 6)
	<ul> <li>9. Announcement of the place and date for the next Ordinary Meeting of the Association</li> <li>10. Any other business raised by a Member, of which notice in writing has been received by the Secretary General two months prior to the date of the meeting</li> <li>11. Any other business raised by consent of the Executive Committee</li> </ul>
14:30 - 15:00	Coffee break
15:00 - 16:00	Ordinary Meeting (Block 7)
	<ul><li>12. President's closing address</li><li>13. Adjournment</li></ul>

Proposed Changes to the ISTA Constitution

# Proposed Changes to the ISTA Constitution

For consideration and decision at the Ordinary Meeting in Bangkok, Thailand, April 25 - 28, 2005

### INTRODUCTION

The Executive Committee of ISTA is suggesting to amend the ISTA Constitution and is therefore seeking approval by the Designated Members of proposed changes in the following sections:

**I.** The introduction of the fixation of the subscription fees for ISTA on an annual basis.

**II.** Changes of the publication of the statement showing the financial position of the Association.

**III.** Changes in the submission and notification period for ISTA Constitution Change Proposals.

At the upcoming Ordinary Meeting 2005 in Bangkok, Thailand, the Designated Members designated by their respective Designated Authority to vote on behalf of the Government in their country, will be asked to vote section by section.

In this document you will find the current text of the Constitution Article of concern in the grey box and the proposed new version of the same Constitution Article in the green box. In order to make the necessary changes in the Constitution in one section, it is possible that more than one Article of the Constitution needs to be changed. However, please keep in mind that voting will not be made Article by Article but as a package section by section.

Please take into consideration that according to ISTA Constitution Articles XII (a) and (b), modifications of the text presented in the Constitution Change Proposals can not be made during the Ordinary Meeting.

The Executive Committee of ISTA recommends all of the presented Constitution Change Proposals for adoption by the Designated Members entitled to vote. According to ISTA Constitution Articles IX (b) and XII (c), the motion to alter the Constitution requires for adoption at least a two-thirds majority of those Designated Members voting, provided a quorum is present.

The following changes and amendments of the current Constitution of the International Seed Testing Association are proposed for adoption at the next Ordinary Meeting of the Association to be held in Bangkok, Thailand, April 2005.

### I. PROPOSED CONSTITUTION CHANGES - FIXATION OF THE SUB-SCRIPTION FEES ON AN ANNUAL BASIS

**I. 1.)** It has been proposed to change Article XI (c) as follows:

Current Version:

### ARTICLE XI

### Finances

(c) The amount of the annual subscription for Designated Members, Members and Accredited Laboratories shall be determined for at least a three year period at an ordinary meeting of the Association, due to consideration being given to statements submitted in accordance with Article VII (c)(9) and paragraph (g) of this Article. Notification of proposals to change the rate of annual subscriptions shall be sent to the Designated Authorities, Designated Members and Members at least six months prior to an Ordinary Meeting.



Proposed Version:

### ARTICLE XI

### Finances

(c) The amount of the annual subscription for Members and <u>the additional fee</u> <u>for</u> Accredited Laboratories shall be determined <u>annually</u> at an ordinary meeting of the Association, due to consideration being given to statements submitted in accordance with Article VII (c)(9) and paragraph (g) of this Article.Notification of proposals to change the rate of annual subscriptions shall be sent to the Designated Authorities and Members <u>of</u> <u>the Association</u> at least <u>two</u> months prior to an Ordinary Meeting.

**Rationale for this change:** At the moment there is no difference between the membership subscription of a Designated Member and a Member. So, in this paragraph the mentioning of a Designated Member is superfluous.

The change to annual determination of membership subscription fees will give more flexibility and speed to the Association in regards to the finances.

By installing annual meetings with annual determinations of the membership subscription fees, a notification period of six months as fixed in the current ISTA Constitution is unworkable. In a 12 months circle a two months notification period seems to be appropriate. Also two months should be in today's business world sufficient time to discuss the proposal with the relevant bodies in the country.

**I. 2.)** It has been proposed to change Article XI (b) as follows:

MEMBERSHIP MEETINGS

Proposed Changes to the ISTA Constitution

Current Version:

### ARTICLE XI

### Finances

(b) The income of the Association shall be derived from annual subscriptions paid by Governments, Designated Authorities, Designated Members, or by Members, and from payments and donations received from persons, organisations, or governments for specified or general purposes.

Proposed Version:

### ARTICLE XI

Finances

(b) The income of the Association shall be derived from annual <u>membership</u> subscriptions, and from payments and donations received from persons, organisations, or governments for specified or general purposes.

**Rationale for this move:** To remain consistent with Article XI (c) and to remove subscription categories which do not exist.

**I. 3.)** It has been proposed to change Article X(e) as follows:

Current Version:

### ARTICLE X

Meetings of the Association

(e) The agenda for an ordinary meeting of the Association shall include:

 (1) Call to order.
 (2) President's address.
 (3) Roll call of Designated Members entitled to vote.
 (4) Reading and acceptance of Minutes.
 (5) Report of the Executive Committee.
 (6) Report of the Secretary General.
 (7) Consideration and adoption of reports.
 (8) A programment of the place and d

(8) Announcement of the place and date for the next ordinary meeting of the Association.

(9) Any other business raised by a Member, of which notice in writing has been received by the Secretary General at least two months prior to the date of the meeting.

(10) Any other business raised by consent of the Executive Committee.(11) President's closing address.

(12) Adjournment.

And additionally at the ordinary meeting held inthe third year after the ordinary meeting at which officers and membersat-large of the Executive Committee were appointed:

(13) Discharge of the Executive

Committee

(14) Election of Officers and membersat-large of the Executive Committee.

(15) Installation of new Officers.

(16) Fixation of annual subscriptions.

Proposed Version:

### ARTICLE X

### Meetings of the Association

(e) The agenda for an ordinary meeting of the Association shall include:

(1) Call to order.

(2) President's address. (3) Roll call of Designated Members entitled to vote. (4) Reading and acceptance of Minutes. (5) Report of the Executive Committee. (6) Report of the Secretary General. (7) Fixation of annual subscriptions. (8) Consideration and adoption of reports. (9) Announcement of the place and date for the next ordinary meeting of the Association. (10) Any other business raised by a Member, of which notice in writing has been received by the Secretary General at least two months prior to the date of the meeting. (11) Any other business raised by consent of the Executive Committee.

(12) President's closing address. (13) Adjournment.

And additionally at the ordinary meeting held inthe third year after the ordinary meeting at which officers and membersat-large of the Executive Committee were appointed:

(14) Discharge of the Executive Committee.

(15) Election of Officers and membersat-large of the Executive Committee. (16) Installation of new Officers. **Rationale for this move:** To be consistent with the proposed Constitution change XI (c).

### II. PROPOSED CONSTITUTION CHANGES - PUBLICATION OF THE STATEMENT SHOWING THE FINANCI-AL POSITION OF THE ASSOCIATION

**II. 1.)** It has been proposed to change Article XI (g) as follows:

Current Version:

### ARTICLE XI

### Finances

(g) A statement showing the financial position of the Association, examined and certified by the Auditor, shall be circulated annually to the Members of the Association and published in "Seed Science and Technology".

Proposed Version:

### ARTICLE XI

### Finances

(g) A statement showing the financial position of the Association, examined and certified by the Auditor, shall be circulated annually to the Members of the Association and published <u>in the "Activity Report of the ISTA Committees"</u>.

**Rationale for this change:** As 'Seed Science and Technology' (SST) today is a purely scientific journal, it is recommended to publish the financial statement in the "Activity Report of the ISTA Committees".

III. PROPOSED CONSTITUTION CHANGES - CHANGES IN THE SUB-MISSION AND NOTIFICATION PERI-OD FOR ISTA CONSTITUTION CHANGE PROPOSALS

**III. 1.)** It has been proposed to change Article XII (a) and (b) as follows:

Proposed Changes to the ISTA Constitution

Current Version:

### ARTICLE XII

### Amendments

(a) Any proposal to alter the provisions of this Constitution must be received in writing by the Secretary General at least <del>four</del> months prior to the date of the meeting of the Association at which it is to be considered.

Proposed Version:

### ARTICLE XII

### Amendments

(a) Any proposal to alter the provisions of this Constitution must be received in writing by the Secretary General at least <u>three</u> months prior to the date of the meeting of the Association at which it is to be considered.

**Rationale for this move:** The cutting of the notification period is necessary to come to a workable and appropriate time frame to allow for annual constitution changes.

Current Version:

### ARTICLE XII

### Amendments

(b) The Secretary General shall communicate any such proposal to each Member of the Association at least <del>three</del> months prior to the date of such meeting of the Association and shall maintain records showing evidence of such communications.

Proposed Version:

### ARTICLE XII

### Amendments

(b) The Secretary General shall communicate any such proposal to each Member of the Association at least <u>two</u> months prior to the date of such meeting of the Association and shall maintain records showing evidence of such communications.

**Rationale for this change:** The cutting of the notification period is necessary to come to a workable and appropriate time frame to allow for annual constitution changes.

# Documents concerning the ISTA Ordinary Meeting 2005

The following documents relevant for the upcoming ISTA Ordinary Meeting 2005 in Bangkok, Thailand have been finalised and distributed to the membership, and/or posted on the ISTA Website at <u>https://www.seedtest.org/en/content---1--1159.html</u>:

### **Final Programme of the Ordinary Meeting 2005**

Lays down all sessions and discussion points during the meeting

### **Minutes of the Ordinary Meeting 2004**

Contains the minutes and participants list of the last Ordinary Meeting held in Budapest 2004, to be adopted at the Ordinary Meeting 2005

### Activity Report 2004 of the International Seed Testing Association

Contains the Activity Reports 2004 of the Executive Committee, the Secretary General, the Editorial Board, the Proficiency Test Committee and the Rules Committee, as well as the Working Programme 2004 - 2007 of the ISTA Technical Committees, to be voted on at the Ordinary Meeting 2005

### **Proposed Changes to the ISTA Constitution**

Contains the proposed changes 2005 to the current ISTA Constitution, to be voted on at the Ordinary Meeting 2005

### Proposed Rules Changes 2005

Contains the proposed changes 2005 to the current ISTA International Rules for Seed Testing, to be voted on at the Ordinary Meeting 2005

# Editorial Merger of Chapter 2 of the International Rules for Seed Testing

Contains the first stage in the process of the Rules amalgamation, the merge of the Rules and Annexes of Chapter 2 on an editorial basis

# Supporting Data and Evidence for the Proposed Rules Changes 2005 - Item 4B

Contains a report of the results and statistical analysis of the comparative test in substrates for sunflower germination written by S. Ducournau and L. Wiesner. The comparative test was organised by a Working Group of the ISTA Germination Committee in order to decide if compost can be used as primary substrate for sunflower germination

### ISTA Method Validation Reports, Volume 2005/1

Contains the details of the method validation study of a new method for detecting *Xanthomonas hortorum* pv. *carotae* on carrot seeds written by M. Asma. This method validation study supports the rules proposal under item 5a: 'Modifications and additions to the validated seed health testing methods' of the Proposed ISTA Rules Changes 2005 (page 36) submitted by the ISTA Seed Health Committee

# Descriptive and Illustrated Collection of Weed Seeds

A new publication from GEVES\* The first seed-book collection

### By Liciniu Dragos, Maria Rosaria Mannino, Joël Léchappé

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### Introduction

The French 'Collection descriptive et illustrée des principales semences de mauvaises herbes' was first published in December 1999 by GEVES. Five years from its publication, the French version continues to be a success in France and is sold abroad (e.g. in Denmark, Germany, Spain, Switzerland and Chile). The English version is now available.

Every year about 17000 purity analysis and 11 000 determinations of other seed by number are carried out at the SNES. In the field of seed training, 60 seed technicians (analysts of private firms) and 80 students participate each year in the training sessions organised by the SNES. On the basis of this experience of seed analyses and training of seed analysts, we became involved with the creaof the 'Descriptive and Illustrated tion Collection of Weed Seeds' with the intention of creating a valid tool for seed laboratories. Seed specialists who carry out purity analyses can find this collection both interesting and useful, its utility being that it can be used not only for identification of weed seeds found in samples, but also for specialist training purposes. This work also represents today our Seed Reference Collection for our Quality Assurance System.

Work on a second volume, describing the seeds of other weeds in expansion, has been started.

### General remarks

The choice of species to be presented and described in 'Descriptive and Illustrated Collection of Weed Seeds' was made with the aim of covering the most common Western European species. The criteria for this selection were based on bibliographic data and the frequency with which these species are found in purity analyses carried out by the French National Seed Testing Station on seed lots produced in Europe and imported lots from outside Europe.

The seeds of the chosen species were obtained by collecting in the wild and from mutual exchanges of seed with some of the 140

international partners of the GEVES botanical garden. In case of seeds for which there was initially an insufficient quantity, these were cultivated in the botanical garden of GEVES.

In order to have a representative sample for each species, seeds from three different sources were generally used. The photographs were taken in such a way that any intraspecific variability is visible.

Finally, to ensure the correct species identification, the seeds were compared with the samples of the Seed Collection of the SNES (17000 samples of different species, created by Mr. Michel Kerguélen, an internationally famous botanist) and also with other seeds found in ISTA referee test samples and correctly identified by the laboratory.

The principal problem in the creation of the book was to find a practical means of presenting the seeds, their close-up photographs and the textual descriptions together on the same page, which would at the same time allow for rapid consultation without requiring a search in different sections of the book.

The best solution found was a book-type format, loose leaf binder using transparent storage sheets, each sheet containing seed samples, photographs and textual descriptions of 4 different species. The samples are stored in flat and rigid self-adhesive containers, with a round, resistant, soft-plastic window in the centre to hold the seeds.

### Description

This Collection Handbook, in A4 format and weighing 2.6kg (5.73lbs), presents and describes the seeds of 175 weeds from 38 botanic families. The book begins with 3 indices; the first with the botanic families represented (which is also the order of the book), the second with the scientific (Latin) stabilised names (genus and species) and the third with the English common names. These indices are followed by a glossary defining 125 botanic terms. Each species occupies one line of 3 pockets including the seed sample, the enlarged colour photograph and a botanic



description.

The dimensions of the seeds of the majority of species presented vary from 1 to 4 mm. The photographs are enlarged up to 10 times to allow the seed to be seen without a magnifying glass; this should avoid eye fatigue caused by peering at small seeds.

For seed comparison studies, the seed sample in its support can be removed from the pocket and observed on both sides by magnifying glass, microscope, profile projector etc.



For further information, you can contact the authors at the following addresses:

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\*GEVES: Groupe d'Etude et de Contrôle des Variétés et des Semences (Variety and Seed Study and Control Group)

\*\*SNES: Station Nationale d'Essais de Semences (French National Seed Testing Station)

Distribution of disease in wheat seed bulks

# Distribution of *Tilletia tritici* and *Microdochium nivale* in wheat seed bulks, and significance for seed sampling strategies

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### Introduction

The most important seed-borne diseases of wheat in the UK are Microdochium nivale, the cause of seedling blight, and Tilletia tritici, the cause of bunt. The former can cause significant loss of plant population in the autumn, whilst the latter can result in the complete loss of a crop due to the production of fishy-smelling bunt balls in place of the grain. The widespread prophylactic use of effective seed treatments has thus been viewed as a reliable way of preventing losses due to these pathogens. However, both surveys of seed stocks and results from advisory seed health tests have indicated that the levels of disease in seed intended for ware production are often very low (Cockerell et al., 2002), and a more sustainable approach to control might be achieved through a combination of seed health testing, and treatment where necessary. The absence of rapid and high through-put testing techniques has prevented the implementation of this approach, since the majority of wheat in the UK is autumn sown, with a very short period between harvest of the seed and drilling of the next crop. However, the development of PCR based techniques now offers an opportunity to overcome this problem, and the majority of wheat seed could be tested for seed-borne diseases in the near future.

The use of health testing and a treatment according to need strategy requires that a small submitted sample, probably of the order of 50 to 100 g is representative of the seed bulk from which it is derived. Sampling systems are needed which can adequately detect the risk from any pockets of high infection, and thus allow confidence limits to be attached to a test result. Little is known about how pathogens such as *M. nivale* and *T. tritici* are distributed in seed bulks. The work described here was undertaken to investigate this in bulks of varying sizes consisting of both certified seed for sale by mer-

chants, and farm processed seed saved by farmers for their own use.

### Materials and methods

### Commercial and farm processed seed sampling

A range of seed bulks from 10 t to about 500 t were sampled with either a 4 chambered "walking stick" sampler, used for smaller bulks, or a single chambered stick sampler capable of extending to a depth of 3 m. Each bulk was sampled at random points over the whole surface area, and at three different depths at each point. 40 primary samples were taken for most bulks, but 80 were taken from some larger ones. Some bulks were selected on the basis of results from samples that had previously been submitted for advisory tests at the OSTS, Cambridge, others were chosen from material made available by merchants and growers, but with no prior knowledge of their health status.

### Sample testing

Primary samples, each weighing about 200g, were processed individually using standard techniques to give working samples. Tests for *M. nivale* were performed by plating 200 seeds on to potato dextrose agar, incubating at 22 °C for 5 days under 12 h uv light and 12 h dark, and recording the percentage of seeds infected. Tests for *T. tritici* were carried out by shaking seeds in water with a wetting agent, filtering the washings, and





counting spores in three repeat sub samples of 300 seeds. Results were expressed as number of spores per seed. A single composite sample for each bulk tested was prepared by combining and dividing down a portion of each primary sample, and working samples from the composite were tested for both diseases.

### Results

Results have been considered in the light of current advisory treatment thresholds. A 5% infection level for M. nivale is regarded as low, and seed could be sown untreated. In the case of bunt, samples with less than one spore per seed may be sown untreated (Cockerell et al., 2001). For M. nivale, where composite samples indicated a low result, all primary samples also showed a low infection level. In general, where the composite sample gave a moderately high result, there was more variability between primary sample results (table 1). This trend was apparent regardless of bulk size and the number of primary samples taken. In the case of bunt, four distinct distribution patterns could be identified. Firstly, a very low composite result with all primary samples having either low or zero spores per seed, secondly, a very high composite result with all primary sample results also being high, thirdly a composite result just above the treatment threshold with all primary samples also above this level, but to varying degrees, and finally a low composite result below the treatment threshold, but with one or more primary samples above the threshold (table 2). The number of bulks examined which could be regarded as uniform (all primary samples indicating the same treatment decision as the composite) or variable (one or more primary samples indicating a different treatment decision) is summarised in table 3 for both diseases.

Bulk size	Number of	Composite	Number of samples in infectionran			ectionranges	
(t)	samples	percentage infection	(percentage of seeds infected)			nfected)	
			0	<5	<10	<20	>20
200	40	1.7	1	39	0	0	0
200	40	9.5	0	0	32	8	0
500	80	11.0	0	11	29	40	0

 Table 1
 Distribution of M. nivale in wheat seed bulks

Table 2 Distribution patterns of T. tritici infection in wheat seed bulks

Bulk size	Number of	Composite (spores/seed)	Number of primary samples in infection ran-					an-	
(1)	samples	(spores/seed)	$\frac{\text{ges (spores/seed)}}{0 < 1 < 5 < 10 < 50 > 50}$						
500	80	0.08	74	6	0	0	0	0	
200	40	0.08	0	0	20	1	0	0	
200	40	2.4	0	0	39	1	0	0	
100	40	46.7	0	0	0	0	30	10	
200	40	0.6	0	36	2	2	0	0	

Table 3 Numbers of bulks defined as having "variable" or "uniform" distributionsof M. nivale and T. tritici in primary samples

Pathogen	Number of bulks	Number "variable"	Number "uniform"
M. nivale	25	3	22
T. tritici	15		14

### Discussion

The consistency of primary sample results for M. nivale suggests that a single composite sample can be used to represent relatively large bulk sizes, though in practice it is unlikely that bulk sizes as large as some of those sampled here would be used. A frequency of 40 primary samples for bulks up to 100 t would probably be adequate, provided that all areas of the bulk were accessible to the sampler. Infection with M. nivale arises when wet weather coincides with anthesis. and inoculum may be derived from a background infection on lower leaves and stem bases. Foci of high infection in the field may be relatively rare, though they may occur, for instances in low lying or wet patches where conditions are particularly conducive to infection. However, even so, the consequences of a pocket of high infection in harvested seed are relatively insignificant. A reduced plant population in one area caused by seedling blight might cause some loss in yield, but over a whole crop, losses would not be serious

In many cases, the distribution of bunt appeared to be very uniform. Seed bulks intended for certification were almost entirely free of spores in all the primary samples, and this was true of relatively small and much larger bulks. Some farm-saved seed also had very low levels of bunt. Paveley *et al.*, concluded that, provided seed was produced from a crop where the input seed had been effecti-

vely treated, there was probably a very low risk of serious bunt developing, provided that there was no contamination of the seed at harvest by spores in machinery, or from neighboring crops. However, several farmsaved bulks did have significant bunt infection, and while it was not possible to determine accurately how this infection was introduced, the seed required treatment before use. In the majority of cases, the distribution appeared relatively uniform, such that any one primary sample indicated the same treatment decision as the composite. However, in one case, this was not true. A significant infection in one area of the seed bulk of 200t was thus diluted by mixing with other uninfected seed, but if the bulk had been sown without treatment, part of the crop would have been bunted, and this could potentially have lead to loss of the whole crop.

Thomas *et al.*, (2001) showed that it was possible to detect a serious area of bunt infection, consisting of about 30 kg of seed, wit-



hin a larger bulk of about 28 t by taking 40 primary samples and mixing to produce a composite which was well over the treatment threshold. However, the infection was severe, and had been deliberately introduced by sowing a strip of bunted wheat within a healthy crop. While a similar primary sampling frequency in a 200 t bulk was able to detect a naturally occuring area of infected seed in the example described, the composite result did not indicate the risk. Further sampling (results not shown) indicated that this situation was the exception and not the rule, and limiting bulk size to a maximum of 30 t with 40 primary samples should ensure that damaging levels of bunt are effectively detected.

### References

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### Acknowledgments

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### TECHNICAL COMMITTEES

3rd ISTA Proficiency Test on GMO

# 3<sup>rd</sup> ISTA Proficiency Test on GMO Testing on *Zea mays* (MON810+T25)

# Summary of Results

**By Bettina Kahlert,** ISTA GMO Proficiency Test Working Group Leader, ISTA GMO Task Force

### 1. AIM

The aim of this 3<sup>rd</sup> ISTA Proficiency Test on GMO Testing is to check the ability of individual laboratories to detect and, on a voluntary basis, to quantify the presence of GM seeds in samples of conventional seed of *Zea* mays L.

The object of data analysis is not be to identify deviating laboratories but to compile the performances in the laboratories and to provide data for the laboratories' internal performance data base.

### 2. EXPERIMENTAL DESIGN

For this proficiency test three different maize seed lots were provided: a non GM, a MON810 GM and a T25 GM seed lot.

Each participating laboratory received a set of 12 maize samples. Samples in each set were given a random number so that, for example, positive samples due to MON810 and T25 sent to laboratory 1 could have the same or a different number as compared to those sent to laboratory 2. Each sample contained about 1500 seeds (determined by weight) and was labelled only with a random sample number and a laboratory number. Three samples were negative (no GM seeds added) and 9 samples were positive. The positive samples were made positive by adding seeds from the MON810 and T25 seed lot to negative seeds. The negative seeds were determinded by weight whereas the positive seeds were counted and weighted. For three samples, 1 seed from the MON810 and 2 seeds from the T25 seed lot were added to approximately 1497 (362.3g) negative seeds so that the expected value for the GM content in these positive samples is 0.2% (Table 1). For three samples, 10 seed from the MON810 and 20 seeds from the T25 seed lot were added to approximately 1470 (355.7g) negative seeds so that the expected value for the GM content in these

positive samples is 2.0%. For three samples, 20 seed from the MON810 and 40 seeds from the T25 seed lot were added to 1440 (348.5g) negative seeds so that the expected value for the GM content in these positive samples is 4.0%.

To avoid cross contamination, the negative samples were prepared first, whereas the positive samples were prepared after sealing all negative bags.

### 2.1 Qualitative Test

Laboratories could use the method they thought appropriate for this test. The results for the qualitative test, i.e. a sample is positive or negative, had been submitted for each sample along with the sample identification number provided by ISTA. Participants were not expected to identify the events in the positive samples.

### 2.2 Quantification of GM Content in Positive Samples

Laboratories could do a quantification of the GM content in the positive samples by either a sub-sampling quantification or by a quantitative test (real time PCR).

### 2.2.1 Sub-sampling Quantification (SemiquantitativeTest)

The participants should report as a result of this test whether the GM content in the test sample was below-equal or above the 1%, the % of GM seeds and the testing plan, i.e. the number of sub-samples tested, the size of sub-samples (number of seeds), the number

Table 1: Overview of the spiking levels of the test samples:

# of samples	A-C	D-F	G-I	K-M
Spiking level	0%	0.2%	2.0%	4.0%
# of negative seeds	1500	1497	1470	1440
# of MON810 seeds	0	1	10	20
# of T25 seeds	0	2	20	40



of positive sub-samples per sample, the false positive and false negative rate which was used for calculation of results, and the decision rule (maximum number of positive samples to accept = 1%). The laboratories could use the method they thought appropriate for this test. The SEEDCALC programme was recommended to use for designing the testing plan (available on the ISTA Website).

### 2.2.2 Quantitative Test

This quantitative test is for checking the ability of the laboratories to quantify the GM content in a sample. The participants should report the quantitative estimated value of the GM content of the test sample (mean % of GM seeds) and classified whether the sample was negative or positive with a GM content below-equal or above 1%. The laboratories could use the method they thought appropriated.

### **3. RESULTS**

kept confidential.

Forty-eight laboratories out of 50 received the samples and 40 submitted their results (Figure 1). All 40 laboratories reported qualitative results that could be evaluated. Seven laboratories performed the sub-sampling testing strategy. Twenty-two laboratories reported quantitative results. Twenty laboratories reported values for the GM content of the seed samples whereas 2 laboratories did not submit values but performed the classification.

samplas

The identity of the individual laboratories is

### **TECHNICAL COMMITTEES**

3<sup>rd</sup> ISTA Proficiency Test on GMO

# 3.1 Descriptive Statistics of the Qualitative Results

Each laboratory reported for the individual sample whether this is a negative sample or a positive sample. There was no identification or quantification requested. So, for a given sample, the result reported by the laboratory can be either correct or false (Figure 2 and Table 2).

Out of the 40 laboratories:

- 33 laboratories reported results without any false results, all 12 tested samples were classified correctly. This are 81% of the laboratories.

- 96.1% of the 491<sup>1</sup> samples were reported correctly by the 40 laboratories.

- In total, 8 laboratories reported false results, 1 laboratory reported both, false positive results and false negative results, 5 laboratories only false negatives and 2 laboratories only false positives.

- 3 laboratories reported false positive results (between 1 and 2 out of the 3 negative samples (1/3) and 2/3) with a total number of 4 out of  $122^1$  negative samples tested. This are 7.5% of the laboratories and 3.3% of the negative samples.

- 6 laboratories reported false negative results (between 2/9 and 3/9) with a total number of 16 out of 369 positive samples tested. This are 15% of the laboratories and 4.3% of the positive samples.

- With respect to the spiking level, 6 laboratories reported false negative results with positive samples of 0.2% GM content. Between 1/3 and 3/3 samples were classified falsely as negative with a total number of 14 samples out of the 123 positive samples of 0.2% GM content. These are 15% of the laboratories and 11.4% of the 0.2% GM content samples.

- With respect to the spiking level, 0 labora-



Figure 1: Performed tests by the participating laboratories.

tories reported false negative results with positive samples of 2% GM content.

- With respect to the spiking level, 2 laboratories reported false negative results with positive samples of 4% GM content. 1/3 sample was classified falsely as negative with a total number of 2 samples out of 123 positive samples of 4% GM content. These are 5% of the laboratories and 1.6% of the 4% GM content samples.

## 3.2 The Sub-sampling Quantification Results

Five laboratories reported for the individual

sample a value for the GM content of the samples. In some cases, due to the testing plan chosen for the testing, a value could not be calculated for the higher GM spiking level, 2% and 4% (see lab 21 and 24, Table 3).

Further, 5 laboratories reported as a result of this test whether the GM content is belowequal or above 1% GM content of the seed sample (Table 4). Two laboratories out of 5, which used this approach, classified all samples correctly. Three laboratories had difficulties in categorising the samples with a GM content of 2%. Between 1 and 3 samples were classified falsely.

<sup>1</sup>One sample was not analysed by a laboratory because it had been damaged during shipment.

Table 2: Number and percentage of all, negative and positive samples reported as false.

		# of samples tested	# of samples reported as false	# of samples reported as false
All samples		491 <sup>1</sup>	19	3.9%
Negative samples		122 <sup>1</sup>	4	3.3%
	all	369	16	4.3%
Positive samples	0.2% GM content	123	14	11.4%
	2% GM content	123	0	0.0%
(r	4% GM content	123	2	1.6%

Table 3: Percentage of GM content reported by the laboratories for the seed samples are given in the table below. The column letters (A to M) correspond to the column letters in Table 1 showing the sample numbers. All laboratories used sub-sampling quantification.

	0%	0%	0%	0.2%	0.2%	0.2%	2%	2%	2%	4%	4%	4%
Lab	Α	в	С	D	Е	F	G	н	1	к	L	м
7	0	0	0	0.07	0.07	0.007	0.7	0.88	0.55	1.97	1.42	1.42
21	0	0	0	0.74	0.54	0.65	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4
24	0	0	0	0.14	0.22	0.22	2.32	>2.59	>2.32	>3.04	>2.59	>2.32
41	0	0	0	0.1	0.2	0.2	2	1.7	2	3.7	4.3	3.8
57	0	0	0	0.08	0.38	0.51	0.51	0.81	0.38	1.95	1.93	2.66

Table 4: The table shows the negative samples (column A to C, white), the samples with a GM content of <1% (0.2%, column D to F, light yellow) and of >1% (2% and 4%, column G to M, orange), respectively. If a laboratory reported that a sample with a GM content of 0.2% has a GM content >1% or that a sample with a GM content of 2% or 4% has a content <=1%, the cells are marked red. If a laboratory reported false positive or false negative results the cell is also marked red and pos and neg, respectively.





Figure 2: Percentage of laboratories reporting correct and false results.

### 3.3. The Quantitative Test Results

Twenty laboratories reported for the individual test sample the estimated value of the GM content as the percentage of e.g. haploid genomes, DNA or seed by mass (Table 5). Twenty-one laboratories reported for each sample if the value was below-equal or

above 1% (Table 6). Two laboratories, 15 and 35, did not report values for the GM content of the samples but

categorised the samples. Eleven laboratories classified correctly. One laboratory reported one sample with the 0.2% GM content as above 1%. Ten laboratories reported the samples with the 2% GM content as below-equal 1%. Between 1/3 and 3/3 samples were reported falsely. No laboratory classified samples with a 4% GM content as below-equal 1%.

### Acknowledgments

I would like to thank S. Zanetti and her staff for the support in preparation of the samples at the Agroscope, FAL Reckenholz, Switzerland. Thanks are also due to Bayer Crop Science for providing T25 GM seeds and Monsanto for MON810 GM seeds for the preparation of positive samples.

Table 5: Percentage of GM content reported by the laboratories for the seed samples are given in the table below. The column letters (A to M) correspond to the column letters in Table 1 showing the sample numbers.

	0%	0%	0%	0.2%	0.2%	0.2%	2%	2%	2%	4%	4%	4%			
Lab	А	в	с	D	Е	F	G	н	т	к	L	м	screening or event- specific	# of flour samples	# of repli- cates
4	0	0	0	0.12	0.12	0.12	0.82	0.87	1.43	1.67	1.92	2	sc	2	2
5	0	0	0	0.22	0.2	0.5	1.5	2	0.88	2.9	2.7	2.9	sc	?	2
11	0	0	0	0.2	0.3	0.1	1.7	1.6	2.3	3.4	3.1	3.6	sc	2	3
16	0	0	0	0.19	0.14	0.19	0.94	1.13	1.06	1.94	3.06	3.54	sc	2	3
17	0	0	0	0.32	0.43	0.17	3.01	4.64	3.97	5.25	6.31	5.25	sc	2	3
22	<0.01	<1	<1	<1	<1	0.1	0.49	0.99	0.87	1.85	1.44	2.18	sc	3	2
23	0	0	0	0.3	0.3	0.3	2.9	2.9	3.2	5.7	6.5	6.4	?	2	2
27	0	0	0	0.08	0.08	0.15	1.16	1.33	1.86	3.01	3.18	1.44	sc/e	2	2
28	0	0	0	0.27	<0.1	0.23	0.97	1.47	0.72	3.87	7.88	2.46	e	2	1
31	0	0	0	0.15	0.15	0.12	1.03	1.1	1.04	3.18	2.38	2.34	sc	3	3
32	0	0	0	0.09	0.09	0.06	0.7	0.8	0.7	1.8	1.4	1.4	sc	2	1
33	0	0	0	0.18	0.3	0.3	3.91	3.07	2.02	6.22	5.79	5.27	e	2	1
36	0	0	0	0.3	0.2	0.2	2.4	2.3	2.7	6.2	5.2	5.1	sc	2	2
37	0	0	0	0.16	0.18	0.12	1.65	1.12	1.25	3	2.53	3.11	sc	2	3
39	0	0	<0.2	<0.2	<0.2	<0.2	0.6	0.7	0.8	1.4	1.8	1.6	sc	2	2
40	0	0	0	0.05	0.07	0.05	1	0.89	1.04	1.67	1.69	1.89	sc	2	4
44	0	0	0	0.14	0.13	0.14	1.57	1.34	1.59	3.27	3.01	3.17	?	2	3
45	0	0	0	0.27	0.23	0.22	2.13	2.11	2.36	5.07	4.57	4.87	e	2	3
46	0	0	0	0.4	0.1	0.1	0.9	0.7	0.8	1.3	1.5	1.6	e	3	1
54	0	0	0	0.25	0.18	0.28	2.29	1.83	2.02	4.31	4.54	4.6	2	2	2

Table 6: The table shows the negative samples (column A to C, white), the samples with a GM content of <1% (0.2%, column D to F, light yellow) and of >1% (2% and 4%, column G to M, orange), respectively. If a laboratory reported that a sample with a GM content of 0.2% has a GM content >1% or that a sample with a GM content of 2% or 4% has a content <=1%, the cells are marked red. If a laboratory reported false positive or false negative results the cell is also marked red and pos and neg, respectively.

	0%	0%	0%	0.2%	0.2%	0.2%	2%	2%	2%	4%	4%	4%
Lab	Α	В	С	D	Е	F	G	Н	I	K	L	М
22		pos	pos									
39			pos	1								
31												
32	1											
46												
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# 4<sup>th</sup> ISTA Proficiency Test on GMO Testing on Glycine max (L.) Merr. Summary of Results

**By Bettina Kahlert,** ISTA GMO Proficiency Test Working Group Leader, ISTA GMO Task Force

1. AIM

The aim of the proficiency test is to check the ability of individual laboratories to detect the presence or absence and to quantify the presence of GM seeds in samples of conventional seed of soybean *Glycine max* (L.) Merr.

The results of the proficiency test rounds are intended to be used by the laboratories for their internal performance evaluation. At this stage performance in the tests, based on voluntary participation, will remain without consequences for the participants. Once GMO testing will be included in the ISTA Accreditation Programme and has become part of a laboratory's intended scope of accreditation, the results from voluntary proficiency tests on GMO testing, maybe taken into account. This will in essence speed up the accreditation process for laboratories that did participate on a high performance level.

### 2. EXPERIMENTAL DESIGN

For this proficiency test two different soybean seed lots were provided: a non GM and a GTS 40-3-2 GM seed lot. For checking genetic purity, 30,000 seeds of the negative seed lot were tested and proved to be negative and 400 seeds from the positive seed lot were individually tested and proved to be positive. These tests were made in the laboratory of Norbert Leist, Staatl. Landw. Untersuchungs- und Forschungsanstalt Augustenberg, Germany. Based on the testing of the negative seed lot, the potential accidental content of positive seed is below 0.01% with 95% confidence.

Each participating laboratory received a set

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### 4<sup>th</sup> ISTA Proficiency Test on GMO

of 12 soybean samples. Samples in each set were given a random number so that, for example, positive samples due to GTS 40-3-2 sent to laboratory 1 could have the same or a different number as compared to those sent to laboratory 2. Each sample contained about 3000 seeds.

Three samples were negative (no GM seeds added) and 9 samples were positive. The positive samples were made positive by adding the exact number of seeds from the GTS 40-3-2 seed lot to the negative seeds to obtain the three spiking levels 0.1%, 0.5% and 1.0% according to seed number. For each sample, the number of non-GM seeds and the number of GM seeds were counted and the weight determined. Table 1 gives detailed information about the spiking levels by number and by mass of seeds, the number and average weight of GM and non-GM seeds per sample. Due to a lower kernel weight of the GTS 40-3-2 seed lot the spiking level by mass was lower than the spiking level by number

To avoid cross contamination, the negative samples were prepared first, whereas the positive samples were prepared after sealing all negative bags.

### 2.1 GM Event and Reference Material

In contrast to previous ISTA Proficiency Tests, in this test round the laboratories received information about the event used for the positive samples and about reference material as followed:

For this proficiency test reference material for roundup ready can be used by the laboratories. GM seeds in the samples have the genes 35S Promotor, the NOS Terminator and the CP4 EPSPS. (The CP4 EPSPS gene encode the 5-enolpyruvylshikimate-3-phosphate synthase, which confers tolerance to glyphosate (Roundup) herbicide.) Certified reference material at different

levels can be obtained through Sigma-Aldrich at

http://www.sigmaaldrich.com/Brands/Fluka \_\_\_\_Riedel\_Home/Analytical/Food\_Analysis .html#round.'

### 2.2 Qualitative Test

As in previous ISTA Proficiency Tests, a qualitative result was requested. The laboratory should report whether a sample was negative or positive. This result could be either derived from the estimation of % made by the laboratory, or from another test on the sample.

### 2.3 Quantification of GMO in Positive Samples

In this 4<sup>th</sup> Proficiency Test, laboratories had to quantify the GMO level in the positive samples either by a semi-quantitative test (sub-sampling strategy) or by a quantitative test.

It was obligatory to report an estimate of % of GM seeds per test sample.

# 2.3.1 In case of Sub-sampling *Quantification*

The laboratory reported the number of subsamples tested, the size of the sub-samples (number of seeds) and the number of positive sub-samples per sample. These elements were used by the laboratory to compute the estimate of the percentage of GM seeds. The SEEDCALC6 programme was recommended to use for designing the testing plan and to perform the computation (freely available on the ISTA Website). The laboratory also reported whether the GM content in the test sample had been below-equal or above the level of 1%.

The laboratories were advised to use all seeds when they created their sub-samples in order to avoid that a GM seed is left out in the test. Laboratories should homogenise the received sample and should try to make each sub-sample of equal size.

### 2.3.2 In case of Quantitative Test

This quantitative test was for checking the ability of the laboratories to quantify the GM content in a sample. The laboratories could use the method they thought appropriated. The laboratories reported the GM content of the test sample. The results were given in percentage of GM seeds in the sample. The laboratory had to indicate the quantification in percentage of seeds by number or percentage of seeds by mass. It was optional to the laboratory to report the value of a positive sample with another unit. This was especially recommended in case the result was obtained with this unit and the percentage by seeds were computed from it.

For each positive sample the laboratory were asked whether they would report it as below-equal or above the level 1%.

### **3. RESULTS**

Fifty-nine laboratories out of 60 received the samples and 51 submitted their results. Three laboratories submitted only qualitative results. Eleven laboratories performed the quantification using the sub-sampling strategy. Thirty-six laboratories reported quantitative results. One laboratory submitted classification data using the sub-sampling strategy and the estimates of percentage of GM seeds using a quantitative test. Eight reported no data (Figure 1).

### Table 1: Overview of the spiking levels of the test samples

Label	A-C	D-F	G-I	K-M
# of samples	3	3	3	3
GM spiking level by # of seeds	0%	0.1%	0.5%	1.0%
# of non-GM seeds	3000	2997	2985	2970
# of GM seeds (GTS 40-3-2)	0	3	15	30
Average GM spiking level by mass of seeds	0%	~0.09%	~0.41%	~0.82%
Weight of non-GM seeds	~524g	~523g	~521g	~516g
Weight of GM seeds (GTS 40-3-2)	0g	~0.45g	~2.15g	~4.29g



Figure 1: Performed tests by the participating laboratories.

The identity of the individual laboratories is kept confidential.

# 3.1 Descriptive Statistics of the Qualitative Results

Each laboratory reported for the individual sample whether this is a negative sample or a positive sample. So, for a given sample, the



Figure 2: Percentage of laboratories reporting correct and false results.

Table 2: Number and percentage of all, negative and positive samples reported as false.

		# of samples tested	# of samples reported as false	# of samples reported as false
All samples		612	18	2.9%
Negative samples		153	8	5.2%
Positive samples	all	459	10	2.2%
	0.1% GM content	153	7	4.6%
	0.5% GM content	153	0	0.0%
-1	1.0% GM content	153	3	2.0%





result reported by the laboratory can be either correct or false (Figure 2 and Table 2).

### Out of the 51 laboratories:

- 42 laboratories reported results without any false results, all 12 tested samples were classified correctly. These are 82.4% of the laboratories.



- In total, 9 laboratories reported false results, 1 laboratory reported both, false positive results and false negative results, 3 laboratories only false positives and 5 laboratories only false negatives.

- 4 laboratories reported false positive results (between 1 and 3 out of the 3 negative samples (1/3) and 3/3)) with a total number of 8 out of 153 negative samples tested. These are 7.8% of the laboratories and 5.2% of the negative samples.

- 6 laboratories reported false negative results (between 1/6 and 2/6) with a total number of 10 out of 459 positive samples tested. These are 11.8% of the laboratories and 2.2% of the positive samples.

- With respect to the spiking level, 6 laboratories reported false negative results for samples with a spiking level of 0.1%. Between 1/3 and 2/3 samples were classified falsely as negative with a total number of 7 samples out of 153 positive samples with 0.1% GM content. These are 11.8% of the laboratories and 4.6% of the 0.1% content samples.

- With respect to the spiking level, 0 laboratories reported false negative results for samples with a spiking level of 0.5%.

- With respect to the spiking level, 3 laboratories reported false negative results for samples with a spiking level of 1.0%. 1/3 samples was classified falsely as negative with a total number of 3 samples out of 153 positive samples with 1.0% GM content. These are 5.9% of the laboratories and 2.0% of the



Figure 3: The results of the sub-sampling quantification: Estimates of the percentage of GM seeds reported by the laboratory (circles) for each sample (three samples per laboratory) of the different spiking levels: 0.1% GM seeds (a), 0.5% GM seeds (b) and 1.0% GM seeds (c) and the mean of these estimates for each laboratory (short line). The mean is only shown in case that all three estimates were reported as values.

### 4<sup>th</sup> ISTA Proficiency Test on GMO

1.0% content samples.

# 3.2 The Results of the Sub-sampling Quantification

Each laboratory reported for the individual sample as a result the number of positive sub-samples; the estimate of the percentage of GM seeds (Figure 3); whether the percentage of GM seeds is below-equal or above 1%; and the testing plan.

Eleven laboratories used the sub-sampling strategy for a quantification and twelve for a classification. All laboratories reported the number of sub-samples, the size of these subsamples and the number of positive sub-samples tested. Almost all laboratories used a testing plan with a high number of sub-sam-







Figure 4: The results of the quantification: Estimates of the percentage of GM seeds reported by the laboratory (circles) for each sample (three samples per laboratory) of the different spiking levels: 0.1% GM seeds by number and ~0.09% GM seeds by mass (a), 0.5% GM seeds by number and ~0.41% GM seeds by mass (b) and 1.0% GM seeds by number and ~0.82% GM seeds by mass (c) and the mean of these estimates for each laboratory (short line). The mean is only shown in case that all three estimates were reported as values. The values of lab58 are 58a (table 7).

ples. Eleven of the twelve laboratories used all 3000 seeds to create the sub-samples. Laboratory 36 used between 1500 and 200 seeds of the sample and did not avoid that a GM seed is left out in the test. The laboratories 36 and 49 chose a testing plan having a different number and a different size of each sub-sample.

### 3.3. The Quantitative Results

Thirty-seven laboratories performed the quantitative test and submitted thirty-nine test series. Thirty-four laboratories reported for the individual test sample the estimated value of the GM content as the percentage seed in number of seeds or mass of seeds. Four laboratories (lab10, lab14, lab26 and lab35) only reported the results in other units, e.g. percentage GM DNA (Figure 4).

# 4. RATING OF LABORATORIES IN CASE OF QUALITATIVE RESULTS

The following figures will give an indication about the performance of the laboratories in this test round (Figure 5) and their overall performance (Figure 6). The overall performance is shown for the laboratories which participated in all four ISTA Proficiency Tests on GMO Testing. Three rating systems are used, rating system 1 compares with an absolute number of misclassification, and rating system 2 and 3 compare with a relative number (a percentage) of miss-classifications. Score class A is the class with the highest score (good results), score class BMP the one with the lowest score (insufficient results; below minimum of performance). Please see for more information regarding these rating systems the article 'ISTA GMO Proficiency Tests - Use of presence/absence (qualitative) results for an overall rating from more than one test,' C. Haldemann and S. Grégoire (Seed Testing International, No. 128, page 8-10).

### 4.1 Rating of Laboratories for the 4<sup>th</sup> Proficiency Test

For **Rating System 1**, there are 42 laboratories assigned in score class A, 7 laboratories in score class B, and 1 laboratory each for score class C, respectively score class BMP.

**Rating System 2** obtains 42 laboratories in score class A, 3 laboratories in score class B, 4 laboratories in score class C and 2 laboratories in score class BMP.

**Rating System 3** includes 42 laboratories in score class A, 7 laboratories in score class B, and 1 laboratory each for score class C, respectively score class BMP.

4th ISTA Proficiency Test on GMO

# 4.2 Rating of Laboratories for the Proficiency Tests PT01 to PT04

The total number of miss-classified samples of the proficiency test round PT01 to PT04 is used as a basis for the overall rating. Table 3 shows for each rate the corresponding number of points (score).

Thirteen laboratories reported qualitative results for all four test rounds. Twelve laboratories obtained an A and one laboratory obtained an B, C or BMP depending on the used rating system.



Figure 5: Comparison of the three rating systems for the laboratories participated in the 4th proficiency test.

Table 3: Rating system for the overall rating based on the ISTA rating system. Range 2, used to show the mechanism with the four test rounds available.

rating	score	Overall rating	Range <sup>1</sup>	Range 3 <sup>2</sup>
A	5	A	28 - 30	19 - 20
В	4	В	21 - 27	14 - 18
С	3	С	16 - 20	11-13
BMP	0	BMP	below 16	below 11



Figure 6: Comparison of the three rating systems for the laboratories participated in all four ISTA Proficiency Tests (13 laboratories).

### Acknowledgment

Thank you very much to N. Leist and his staff at the Staatl. Landw. Untersuchungs- und Forschungsanstalt, Augustenberg, Germany, for the preparation and shipment of the proficiency test samples. I would also like to thank Monsanto for the MON810 GM seed material used in this test round for the preparation of positive samples. I thank C. Haldemann for producing figures for paragraph 4.2.

## Announcement 5<sup>th</sup> ISTA Proficiency Test on GMO Testing

We would like to address this announcement for participation in the 5<sup>th</sup> ISTA Proficiency Test on GMO Testing to all laboratories which participated in a previous round or are interested in the fifth round.

The aim of the proficiency test is to check the ability of individual laboratories to detect the presence or absence and to quantify the presence of GM seeds in samples of conventional seed of *Glycine max* (L.) Merr.

Each participating laboratory will receive 12 soybean test samples of 3000 seeds. Some of the samples will be positive (i.e. contain GM seeds) and others will be negative (i.e. contain no GM seeds). Laboratories will have to prepare the flours from seed samples.

## **OBLIGATORY QUALITATIVE TEST:**

A qualitative result is requested. This result can be either derived from the estimation of % made by the laboratory, or from another test on the sample.

OBLIGATORY QUANTIFICATION OF GMO IN POSITIVE SAMPLES:

Also in this 5<sup>th</sup> Proficiency Test, laboratories must quantify the GM seed material in the positive samples either by a sub-sampling quantification or by a quantitative test (real time PCR).

It is obligatory to report an estimate of % of GM seeds per test sample.

Please inform the ISTA Secretariat together with your application, whether you are going to perform a sub-sampling quantification or a quantitative test.

Laboratories interested in participating should please contact the ISTA Secretariat

Email: (ista.office@ista.ch) or Fax (+41-44-838-6001)

More details can be found on the ISTA Website at **www.seedtest.org**  Overview ISTA Proficiency Test on GMO

# **ISTA Proficiency Test on GMO Testing** A Brief Overview

By Bettina Kahlert, ISTA GMO Task Force

In May 2002, the International Seed Testing Association initiated its first international proficiency test on GMO testing. Since then, 90 laboratories from 30 countries have participated in the four rounds of the ISTA Proficiency Tests on GMO Testing showing their ability to detect the presence and absence of GM seeds in samples of conventional seed lots and to quantify the presence of GM seeds.

### **1. EXPERIMENTAL DESIGN**

In the first proficiency test (PT01) only a qualitative test, i.e. the detection of GM seeds in conventional seed lots, was required. In PT02 and PT03, a quantitative in addition to the qualitative test was optional, whereas, in PT04 a quantitative test was obligatory. (See page 10 and 13 for more details of the qualitative and quantitative tests.) In order to assess the laboratories' performance in routinely testing on GMO, they were allowed to use their standard methods for testing on GMO.

The crop kind under test was maize in the first three test rounds, one conventional seed lot and two GM seed lots: MON810 (PT01 to



Sample sets with eight different spiking levels between 0.1% and 4.0% and sample sets containing no GM seeds were prepared (Table 1). Prior to dispatch, the genetic purity of the seed material used was tested using the same procedure as used in previous test rounds.

For rating the laboratory's performance acceptance criteria such as the maximum tolerated error rate are needed. In order to detect systematic testing errors, the laboratories were required to test a comparatively high number of samples each containing 300 seeds in test round PT01. However, it turned out that this small sample size was not adequate for common testing procedures and sample handling needed to be adjusted by the laboratories. Thus, it was agreed to increase the sample size (1500 to 3000 seeds) in subsequent test rounds and instead to adjust the number of samples in order not to increase the sample package weight. Moreover, an increased sample size enabled the laboratories to use the sub-sampling strategy for a quantification in addition to qualitative and quantitative tests.

### 2. PARTICIPATION

Between 43 and 60 laboratories participated per test round in total 90 laboratories from 30

countries (Table 1). Two thirds of the participants are from Europe (Figure 1). Thirteen of 90 laboratories participated in all four test rounds.

Between 10% and 17% of the participants have either not returned any results or their result sheets could not be evaluated as some were e.g. incomplete or faulty. 53% of the participants of test round PT02 and 60% of the participants of test round PT03 carried out a quantitative test. Although it was obligatory to report quantitative test results in proficiency test round PT04, five percent of the laboratories only reported qualitative test results (Table 2).

### 3. RESULTS

In the first proficiency test, where the high number of samples was tested, 70% reported no false results. (Table 3). In subsequent proficiency tests, between 81% and 85% of the laboratories reported all results correctly. The proportion of laboratories that reported false negative results was higher than the proportion of laboratories that reported false positive results. False negative results were more frequently reported on samples with a spiking level lower than 1 % and the event T25. The percentage of correctly reported results varied between 95.4% and 97.1%. There was no significant difference in the percentage of false negative or false positive results. Again, most false negative results were reported on samples with a low spiking



Figure 1: Region of participating laboratories

### Table 1: Design of the four ISTA Proficiency Tests (PTs)

		_		
Proficiency Test	PT01	PT02	PT03	PT04
# of laboratories	43	52	50	60
# of samples per	30	10	12	12
lab				
negative	12	3	3	3
positive	18	7	9	9
0.1 %	-	-	-	3
0.2 %	-	-	3	-
0.5 %	-	-	-	3
0.7 %	-	3	-	-
1.0 %	18	-	-	3
1.4 %	-	4	-	-
2.0 %	-	-	3	-
4.0 %	-	-	3	-
Events	T25/Mon810	Mon810	T25/Mon810	GTS-40-3-2
	(maize)	(maize)	(maize)	(soybean)
Sample size seeds	300	3000	1500	3000
Qualitative	X	X	X	X
Sub-sampling	-	X*	X*	X
Quantitative	-	X*	X*	X

# Table 2: Performed tests by laboratories: qualitative test (qual.) and/or quantitative test using the sub-sampling strategy (sub-sampl.) or the quantification (quant.)

	PT01	PT02	PT03	PT04
only qual.	89%	29%	23%	5%
qual. & sub-sampl.	-	23%	15%	18%
qual. & quant.	-	30%	45%	60%
qual. & sub-sampl. & quant.	-	8%	0%	2%
not submitted/non-evaluable results	11%	10%	17%	15%

Table 3: Percentage of laboratories reporting correct or false results.

	PT01	PT02	PT03	PT04
Correct results	70%	85%	81%	82%
False results	30%	15%	19%	18%
False negative	19%	4%	12%	10%
False positve	7%	4%	5%	6%
Both false positive & negative	5%	6%	2%	2%

Table 4: Percentage of samples reported correct or false

	PT01	PT02	PT03	PT04
Correct results	95.4%	96.4%	95.9%	97.1%
False positive	1.7%	5.0%	3.3%	5.2%
False negative	6.5%	3.0%	4.3%	2.2%

Table 5: The (overall) mean of the quantitative test results for each spiking level, the standard deviation and the variation coefficient among the samples within each spiking level.

	Spiking level	Mean ± SD	Variation coefficient
PT02	0.7%	$0.73 \pm 0.37$ %	50%
PT02	1.4%	$1.47 \pm 0.91$ %	62%
PT03	0.2%	$0.19 \pm 0.10$ %	51%
PT03	2.0%	1.62 ± 0.92 %	57%
PT03	4.0%	3.37 ± 1.68 %	50%
PT04	0.1%	0.13 ± 0.11 %	87%
PT04	0.5%	0.47 ± 0.29 %	61%
PT04	1.0%	$0.87 \pm 0.32$ %	37%

level (Table 4).

Figure 2 shows box plots for the results of the quantitative test results. Laboratories reported an estimated value for the percentage of GM seeds of each sample. In every proficiency test round a laboratory received between three and six samples of one spiking level. For the box plots the laboratories' values of the different spiking levels are plotted. Dots represent outliers, i.e. results of laboratories that differ significantly from the Generally, mean there was a tendency to overestimate the percentage of GM seeds. Table 5 shows the (overall) mean of the quantitative test results for each spiking level, the standard deviation and the variation coefficient among the samples within each spiking level. The lowest variation coefficient was calculated for the

1.0% spiking level with 37% and the highest for the 0.1% spiking level 87%. 0.1% is for most of the laboratories the limit of quantification. For the other spiking levels the variation coefficient is between 50% and 60%. (The standard deviation and the variation coefficient in Table 5 are related to the single results per sample and not to the laboratories' means.)

### 4. PROSPECT

There were no major differences between the proficiency tests rounds regarding the correct classification of the samples as positive or negative. The proficiency test rounds provided sufficient data material to prepare a proposal for a rating system of the laboratories' performance to detect the presence and absence of GM seeds in conventional seed lots (STI No. 128, page 8-10). It showed that only few laboratories had serious problems with the qualitative testing of the samples. The rating system will be applied in future ISTA Proficiency Tests on GMO Testing. The high variation within and between the laboratories' quantitative results makes it more complicated to evaluate the performance of the laboratories. Intensive work has been carried out to define fair and reliable systems of rating for quantitative estimates. Both presence/absence rating and quantitative rating proposals are presented at the Ordinary Meeting in Bangkok.

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Figure 2: The laboratories mean test result in % of GM seeds of the quantitative test: (a) PT02, (b) PT03 and (c) PT04.

Task Force on Tropical Seed

# Task Force on Tropical Seed

By Grethe Tarp, Task Force on Tropical Seed Chair Danish Plant Directorate, Skovbrynet 20, 2800 Kgs. Lyngby, Denmark, e-mail: gta@pdir.dk

The ISTA Task Force on Tropical Seed has investigated the need for inclusion of new species in the ISTA Rules.

The task force was formed at the 2004 ISTA Congress to highlight the importance of tropical seed in the ISTA Rules for germination and purity analysis. The mandate was to find the relevant species and speed up the process of including them. Kae-Kang Hwu (Taiwan), Mei-Hsuan Lin (Taiwan), Doris Groth (Brazil), Joseph Ahendra (Kenya) and Michael Muschick (ISTA Secretariat) are members of the task force, which Grethe Tarp (Denmark) is chairing.

A questionnaire was sent out in November 2004 to review the need to include more tropical species into the ISTA Rules. Five countries filled in the questionnaire indicating a variety of interests in supplementing the tropical species included in the ISTA Rules. The table shows the suggested species and for some of them suggestions for germination conditions.

Latin name	Common name(s)	Suggested germination con- ditions (Germination method, temperature, first- and final count)
Cleome hassleriana	Spider flower	TP: 20-30°C7, 21 days
Solanum nigrum	Black nightshade	TP: 20-30°C7, 14 days
Physalis peruviana	Cape Gooseberry, Ground Cherry, Golden Berry	TP: 20-30°C7, 21 days
Passiflora edulis	Purple Passion Fruit	Sand: 20-30°C7, 14 days
Carica papaya	Papaya, papaw, fruta bomba, lechosa	Sand: 20-30°C7, 28 days
Bupleurum rotundifolium	Common hare's ear	TP: 20-30°C7, 28 days
Zantedeschia spp.	Zantedeschia	TP: 20-30°C7, 42 days
Anacardium occidentale	Cashewnut	
Coffea arabica	Coffee	
Jatropha curcas L	Barbados nut, Curcas bean, Physic nut, Purging nut	

The list has been presented to the Purity and Germination Committees, which will make plans for evaluation of the suggested species. When the committees have made a through investigation, a vote on the inclusion into the ISTA Rules will then be made at one of the annual ISTA Meetings.

### NIAB's Seed Identification Handbook now available



The  $2^{nd}$  edition of this handbook (printed August 2004) is greatly improved. The lie-flat format is very convenient for use in the laboratory.

A comprehensive guide to some 200 species of agricultural, horticultural and weed seeds commonly seen in the UK. Many species are also found worldwide. The 94-page book is a useful tool for seed analysts, biologists and plant physiologists. The information for each species comprises a magnified colour photograph of the seeds, two or three sentences giving some background and a description of seed shape, size and colour. A white inset 10mm in diameter within each photograph provides life-size silhouettes of the seeds to aid identification. The arrangement of species is alphabetical within families. Six pages of life-size silhouettes then follow in order of ascending size.

The handbook concludes with an index of botanical names (following the International Seed Testing Association List of Stabilised Plant Names 2001), an index of (UK) common names, a substantial bibliography and reference list, plus a few relevant websites.

The handbook is available at £35 plus post and packaging direct from NIAB. Order online from the 'NIAB shop' at www.niab.com



Common name: Black Nightshade, Latin: Solanum nigrum Picture: seedimages.com

### TECHNICAL COMMITTEES

### ISTA Pure Seed Definition (PSD) Handbook

# ISTA Pure Seed Definition (PSD) Handbook

# **Undergoing Review**

By Steve Jones<sup>1</sup>, Jane Taylor<sup>1</sup>, Maria Rosaria Mannino<sup>2</sup>, Corinne Sahuguède<sup>2</sup>, Fabio Ferrari<sup>3</sup>, Zita Ripka<sup>4</sup>, ISTA Purity Committee Members

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Within the ISTA Rules there are major chapters that describe procedures in daily use by seed analysts worldwide. As a prerequisite to many of these tests the analysts need to know how to apply the definition of a pure seed for any given species; not only the species being tested but those found as contaminants in the sample.

This is particularly significant in case of a purity test. Retrieval and identification of the various species of seeds and inert particles represent one of the objects of the test and to help this process laboratories have reference collections and spend a considerable amount of time training people in the visual identification of seed species. A very important task is the correct application of the relevant Pure Seed Definition to separate the working sample in the right way and to calculate the proper percentage composition by weight of the sample. ISTA has tried to help this process by including the 63 PSDs in the ISTA Rules and producing a PSD Handbook to act as more detailed guide. The first edition of the PSD Handbook was produced in 1983 and the second in 1987. As an important ISTA document it has been scheduled for review. A working group of the Purity Committee met at NIAB in Cambridge during August 2004 to review the existing handbook and if necessary commission a 3<sup>rd</sup> Edition.

The working group consists of Maria Rosaria Mannino, Corinne Sahuguède, Fabio Ferrari, Zita Ripka, Jane Taylor, Helen Appleyard, Ken Allison, Debbie Meyer, Steve Jones and a member from the Forest Tree and Shrub committee (not yet named). Most were able to attend the August meeting.

The conclusion of the meeting was that the handbook could benefit from review and should cover all the PSDs in the ISTA Rules. It was decided to present each of the pure seed definitions, in numeric order as specified in the ISTA Rules, together with a list of all the families and genera that are covered by that number. An illustration of the most relevant genera within a pure seed definition will be given to provide practical guidance on the application of a definition. Also each PSD will be illustrated with a scaled, colour photograph or line drawings of seed to help in the understanding the descriptions. The handbook will also have a glossary.

Other features to be included would be:

- Loose-leaf format for easy laboratory use. - Statements about which genera has species that cannot be easily distinguished, e.g. *Brassica* spp., *Lolium* spp.

- PSD number on the side of pages as in TEZ handbook for easy access.

- Up to six images per page.
- Include images of seed from tropical countries wherever possible.



Since the meeting last year the working group has made good progress and the group is hoping to have an early draft ready for presentation and discussion at the meeting in Thailand. To help people understand what we are planning we have included an example of the PSD tables and a draft page of pictures from the new handbook for PSD10.

If you have any thoughts or suggestions about this project please contact Maria Rosaria Mannino (Chair of the Purity Committee) or Steve Jones (Leader of the PSD review team).

### **Contact details:**

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Figure 1. Two pages of the revised ISTA Pure Seed Definitions Handbook presenting PSD 10: 1.A. introduction to the PSD (1st page) 1.B. species covered by the PSD (some examples).





Figure 1.A

Figure 1.B

# Towards the validation of the controlled deterioration vigour test for small seeded vegetables

### By Alison A. Powell and Stan Matthews

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The validation procedures for new laboratory tests of seed quality by ISTA have been formally laid out in the Handbook of Method Validation (ISTA, 2005) In the ISTA context, there are two main components of method validation applicable to vigour tests. First, the description of the method should be clear and complete, so as to give reliable and reproducible results, and second, the relationship between the results of the vigour tests and a practical expression of vigour should be confirmed. Currently two vigour tests (conductivity for Pisum sativum and accelerated ageing for Glycine max) have been validated by inclusion in the ISTA Rules. This article describes the work that has been completed at the University of Aberdeen and by members of the Controlled Deterioration Working Group of the ISTA Vigour Committee towards the validation of a third vigour test, the Controlled Deterioration test for small seeded vegetable species.

The history of the Controlled Deterioration (CD) vigour test goes back more than 25 years (Matthews, 1980). As the name suggests the test involves the deterioration of samples of seeds from seed lots in a precise and controlled manner at an elevated moisture content (dependent on the species, often 20%) and temperature ( $45^{\circ}$ C) for a defined duration; for convenience 24h is preferred (Figure 1).

### Theoretical basis of CD:

The theoretical basis of the test lies in the seed survival curve (Figure 2). Seed lots having similarly high and commercially acceptable levels of germination would be located on the slow initial decline of the curve (e.g. A, B and C). The small differences in mean germination of such seed lots are not, however, clearly differentiated on the basis of the 400 seed sample used to estimate the population mean. However, if samples of lots A, B and C are deteriorated rapidly to exactly the same extent, then their germinations after deterioration are contrasted (Figure 2). For example, lot A compared with C: after a period of deterioration C would be placed well down the slope of the survival curve while A would remain near the top.





Figure 1. Stages in the completion of the Controlled Deterioration vigour test



Figure 2. The theoretical basis of the Controlled Deterioration test lies in the seed survival curve and seed ageing.

Thus, CD increases the range of germination between the seed lots and the differences in germination after deterioration reflect the initial position of the seed lot on the survival curve i.e. the degree of ageing within the sample. Since ageing is the main cause of differences in seed vigour, the germination after CD reflects the vigour of the seed lot.

### Relationship of CD test results to expressions of vigour:

Clear differences in germination after CD have been reported amongst commercial seed lots with high germination (Matthews, 1980). The CD results were statistically significant indicators of field emergence in each of two years in nine crops, including a number of small seeded vegetable species (turnip, swede, kale, sprouts, carrot, lettuce and onion). Standard laboratory germination was also often significantly related to field emergence, but, in general, not as closely as CD germination, which gave a much clearer separation between lots emerging well and those emerging poorly (Matthews, 1980). The relationship between field emergence and CD was supported by the work of the CD Working Group, when three laboratories carried out emergence trials in 1999, and by the work of Powell and Dutton (1984). In addition the CD Working Group noted a correlation between the CD test result and the rate of emergence, as also reported in oilseed rape (Brassica napus subsp oleifera) (Larsen et al.). Correlations between CD results and field emergence have also been seen in red clover (Wang et al, 1994), vining peas (Bustamente et al, 1984), combining peas (Powell et al., 1997) and Italian ryegrass (Lolium multiflorum; Marshall and Naylor, 1985) and in the emergence of cabbage in compost trays (Strydom and Van der Wenter, 1998).

Vigour differences not only cause problems in field sowings, but also under controlled glasshouse production. Comparisons of commercially acceptable lots of Brassica crops (cauliflower, sprouts, cabbage and calabrese) in glasshouse modules showed how germination after CD was related to seedling performance (emergence, rate and spread of emergence, and variation in seedling size) (Powell et al., 1991). Similar findings were reported recently for aubergine (Demir et al., 2005).

Another practical expression of seed quality indicated by the CD test is seed longevity (Figure 3) with a clear relationship reported between the results of the CD test before storage and germination after commercial storage of 15 seed lots of onions (Powell and Matthews, 1984a) and 29 lots of Brussels sprouts (Powell and Matthews, 1984b). The



Figure 3. The relationship between the results of the CD test before storage and germination of seed lots of Brussels sprouts and onions after storage for over 2 years in commercial storage conditions.

storage potential of rye during 80 days natural storage was also predicted by CD (Steiner and Stahl, 2002), as was that of *Arabidopsis thaliana* (Tesnier et al., 2002).

### Precision, repeatability and reproducibility:

The reliability of the CD test was first shown by Powell and Matthews (1981). Subsequently evidence from six laboratories from official seed testing stations and seed companies also demonstrated repeatability within, and reproducibility between, laboratories when ten seed lots each of swede and onions were tested in each of three test runs (Powell et al., 1984). Subsequent work by members of the CD Working Group over nine years (Table 1) has confirmed the repeatability and reproducibility of the test using a range of Brassica seed lots.

As in any vigour test, precision is required in aspects of the CD test. Precision is required to achieve the same seed moisture contents (MC) in seed lots at laboratory temperature before seeds are deteriorated rapidly at the high temperature (45°C). The adjustment of seed MC before deterioration (Figure 4) was adopted following the observation that when small seeded vegetables were deteriorated in a moist atmosphere at high temperature, as in the accelerated ageing test, the rate of increase in moisture differed between lots. This resulted in differences in the extent of deterioration (Powell, 1995). Precision is needed in the adjustment of the MC, since a 1% difference in MC has a clear and striking effect

on the final germination (Table 2).

Different methods of raising seed MC content have been investigated in three series of comparative tests (Table 1). All methods require determination of the initial seed MC, then calculation of the weight that a sample of seeds would have at the desired MC. The attainment of this MC (seed weight) was achieved by imbibition from moist filter paper, or by addition of the required amount of water to achieve this weight increase, eit-



Figure 4. Raising the seed moisture content for the CD test

### **TECHNICAL COMMITTEES**

### Controlled Deterioration Vigour Test

Experimental series	Laboratories <sup>1</sup>	Crops	No. of lots	No. of repeat runs	Aspects of the test evaluated
1996-1998	DK, FR, H, K, GB1	Brassica napus var napobrassica	5	3	<ul> <li>Method of raising seed MC (filter paper and water added to foil packet)</li> <li>Repeatability</li> </ul>
		Allium cepa	4		<ul> <li>Reproducibility</li> <li>Counts of radicle emergence vs normal germination</li> </ul>
1998-2001	DK, FR,K, GB1	B.napus var napobrassica	5	3	<ul> <li>Method of raising seed MC (filter paper and water added to foil packet)</li> <li>Repeatability.</li> <li>Reproducibility.</li> <li>Counts of radicle emergence vs normal germination</li> <li>Relationship between CD results to sto rage potential and field emergence.</li> </ul>
2001-2004	DK, FR,GB1, GB2, IT	B. napus subsp oleifera	6	2	<ul> <li>Method of raising seed MC (filter paper and water added to revolving container)</li> <li>Repeatability.</li> <li>Reproducibility.</li> <li>Counts of radicle emergence vs normal germination.</li> <li>Relationship between CD results to field emergencee.</li> </ul>

### Table 1. Comparative tests of CD conducted by the ISTA Vigour Committee

<sup>1</sup>DK: Plant Directorate Copenhagen; Fr: GEVES-SNES, Angers; H: OMMI, Budapest; K: Dept of Agronomy, Gyeongsand National University; GB1: Dept of Agriculture and Forestry, University of Aberdeen; GB2: OSTS, Cambridge; It: LaRAS, University of Bologna

her with the seeds retained in a foil packet during equilibration, or in a constantly revolving container. Comparative tests revealed that increasing the seed MC by imbibition on filter papers gave consistently the most repeatable results. This was recently confirmed by Wagner et al. (2004) who found that the filter paper method gave the overall best results for achieving any seed MC in four species, including rape seed.

A second aspect of precision that is important in the CD test is achievement of the high temperature of 45°C. Close temperature control is necessary to ensure that the same degree of deterioration is imposed on all seed samples, particularly when the seeds have a raised MC. This is accurately achieved ( $\pm$ 0.5°C) using a water bath, a readily available and relatively inexpensive item of equipment.

In their work on standardisation of the CD test (Table 1), the Vigour Committee have also examined the assessment criteria for germination after CD, as total germination (radicle emergence) or as normal germination. Both methods of evaluation resulted in good repeatability and reproducibility, with, predictably, a greater range in normal than total germination. Both germination assess-

ments correlated with field emergence in 1999, with neither method of assessment giving a better correlation with field emergence, as was also found in red clover (Wang et al., 1994). The recommendation from these observations is that both normal and total germination should be reported. The spread in normal germinations is likely to be greater when the lots being compared are not very different in vigour and the moisture content during deterioration is not sufficiently high to clearly distinguish seed lots in terms of total germination (Table 2). Reporting both assessments would also confirm that differences in vigour are related to the relative position of lots on the survival curve.

### Species applicability:

CD has identified differences in vigour in a range of small seeded vegetable species (Matthews, 1980; Powell et al., 1981; Powell and Matthews, 1981a,b; Demir et al., 2005), grasses (Marshall and Naylor, 1985) and red clover (Wang et al., 1994). However, the test is likely to be suitable for any species whose shape or size allows imbibition of water to an appropriate seed MC within a few hours. A number of authors have applied the test to large seeded species, such as peas and soyabean. Our experience in Aberdeen is that while this may be useful in research, the time taken to raise seed MC makes use of the test for large seeded species inappropriate in a routine seed testing context, especially when convenient alternative vigour tests are available.

Table 2. Effect of seed moisture content during CD test on total germination (% radicle emergence) of turnip seed (taken from Powell and Matthews, 1981 with permission of Seed Science and Technology).

Seed lo	ot	Seed moistu	re content		
	Initial (7%)	20%	21%	22%	
1	100	100	96	89	
2	98	96	92	87	
3	97	94	89	78	
4	99	96	80	72	
5	96	80	43	25	
6	90	24	3	1	

### **TECHNICAL COMMITTEES**

Controlled Deterioration Vigour Test

### The future:

There is detailed evidence that the CD test satisfies the criteria required for validation as an ISTA test. The evidence summarised above is especially convincing and extensive for Brassica species. The Vigour Committee is therefore currently preparing a submission to the ISTA Method Validation Programme for application of the Controlled Deterioration test to Brassica species.

### Acknowledgements

Many thanks to past and present members of the CD Working Group of the

Vigour Committee and their colleagues who have contributed to the completion of the comparative testing of the CD test.

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Introduction to new ISTA Technical Committee Chairs

# Introduction to new ISTA Technical Committee Chairpersons

# Ronald Don

ISTA Germination Committee Chair



Hi, I am the Chairman of the Germination Committee, Vice-Chair of the Tetrazolium Committee and a member of the Moisture, Proficiency and Rules Committees. I have been involved in seed testing since leaving University in 1974. I am a hands on person and enjoy the technical challenges of seed testing rather than the politics. Although I am the Chief Officer of the Official Seed Testing Station for Scotland (OSTS), I maintain my seed testing skills and within the ISTA and ISO quality systems, under which the OSTS operates, I am authorised to carry out germination, moisture, tetrazolium and vigour tests.

I enjoy teaching and am involved in the training of UK and overseas students and technicians. The OSTS is renowned for its expertise in training and has been used by a range of donors (e.g. British Council, EU, EU Tacis, GTZ/DAAD, FAO and World Bank) to provide training in seed testing and technology. Training courses range from basic technician training to laboratory management and the monitoring of private sector involvement in seed certification. In addition to bespoke training courses I have also organised International Workshops in Tetrazolium Testing and Quality Assurance for ISTA.

I am a fully trained Management and Organisational consultant and have used

these skills in conjunction with my Seed Testing and Seed Technology background on a range of overseas development projects in countries as diverse as Nepal and Turkmenistan. At the present time I am involved in an EU Development project in Kosovo where I am updating local seed testing skills and making recommendations on the upgrade of seed certification services that would permit the ISTA accreditation of the Seed Laboratory in Peje. To date my proudest achievement in Seed Testing has been the publication of the third edition of the Seedling Evaluation Handbook, which I edited, and the Guidance to Temperature Monitoring, published in Seed Testing International, that I compiled.

Out of the laboratory my main interests are gardening, I have two vegetable allotments, computing, football (spectating only) and food.

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# Günter Müller

ISTA Proficiency Test Committee Chair



I believe most ISTA members know me because I am a member of the Germination Committee and Proficiency Test Committee since 1992. In this time I organized five ISTA Proficiency Tests with different agricultural and horticultural species.

I am the head of the seed testing station in Jena. In the same way, I am responsible for official Field Inspection, Certification and Marketing Control of Seeds in the German federal state Thuringia. These are many tasks we have to do but the laboratory staff and the offices are very motivated and well trained.

Besides this I am working in two seed research projects. Seed research is the salt in the soup! One project deals with the influence of chemical treatments on the germination potential of sprouted wheat seed and the other deals with health testing on peas seed.

Our seed testing station is a small one because we have only 12 employees. But it has an old tradition. For the first time seed was officially tested in Jena in the year 1875. Thuringia is situated right in the centre of Germany and Europe. It has 2.4 million inhabitants and an area of 16172 square kilometres. Jena is known because of the precision engineering and optical industry. Carl Zeiss the father of the German optics lived and worked there in the 19<sup>th</sup> century.

I am glad to work for ISTA because we are one big family and all members profit from our mutual work.

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### **TECHNICAL COMMITTEES**

### Introduction to new ISTA Technical Committee Chairs

# Maria-Rosaria Mannino

ISTA Purity Committee Chair



Maria Rosaria Mannino started to participate to the work of the Purity Committee in 1995, at first as member of working groups (PSD, blowing), then as Vice-Chairperson and finally, since the Ordinary Meeting in Budapest in 2004, as Chairperson.

M. R. Mannino is an Agronomist and obtained a PhD on Biology and Productivity of Cultivated Plants in 1989.

She is the Head of the Physical Analysis Laboratory of the GEVES-SNES, the French National Seed Testing Station where she has been working since 1993. In this laboratory she manages between 19 and 30 persons, depending on the activity seasonal charges. Responsible of the Purity Analysis (17000 samples per year), Other Seed Determination by Number (11000 samples per year), Weight Determination (100 samples per year), Seed Cleaning (2000 samples per year), Moisture Determination (4000 samples per year) and X-Ray (20 samples per year), she also coordinates the activities of the SNES botanist in charge of the botanical garden and seed collections management.

### **Contact information:**

Dr. Maria Rosaria Mannino Head of the Physical Analysis Laboratory GEVES-SNES National Seed Testing Station Rue Georges Morel - B.P. 90024 49071 - Beaucouzé Cedex - France Tel. +33 2 41 22 58 40 Fax +33 2 41 22 58 01 maria-rosaria.mannino@geves.fr Maria Rosaria Mannino is also involved in theoretical and practical training sessions of about 60 seed specialists and 80 students, annually organised at the SNES. She operates as auditor on the process of "authorisation of seed laboratories for seed testing under official supervision".

# Valerie Cockerell

ISTA Seed Health Committee Chair



It has been 25 years since I first started work at the Official Seed Testing Station for Scotland (OSTS) as a trainee seed analyst and 17 years since my first introduction to ISTA when the OSTS held the 4<sup>th</sup> ISTA/PDC Training Course. Although an exceptionally busy time this was a wonderful introduction to the work of the ISTA Plant Disease Committee, now the Seed Health Committee (SHC) of ISTA. At that time Bill Rennie was the Seed Pathologist at the OSTS and proved an excellent mentor for the job I was to take over in 1990.

Cereals are the most common arable crops in Scotland and Scottish climatic conditions are often conducive for the development, multiplication and spread of cereal seed-borne pathogens. Therefore an important aspect of my job is to manage and direct the research and development work on the epidemiology and management of cereal seed-borne diseases. Most recently I have led a UK project that required to put in place the tools (rapid diagnostic tests, thresholds and sampling procedures) to allow growers/producers to consider using a 'treatment according to need strategy' to manage seed health in UK wheat seed lots.

At the OSTS seed health tests are made for the purposes of seed certification, enforcing the Seeds Regulations and advising growers, producers and seed merchants. The health laboratory tests for fungal pathogens on a range of crops with expertise centred on cereal seed-borne pathogens. The laboratory also has some experience of bacterial testing. As manager of the laboratory I must ensure that our service is supported by properly validated methods and advise on the interpretation of seed health test results. In addition as seed pathologist I must provide advice to government on policy issues related to seed-borne pathogens.

I am also the Quality Manger and Deputy Technical Manger for the OSTS. My experience includes introducing a quality system for all aspects of seed testing including seed health, both internal and external auditing and organisation of an ISTA Quality Assurance Workshop in Edinburgh. Together with a dedicated staff I have ensured our laboratory has maintained accreditation to both ISTA and UKAS (17025 standard) since 1998.

Invitations to speak at the ISTA/PDC Seed Health Symposium, Cambridge, 1996 and the ISTA Mycology Workshop, Ottawa 1997 led to my acceptance of a position on the Seed Health Committee as Quality Assurance Working Group Leader in 1998. Since then I have enjoyed working with a variety of great people and visited many beautiful countries, but the highlight of my work with ISTA so far has been working with Jim Sheppard on the complete overhaul of Chapter 7 of the International Rules for Seed Testing and the subsequent acceptance at the 2001 ISTA Congress in Angers of the ISTA Seed Health Method Validation Programme.

It should never be underestimated the time, effort and sacrifices Jim made to ensure that the Seed Health Committee could move forward and meet the challenges of the 21<sup>st</sup> Century. I look forward to working with my new committee to consolidate this work and to take forward an ambitious programme including: the careful planning and implementation of a proficiency test programme for seed health testing; providing a 'Seed Health Testing Handbook' which supports Chapter 7 of the ISTA Rules; and liasing with other organisations with an interest in seed health testing such as IPPC and ISHI.

I also very much look forward to the 5<sup>th</sup> ISTA Seed Health Symposium in Angers, France (9-12 May 2005). This is a great opportunity to make a contribution to the future of seed health by presenting your work or through participation in the audience or working groups.

Hope to see you there!

### **TECHNICAL COMMITTEES**

### Introduction to new ISTA Technical Committee Chairs

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# Rainer Knoblauch

ISTA Variety Committee Chair



My position is Senior Analyst in the department for Seed Testing and Applied Botany at the governmental institute Augustenberg in Southern Germany.

As a seed tester my specific work was mainly Seed Sampling, Testing of Purity, Germination, Tetrazolium, Seed Health and Variety testing from 1974-1989.

Then I was starting with the electrophoretical work in IEF, SDS, PAGE, Isozymes and so I got the responsibility for variety testing in our department. In this time I developed different seed-crushing-mashines (Kataskapt) for easy sample preparation for our electrophoresis, together with a colleague. I am managing the technical questions in our laboratory, take care for seed storage, I am on the way as ISTA-seed-sampler and also as lecturer for seed sampling.

Besides all this I have to take care of the operational safety and as specialist I am guiding master students and technicians from outside.

It gives me special pleasure to develop or adjust methods to solve sophisticated seed problems and to spread knowledge which I could realise in seven great ISTA/FAO-Workshops worldwide (Agentina, South Africa, Thailand, Slovenia, Egypt, China, Jamaica).

My ideas while chairing the Committee from 2005-2007 is to get more focus to explore new Committee Members and to start Proficiency Tests for different variety methods. This is a special goal for our Committee. I would like to check who is using wich method and for what, to be able to include new methods into the ISTA Rules. As I know many methods are in use in the seed testing labs worldwide and it is my expectation to check these methods and validate together with their developer for the benefit of all ISTA members. In this respect, besides workshops, a special focus shall be given to a new handbook or working sheets. I hope to meet your interest and your support for this Committee work.

As you realise I like high precision, no wonder that my sporting activity is achery. But for relaxing I take care of my garden with flowers, fruit trees, vegetables and my 5 chicken.

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# Norbert Leist

ISTA GMO Task Force Chair



My position is Director of Biology and as such I am the Head of the Department of Agrobiology with the special responsibility for the Section Seed Testing and Applied Botany at the governmental institute Augustenberg in southern Germany. Besides this I am teaching at the University of Karlsruhe (TH) about plant systematics, vegetation systems and seed testing.

The specific work is presented in 120 publications mainly about seeds, with a concentration to the genera Zea and Avena and the topics seed technology, variety testing with Protein and DNA, seed health testing and tetrazolium testing. In this area 55 diploma thesis and 3 doctorate degrees have been absolved.

I have served the ISTA as Chair of the Tetrazolium Committee, member of the committees for purity, variety, tree and shrub seeds, rules and as President from 2000-2004. Besides this I am available as consultant and auditor on Quality Management System for ISTA and DAP.

My graduation as doctor rerum naturarum was at the Ruprecht Karl University of Heidelberg, Germany, after studies and state examination in Botany, Zoology, Chemistry and Physics. This explains my further activities in plant systematics, limnological aspects supported by my diving sport, arachnological activities regarding their systematics and distribution. Due to a postgraduate work in the tropical rainforests in South Colombia I am fascinated and engaged in the vegetation of the tropics. This leads to the hobbies being photographer, diver and hiker.

The work in the GMO Task Force has been setup 2001 and the aim is presented in the respective position paper on the ISTA website.

In three working groups most of the work is initiated, running and the new chapter for GMO testing is prepared for voting at the Ordinary Meeting 2005 in Bangkok. There are mainly 4 engaged colleagues in the Task Force: Prof. Dr. Michael Kruse, Mr. Sylvain Gregoire, Dr. Bettina Kahlert, Dr. Enrico Noli who did most of all that work for the Association.

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### Introduction to new ISTA Technical Committee Chairs

**TECHNICAL COMMITTEES** 

# Stefanie Krämer

ISTA Tetrazolium Committee Chair



I am working as a Senior Analyst in the department of Agrobiology in the governmental Seed Testing Station in Karlsruhe, South-Germany.

My education in seed testing started here in 1978 and since that time I am working mainly in the fields of purity testing, germination testing and viability testing. From January 2005 I am also responsible for the seed health testing in our laboratory. For me, this is a totally new but very interesting work with seeds.

My first official contact with the Tetrazolium Committee was in 1993. There was a mee-

ting in Stuttgart- Hohenheim, Germany, where we created the idea to prepare new Tetrazolium working sheets. It took nearly ten years to bring this idea to reality and in 2003 ISTA was able to publish the working sheets.

Since 1999 I am a member of the Tetrazolium Committee and also of the Flower Seed Testing Committee and the Forest Tree and Shrub Seed Committee.

In the last years I have been involved in the organisation and practical education in several ISTA Tetrazolium workshops and national workshops on Seed Testing, which gives me the possibility to spread my practical knowledge about Tetrazolium testing.

In my private time I follow my fascination of orchids and other tropical plants - you see there is no real border between work and fun.

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# Non-orthodox Seed Moisture Survey

ISTA Moisture Committee

A survey to determine which non-orthodox species are being tested for seed moisture content, and the methodology used to assess the seed moisture content, has been distributed to ISTA Member laboratories. The information gained from this survey will be used to help bring nonorthodox species into the Seed Moisture Chapter in the International Rules for Seed Testing.

If you would like to participate in this survey please contact:

Craig McGill Centre for Plant Reproduction & Seed Technology Institute of Natural Resources Massey University P.B. 11 222 5301 Palmerston North, New Zealand Phone: +64 6 3569099 ext 7841 Fax: +64 6 3505649 E-mail: C.R.McGill@massey.ac.nz

A copy of the survey can also be ordered from the ISTA Secretariat.

# S S E P

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# A comment on a shock

### By N. Leist, M. Kruse and A. M. Steiner, former ISTA TEZ Committee Members

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With great interest and pleasure we saw in Seed Testing International No. 128 the report on The ISTA Tetrazolium Workshop in Tunica, US. However, with some surprise we read (citation): "The shock of the meeting was..[the]..presentation on Glycine seeds which had been stained with .075 % TZ and then were grown out to see the seedlings these staining patterns produced! This technique has enormous potential value in standardizing our evaluations." So far so good, but why a shock? This phenomenon is known for more than 60 years. This finding dates back to the very early days of tetrazolium testing.

After Nobel prize laureate R. Kuhn at Heidelberg had informed G. Lakon at Hohenheim about the TZ salts for replacing the poisonous selenium salts, in 1942 Lakon published his topographical TZ test and Kuhn and his co-worker D. Jerchel continued research on TZ salt reduction in living tissues. Already then they had observed that after TZ staining garden cress seeds may develop into seedlings losing formazan colouration during greening. The germination of seeds having faintly been stained at low TZ concentrations is easily comprehensible. Admittedly, TZ salts are toxic by detracting electrons from the respiratory chain, the generally used TTC at the level of the cytochromes. Though, as long as respiration is not impaired too much, and as long as the cells are not overloaded by formazan crystals, and as long as species specifically the tissue is able to bear with these stresses and to recover, why should it not be possible for a seed to form a seedling? However, for the improvement of TZ testing this finding was not conducive. So it was not until J. Schubert that TZ staining pattern and seedling development was studied again in one and the same seeds in black locust as communicated in 1960 and 1961 in the Proceedings of ISTA. Albeit that this furthered insight, again it did not add to TZ testing. Therefore, supposedly, no subsequent publications appeared on that approach.

Nevertheless, further observations were made thanks to an advantageous lack of orderliness. For having evaluated seeds for TZ viability every now and then these were not disposed, but were forgotten in the boxes where they had been displayed on paper for evaluation. Thus, appropriately stained seeds got a chance to germinate at room temperature in daylight. This way it was observed that species of legume seeds and crucifer seeds may develop into seedlings whereas grass seeds may not.

Returning to the outset: a shock? Well, may the rediscovery of a forgotten observation be regarded as shock, but then hopefully as a beneficial shock. May it initiate restudying the long ago reported findings and may it encourage new studies to assess the usefulness of this approach. References are found e. g. in the Tetrazolium Testing Handbooks of AOSA and ISTA.

# ISTA /APSA Training Course in Seed Testing Hanoi, Vietnam, 22-26 November 2004

By Dr. Michael Turner, Senior Seed Adviser

Danish International Development Assistance (Danida), Vietnam

In late November, the third in a series of ISTA/APSA regional training courses in seed testing took place in Hanoi. This course was hosted by the Seed Component of the Danida Agriculture Sector Programme in Vietnam and was held in the main seed laboratory of the National Centre for Variety Evaluation and Seed Certification, (NCVESC). The ISTA lecturers were Prof. Dr. Norbert Leist and Ms. Andrea Jonitz, both from the State Seed Testing Laboratory in Karlsruhe, Germany.

The course attracted 19 participants from 7 countries of the region, namely Cambodia (2), China (1), Indonesia (3), Japan (2), Laos (2), Philippines (1), and 8 from Vietnam, all employed in public or private seed testing labs. Several late applications had to be rejected, due to space limitations, showing the keen interest in seed testing within the region.

In the introductory session Dr. Michael Turner, Senior Seed Adviser and Dr. Tran Dinh Nhat Dung, Deputy-Director of NCVESC welcomed the lecturers and participants and explained the work of the Danida Seed Component, which provides support to many different parts of the national seed system in Vietnam. Strengthening seed quality management and variety testing are key elements in this support, for which NCVESC is the responsible technical agency.

Prof. Leist then introduced the work of ISTA, explaining the key role which ISTA plays in international seed trading and the many new challenges faced in the past decade, mainly as a result of globalization and devolution of regulatory tasks from government to the private sector in many countries. Meanwhile, the development of biotechnology in the last 20 years has presented new tools for testing but also opened the whole



new area of detecting GMOs in seed samples which can be very sensitive in some countries.

This set the scene for the main part of course which covered the core activities of sampling, then testing purity and germination. The fundamental importance of correct sampling procedures was strongly emphasized, since all subsequent analytical work in the laboratory is based on the assumption that the sample truly represents the entire seed lot. Finally, participants were introduced to the ISTA accreditation standard, which is now a major platform for quality management in seed testing laboratories. The practical materials represented the main field crops of the region, namely rice, maize, soyabean, groundnut and a range of vegetable crops including tomato, cucurbits and brassicas. Dr. Dung and his staff made a tremendous effort to prepare a large amount of practical material and to find some interesting samples which gave rise to plenty of discussion. The lecturers had also prepared a comprehensive set of reference materials for the participants to take home. The participants made full use of the ISTA experts to discuss a wide range of topics and problems relating to their daily work.

This course forms part of a continuing effort to increase awareness about seed quality issues among APSA members, both in terms of technical procedures for quality assessment and the managerial framework within which they are carried out in seed laboratories. The support of Danida and Vietnam to this effort was greatly appreciated, not only for the technical information which participants can gain from such a course, but also for the opportunities to establish professional contacts between staff of seed labs in the region. In this way, we may hope to strengthen the 'ISTA family' in South-East Asia and promote the exchange of experience in the crops of the region. The 2005 ISTA Annual Meeting in Bangkok will provide another opportunity for those involved in seed quality work within the region to get together and participate in the Association.

In the final discussion, there were requests from the participants for further courses of this kind in other countries of the region but that will depend on finding additional sponsorship, probably from development agencies. However, a regional organization like APSA can provide coordination for training courses which can be very helpful to donors.

In this discussion, it was noted that APSA itself was born out of a regional FAO seed project which was financed by the Government of Denmark from 1982 - 1998. This project developed a strong network of seed industry contacts and in its final phase, laid secure foundation for the launch of APSA at Chang Mai in 1994. The subsequent growth of APSA as an important organization in the international seed scene is well known and widely recognized.

APSA and ISTA expect to organize further courses on general or specialized topics and hopes that international development agencies can offer support. A strong domestic seed sector is vital both to ensure food security for poorer farmers and to develop commercial export markets, and both are priorities in the diverse countries of Asia.

# 2<sup>nd</sup> ISTA Workshop on Statistical Aspects of GMO Detection

# St. Louis, Missouri, United States November 17 - 19, 2004

By Kirk Remund, ISTA Statistics Committee Vice-Chair Monsanto Company, St. Louis, USA, email: kirk.m.remund@monsanto.com



The second ISTA Workshop on Statistical Aspects of GMO Detection was held in St. Louis (USA) on November 17-19, 2004, hosted by Monsanto Company. Sylvain GREGOIRE, Jean-Louis LAFFONT and Kirk REMUND made presentations on the following 11 topics:

- statistical distributions
- statistical tests
- uncertainty
- designing test plans
- robustness
- regulatory example
- data checking
- GM estimation
- checking purity of proficency test material
- repeatability
- LOD/LOQ calculations.

Free software including Seedcalc and Qualstat were demonstrated to participants through numerous exercises. Working sessions were very intensive. The participants took an afternoon tour of a Monsanto biotech facility and enjoyed an evening dinner in the city of Creve Coeur.

Twenty-three individuals from academia, government and industry participated in the workshop from North America, Europe, and Australia. In general the participants were pleased with the content and presentation of the workshop topics. Due to the limited number of participants, not all persons that were interested in the workshop were able to attend. A similar workshop will be held in Buenos Aires (Argentina) in the fall of 2005. ■



# 5<sup>th</sup> ISTA/FAO Workshop on **Electrophoretic Methods** and PCR-Techniques for Variety Verification and GMO Detection



Giza, Egypt, December 18 -22, 2004

By Kasem Zaki Ahmed

Minia University, Egypt

This workshop was for seed testing analysts from laboratories located in the Near East region. The aim of the workshop was to train the seed technicians in methods for the verification of species, cultivars and hybrids as well as for qualitative and quantitative GMO detection. The workshop was organized and carried out by ISTA with financial support of FAO.

Despite the busy time of year, twenty participants of 5th ISTA/FAO Workshop on Electrophoretic Methods and PCR-Techniques for Variety Verification and GMO Detection, attended the meeting at Giza, EGYPT, December 18-22, 2004. The workshop participants were actual technicians, with at least a basic knowledge or experience in the field of variety verification or GMO detection. They were from Egypt, Ethiopia, Jordan, Kuwait, Lebanon, Morocco, Oman, Pakistan, Sudan, Syria and United Arab Emirates aimed to Agricultural Genetic Research Engineering Research Institute (AGERI), Agric. Res. Center, Giza, Egypt, very close to the Pyramids (one of the 7 World-Wonders, and the only one that still stands), to trainee and converse electrophoretic and PCR-based methods in this training course

In the first session, the participants gave short presentations about their results, experiences and questions that arose during the testing. For example, they presented what had caused in their case the variation from the true values of the GMO content or what they had done or they might do to improve results in the future. These presentations were essential to this round table and served as a discussion basis.

The workshop was in two parts: the first part: **Electrophoretic Methods for Varietal** 

Verification, Was carried out by Prof. Dr. Norbert Leist, ISTA GMO TF Chair (the theoretical section), and Mr. Rainer Knoblauch, ISTA Variety Committee Vice Chair (the practical work) Staatl. Landw. Untersuchungs- und Forschungsanstalt Augustenberg, Karlsruhe, Germany. While the second part: PCR based Methods for GMO Detection was carried out by Dr. Enrico Noli, ISTA GMO TF Member Lab. di Ricerca e Analisi, Sementi LaRAS, DiSTA, Università di Bologna, Bologna, Italy.

The workshop provided general information regarding the possible applications and hands-on experience of electrophoresis and PCR-based methods in the field of variety verification and GMO detection. Modern Methods for Varietal Verification such as Isoelectric Focusing in Ultrathin layer (IEF) of seed storage proteins and testing of seed storage proteins with PAGE, SDS and Isozymes were applied with wide range of crops e.g. Zea mays, Triticum aestivum, Cucumis sativa, and Orvza sativa. For the second part of the workshop "PCR based Methods for GMO Detection" many theoretical (Sample preparation and DNA extraction - PCR analysis: basis, molecular markers and PCR-based methods for GMO detection - introduction in other methods such as ELISA and bioassay) and practical subjects (DNA extraction, UV spectrometry

- gelpreparation, fixation, staining, destaining and evaluation of the gels - DNA extraction, UV spectrometry - Agarose gel electrophoresis of extracted DNA - Amplification of endogenous invertase gene -PCR for qualitative detection of CaMV 35S promotor, epsps gene and cryIA(b)gene) were studied.

All participants were thankful to Dr.

Enrico Noli for introducing them to the new a Real time PCR method and special thanks to Dr. Gihan M. El-Moghazy (Central Lab. For Food & Feed, Agric. Res. Center, Giza) for organize a Real time machine and practice in her lab.

All information and power point presentations were given on CD-Rom and on a printed version to the participants. Many related websites were visited. Working sessions were very intensive, allowing only a short visit to the AGERI laboratories and a walk through Khan el-Khalili (the old Egyptian down town Souk) with an evening meal in the Nile City Ship swim in the Nile River in beautiful Cairo.

We are grateful to the members of the Local Organizers Committee, Prof. Dr. Haniya El-Itraby (Director of AGERI) and Dr. Hesham El-Sharnoby and all staff members of AGERI (Giza, Egypt), for their many-sided support during this training course. Our thanks are also due to Dr. Kakoli Ghosh (Agriculture Officer for capacity building from the Seed and Pant Genetic Resources Servers of the Agriculture Department, FAO) and Ms. Branislava Opra (Head of Membership Department, ISTA) for organizing, communication, offering valuable information and for making this workshop successful.



# 5<sup>th</sup> ISTA Seed Health Symposium

# Angers, France, May 10 - 13, 2005

Joël Léchappé, President of the Organising Committee







This second announcement of the  $5^{\text{th}}$  ISTA – SHC Seed Health Symposium includes the scientific programme, the registration form and additional information about the venue and your stay in Angers.

By the end of September 2004, more than 100 people from 45 countries had indicated their interest and had pre-registered. We thank them very much.

We would be very grateful to you for a final registration at your earliest convenience, and by February  $28^{th}$ , 2005 at the latest. After this date, the registration fees will be increased.

Your presence will contribute to the success of the Symposium.

The Organising Committee is looking forward to welcoming you to Angers, France in May 2005.

Joël LÉCHAPPÉ

President of the Organising Committee

### DATE AND VENUE

The 5<sup>th</sup> ISTA – SHC Seed Health Symposium will take place from May 10<sup>th</sup> to May 12<sup>th</sup>, 2005 at the Angers Congress Centre which is in the very heart of the city, at walking distance from historical sites and from the selected hotels (no transportation will be provided from hotels). Seed health committee and working group meetings will be organised at SNES on May 13<sup>th</sup>.

All facilities will be centralised at the Congress Centre: commission room, exhibition space, posters space, lunch area and reception offices with secretariat services. All participants will check in at the Congress Centre, the registration desk of which will be opened on May,  $10^{th}$  at 11:00 a.m.

### Languages

The working language of the Symposium will be English. There will be no translation.

### Climate

Angers has a temperate climate. Weather in May is generally warm and sunny (17 to 22°C). Evening may be cooler. Rainfall, which is necessary after all for an attractive vegetation-covered landscape, may sometimes occur.

### Currency and finance

France uses the Euro. The registration fees must be paid solely in Euro ( $1 \in = 1,25 \text{ US }$  s approx.). Major credit cards are accepted in hotels, shops and restaurants. Travellers cheques and foreign bank notes of all major currencies can be exchanged in any commercial banks and in hotels. Numerous cash-machines are available to withdraw Euros using credit cards.

### ORGANISATION

### **Hosting Institute**

GEVES: Groupe d'Étude et de contrôle des Variétés et des Semences

### **Organising Committee**

- GEVES
- INRA
- Plant Health Service/National Laboratory
- for Plant Health
- Angers University

### Registration

Centre de Congrès d'Angers 5th ISTA - SHC Seed Health Symposium 33 Boulevard Carnot 49100 ANGERS France Phone: +33 (0)2 41 96 32 32 – Fax: +33 (0)2 41 96 32 33 E-Mail: ista@angers-expo-congres.com Website: http://www.angers-congres.com/ISTA/

### Information

GEVES - SNES 5<sup>th</sup> ISTA - SHC Seed Health Symposium Rue Georges Morel BP 90024 49071 BEAUCOUZE Cedex France Phone: +33 (0)2 41 22 58 03 Fax: +33(0)2 41 22 58 01 E-Mail: Veronique.binoit@geves.fr Website: http://geves.zarcrom.fr/rubrique.php?rub\_id =99042&site\_graph=

### **REGISTRATION FEES**

Online registration and payment facilities are available at:

http://www.angers-congres.com/ISTA/

The symposium registration fee is  $395 \in (1 \text{ Euro} = 1,25 \text{ US } \text{ approx.})$ . It includes attendance at all meetings, the welcoming reception, coffe/tea breaks, lunches, symposium dinner, shuttles (for the symposium dinner and the meetings held at SNES) and abstract booklet.

There is no program for accompanying persons but they can participate to the symposium dinner for  $65 \in .$ 

### Seed Testing International No. 129 April 2005

### **TECHNICAL COMMITTEES**

5th ISTA Seed Health Symposium

### EXHIBITION

An exhibition of technical equipment will be organised. It will be located in an area leading to the main conference and poster halls. This will offer an excellent opportunity for international exhibition and direct communications between the suppliers and users of equipment.

### ACKNOWLEDGEMENTS

The  $5^{\text{th}}$  ISTA-SHC Seed Health Symposium receive financial support from several institutions, companies and seed associations. The organisers are most grateful to all the sponsors and donors, whose contributions will make the  $5^{\text{th}}$  ISTA-SHC Seed Health Symposium a successful meeting.

### The National Seed Testing Station

The National Seed Testing Station which was established in France in 1884, moved from the Paris area to Angers in 1993. It is now housed in a new building specially designed for the development of the Station's major activities: seed testing, perfecting methods and training. It has 72 permanent members of staff working in various areas of seed quality and testing: sample checking, purity, germination, seed-borne pathogens and molecular biology.

The National Seed testing Station is part of GEVES, which is involved in the study and control of varieties for registration purposes and plant breeders' rights, the management

of genetic resources and the setting up of applied research programmes focusing parti-

applied research programmes focusing particularly on characterisation, experimental design, data treatment and management.

### ACCESS TO ANGERS FROM PARIS

### From Roissy-Charles de Gaulle airport

- Direct link by TGV (high speed train) to Angers (2:10 hours travel). 3 TGV per day from the airport terminal 2. Advance seat reservation necessary (return ticket costs 100 to  $130 \in \text{ in } 2^{nd} \text{ class}$ ).

- Air France bus shuttle service, leaving from terminal 1 and 2 every 30 minutes (from 7:00 to 21:00) to Montparnasse railway station in the city of Paris (one way ticket costs 11,50 €)

- Taxi between Roissy and Montparnasse costs approximately 60 €.

**From Orly-Sud and Orly-Ouest airports** - Air France bus shuttle service, leaving from the airports every 15 minutes (from 6:00 to 23:00) to Montparnasse railway station (one way ticket costs 7,50  $\in$ ). - Taxi between Orly and Montparnasse costs approximately 30  $\in$ .

### From Montparnasse railway station

- 15 TGV per day to Angers (1:30 hours travel). Advance seat reservation necessary (return ticket costs 84 to 108

 $\in$  in 2<sup>nd</sup> class).

### By road

- A10 and then A11 motorway from Paris to Angers. 3:00 hours drive.

FOR MORE INFORMATION ABOUT





### 5<sup>th</sup> ISTA - SHC Seed Health Symposium Reference to be quoted: AXZE SE 41085 Offer valid from 7 May 2005 to 15 May 2005

Preferential fares (subject to conditions) on the regular full fare in business or economy class on a round-trip journey are made available for this event.

To make use of this offer, make reservations and have electronic tickets issued, please contact the address below , giving details of the event.

### e-mail : mail.espaff2cdg@airfrance.fr

TELEPHONE: +33.(1).48.64.37.81 FAX:+33.(1).48.64.66.38

This document is mandatory for the issuance and collection of the ticket and will be required at any point of the journey.

Société Air France , société anonyme au capital de 2 289 759 903 euros – RCS Bobigny 552 043 002 Siège social : 45 rue de Paris, F95704 Roissy Charles de Gaulle cedex, France

### Symposium Programme 16:00 - 17:00 Session 4: Seed Health and the international movement of seed 16.00 - 16.30 Thakur Ram - seedborne and quarantine significant fungal pathogens of sorghum, pearl millet, chickpea, At the SNES laboratory pigeonpea and groundnut 16:30 - 17:00 Lamka Gregory - Successful implementation of the National Seed Health System in the USA Monday, May 9 17:00 - 18:00 Session posters Seed Health Committee meeting 09:00 - 17:00 19:00 - 20:00 Welcoming reception by the Mayor of Angers At the Congress Centre 20:00 - 23:00 Symposium dinner **Tuesday, May 10** Thursday, May 12 14:00 - 15:30 Opening ceremony 08:30 - 10:00 Session 5: New disease and emerging seed-Pieter Oosterveld, President of ISTA borne pathogens Guy Riba, President of GEVES Shakya Dilli - Detection of Fusarium solani in 08:30 - 09:00 Dalbergia sissoo pods and seedlings 15:30 - 17:00 Session 1: Methods of standardisation and 09.00 - 09.30Duvnjak Tomislav - Soybean seed decay in Croatia quality assurance in seed health testing 09:30 - 10:00 Mortensen Carmen Nieves - Emerging seed-borne Zoina Astolfo - Evaluation and improvement of the 15.30 - 16.00bacterial diseases in developing countries of Africa official methods for the detection of quarantine and Asia bacteria in potato seed tubers Break 16:00 - 16:30 Gitaitis Ronald D. - Alognormal distribution of phy Session 6: Innovation and technical improve 10:30 - 12:30 topathogenic bacteria in seeds and its potential impact ment in seed health testing on quality assurance of seed health assays 10:30 - 11:00 Sahin Fikrettin - Observation of bacterial spot causing 16:30 - 17:00 Thomas Jane - Sampling strategies for effective organism from naturally infected pepper seeds in detection of Tilletia tritici in wheat seed bulks Turkey Break 11:00 - 11:30 McNeil Marian - Improving the reliability of real-time 17:30 - 19:30 Workshop on Validation Method PCR assays for Microdochium nivale and Tilletia 17:30 - 17:40 Introduction to method validation caries 17:40 - 18:00 The test plan 11:30 - 12:00 van der Wolf Jan - Detection of seedborne pathogens 18:00 - 18:30 Analysis of results by flow cytometry or by the Luminex MAPS 18:30 - 18:50 The test report technology 18:50 - 19:00 Review and approval of methods 12:00 - 12:30 Fargier Emilie - Detection of living cells of 19:00 - 19:30 O&A session Xanthomonas campestris pathovar campestris in crucifer seeds Wednesday, May 11 Lunch 08:30 - 10:00 Session 2: Seed contamination 14:00 - 15:30 Session 6: Innovation and technical improvement 08:30 - 09:00 Cockerell Valerie - Integrated field experimentation in seed health testing and modelling of the bunt (Tilletia caries) life cycle 14:00 - 14:30 Thomas Jane - Development of PCR based approach 09:00 - 09:30 Pinnschmidt Hans - Quantitative relationships in the for detection of Xanthomonas campestris pv infection cycle of seed-borne net blotch campestris in brassica seed 09:30 - 10:00 Pradhanang Prakash - Tobacco mosaic virus: does it 14:30 - 15:00 Justesen Annemarie Fejer - Quantification of leaf really transmit through tomato seeds? stripe, Pyrenophora graminea, in barley seed by Break real-time PCR 10:30 - 12:30 Session 2 : Seed contamination Session 7: Chemical and physical seed treatment 10:30 - 11:00 Walcott Ron - The role of blossoms in waterlemon 15:00 - 15:30 Borgen Anders - Brush cleaning to remove fungal seed infestation by Acidovorax avenae subsp. Citrulli, spores from seed lots causal agent of bacterial fruit blotch 15:30 - 16:00 Jahn Marga - Evaluation of hot water, hot air and 11:00 - 11:30 Jacques Marie-Agnès - Seed transmission of electron treatment for seed sanitation in organic Xanthomonas axonopodis pv. phaseoli, agent of vegetable production common bacterial blight, on symptomless beans Break Session 3: Characterisation of population of 16:30 - 18:30 Session 7: Chemical and physical seed treatment seed pathogens 16:30 - 17:00 Pradhanang Prakash - Acid seed treatment is an 11:30 - 12:00 Aboul-Ata Elnady - Seed-Borne Barley stripe mosaic effective tool for management of bacterial canker virus in Egypt: incidence, effect of virus and seeddisease of tomato caused by Clavibacter transmisibility michiganensis subsp. michiganensis in California, Gaurav Shailendra Singh Effect of loose smut 12:00 - 12:30 USA (Ustilago segetum var. tritici) on yield components of 17:00 - 17:30 van der Wolf Jan - Natural compounds for wheat disinfection of vegetable seed Lunch 17:30 - 18:00 Koch Eckhard - EU project seed treatments for 14:00 - 15:30 Session 3: Characterisation of population of organic vegetable production seed pathogens Ilyas Satriyas - Effect of clove flower powder as seed 18.00 - 18.30 14:00 - 14:30 Avenot Hervé - Effects of mutations in the histidine treatment on fungi contamination, seed viability and kinase gene AbNIK1 from the seed-borne pathogen vigour of soybean during storage Alternaria brassicicola on iprodione resistance, osmo sensivity and fitness At the SNES laboratory 14:30 - 15:00 Alavi Seyed Mehdi - Genetic caracterisation of Xanthomonas axonopodis pathovar phaseoli responsi Friday, May 13 ble of common bacterial blight on bean 09:00 - 12:30 Seed Health Committee meeting and Working 15:00 - 15:30 Riccioni Luca - PCR-RFLP identification of the group meetings Phomopsis/Diaporthe species on soybean seeds

Break

# 6<sup>th</sup> ISTA/FAO Workshop on Electrophoretic Methods and PCR-Techniques for Variety Verification and GMO Detection

University of West Indies (UWI), Kingston, Jamaica May 9 - 13, 2005

The International Seed Testing Association (ISTA) and the Food and Agriculture Organization of the United Nations (FAO) have great pleasure to announce the 6th ISTA/FAO Workshop on Electrophoretic Methods and PCR-Techniques for Variety Verification and GMO Detection. This workshop is for seed testing analysts from laboratories located in the Caribbean and Central American region. The aim of the workshop is to train the seed technicians in methods for the verification of species, cultivars and hybrids as well as for qualitative and quantitative GMO detection. The workshop will be made up of lectures and practical work.

### Lecturers:

# Part 1: Electrophoretic Methods for Varietal Verification

- Ms. Andrea Jonitz,

- Mr. Rainer Knoblauch, ISTA Variety Committee Chair

Staatl. Landw. Untersuchungs- und Forschungsanstalt Augustenberg, Karlsruhe, Germany

# Part 2: PCR based Methods for GMO Detection

- Dr. Christoph Haldemann, ISTA GMO Task Force Member

Swiss Federal Research Station for Animal Production RAP, Switzerland

### **Considered Workshop Content:**

The workshop shall provide general information regarding the possible applications and hand-on experience of electrophoresis and PCR-based methods in the field of variety verification and GMO detection. These will cover:

- Object, field of application and general principles of the verification of species and



### cultivars

- Seed storage proteins analysis
- Testing of seed storage proteins with IEF, PAGE; SDS; Isozymes: Protein extraction, gel preparation, fixation, staining, destaining and evaluation of the gels
- Sample preparation and DNA extraction

- PCR analysis: 1) Basis, 2) Molecular markers, 3) PCR-based methods for GMO detection

- Introduction in other methods such as ELISA and bioassay

### **Considered Practical Work:**

- Isoelectric Focusing in Ultrathin layer (IEF) of seed storage proteins:

- Extraction of different protein classes
- Production, loading and running of gels
- Evaluation of results

- ISTA Method for maize and sunflower and adaption of methods for special questions

- DNA extraction, UV spectrometry - photometry

- Agarose gel electrophoresis of amplified DNA

- PCR for qualitative detection of CaMV 35S promotor, epsps gene and cryIA(b) gene

### **Participants:**

Considering the above, the workshop participants should be actual technicians, with at least a basic knowledge or experience in the field of variety verification or GMO detection.

### **Costs:**

Kindly note that funds can be secured for the workshop expenses covering accommodation for 5 nights, food during the workshop period as well as a maximum of US\$400.for travel expenses.

### **Registration:**

The workshop is limited to 20 participants! Seed analysts can apply for the participation with the attached registration form and a short curriculum vitae about their working experiences and technical duties which they performed in their laboratory. Please send the preliminary registration form as soon as possible to the ISTA Secretariat. For further information please do not hesitate to contact the ISTA Secretariat.

### **Contact persons from ISTA and FAO** Dr. Kakoli Ghosh

FAO, Seed and Plant Genetic Resources Service, Capacity Building Email: kakoli.ghosh@fao.org

Branislava Opra, M.Sc. ISTA, Head of Membership Department Email: branislava.opra@ista.ch



# 10<sup>th</sup> Workshop on Tetrazolium Testing on Tree and Shrub Seeds

LUFA Augustenberg, Karlsruhe, Germany July 11 - 15, 2005

Stefanie Krämer, ISTA Tetrazolium Committee Chair

### Location

State Agricultural Testing and Research Institute, Department Seed Testing and Applied Botany Nesslerstr. 23 D-76227 Karlsruhe Germany

### Participants

16 at maximum

### Participation fee

- 350 Euro, ISTA Members
- 420 Euro, Non-ISTA Members
Hotel available for 50 Euro/night

### Programme

- Lectures on History and development of Tetrazolium Testing and FTS in ISTA, Viability - germination - vigour, general and special aspects of Tetrazolium Testing.

- Tetrazolium test in woody plants, Physiological aspects.

- Practical Work with the ISTA Rules and Tetrazolium Working Sheets on *Abies, Larix, Pinus* large and small seeded; *Fagus, Genista, Prunus, Quercus, Sophora* 

- Half day excursion in the black forest.



### For final registration

Please send the filled registration form to:

Ms. Stefanie Krämer or Prof. Norbert Leist E-mail: Stefanie.Kraemer@lufa.bwl.de, Norbert.Leist@bio-geo.uni-karlsruhe.de Fax: 0049 (0) 721 9468 387 Phone: 0049 (0) 721 9468 150 LUFA Augustenberg, Nesslerstr. 23, D- 76227 Karlsruhe, Germany

# Register Online for Workshops at

# www.seedtest.org

# 7<sup>th</sup> Seminar on Statistics in Seed Testing

Stuttgart, Germany August 29 -September 2, 2005

Call for registration to the 7<sup>th</sup> ISTA Seminar on Statistics in Seed Testing

### Location

University of Hohenheim Institute for Plant Breeding, Seed Science and Population Genetics 70593 Stuttgart Germany

### **Preliminary Participation fee** Euro 150.

### Programme

1. Workshop on practical applications of statistics in seed testing like tolerances, proficiency tests, seed calc ....

2. Presentation and discussion of STA work, Excursion

3. Scientific Symposium on new statistical approaches for seed testing, statistics of GMO testing, statistics for scientific research

For the further planning of the seminar we need your binding registration and, if you like, your title of an oral or poster contribution to the seminar. After receiving the registrations, we will send you further information, including the program and travel information.

For travel-information please refer to the homepage of the University of Hohenheim (www.uni-hohenheim.de), (click on the English flag and then "how to find us"). Stuttgart airport (STR) as well as highways is very close to the campus (about 10 minutes by car).

We reserved a contingent of 20 rooms in "Christkönigsheim" that is very close to the campus (45€ per night including breakfast). When we receive your registration we will book the rooms for you. ■

### deadline for receiving your registration form: May 10

### Binding registration for the 7<sup>th</sup> ISTA Seminar on Statistics 2005

Last Name	Address			
First Name				
Institution / Company	Country			
	Phone			
	Fax			
	e-mail			
I will attend the meeting during the following days (tick relevant boxes)	:			
Monday, August 29Image: Constraint of the sector of the secto	workshop um			
I would like to present an oral D poster D presentation with the following title :				
in the practical workshop 🛛 🛛 in the symposit	um 🗖			
I will arrive on and leave on I will travel by airplane □, train □, car □.	(dates).			
I have ordered the participation fee of 150€ to	be transferred to:			
Bank:Baden-WuerttembergiscBank Code (BLZ):600 200 30account No. :105 455 40700purpose:BA 350 501 ISTA Seminal	the Bank Stuttgart			
My field of work is:	Application of statistics:			
□ seed testing	Lis my daily work			
L seed research	□ am interested to learn			
	L is my field of research			
- 00200				

ISTA Tetrazolium Proficiency Test 04-1

# ISTA Tetrazolium Proficiency Test 04-1

By Ronald Don, ISTA Proficiency Test Leader

2004 marked a new milestone in tetrazolium history with the first ISTA proficiency test. The results have now been analysed and laboratories have been informed of their performance. So how well did laboratories perform and how close were we to achieving uniformity in tetrazolium testing within ISTA?

Eighty-four laboratories took part in the proficiency test and of these 64 were accredited laboratories and 20 volunteer laboratories. Here is a brief resumé of the results which have been subject to statistical analysis at the ISTA Secretariat using the same computer program that is used to analyse purity, germination and moisture content results.

The mean viability results obtained are shown in Table 1.

When reporting the results to ISTA all 84 did this correctly with replicate results for viable seeds of individual lots being in tolerance with one another. An examination of the results show that they were distributed around the mean in a similar way to germination and purity proficiency test results. However, for all three samples the mean value for viable seeds was skewed to the lower end of the distribution with the sample modes and medians being higher than the means (Figure 1).



Figure 1 Frequency distribution of results reported on the three wheat samples by laboratories participating in the first ISTA Tetrazolium proficiency test

There was also considerable variation in the results, which was particularly noticeable in Samples 1 and 3 (see Table 2).

It was decided to analyse the results using the standard ISTA program that is used to assess the performance of laboratories in ISTA purity, germination and moisture content proficiency tests. Because of the nature of seed and its variability, even with a homogenous referee sample, repeated tests in the same laboratory will produce a range of results and 2.5% of these will have z-scores that are less than (<) minus 2 and 2.5% will



have a z-scores greater than 2 (>). No laboratory had a z-score of >+2.0 but 9.5% of laboratories had a z-score of <-2.0. This indicates that a significant proportion of laboratories tended to be too severe in their assessment of the samples. This assessment problem was particularly marked in sample 1 with 20% of volunteer laboratories and 11.9% of accredited laboratories having a zscore of <-2.0 (Table 3).

Table 1	Mean	results	obtained	in	first	ISTA	Tetrazoliu	m Pr	oficiency	Test
Table L.	witan	ICSUITS	obtaincu	111	111 30	INIA	I CU azonu	111 1 1	υπαιτική	1030

Sample	Mean of all Labs (% Viable)	Mean of Volunteer Labs (% Viable)	Mean of Accredited Labs (% Viable)
1	89	87	89
2	94	92	95
3	95	94	95

### Table 2. Descriptive Statistical Analysis of the first Tetrazolium Proficiency Test

Statistical Function	Sample 1	Sample 2	Sample 3
Mean	88.90	94.40	94.76
Median	90.75	95.5	96.75
Mode	91.5	97.5	97.5
Minimum	60.5	77.75	44.25
Maximum	98	99	99.25
Range	37.5	21.25	55
Sample Variance	47.4145	15.8299	50.8191
Standard Deviation	6.8858	3.9787	7.1288
Standard Error	0.7513	0.4341	0.7778
Confidence Limit (95.0%)	1.49	0.86	1.55

### Table 3. Details of Analysis of Z-Scores obtained in Tetrazolium Proficiency Test

Statistical Function	Sample 1	Sample 2	Sample 3	Average
Sample Results with z-scores <-2.0	10	7	7	8
Sample Results with z-scores $>+2.0$	0	0	0	0
Total of Sample Results with z-scores				
<-2.0 and >+2.0	10	7	7	8
Proportion of all Laboratories with z-				
scores <-2.0 and >+2.0	11.9%	8.33%	8.33%	9.5%
Proportion of Volunteer Laboratories				
with z-scores <-2.0 and >+2.0	20%	10%	10%	13.33%
Proportion of Accredited Laboratories				
with z-scores $<-2.0$ and $>+2.0$	9.4%	7.8%	7.8%	8.33%

### ISTA Tetrazolium Proficiency Test 04-1

A plot of the z-score distribution confirms this finding. Results are skewed to the lower end of the distribution with some laboratories being more severe in their assessment of the samples and obtaining fewer viable seeds (Figure 2).



Figure 2 Distribution of z-scores for the three samples tested in the tetrazolium proficiency test

In general, volunteer laboratories were more severe in their assessments than accredited laboratories with significantly lower numbers of viable seeds being obtained in all 3 samples by volunteers laboratories (Figure 3).



Figure 3 Mean tetrazolium viabilities obtained by volunteer and accredited laboratories on the three wheat samples tested in the first ISTA tetrazolium proficiency test

Eighty-two percent of participants used the longitudinal cut method of preparation and those using this method tended to get lower viabilities than those using the embryo excision method (Figure 4).



Figure 4 Mean tetrazolium viabilities obtained by participating laboratories using embryo excision and longitudinal cut methods of preparation on the three wheat samples tested in the first ISTA tetrazolium proficiency test However, there was no significant difference between the results of laboratories using the embryo excision and longitudinal cut methods and the lower results obtained using the longitudinal cut method could due to the high proportion of participants using this method. Of the 20 volunteer laboratories, only 2 used the embryo excision method.

In terms of ratings the performance of laboratories is similar or better than that found in other proficiency tests (Figure 5) (see also report on Moisture Content proficiency test in this issue of Seed Testing International).



Figure 5 Ratings obtained by laboratories in ISTA tetrazolium proficiency tests on Triticum

Ninety percent of accredited laboratories achieved an A or a B rating with 5% obtaining a BMP rating. The proportion of A and B ratings from volunteer laboratories was lower (70%) and 20% of them obtained a BMP rating. All laboratories that obtained a BMP rating have been offered a new set of samples and they will be provided with detailed feedback on their performance. In addition, some laboratories with A, B and C ratings were also offered further samples particularly where their performance showed inconsistency between samples.

Laboratories should grasp the chance to test the additional samples should they have been offered. It is hoped that practice on the additional samples, the test leader's comments and recommendations that were issued to laboratories with their results will assist in improving the overall performance of laboratories in the next Tetrazolium proficiency test.

Laboratories have to be congratulated on their performance in this proficiency test. The result obtained, with only 5% of accredited laboratories having a BMP rating, give our customers assurance of the tetrazolium results reported on its International Certificates. I would like to thank all of the laboratories that took part in the proficiency test and also thank Martina and Gerhard of the Secretariat Accreditation Department for their timely work in the analysis of the results. Additional thanks are also given to those laboratories that voluntarily carried out a tetrazolium vigour assessment of the three samples. Preliminary analysis of the results of this assessment has now been completed and is the subject of another report in this issue of Seed Testing International.



Viable Wheat seed - perfect staining of all essential structures



Non-viable Wheat seed - damage to root and shoot



Non-viable Wheat seed - sprouted with no live roots and some dead coleoptile tissue



Viable Wheat seed - although there is damage to root, there is sufficient to allow normal development

# ISTA Moisture Content Proficiency Test 04-1

By Ronald Don, ISTA Proficiency Test Leader

The results of the first moisture content proficiency test (03-1) were very encouraging with a distribution of results similar to those obtained in Purity and Germination proficiency tests but with a better overall performance from participating laboratories.

"Yes but that was the easy one", said a cynical Chair of the Moisture Committee. "No grinding was required for the Trifolium samples - wait till the next proficiency test before you offer your congratulations. It will be more of a challenge to laboratories as it will involve testing a species that requires grinding prior to oven drying." Well was he correct with his reservations?

The results of the 04-1 Moisture Proficiency test on Triticum have been finalised and I am pleased to report that the results are much better than Harry anticipated with 81% of accredited laboratories achieving an "A" score. But how did the results of the Trifolium and the Triticum Moisture Content proficiency tests compare?

Here is a brief resume of the results which have been subject to statistical analysis at the ISTA Secretariat using the same computer program that is used to analyse purity and germination results. One hundred and three laboratories took part in the proficiency test and of these 81 were accredited laboratories and 22 volunteer laboratories.

The mean results of the samples were (Table 1):

When reporting the results to ISTA, 90 laboratories did this correctly. Eight laboratories reported results to two or more decimal places and one laboratory reported results to the nearest whole number (the ISTA Rules state that: moisture content must be reported to the nearest 0.1%). Six laboratories reported replicate results that differed by more than 0.2% (the ISTA Rules state that: Take as the result the arithmetic mean of the duplicate determinations carried out on a sample if the difference between the two determinations does not exceed 0.2%. Otherwise, repeat the determination in duplicate)

An examination of the results show that they were distributed around the mean in a similar way to germination and purity proficiency test results (Figure 1). However, there is a tendency for the mean to be skewed towards the lower side of the distribution.

A similar effect is observed when examining the distribution of z-scores (Figure 2) where the predominance of scores with values greater than 2 occurs at the negative end of the distribution.

So was Harry correct in thinking that the Triticum Moisture content proficiency test would be more difficult than the Trifolium one? Well on the evidence of the scores that the laboratories achieved this was definitely the case (Figure 3).

In the Trifolium proficiency test no accredited laboratory obtained a BMP rating and 96% of accredited laboratories obtained either an A or a B rating. In the Triticum proficiency test 6% of accredited laboratories were rated BMP and the proportion of laboratories with an A or a B rating was 92%. The decrease in rating was even more pronounced with volunteer laboratories. For the Trifolium proficiency test the proportion of volunteers with a BMP rating was 12% and 88% of volunteers had an A or B rating. In the Triticum the proportion of BMPs increa-

 
 Table 1 Mean Moisture Content results in the ISTA Moisture Content Proficiency Test 04-1

Sample	Mean of all Laboratories (% Moisture Content)	Mean of Accredited Laboratories (% Moisture Content)
1	13.9	13.9
2	6.4	6.3
3	16.2	16.2

sed to 27% and the As and Bs decreased to 64%.

So why did the performance of laboratories decrease in the Triticum proficiency test? The need to grind the Triticum samples introduces another variable that can have a direct



Figure 1 Frequency distribution of results reported on the three wheat samples by laboratories participating in the second ISTA Moisture Content proficiency test



Figure 2 Distribution of z-scores for the three samples tested in the moisture proficiency test



Figure 3 Ratings obtained by laboratories in ISTA Moisture Content proficiency tests on Trifolium and Triticum

effect on the results of Moisture Content tests. Laboratories whose ratings decreased should look very carefully at their procedures:

# Is their grinder calibrated and did they grind their samples to the required particle size?

- particle size will have an influence on the rate of drying in the oven and on the moisture content reported. Large particle size will lead to lower moisture contents.

### Does their grinder heat up during operation?

- if the grinder heats up significantly this will cause the sample to heat up and this will result in moisture loss and lower moisture contents being reported.

### Is the grinding rapid and the exposure of the ground material to the air restricted?

- prolonged grinding and exposure of the ground sample to the air will result in lower moisture contents.

The test leader has offered laboratories with BMP ratings the opportunity to test additional prepared Triticum samples and receive feedback on their results. They are encouraged to accept this opportunity and use it to refine their procedure to ensure that they improve their rating in the next Moisture Content proficiency test (05-1), that will also involve a species that requires grinding.

Overall laboratories should be congratulated for their performance in both Moisture Content proficiency tests. An examination of the proportion of accredited laboratories obtaining BMP ratings (Figure 4) shows that the moisture content test compares favourably with the germination test and that both moisture content and germination tests have lower proportions of BMP rating than analytical purity and other seed determination tests.



Figure 4 Proportion of accredited laboratories obtaining BMP ratings in Proficiency tests

# ISTA Tetrazolium Vigour Comparative Test

**By Ronald Don,** (Tetrazolium Committee) and **Gillian McLaren** (Vigour Committee), ISTA Proficiency Test Leaders

Surveys of both ISTA and AOSA seed testing laboratories have shown that about two thirds of those responding provide a seed vigour testing service with 16% of ISTA laboratories and 43% of AOSA laboratories carrying out more than 500 tests per year. The main reason for the tests was customers' requirements for vigour tests with quality assurance and research being other major reasons. Most of the vigour tests carried out have not been standardised and information on intra and inter laboratory variation is limited.

Vigour tests in the ISTA Rules are limited to the conductivity test for Pisum sativum and the Accelerated Ageing test for Glycine max. Both of these tests require specialist equipment and only limited numbers of laboratories are accredited to carry out the tests. Within the ISTA "Handbook of Vigour Test Methods" there are numerous other vigour testing methods and the Vigour Testing Committee has been tasked with the validation of vigour testing procedures that can be included in the ISTA Rules.

Over 60 ISTA laboratories are accredited to test seed viability using the Tetrazolium test and the first ISTA Tetrazolium Proficiency test in 2004 provided the ISTA Tetrazolium and Vigour Committees with a unique opportunity not only to gauge the performance of laboratories in carrying out Tetrazolium viability tests but also to assess the potential of the Tetrazolium test as vigour testing method. Laboratories taking part in the Tetrazolium Proficiency test on wheat seed were asked to voluntarily assess the seed for vigour as well as viability.

When using the Tetrazolium test to assess viability, seeds are classified as viable or non-viable according to the staining pattern. An embryo need not be completely stained to be classed as viable and the ISTA Rules give details of the maximum area of unstained, flaccid or necrotic tissue that is permitted in viable seed. As far back as 1955 viable seed have been classified as vigorous or of low vigour according to the tetrazolium staining pattern. In this comparative test participants were asked to classify the three wheat seed lots for vigour and give each seed a score of 1 to 5 according to their staining pattern.



Category 1: Viable Seed - fully stained



**Category 2:** Viable Seed - some unstained tissue in non-critical areas



**Category 3:** Non-Viable Seed - more than 50% stained but unstained areas in critical areas (This seed exhibits heat damage characteristics)



**Category 4:** Non-Viable Seed - Less than 50% stained

### ISTA Tetrazolium Vigour Comparative Test



Category 5: Dead Seed - No staining.

Sixty-three laboratories of the 84 laboratories who participated in the ISTA Tetrazolium Proficiency Test 04-01 voluntarily scored the three wheat lots for vigour. A preliminary statistical analysis of the results has now been completed.

For each lot an average vigour score was calculated:

For example:

Category	Number of Seed in Category (SCat)
1	20
2	40
3	20
4	15
5	5

Vigour Score
= ((20 + (2*40) + (3*20) + (4*15) +
(5*5))/100
=(20+80+60+60+25)/100
= 2.45

The mean vigour score results obtained are shown below:

Seed Lot	Mean of all Laboratories (Vigour Score)
1	1.43
2	1.28
3	1.26

An examination of the results show that they were distributed around the mean in a similar way to germination and purity proficiency test results. In all three samples the mean value for the vigour score was skewed to the lower end of the distribution with the sample modes and medians being higher than the means (Figure 1).

There was also considerable variation in the



Figure 1 Frequency distribution of results reported on the three wheat samples by laboratories participating in the ISTA Tetrazolium Vigour Comparative test

Table 1.	Descriptive	Statistical	Analysis	of the fi	rst Tetrazolium	Proficiency	7 Test
			, ~-~			,	

Statistical Function	Sample 1	Sample 2	Sample 3
Mean	1.43	1.28	1.26
Median	1.38	1.20	1.17
Mode	1.28	1.16	1.12
Minimum	1.08	1.04	1.03
Maximum	2.42	2.64	2.50
Range	1.34	1.60	1.47
Sample Variance	0.0629	0.0612	0.0780
Standard Deviation	0.2508	0.2473	0.2792
Standard Error	0.0316	0.0312	0.0352
Confidence Limit (95.0%)	0.06	0.06	0.07

results, which was particularly noticeable in Samples 1 and 3 (see Table 1). The results from one laboratory were particularly divergent from other participants with scores being on average 92% higher than the mean values.

The results were analysed using the procedures that are used to assess the performance of laboratories in ISTA purity, germination, moisture content and tetrazolium viability proficiency tests. Normally z-scores are calculated using the mean and standard deviation calculated from the results of accredited laboratories. Since none of the laboratories is accredited for Tetrazolium Vigour, the zscore was calculated using the mean and standard deviation of the results of all participants:

Lab z-score =
(Lab Mean for a Sample - Overall Mean for a Sample)
Standard Deviation for Sample

Because of the nature of seed and its variabi-

lity, even with a homogenous comparative test sample, repeated tests in the same laboratory will produce a range of results and 2.5% of these will have z-scores that are less than (<) minus 2 and 2.5% will have a z-scores greater than 2 (>). No laboratory had a z-score of <-2.0 but 3.6% of laboratories had a z-score of >2.0 (Table 2). This excellent set of results which indicate that in general, laboratories are interpreting the tetrazolium staining patterns in a similar way with little evidence of significant differences between the laboratories.

A plot of the z-score distribution confirms this finding. Results are skewed to the higher end of the distribution with some laboratories being more severe in their assessment of the tetrazolium staining and obtaining higher scores which is equivalent to a lower vigour (Figure 2).

The integer addition of z-scores obtained by a laboratory on the three samples in any particular proficiency test is used to award the laboratory an overall rating for that particular

### ISTA Tetrazolium Vigour Comparative Test

proficiency test. If the integer addition is:

 Table 2. Details of analysis of z-scores obtained in Tetrazolium Vigour comparative test

	Sample 1	Sample 2	Sample 3	Average
Sample Results with z-scores <-2.0	0	0	0	0
Sample Results with z-scores $>+2.0$	4	2	3	3
Total of Sample Results with z-scores				
<-2.0 and >+2.0	4	2	3	3
Proportion of all Laboratories with z- scores <-2.0 and >+2.0	4.8%	2.4%	3.6%	3.6%



Figure 2 Distribution of z-scores for the three samples tested in the tetrazolium vigour comparative test



Figure 3 Integer total of z-scores obtained in the tetrazolium vigour comparative test and the equivalent performance ratings

3.5	A is awarded
3.5 < 5.3	B is awarded
-5.3 < 7	C is awarded
-7	the laboratory is given
	a BMP rating.
	-

N N N

As can be seen from Figure 3 only 2 laboratories have a BMP rating using this criteria and 93% achieved an A rating. This performance exceeds that achieved in any of the ISTA proficiency tests assessed in this manner and amazingly it is better than the performance achieved in the Tetrazolium viability proficiency test.

Laboratories have to be congratulated on their performance in this comparative test. The results are much better than expected and indicate that laboratories can achieve reproducible results in the assessment of Tetrazolium Staining patterns of wheat seed. In the UK GBDL01, GBDL04 and many private sector laboratories offer customers a test similar to this. Customers use the test mainly for quality assurance and stock control purposes. According to the tetrazolium vigour score, seed lots are assessed as high, medium or low vigour according to the following:

Tetrazolium Vigour Score	Vigour
1.00 - 1.34	High Vigour
1.35 - 1.64	Medium Vigour
1.65 and above	Low Vigour

The results of this ISTA comparative test indicate that it should be possible to validate this test and have it included in the ISTA Rules should there be a demand.

This is only a preliminary statistical analysis of the results and more needs to be done. In particular an examination of the inter and intra laboratory variation should enable the drafting of tolerance tables to be used in conjunction with this vigour test. We would like to thank the laboratories that took part in this comparative test. Participating laboratories that would like to have details of their individual score should contact Gillian directly at:

### Gillian.McLaren@sasa.gsi.gov.uk

Gillian would also be pleased to hear from any laboratory who would like to take part in a validation study for this method of assessing vigour through the assessment of tetrazolium staining patterns.

# ISTA Accreditation Document Update

By Gerhard Schuon, ISTA Accreditation Department

A new version (2.0) of the "Procedure for Termination, Suspension and Withdrawal of ISTA Accreditation" has come to replace the previous version (1.1, from July 2002). This document, as its predecessor, defines how laboratory accreditation can be ended or reinstated after a limited period of discontinuation. It points out responsibilities and time limits for responding.

The review was felt necessary after some experiences had been collected and the need to clarify more details had become apparent. The aspects of ending accreditation on the initiative of the laboratory or due to non-fulfilment of requirements are explored systematically. Particularly how the arrears in payments, be it membership fees, accreditation fees or audit visit fees have a bearing on obligations governing the relation between ISTA and accredited member laboratories.

The possibility for laboratories to appeal against accreditation related decisions has been taken up with a reference to a new document, the "Appeals and Complaints Procedure".

This document provides the framework for channelling suggestions and comments in relation to the ISTA Accreditation Programme. It ensures that laboratories can make themselves heard and that a formal way of responding to comments, suggestions and complaints is systematically followed. Appeal against accreditation decisions as a particular form of interaction between ISTA as an accreditation body and auditees is covered in depth.

The document specifies that the ISTA Executive Committee or a panel appointed by it shall handle appeals against decisions related to the accreditation process within a defined time span. It further defines how an appeals panel is established and who qualifies for being appointed to it.

ISTA excludes any liability for claims, damages or expenses in relation to suspension or withdrawal of accreditation. On the other hand, organisations filing an appeal do only have to reimburse ISTA for costs incurred by the Executive Committee or the Appeals Panel in case the appeal is repudiated. Laboratories will be instructed accordingly before any activities are initiated that may be charged to them.

Electronic copies of the documents were sent to ISTA members and can be downloaded from the website. All the other accreditation documents are available for download as well. They have been routinely updated and do, in their current versions, reflect the decisions made at the Budapest meeting in 2004.



# **Publications**

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# Congratulations to Alison Powell!

### **By Stan Matthews**

Dr. Powell, currently the Chair of the ISTA Vigour Committee and Convenor of the ISTA Seed Symposium has been awarded the degree of Doctor of Science (DSc) from the University of Stirling, where she graduated and completed her PhD. The award was for her contribution to seed science and technology, largely based on the external expert assessment of her collection of published work on Physiological Studies of Seed Quality, Its Evaluation and Improvement. Presently she holds an honorary research position in the School of Biological Sciences at the University of Aberdeen and is on the Editorial Board of four international plant science journals, including Seed Science and Technology.



# **ISTA Website Update**

Almost a year ago, ISTA launched the new design of our website www.seedtest.org. We are sure that you all agree that the new look has been a great improvement, and we are glad to see that members and non-members are very frequently using the online bookstore, the online registration for workshops and the many documents that can be downloaded for free from the website.

### New 'search' function

In order to help you find the requested information even faster, we have implemented a new 'search' function on all pages of the website. You may enter any word or phrase in the search box, hit the button and all documents containing said keywords will be listed for you.



# Keep updated and visit ISTA Online at www.seedtest.org

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ISTA Membership

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### YU - Serbia & Montenegro

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### ATDL02

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### AU - Australia

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### IT - Italy

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e-mail: abednie@inspection.gc.ca

Fax: +1 613 7591260

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### ZMDM01

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### **International Seed Testing Association**

- develops, adopts and publishes standard procedures for sampling and testing seeds
- promotes uniform application of these procedures for evaluation of seeds moving in international trade
- promotes research in all areas of seed science and technology
- Accredits Member Laboratories
- to participate in conferences and training courses
- has established & maintains liaison with other organisations having common or related interests in seed

### ISTA Membership offers you

free access to the 'International Rules for Seed Testing', an internationally standardised publication containing seed testing procedures and techniques, which is constantly revised and updated



valuable information through all ISTA publications, including Seed Science Technology and Technical Handbooks, which are free for members

involvement in seed testing methodology development

ISTA proficiency testing, quality assurance standards and auditing services, which assist you in attaining the highest quality assurance levels in today's business environment

the possibility of issuing ISTA international certificates

easy access to leading seed experts worldwide

### "ISTA, providing methods & services for the testing of seed moving in international trade..."



International Seed Testing Association Association Internationale d'Essais de Semences Internationale Vereinigung für Saatgutprüfung

# **REQUEST FORM**

All interested persons are invi- ted to forward the attached request form to the ISTA Secretariat, PO Box 308, 8303 Bassersdorf, CH-Switzerland, phone +41 44 838 6000 or fax +41 44 838 6001, E-mail ista.offi- ca@iita.ch
fax +41 44 838 6001, E-mail ista.offi- ce@ista.ch to receive a membership information package.

Contact Person	
Organisation	

Yes, please send me more information on how to become an ISTA Member.

Organisation	 
Address	
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# **ISTA Seed Symposium Awards**

# Awards for excellence in oral and poster presentations

By Alison Powell, ISTA Seed Symposium Convenor

Awards are given to three oral and three poster papers at each ISTA Seed Symposium. The primary purpose of these awards is to help raise the general standard of papers and in addition the individual who has presented the paper is recognised. The papers receiving awards are acknowledged as having held the attention of their audience or readers and presented new and significant information in a clear and logical way.

The recipients of the awards are decided by two panels of seven judges, each of whom makes an independent assessment of the oral or poster presentations. The award winners receive a certificate signed by the ISTA President to acknowledge their contribution.

The recipients of the award in 2004 were (in alphabetical order of first author)

Oral papers (\*Presenter of the oral paper) Norberto De Atrip, Stan Matthews\* and Alison A Powell. The use of rapid ageing and controlled deterioration to evaluate iodine vapour treatments to improve seed storage potential.

Gillian McLaren\* and Ronald Don. The effect of glyphosate treatment on the germination of barley seed

Marie-Helene Wagner\*, Anne Preveaux, Elise Moizan, Matthieu Beaulaton and Sylvie Ducornau. Vigour testing: Towards an extended use of the conductivity test.

### Poster papers

M Kerkoud, C Gumier, M Guenard, J Lechappe and M Esquibet. Identification of the stem lucerne nematode *Ditylenchus dipsaci* by PCR.

**P Valancogne and Marie-Helene Wagner** Relationships between microclimate, desiccation rate and seed vigour in beans (*Phaseolus vulgaris* L.)

Marie-Helene Wagner, Anne Preveaux, Matthieu Beaulaton and Sylvie Ducornau. Comparison of three methods of moisture content adjustment: their impact on germination and vigour testing.



M Kerkoud, C Gumier, M Guenard, J Lechappe and M Esquibet.



P Valancogne and Marie-Helene Wagner



Marie-Helene Wagner, Anne Preveaux, Matthieu Beaulaton and Sylvie Ducornau

# Announcement of the 28<sup>th</sup> ISTA Seed Symposium

### At the ISTA Congress 2007 in Iguassu Falls, Brazil

The next ISTA Seed Symposium will include:

- Six lead speakers
- Six sessions of oral papers on aspects of seed science and technology
- Two poster sessions

Now is the time to focus the attention of your research on topics suitable for presentation!

More details of the symposium and call for papers will be available from September 2005 and in the next issue of Seed Testing International. We shall look forward to receiving your proposed papers!

# Seed Science Resources from CABI Publishing

### 20% discount on the following books when quoting reference JBP20



### Seed Science Research

Seed Science Research is the official journal of the International Society for Seed Science. It is a leading international journal featuring high quality, original papers on the fundamental aspects of seed research, reviewed by international distinguished editors.

The emphasis is on the physiology, biochemistry, molecular biology and ecology of seeds, including embryo development, biotechnology, maturation, dormancy, germination, seed-soil and seed-animal interactions, and computer modelling. Frequency: Quarterly ISSN (Print): 0960-2585 ISSN (Online): 1475-2735

For more information please visit: www.cabi-publishing.org/ssr

# The Encyclopedia of Seeds: Science, Technology and Uses

	Edited by J D Bewley, M Black and P Halmer	
Coming late 2005	November 2005 c. 800 pages HB ISBN 0 85199 723 6 <del>Approx. £150.00 (US\$250.00)</del>	
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of the subject of seeds. This one-volume book gives a unique and authoritative coverage of fundamental seed science alongside the applied seed technologies involved in raising the next crop and using grain. The encyclopedia is composed of short descriptive articles and brief definitions, contributed by over 100 expert authors worldwide, and illustrated with plentiful figures,

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Edited by M B McDonald and F Y Kwong December 2004 400 pages HB ISBN 0 85199 906 9

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This book provides a unique and much-needed resource of information on the biology and technology of flower seeds. It presents indepth information on the history and evolution of the ornamental and wild flower seed industries followed by recommendations for

successful breed and production programs.

# Seed Fate: Predation, Dispersal and Seedling Establishment



Edited by P M Forget, J E Lambert, P E Hulme, and S B Vander Wall

December 2004 432 pages HB ISBN 0 85199 806 2

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This book presents current knowledge of seed fate in both natural and humandisturbed landscapes, from various regions of the world. Particular attention is paid to

plant diversity conservation when seed removal is affected by factors such as hunting, habitat fragmentation or intensive logging.

### Saving Seeds: The Economics of Conserving Crop Genetic Resources Ex Situ in the Future Harvest Centres of CGIAR



B Koo, P G Pardey, B D Wright, and others October 2004 232 pages HB ISBN 0 85199 859 3

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The genebanks of the Consultative Group on International Agricultural Research (CGIAR) are pivotal to the global conservation of genetic resources. This book discusses the economic issues and

implications surrounding these centres through examination of a series of detailed economic case studies.

### For more information please visit www.cabi-publishing.org/bookshop

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Publications

# Guide to ISTA

New ISTA Information Pamphlet

The new Guide to ISTA gives an introduction and overview of the Objectives of the Association, the Management, Membership, Accreditation, Technical Committees and the ISTA Services.

The Guide to ISTA is primarily aimed at prospective ISTA members and persons interested in the International Seed Testing Association.

The information pamphlet is available from the **ISTA Secretariat** free of charge.

It can also be downloaded from the ISTA Website at **www.seedtest.org**.

d in the International Seed Testing

International Rules for Seed Testing Edition 2005

Complete set in two parts (Rules & Annexe to Chapter 7, including Amendments 2005)

ISTA's primary instrument in promoting uniformity in seed testing procedures is the 'International Rules for Seed Testing', which lays down detailed standard techniques and procedures. The publication includes 17 Chapters and Appendices describing principles and definitions in detail, assisted by many tables and the methods to be used. The 'International Rules for Seed Testing' is designed for the principal crop species of the world, but apply in general, if not in detail, to any species of crop plant, even including those not mentioned in the text.

### Valid as per January 1<sup>st</sup>, 2005 PRICE: Swiss Francs CHF 376.00 plus postage (approx. US \$ 316.00/EUR 246.00)

# Amendments 2005

Includes 89 pages to replace current pages in the Rules & Annexe to Chapter 7, Edition 2004

The Rules Amendments were adopted at the Ordinary Meeting held in Budapest, Hungary, May 20 - 21, 2004. Only the changed pages are inlcuded in this package, which is made up of 89 pages. The chapters and pages to be replaced are clearly specified in a table in the preface. The pages from the Edition 2004 that are to be replaced will be no longer **valid as of January 1<sup>st</sup>**, **2005**.

PRICE: Swiss Francs CHF 102.00 plus postage (approx. US \$ 89.00/EUR 68.00)





# CALENDAR 2005

# April

24-30 ISTA/FAO/ICAR Training Course on

Seed Quality Testing and Evaluation for Selected Asian Countries (New Delhi, India)

25-28 ISTA Ordinary Meeting 2005 (Bangkok, Thailand)

# Мау

- 08-13 ISSS Triannual Meeting (Brisbane, Australia)
- 09-13 6th ISTA/FAO Workshop on Electrophoretic Methods and PCR-Techniques for Variety Verification and GMO Detection (Kingston, Jamaica)
- 10-13 5<sup>th</sup> ISTA SHC Seed Health Symposium (Angers, France)
- 30-02 ISF Congress (Santiago, Chile)

# June

- 15-22 AOSA/SCST Annual Meeting (Saskatoon, Canada)
- 18-22 ASTA 122<sup>nd</sup> Annual Convention (Seattle, USA)

# July

- 11-15 10<sup>th</sup> ISTA Workshop on Tetrazolium Testing on Tree and Shrub Seeds (Karlsruhe, Germany)
- 18-23 IBC XVII International Botanical Congress (Vienna, Austria)

# August

29-02 7<sup>th</sup> ISTA Seminar on Statistics in Seed Testing (Stuttgart, Germany)

# September

27-30 OECD Annual Meeting (Paris, France)

# October

- 09-11 ESA Annual Meeting (Brussels, Belgium)
- 27 UPOV Annual Meeting (Geneva, Switzerland)

# November

- 07-11 APSA Annual Meeting (Shanghai, China)
- 13-15 EESNET Annual Meeting (Sofia, Bulgaria)

# December

07-09 ASTA Annual Meeting, Corn & Sorhum (Chicago, USA)



# International Seed Testing Association (ISTA)

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