Seed Testing
INTERNATIONAL

ISTA News Bulletin No. 141 April 2011

4 Teaching the teachers

7 Annual Meeting 2011

EDITORIAL

Dear Reader,

you have in your hands the latest issue of Seed Testing International. As usual, you will find interesting articles from all over the world on technical developments, from seed health testing methods and germination testing to forest seed testing. It gives you an indication of the variety of different topics being discussed within ISTA, and provides an explanation why working with ISTA is so interesting.

In this editorial, however, I would like to draw your attention to two topics: the upcoming ISTA Annual Meeting and the closing of the Official Seed Testing Station in Denmark.

Unfortunately this year's Annual Meeting has been strongly affected by the catastrophe that has hit Japan so unexpectedly and so badly. We all feel for the people in Japan and wish them all the best in the near future. Despite this catastrophe, the ISTA Annual Meeting will take place, unfortunately not in Japan, but in Zurich, Switzerland. The meeting will start with a one-day germination seminar. The programme is highly interesting, and the Germination Committee has worked long and hard to organize such an interesting seminar.

Next to the reports of all the ISTA Committees, the Ordinary Meeting will also provide interesting discussions on many different topics, including the current ISTA Accreditation programme. Please find more detailed information about the ISTA Annual Meeting 2011 on pages 7–13.

The closing down of the Official Seed Testing Station in Denmark has come as a shock to us. This station has played an outstanding role in our Association. It was the station of the first ISTA President, and the home of the ISTA Secretariat for a long period in the first years of the Association. It is not only an official monitoring institute which has been closed down, but also an excellent centre for applied research in seed science and technology, and an internationally recognized training centre. This will create a major gap. This development should be of concern for all of us, and indicates that ISTA's voice needs to be raised more intensively, obviously also at the political level.

In closing, I wish you a lot of fun in reading this issue, and I look forward to meeting you in person at the upcoming ISTA Annual Meeting in Zurich, Switzerland.

Yours sincerely,

Michael Muschick

Seed Testing International
No. 141 April 2011

Produced on behalf of the
ISTA Executive Committee

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Other ISTA publications
For information on and orders for other ISTA publications, please contact the ISTA Secretariat at the above address, or visit our web site at www.seedtest.org.

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President's Report

Joël Léchappé

When preparing the present report, I was very pleased and honoured to refer to Tsukuba, the venue of our next Annual Meeting in June 2011.

Unfortunately, with the very strong earthquake and its devastating consequences, tragedy has befallen the people of Japan.

With the Executive Committee members, I wish to express our support and admiration for the courage of the Japanese people and our ISTA colleagues in Japan. In this very difficult situation, the National Organization Committee of Japan was willing to continue with the organization and implementation of the Annual Meeting. This dedication is outstanding and greatly appreciated.

However, in close collaboration with the National Organizing Committee and Masatoshi Sato, the ECOM decided to transfer the venue of the Annual Meeting to Zurich.

The programme as scheduled for Japan will be retained. I hope that you will attend the meeting, and I look forward to meeting you in Zurich in June.

At the ISTA Congress in Cologne in June, 2010, the new ISTA strategic plan, with its vision and mission, was accepted by the membership:

"We, as ISTA members, work together to achieve our vision. Our Association produces internationally agreed rules for seed sampling and testing, accredits laboratories, promotes research, provides international seed analysis certificates and training, and disseminates knowledge in seed science and technology. This facilitates seed trading nationally and internationally, and also contributes to food security."

2010 was a very important year for ISTA, with the 29th Congress, and some major questions from Members, for example regarding the accreditation system.

We were also faced with the unexpected resignation of President Prof. John Hampton four months after the Congress.

John served the Association for many years as an active Chair, Vice-Chair and member of several committees, as an Associate Editor of Seed Science and Technology, and in the ECOM since 2001. I would like to acknowledge this service, and to thank him for his dedication and work for the Association.

The ISTA Constitution, Article VI (b) states: "The Vice-President shall assist the President and, in the event of the inability of the President to serve, shall carry out such duties as pertain to the office of the President."

Although this has come completely unexpectedly, I have in agreement with the ECOM accepted the position of President. I will be acting as President for almost three years before my own still planned term as President. This certainly has implications in my professional and family life, but I have excellent support from my family and my employer GEVES. Also, with the ECOM and the Secretariat, I am very well supported and motivated. Together we work as a team, and it's a real pleasure to serve the Association.

Changes to the ECOM

At the ECOM meeting in November 2010 and February 2011 we worked on many topics. Francisco Carlos Krzyzanowski was appointed as an Officer. His function is to assist the President to compensate for the vacancy of Vice-President.

The vacant position for a member-at-large was filled according to Article VII (c) (3) of the Constitution. On behalf of the ECOM I have the pleasure to welcome Craig McGill, of Massey University, New Zealand, to the ECOM. All important areas of the world are once again represented within the ECOM.

Activities

The strategic plan approved in Cologne lists seven key areas of activity:

1. Membership

Membership (up by 6 laboratories in 2010) and participation in ISTA activities are increasing. Despite a somewhat unfavourable economic background more and more volunteers are contributing to ISTA (212 TCOM members). It is very encouraging and positive that many members from Africa, Asia (India) and Russia regularly attend meetings and are supported by their ECOM representatives. Membership activities are efficiently supported by the Secretariat.

In the follow-up project to the 2nd World Seed Conference in 2009 of the FAO, the OECD, UPOV, the ISF and ISTA, we not only wish to strengthen collaboration with our partner organizations, but also actively help particularly developing countries to join the ISTA family and build their technical capacities. A first focus of this joint project wil be on Africa.

2. Development of methods and Rules

The annual rhythm of ordinary meetings continues, with new methods and Rules proposals. Those to be discussed this year anticipate or reply to requests from the seed sector, governments and the seed industry. Topics include seed mixtures, the detection of *Orobanche* (broomrape), the detection of *Ustilago nuda* and *Pyrenophora teres* on *Hordeum*, and the motion from the Netherlands on the use of multiple Orange Certificates for sublots. An overview of the important technical work of 2010 will be presented as usual during the TCOM meetings. Proposals are supported where required by fully documented validation reports, showing the close collaboration between the TCOMs and the Statistics and Rules Committees.

3. Accreditation

The number of ISTA-accredited laboratories is increasing (from 96 in 2004 to 120 in 2010) and represents a continuous challenge for the Accreditation Department.

On an international level, ISTA accreditation is comparable to ISO 17025, and is both appropriate and a specific fit to the needs of the seed sector. The ECOM, with the Accreditation Department, tries to match QA requirements with reality by attending to the needs of Members and stakeholders. For example, two major topics are studied very carefully:

– The adoption in Cologne by the Membership of the motion of New Zealand and Australia to review the audit process. The results of the in-depth analysis carried out by the Secretariat and the ECOM will be presented and discussed at the ordinary meeting in June.
– To what extent must accredited laboratories implement check sampling to monitor samplers, e.g. cost versus guarantee of sampling. Sampling and monitoring of samplers is specific to the ISTA Accreditation Standard, and is the basis of the added value of the Orange International Seed Lot Certificate. This question therefore requires careful consideration and detailed study.

4. Facilitation of seed movement

The Association is listening carefully to stakeholders' needs, with the ECOM and TCOMs working very closely together to set priorities. The following examples illustrate important topics studied in response to demands from the seed trade.

– ISTA/ISF grass seed lot size, seed mixture experiment (2011, rules proposal); Use of multiple original Orange International Seed Lot Certificates to facilitate the trade of seed lot fractions (discussion at the ordinary meeting)
– New demand and new needs identified: sampling expensive small seed lots seed for health and/or germination; sampling for detection of *Orobanche*.
– A new issue: the need for a standard "dust test" in seed lots has to be discussed.

These topics have been included in the TCOM working programmes.

5. Dissemination of knowledge

Two activities should be highlighted:

– The close collaboration between ISTA and the International Society for Seed Science, and between ISTA and Kew Gardens in UK under the leadership of Alison Powell and the working group on seed science.
– Follow-up on training after the Bologna seminar, with the publication on the ISTA web site of training objectives, strongly supported by ECOM policy, and handbook production and workshop organization by the TCOMs.

6. Communication

Relationships with other international or regional organizations are subject to special efforts. Secretary General Michael Muschick represents the Association as much as possible at meetings of the OECD, the ISF, UPOV, the FAO, ESA and other important associations. Also, ECOM members have for several years now been representing ISTA in their regions. You are welcome to contact them.

After several meetings I have the impression with some organizations that ISTA activities are poorly known. In some regions, better understanding would avoid overlapping and contribute to better service and optimization of seed quality control. Surprisingly, among them is Europe. We as ECOM members will work to improve communication with these organizations.

7. Management of ISTA affairs

ISTA is and should remain an association of Members, dependent on the input of volunteers for smooth functioning. The success of our Association is therefore in your hands. With every task taken on by an additional person, ISTA and seed quality determination will become stronger.

Cooperation between the ECOM and the TCOMs has been significantly improved by several actions started by Katalin Ertsey and John Hampton:

– strengthening of the support role, with each ECOM member being a contact to a TCOM;
– the guideline "Responsibilities of ISTA TCOMs" available on the ISTA web site;
– the validation by the ECOM of the TCOMs' working programmes in February 2011.

The Secretariat has also been engaged in a detailed review and reorganization of its work, with the goal of managing the excessive workloads but remaining responsive to Members' needs. A preliminary analysis was made with the assistance of a consulting firm, approved by the ECOM, which has led to a new organization structure to meet current and future needs. I am confident of the ability, professionalism and experience of the Secretariat to carry out this important change. Following this, a detailed cost-accounting system will be implemented to allow financial tools adapted to the activity and to guarantee complete transparency.

My ISTA vision

Despite the importance and strategic relevance of seed quality determination, seed testing today unfortunately does not receive the attention it deserves. We have to ensure that we have done everything to communicate the relevance of seed testing appropriately at the right places.

My primary aim is to build on the excellent foundation built by Katalin and John. ISTA is a dynamic and productive association with the potential to grow and evolve. I wish to continue with the strategic and technical developments through symposia, publications, the work of the TCOMs and the Rules. These together form the heart of ISTA. ISTA must continue to develop new areas of testing, such as GMOs, dust testing, *Orobanche*, mixtures, sampling, molecular tests for seed health, identification of species and vigour tests.

Laboratories from all parts of the world must be able to participate in technical development, not only in Europe, North America and Asia. Competent laboratories should recognize ISTA as their organization for seed quality determination, but also for seed conservation and biodiversity. Relationships with other organizations and especially governments are fundamental, and need to be strengthened. It is a priority to increase ISTA's presence and contribution in areas of the world such as Africa, Asia, Eastern Europe and South America. To achieve this, organizing and focusing the Association's efforts are very important, and will take time. Furthermore, ISTA's development must take the environmental and economic constraints into account with the necessary optimism.

As your Vice-President and President, I am well motivated and very well supported to help lead our association into the future, and welcome your thoughts and comments. ■

A three-step teaching experience in Indonesia, or how to transfer your message when you do not speak the students' language

Joost van der Burg[1], Dina Daryono[2], Karin van Ettinger[3] and Yanti Yusuf[4]

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Participants and trainers of the first HORTSYS course.

During 2008–2010, the HORTSYS project was assisting the Indonesian authorities with policy support, and the national and provincial seed testing labs with technical support. We will not go into technical details in this short report, other than saying that we hope that eventually this support may lead to the accreditation of the national lab (Balai Besar Pengembangan Pengujian Mutu Benih Tanaman Pangan dan Hortikultura, Cimanggis (BB-PPMB-TPH, or BPMB for short). This is to show how we managed to overcome the language barrier.

A three-step approach

Teaching the principles of seed testing and certification is often done by people from another country, so often in a international language such as English. This has the disadvantage that you can effectively reach only those people with good English. In a country such as Indonesia, where there are hundreds of laboratory staff, this means that you can only teach a small selection of them, usually the younger generation (but not exclusively), and missing out the vast majority. In Indonesia, well over 90% of the people do not understand English sufficiently. In addition, the country is vast, and it is impossible to get them all together let alone to visit them all.

So we resorted to teaching the ones with good English and turning them into teachers, the Training of Teachers approach, or TOT.

Of course, the principle of TOT is not new, and it has proven its worth. A disadvantage, however, is that often inexperienced people are supposed to teach the more knowledgeable persons later on, just because they can speak English. Due to their limited knowledge of the teaching material, this may result in questionable or distorted messages, just like in the Chinese whispers game, in which people sitting in a circle whisper a sentence in the next person's ear. The message always gets distorted or is even completely changed. So our additional challenge was to try to overcome this effect, because we wanted the knowledgeable, more experienced people to be involved from the start.

A third challenge was to reach as many labs as possible. In a country as vast as Indonesia, with all its provinces and islands, it is quite a problem to get the message out everywhere. This is necessary, because the methods used by the various labs must be uniform. To this end, a central lab in West Java near Jakarta, the BPMB, has been given the authority to create this level playing field, through teaching and organizing ring tests, and by serving as an expert station.

How were these challenges overcome?

The training component of the HORTSYS project was devised using a three-step approach.

As the first step, a course was run in English at the BPMB, using a training manual also in English. Some of the participants were more experienced staff, some with very good English, others were senior staff understanding English for the most part. Also invited were a few senior staff from the major labs in the country (from Java as well as from outside Java).

The main teaching was done by English-speaking experts from abroad (and some presentations by local officials), and all material was provided in print. These experts were supported by senior BPMB staff, trainees themselves, who provided extra translation if needed. In this way we managed to get the main messages through, we think.

Discussing post control of yardlong beans.

Lecture during TOT II (step 2).

The course material contained more than just the testing and certification manuals; it also included presentations providing a wider perspective, such as the functioning of international organizations involved with seed, such as ISTA, the OECD and UPOV.

For the second course, also held at the BPMB, the core papers on seed testing and certification were translated into the national language, Bahasa Indonesia. This was done by the experienced staff with good knowledge of English. The course was now given in Bahasa by a number of the former trainees, each expert in their own field. One of the foreign teachers was only present in an adjoining room as an emergency back-up. Knowledge questions were collected on the blackboard, and at the end of the day, the foreign teacher would come in for a questions and answers session.

This course was attended by participants of the main regional labs, to generate enough critical mass there and to give them an idea of how to run a course like this. This was done to enable step 3.

In year 3 of the project, four courses were held at four locations strategically selected all over Indonesia (East Java, Sumatra, Kalimantan and Sulawesi). Participants from all other provinces and islands with labs of relevance were invited to these places. This served a number of purposes: the equipment was brought to a level suitable for providing training courses, local staff gained experience in organizing such events, the participants did not have to travel too far, and the project was able to save on travelling costs. Two participants of the previous courses, who had assisted the lab during the preparations, also came over for some teaching. No foreign trainers were involved at this stage; it was an entirely Indonesian affair. In the end we trained 115 participants from 23 provinces.

With this setup we managed to create an all-Indonesian training facility, with a whole system of resource persons from the various labs with the confidence that they are on the right track. We hope to have thus created a sustainable system of knowledge exchange in Indonesia.

The first signs are positive: a training course has already been given on request of a few small companies, and the national authorities have promised to reserve funds for annual repeater and refresher courses. This is important because the ISTA Rules as well as the national regulations are subject to constant improvement and amendment, and there is a need for the training of new and transferred staff. ■

ISTA Annual Meeting 2011: change of venue

Glattpark-Opfikon (Zurich), Switzerland, 13–16 June 2011

Due to the very difficult situation in Japan, the ISTA Executive Committee, with deep regrets, has decided to change the venue of the ISTA Annual Meeting 2011 from Tsukuba, Japan to Zurich, Switzerland.

The dates, registration fees and, as far as possible, the programme for the ISTA Annual Meeting 2011 will remain as previously announced. Registration for the meeting is continuously possible. For registration and the latest information please consult the corresponding sites.

The International Seed Testing Association (ISTA) takes pleasure in inviting you to its Annual Meeting, to be held in Glattpark (Zurich), Switzerland, from 13 to 16 June 2011.

The ISTA Annual Meeting provides the opportunity to meet other seed experts and to exchange experiences. The aim of the meeting is to discuss and decide on proposals for changes to the ISTA International Rules for Seed Testing, and business items of the Association, with the international participation of ISTA delegates and representatives from both the seed industry and governments, including experts in seed technology, scientific research and laboratory accreditation.

Registration

Registration for the full Annual Meeting includes the Germination Seminar on 13 June, the technical presentations on 14 and 15 June, and the Ordinary Meeting on 16 June.

Registration is also possible for the Germination Seminar only. Students may benefit from a reduced fee for the Seminar.

Provision is made for both Members and non-members of ISTA.

Online registration is open at www.seedtest.org.

Registration will close on 15 May 2011.

Accompanying persons

The category 'accompanying persons' is applicable only for the spouse, companion and/or children of a delegate. Registration as an accompanying person does NOT include participation in any of the meetings or sessions, but only to social events, lunches and coffee breaks, the Welcome Reception and the Official Dinner.

Trade exhibition

Reach seed professionals from laboratories and organizations worldwide. Only a limited number of exhibitions stands are available. The registration fee includes 1 booth (10 m^2) and 1 exhibitor at social events, lunches and coffee breaks, the Welcome Reception and the Official Dinner. An additional person may register as an accompanying person.

Registration fees

Periods	Events	Registration (closes 15 May 2011)
ISTA Members		
13–16 June	Annual Meeting incl. Seminar	660 €
13 June	Seminar only	200 €
Non-members		
13–16 June	Annual Meeting incl. Seminar	1200 €
13 June	Seminar only	250 €
Students		
13 June	Seminar only	40 €
Accompanying persons		
13–16 June	Annual Meeting	200 €
Exhibitors (Members)		
13–16 June	Exhibition booth	1400 €
Exhibitors (non-members)		
13–16 June	Exhibition booth	1600 €

Programme overview

Sunday 12 June 2011

19:00 **Registration and Welcome Reception**

Monday 13 June 2011

08:30–18:00 **ISTA Seminar on Germination**

Tuesday, 14 June 2011

08:30–18:30 **Presentation of ISTA's technical work**

Wednesday, 15 June 2011

08:30–18:00 **Presentation of ISTA's technical work (cont.)**

19:00 **Official Dinner**

Thursday, 16 June 2011

09:00–17:30 **ISTA Ordinary Meeting**

Accommodation

Novotel (Annual Meeting venue)

Lindberghplatz 1, 8152 Glattpark-Opfikon-Zurich, Switzerland, phone: +41 (0)44 829 90 00, www.novotel.com
Special room rate: CHF 235 (incl. breakfast and taxes)

For reservations, please fax the reservation form (www.seedtest.org/am2011) to the Secretariat on +41 44 838 60 01 before 10 May 2011.

The Novotel Zurich Airport Messe is conveniently situated within the Glattpark international office and commercial development and World Trade Center, close to the A1/A4/A53 highway with ideal tram and rail links to Zurich city centre and Zurich Airport.

Ibis Hotel Zurich Messe-Airport

Heidi Abel-Weg 5, 8050 Zurich, Switzerland, phone: +41 (0)44 307 47 00, www.ibishotel.com
Special room rate: CHF 146.50 (incl. breakfast and taxes)

For reservations, please fax the reservation form (www.seedtest.org/am2011) to the Secretariat on +41 44 838 60 01 before 10 May 2011.

Getting there

By air

Regular scheduled flights from every continent and most countries and major cities of the world land at Zurich's international airport. A train service every quarter of an hour whisks passengers to the Zurich city centre in just ten minutes.

The Ibis Hotel provides free airport transfers from Zurich airport from 05:40 to 23:10 every 30 minutes (5 minutes travel) from Terminal 2, Zone 2.

For the Novotel, Trams 10 or 12 (Glattalbahn line) from Zurich airport both stop at Lindberghplatz.

By public transport

Over a thousand trains stop daily at Zurich's centrally located main railway station. Direct and frequent services to all large Swiss cities and major European destinations guarantee a pleasant journey.

The Novotel (meeting venue) is served by the No. 10 tram, which runs from Zurich main station to the airport and stops at Lindberghplatz, directly in front of the Novotel.

The Ibis Hotel is served by the No. 781 bus, which runs from Oerlikon station and stops at Riedbach, 3 minutes from the hotel. The No. 781 also stops at Lindberghplatz, thus connecting the two hotels.

Both the No. 10 tram and the No. 781 bus also stop at Oerlikon, Opfikon and Glattbrugg stations. From these stations there are also numerous connections on various suburban train lines to Zurich main station and the airport.

By car

Novotel (meeting venue)

From all directions (Zurich city centre, A1, A3 or A4 highways), follow signs to the airport (Flughafen), then head north towards the A51 highway and Flughafen. Take exit No. 8 towards Glattbrugg. Cross the highway bridge and immediate turn right towards Zurich and the A51 highway (heading south). Take the next exit, No. 9, and head towards Oerlikon. After 600 m, the Novotel is on the right-hand side.

Ibis Hotel

From Bern/ Basel/ Zurich Airport/Winterthur: direction St. Gallen, take exit Wallisellen, direction Zurich-Oerlikon 3 km straight ahead. From Zurich City: highway direction Flughafen, take exit Schwamendingen and follow Messe-Oerlikon.

By taxi

The taxi journey from the airport to the Novotel and Ibis Hotel takes about 5 minutes and should cost approx. CHF 30. A taxi from the train station or city centre to either hotel will cost you approx. CHF 50.

Currencies

Accepted currencies in Zurich (in most shops, restaurants, hotels): Swiss franc (CHF), euro (€), major credit cards such as Mastercard, VISA, Eurocard, American Express.

Visas and letters of invitation

The Secretariat will send out letters of invitation to participants upon written request. However, it should be understood that this letter is only to help delegates to raise travel funds or to obtain a visa, and is not a commitment on the part of the organizers to provide any financial support.

Delegates requiring invitations for visa applications must register and pay the registration fee before the invitation letter is be issued. Please take into consideration that the Secretariat will NOT deal directly with embassies for visa requests for participants.

Need more information? Visit out web site or e-mail us at meetings@ista.ch. ■

Final programme

Meeting venue: Novotel Zurich Airport Messe, Lindberghplatz 1, 8152 Glattpark-Opfikon, Switzerland

Sunday, 12 June

18:00–19:00 Registration desk open

19:00 **Welcome Reception (all delegates welcome)**

Monday, 13 June

08:00–18:00 Registration desk open

ISTA Seminar on Germination

08:30–08:45 **Welcome by the ISTA President, Joël Léchappé (France)**

Welcome by the Chair of the Seminar, Sylvie Ducournau (France)

08:45–09:15 **Physiology of seed germination and dormancy**
Alison Powell (United Kingdom)

09:15–09:45 **Germination characteristics of tropical and sub-tropical species**
Karen Ann Hill (Australia)

09:45–10:15 **Germination characteristics of flower species**
Rita Zecchinelli (Italy)

10:15–10:45 Coffee break

10:45–11:15 **Influence of seed health on the germination quality of seeds**
Valerie Cockerell (United Kingdom)

11:15–11:45 **Relationship between tetrazolium and germination tests**
Stefanie Krämer (Germany)

11:45–12:30 **Recent developments in seedling evaluation**
Takayuki Okuda (Japan), Gillian McLaren (United Kingdom), Sylvie Ducournau (France)

12:30–13:30 Lunch break

13:30–15:30 **Calculation and statistical tools for germination testing**
Jean-Louis Laffont (France)

Presentation of the tools

Demonstrations and practicals
Participants of the Seminar are kindly requested to bring their laptops, if they would like to take an active part in this exercise. Participants are also requested to download the Excel tools from the ISTA web site before the Seminar from 1 month before the Seminar (www.seedtest.org/ger11).

15:30–16:00 Coffee break

16:00–16:30 **Quality control in germination testing**
Rasha El-Khadem (ISTA Secretariat)

16:30–17:00 **Evolution in germination testing**
Joël Léchappé (France)

17:00–17:30 **New technologies for germination testing**
Bert van Duijn (Netherlands)

17:30–18:00 **Discussion forum and conclusion of Seminar**

Tuesday, 14 June

08:00–18:00 Registration desk open

Presentation of ISTA's technical work

08:30 **Opening by the ISTA President, Joël Léchappé (France)**

08:30–10:00 **Session 1:**
a. Report of the Purity Committee: Adriel Garay (United States) (tbc)
b. Report of the Germination Committee: Sylvie Ducournau (France), Lea Mazor (Israel)
c. Report of the Moisture Committee: Craig McGill (New Zealand)

10:00–10:30 Coffee break

10:30–12:30 **Session 2:**
a. Report of the Tetrazolium Committee: Stefanie Krämer (Germany)
b. Report of the Vigour Committee: Alison Powell (United Kingdom)
c. Report of the Seed Health Committee (Theresa Aveling (South Africa)
d. Report of the Variety Committee: Berta Killermann (Germany), Cheryl Dollard (Canada), Beni Kaufman (United States) (tbc)

12:30–13:30 Lunch break

13:30–14:00 e. Report of the GMO Committee: Christoph Haldemann (Switzerland)

14:00–15:00 **Session 3:**
a. Report of the Flower Seed Committee: Rita Zecchinelli (Italy)
b. Report of the Forest Tree and Shrub Seed Committee (tbc)

15:00–15:30 **Session 4:**
a. Report of the Editorial Board of Seed Science & Technology: Alison Powell (United Kingdom)
b. Report on the ISTA session at the ISSS Meeting: Alison Powell (United Kingdom)

15:30–16:00 Official photo session followed by coffee break

16:00–18:30 Time allocated for meetings of ISTA Committees

Wednesday, 15 June

08:30–18:30 **Presentation of ISTA's Technical Work**

08:30 **Opening by the ISTA President**

08:30–10:00 **Session 5:**
a. Report of the Bulking and Sampling Committee: Leena Pietilä (Finland), Christoph Reinhardt (Germany), Lotta Claesson (Sweden)
b. Report of the Statistics Committee: Jean-Louis Laffont (France), Kirk Remund (United States)
c. Report of the Nomenclature Committee: John Wiersema (United States) (tbc)

10:00–10:30 Coffee break

10:30–11:30 **Session 5 (continued):**
d. Report of the Storage Committee: Alison Powell (United Kingdom) (tbc)
e. Report of the Committee on Advanced Technologies: Bert van Duijn (Netherlands)

11:30–12:30 **Session 6:**
a. Report of the Proficiency Test Committee: Günter Müller (Germany)
b. Report of the Audit Programme: Rasha El-Khadem (ISTA Secretariat)

12:30–13:30 Lunch break

13:30–14:00 **Session 7:**
a. Seed Analyst Training Committee: Alison Powell (United Kingdom)

14:00–15:30 **Session 8:**
a. Meeting of the Rules Committee: Steve Jones (Canada)

15:30–16:00 Coffee break

16:00–18:00 **Session 8 (continued):**
a. Meeting of the Rules Committee: Steve Jones (Canada)

19:00 **Official Dinner at the Uto Kulm**

Thursday, 16 June

09:00–17:30 **ISTA Ordinary Meeting**

09:00–10:00 **Welcome by the ISTA President**

Official Address by Mr. Junya Endo, Director of the Intellectual Property Division, Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan

Presentation: The Development of the Seed Industry in Japan (Mr. Madoka Koshibe, Chairman of the Board, Mikado Kyowa Seed Co. Ltd., Japan)

10:00–10:30 Coffee break

10:30–12:30 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
a) Executive Committee response to the Ordinary Meeting decision to investigate the possibility of issuing of multiple seed certificates (motion from the Netherlands, OM2010)
b) Executive Committee response to the Ordinary Meeting decision to review the audit process (motion from Australia and New Zealand, OM2010)
6. Report of the Secretary General

12:30–13:30 Lunch break

13:30–14:30 7. Constitution changes
8. Fixation of annual subscriptions
a) Proposal for the membership fees 2012
9. Consideration and adoption of the proposed Rules changes 2012

15:00–15:30 Coffee break

15:30–17:30 10. Consideration and adoption of reports
11. Announcement of the place and date of the next ordinary meeting of the Association
12. 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
13. Any other business raised by consent of the Executive Committee
14. President's closing address
15. Adjournment

Constitution change proposals 2011

The following four proposals to change the ISTA Constitution were circulated for consideration two months before the ISTA ordinary meeting on 16 June 2011.

The Executive Committee has made these proposals, unanimously supports them and would like the membership to vote in acceptance of them at the ordinary meeting.

The Executive Committee would welcome comments or suggestions about these proposals before or during the ordinary meeting.

Proposal 1

This proposal is to clarify the procedures to be followed when a serving President resigns during their term of Presidency.

The current Article VI (b) states what happens if a President cannot continue to serve:

"(b) The Vice-President shall assist the President and, in the event of the inability of the President to serve, shall carry out such duties as pertain to the office of the President."

Therefore the Vice-President carries out the duties, but perhaps is not the President per se.

The phrase 'in the event of the inability of the President to serve' could be simply referring to when a President is in hospital, on an extended vacation, or on other work duties for a short period of time. It is not clear whether this statement also covers the resignation of the President.

The Executive Committee has studied the Constitution, and considers the following interpretation to be justified:

– when a serving President resigns during their term of office, the Vice-President takes over the role of President and should be referred to as President;
– the Vice-President, although acting as President, should not be required to step down as President until the end of their own planned Presidency.

In order to clarify the interpretation, the following addition (bold) to Article VI (b) is proposed. If accepted by the voting delegates, the proposed change shall come into force with immediate effect.

Current version

ARTICLE VI

Functions of Officers

(b) The Vice-President shall assist the President and, in the event of the inability of the President to serve, shall carry out such duties as pertain to the office of the President.

Proposed version

(b) The Vice-President shall assist the President and, in the event of the inability of the President to serve, shall carry out such duties as pertain to the office of the President. **In the event that a President cannot continue in office for the remainder of his/her term, the Vice President will be referred to as the President for the remaining period of that Presidency and will also serve for the expected period of his/her own Presidency.**

Proposal 2

While reviewing the Constitution the Executive Committee felt that it was unclear how ISTA could operate if an ordinary meeting were not quorate. One important decision that affects ISTA at the ordinary meeting at which elections are held is the discharge of the outgoing Executive Committee, the election of the new Executive Committee and Vice-President, and the status of the incoming and outgoing Presidents. The following addition (bold) to Article V (b) is therefore proposed to clarify which procedure would be followed if an ISTA ordinary meeting where elections are held were not quorate.

If accepted by the voting delegates, the proposed change shall come into force with immediate effect.

Current version

ARTICLE V

Officers

(b) The tenure of office of the President and Vice-President shall be from the adjournment of the ordinary meeting at which they were appointed to the adjournment of the ordinary meeting held in the third year after the ordinary meeting at which they were appointed.

Proposed version

(b) The tenure of office of the President and Vice-President shall be from the adjournment of the ordinary meeting at which they were appointed to the adjournment of the ordinary meeting held in the third year after the ordinary meeting at which they were appointed.

If the ordinary meeting at which elections are held is not quorate the tenure of the existing Executive Committee will continue until a 'by correspondence' vote can be held to discharge the Executive Committee and to appoint a new Vice-President and new Executive Committee.

Proposal 3

If the ordinary meeting is not quorate, some agenda items could not be voted on, which would prevent the effective operation of the Association. The following addition (bold) to Article X (d) is therefore proposed to clarify which procedure would be followed if an ISTA ordinary meeting were not quorate.

If accepted by the voting delegates, the proposed changes shall come into force with immediate effect.

Current version

ARTICLE X

Meetings of the Association

(d) Designated Members designated by forty percent of the Designated Authorities shall constitute a quorum at meetings of the Association. In determining the percentage, fractions less than 0.50 shall be dropped and those 0.50 or greater shall be regarded as a whole number.

Proposed version

ARTICLE X

Meetings of the Association

(d) Designated Members designated by forty percent of the Designated Authorities shall constitute a quorum at meetings of the Association. In determining the percentage, fractions less than 0.50 shall be dropped and those 0.50 or greater shall be regarded as a whole number.

If the ordinary meeting is not quorate a 'by correspondence' vote will be held to allow the adoption of ordinary meeting agenda items.

Proposal 4

The Executive Committee would like to obtain membership approval of the Executive Committee version of the minutes of the ordinary meeting earlier than the next meeting. This would allow formal acceptance of the minutes, and therefore any actions could be implemented as soon as possible without any challenge.

The minutes would be published on the ISTA web site within 2 months of the ordinary meeting. If there were no comments requiring amendment to the minutes within the subsequent 2-month period, the minutes would be considered approved. If there were comments, and the comments were accepted by the Executive Committee, the minutes, including the comments, would be considered to be approved, and would be published on the ISTA web site.

Any comments about the minutes of the previous minutes will be considered at the next meeting under the revised agenda item 4.

If accepted by the voting delegates, the proposed changes shall come into force with immediate effect.

Current version

ARTICLE X

Meetings of the Association

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

...

(4) Reading and acceptance of Minutes.

...

Proposed version

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

...

(4) **Comments about the minutes of the previous meeting.**

...

(f) The Executive Committee approved minutes of the ordinary meeting will be published on the ISTA website within two months of the ordinary meeting. If there are no comments requiring amendment to the minutes within the subsequent two month period, the minutes will be considered approved. If there are comments and the comments are accepted by the Executive Committee, then the minutes including the comments will be considered approved and published on the ISTA website.

Any comments about the minutes of the previous meeting will be considered at the next ordinary meeting under agenda Article X (e) 4. ■

Preparatory documents for the Ordinary Meeting

The following documents have been endorsed by the ISTA Executive Committee to be submitted to the ISTA Ordinary Meeting 2011 for acceptance by the nominated ISTA Designated Members voting on behalf of their respective Governments.

- OM11-01 Agenda for the Ordinary Meeting 2011 [information document]
- OM11-02 Draft Minutes of the Ordinary Meeting 2010 [voting document]
- OM11-03 Draft Activity Report of the ISTA Committees 2010 [voting document]
- OM11-04 Proposal for the Membership Fees 2012 [voting document]
- OM11-05 Proposed Changes to the ISTA International Rules for Seed Testing 2012 Edition [voting document]
- OM11-06 Method Validation Reports on Proposed Changes to the ISTA International Rules for Seed Testing 2012 Edition [supporting document to voting document OM11-05]
- OM11-07 Constitution Change Proposals 2011 [voting document]
- OM11-08 Discussion Paper on the Ordinary Meeting decision to investigate on the possibility of issuing of multiple seed certificates of the same status and value for one seed lot [discussion document]

Please note that only a very limited number of paper copies of the meeting documents will be available at the meeting.

The documents have been posted on the ISTA web site at www.seedtest.org/OM11. ■

Changes to the *International Rules for Seed Testing* 2012 Edition

Again this year, a number of proposals for changes and amendments to the ISTA *International Rules for Seed Testing* will be submitted for voting by the nominated ISTA Designated Members on behalf of their respective Governments, under Agenda point 9.

Among the changes are the following:

Chapter 1: Certificates
- Reporting the methods for evaluating fresh seeds

Chapter 2: Sampling
- Addition of *Prunus persica* to Table 2A. Part 2
- More general description of sampling stick, to include sticks which open with a push-pull motion
- Sampling stick partitions obligatory for diagonal use
- Hand sampling: deletion of misleading example genera
- Obtaining the composite sample: clarification
- Sampler not required to dispatch submitted sample
- Separate procedures for obtaining the submitted sample and working sample
- Spoon method for *Arachis, Glycine* and *Phaseolus* and *Abies, Cedrus* and *Pseudotsuga*
- Hand halving method for *Arachis, Fagus, Glycine, Phaseolus, Pinus cembra* and *Pinus pinea*

Chapter 3: The Purity Analysis
- Addition of *Tripleurospermum* and *Althaea* to Table 3B Part 1
- Harmonization of Pure Seed Definitions with PSD Handbook

Chapter 4: Determination of Other Seeds by Number
- Inclusion of a test for *Orobanche* spp.
- Reporting of actual sample weight

Chapter 5: The Germination Test
- Use of vacuum counters clarified
- Reporting the method for evaluating fresh seeds
- New germination method for *Solanum nigrum*

Chapter 6: The Tetrazolium Test
- Clarification of test definition
- Clarification of premoistening procedure
- Deviations from standard staining temperatures
- Procedures with hard seeds
- Changes to Table 6A for clarification

Annex to Chapter 7: Seed Health testing Methods
- New seed health method 7-013b: *Ustilago nuda* on *Hordeum vulgare*
- New seed health method 7-027: Osmotic method for *Pyrenophora teres* and *P. graminea* on *Hordeum vulgare*

Chapter 9: Moisture Content
- Correction to reporting of moisture meter results

Chapter 11: Testing Coated Seeds
- Merging of Chapter 11 with Annex

Chapter 15: Seed Vigour Testing
- New radicle emergence test for *Zea mays*

Chapter 18: Testing Seed Mixtures
- New Rules Chapter

New ECOM member-at-large

Craig McGill

Craig McGill, from New Zealand, is a Research Officer in Seed Science and Technology at the Institute of Natural Resources at Massey University, Palmerston North. He completed a Bachelor's Degree in Science at Massey University, and in 1986 joined the Massey University Seed Technology Centre. Since then, Craig has completed a Master's of Applied Science in Plant Science, also at Massey University.

Craig teaches seed quality and storage at both undergraduate and postgraduate levels. He has also supervised a number of postgraduate students in these areas, with a focus on germination and storage problems in seed of the New Zealand flora, particularly desiccation tolerance and non-orthodox storage behaviour. In addition, Craig has organized training courses for industry in seed technology.

The first ISTA Congress Craig attended was in Angers in 2001, where he joined the Moisture Committee. He is also a member of the Rules Committee, and has participated in a number of ISTA workshops. Craig's involvement in ISTA has provided the opportunity to meet and work with many different people throughout the world with the common interest in seeds, but from this common interest many friendships have developed.

In his spare time Craig is kept busy with vintage car restoration and is a keen participant in vintage car rallies.

ISTA Handbook on Pure Seed Definitions, 3rd Edition, 2010

By the ISTA Purity Committee: editors M.R. Mannino, J. Taylor and S. Jones

This handbook expands on and illustrates the pure seed definitions (PSDs) of the International Rules for Seed Testing. This will help with training in purity testing according to international principles. Illustrations of the most relevant genera within a PSD provide practical guidance on the application of each definition. Each PSD is illustrated with scaled colour photographs.

Also included is a comprehensive glossary of scientific terms applying to seed purity.

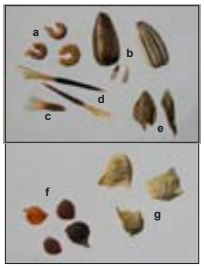
CHF 284.00 (approx. USD 323.00/EUR 222.00)

Now available from the ISTA Secretariat (for contact details, see back cover)

Glossary

► indicates a term defined elsewhere in the Glossary

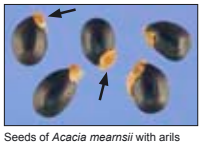
achene, achenium a ►dry, ►indehiscent, one-seeded fruit, formed strictly from one free ►carpel, and with the ►testa distinct from the fruit wall, e.g. *Ranunculaceae, Geum*; occasionally consisting of more than one ►carpel, e.g. *Asteraceae*



Achenes. **a** *Calendula officinalis*. **b** *Helianthus annuus*. **c** *Amberboa moschata*. **d** *Tagetes* sp. **e** *Zinnia elegans*. **f** *Ranunculus sardous*. **g** *Adonis aestivalis*.

anther the pollen-producing part of the ►stamen, borne at the top of the ►filament or ►stalk

aril, arillus [pl. arilli] a fleshy, often coloured covering or appendage of some seeds; an outgrowth



Seeds of *Acacia mearnsii* with arils (arrows). (Steve Hurst, USDA)

of the ►funicle or base of the ►ovule (►caruncle, strophiole)

arista [pl. aristae] a slender, straight or bent ►bristle

aristate surface covered with ►aristae

awn a slender, straight or bent ►bristle. In grasses: usually a continuation of the mid-nerve of the ►lemma or ►glumes

Seeds with awns. Left to right: *Dactylis glomerata, Festuca rubra, Agropyron cristatum, Arrhenatherum elatius, Lolium multiflorum, Oryza sativa, Hordeum vulgare*.

beak a long, pointed prolongation of a fruit (e.g. *Anemone, Geranium, Geum*)

Seeds with beaks. **a** *Adonis aestivalis*. **b** *Cosmos bipennatus*. **c** *Ranunculus sardous*.

berry a many-seeded fleshy ►indehiscent fruit. The ►pericarp usually forms a tough outer skin and the ►mesocarp becomes massive and fleshy. and ►mesocarp ma coloured to attract t act as agents of disp

bract a reduced leaf structure subtending grass ►spikelet in

Bracts of *Amorpha fruti*

bristle a stiff hair; the an bent ►awn

bulb a short, shoot w thickened leaves, de food-storage organs

bulbil a small ►bulb axillary or appearing flowers as in *Poa bu* bulblet

Bulbils of *Poa bulbosa*.

burr a fruit enclosed or prickly ►pericar ent ►calyx, or ►in hooks or prickles

Pure Seed Definition 50

Seed, provided a portion of the testa is attached, with or without aril.
Piece of seed larger than one-half the original size, provided a portion of the testa is attached.

Table 50.1. Genera covered by pure seed definition 50

Family	Genus	Chaffy Table 2A
Berberidaceae	*Mahonia*	Pt 2
Fabaceae	*Acacia*	Pt 2
Fabaceae	*Cytisus*	Pt 2
Taxaceae	*Taxus*	Pt 2

Berberidaceae

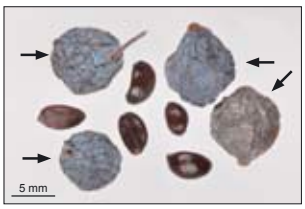


Figure 50.1. Seeds and berries (arrows) of *Mahonia aquifolium*. ×2.5. The berries must be opened and the fruit remains added to the inert matter fraction. (©2010 Her Majesty the Queen in Right of Canada (Canadian Food Inspection Agency))

Fabaceae

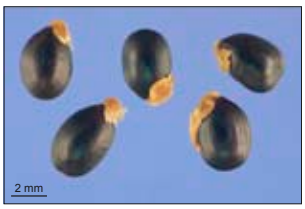


Figure 50.2. Seeds of *Acacia mearnsii*. ×5. (Steve Hurst, USDA)

Taxaceae

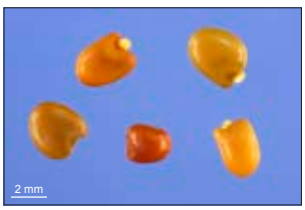


Figure 50.3. Seeds of *Cytisus scoparius*. ×5. (Steve Hurst, USDA)

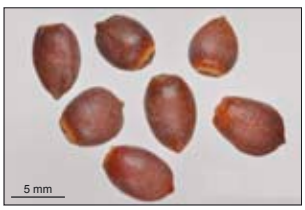


Figure 50.4. Seeds of *Taxus baccata*. ×3. (©2010 Her Majesty the Queen in Right of Canada (Canadian Food Inspection Agency))

PSD 50

140 years of official laboratory seed testing come to an end in Denmark

Dr. agro. Hans Arne Jensen[1] and Ms. Grethe Tarp[2]
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Official laboratory seed testing, which began in Denmark some 140 years ago, will come to end on 1 May 2011. The Danish Plant Directorate will on this date be closing its seed laboratory.

The background is that a gradual expansion in the accreditation of private-sector seed laboratories has led to a drastic reduction in the number of samples tested by the Directorate. The implication is that it is no longer economically possible to continue the official testing for purity, number count, germination, moisture and laboratory cleaning. However, official Danish seed sampling, testing for varietal purity and seed certification will continue at the Plant Directorate,

The aim of this paper is to briefly describe the formation of the official Danish seed testing station, and the Danish contributions to the establishment of ISTA, the ISTA Rules and the continuing development of the organization. We finally outline some of the implications of the closure of the official Danish seed testing laboratory with a view to the Danish seed sector as well as to ISTA and other organizations.

Formation of the official seed testing station

The Danish Seed Testing Station began its activities in Copenhagen in 1871 under the name "Dansk Markfrøkontrol". The founder, E. Møller-Holst, was a trained surveyor, who after extensive travel throughout Europe became a well-known editor of agricultural journals and handbooks.

Through his national and international networks, Møller-Holst became aware of the low quality of seeds sold to farmers, and he must also have learned about Prof. F. Nobbe's seed testing station in Tharandt, Germany. Møller-Holst visited professor Nobbe in 1870, and got ideas how to establish a seed testing station in Denmark. On 27 February 1871 the first samples were received, and the Danish Seed Testing Station began its activities as the second seed testing station in the world (1).

The international contact and exchange of ideas and testing methods continued in the years to come.

It is of considerable interest to note that international cooperation between seed testing stations existed from the very beginning.

From private enterprise to official seed testing station

E. Møller-Holst founded "Dansk Markfrøkontrol" as a private enterprise, but a few years later it was supported and supervised by the Royal Danish Agricultural Society.

After the death of E. Møller-Holst in 1889, the institute was taken over in 1891 by the Danish State, under the name "Statsanstalten Dansk Frøkontrol", from 1916 "Statsfrøkontrollen" (Danish State Seed Testing Station). In 1990 it became part of "Plantedirektoratet" (Danish Plant Directorate) – a new institute working on a range of control issues related to plants.

Scandinavian cooperation

In Sweden, the first of more than 20 local seed testing stations was established in 1876, and in Norway the first official seed testing station was founded in 1884.

Mutual cooperation between the seed testing stations in Denmark, Norway and Sweden began very early. A Scandinavian Seed Testing Committee was formed in 1890, and the first Scandinavian Rules for Seed Testing were accepted in 1892–94 (9).

The founding of ISTA

During the late 19th and early 20th century, cooperation between the European seed testing stations became increasingly important, not least due to expanding seed production and international seed trade.

Experiences in growing and testing seeds were discussed at European seed conferences in Hamburg in 1906 and in Münster and Wageningen in 1910, and at an international seed conference in Copenhagen in 1921 the European Seed Testing Association was established. A few years later, in 1924, ISTA was founded at a similar conference in Cambridge, United Kingdom.

During the formative years of these organisations the Danish director K. Dorph-Petersen (Fig. 1) was very active, and he became the first President of ISTA from 1924 until his death in 1937.

Founding a European seed organisation only three years after the end of the

Figure 1. K. Dorph-Petersen, director of the Danish State Seed Testing Station 1903–1937, and President of ISTA 1924–1937. He was very active and had frequent connection with colleagues in many parts of the world. His very interesting correspondence regarding the foundation of ISTA and relations between the organization and various countries is now kept at the ISTA Secretariat.

First World War would not have been possible without the open minds and sincere willingness to cooperate that characterized Dorph-Petersen and his international colleagues.

The collaborative spirit of the ISTA family was present from the very beginning.

The ISTA Secretariat and journal

From 1924 to 1937, Dorph-Petersen was active both as President of ISTA and as editor of the ISTA journal *Proceedings of the International Seed Testing Association.*

Ms. Kaja Sjelby, scientific assistant at the Danish Seed Testing Station, was in the same period the secretary of the organisation, and she continued as such until 1939.

During the Second World War, the secretariat was located in Sweden, but in 1946 it returned to Denmark, where it remained until 1959.

From 1946 to 1959, the director of the Danish Seed Testing Station, Chr. Stahl, was editor of the *Proceedings of the International Seed Testing Association,* and Ms. Sjelby resumed her former activity as secretary of ISTA.

Danish participation in the activities of ISTA

Due to the extensive Danish seed production, not least of herbage grasses, sugar beet seeds and vegetables, Denmark had from the very beginning both a scientific and an economic interest in the function of ISTA as well as in the formulation of the ISTA Rules for Seed Testing.

Consequently, staff from the Danish State Seed Testing Station and Danish Plant Directorate have over the years served in the following positions in ISTA:

President: K. Dorph-Petersen (1924–1937), Erik Madsen (1989–1992)
1st Vice-President: Erik Madsen (1986–1989)
2nd Vice-President: Hans Arne Jensen (1992–1995)
ISTA Executive Committee: P. Norup Pedersen (1973–1977); Erik Madsen (1983–1986); Grethe Tarp (1995–present).
Chairpersons, technical committees:
Equipment: Erik Madsen (1980–1989); Grethe Tarp (1992–1995)
Flower Seed: Hans Arne Jensen (1980–1986)

Figure 2. As an expression of many years close cooperation between Professor F. Nobbe, Tharandt, Sachsen and The Danish State Seed Testing Station the institute received in 1922 the Nobbe Seed Collection containing ca. 9000 seed samples from all over the world.

Moisture: Grethe Tarp (1995–2000)
Plant Disease: P. Nedergaard (1956–1974)
Purity: Hans Arne Jensen (1986–1998)
Referee testing: P. Norup Pedersen (1956–1965)
Variety: H.C. Baekgaard (1956–1965)

Danish contributions to the ISTA Rules

The methods included in the Nordic Rules for Seed Testing from 1892 to 1894, used in Denmark, Norway and Sweden, clearly influenced the formulation of the first draft of the ISTA Rules for Seed Testing.

Subsequently, Danish seed scientists have contributed considerably to the development of the ISTA Rules through various working groups and technical committees. The Rules for Cleaning on Small Scale Machines, for instance, were developed by Denmark in cooperation with the Netherlands.

Details on the Danish contributions can be found in the Historical Papers on Purity (5), Germination (6) and the Plant Disease Committee (7), and in the Congress Reports from the various technical committees.

Training in seed analyses

During its 140 years of existence, the seed laboratory has played a key role in the training of seed analysts and seed samplers in Denmark. For instance, the Plant Directorate was active in preparing the Danish private seed laboratories to be accredited by ISTA (4).

The development of seed testing methods and mutual training in seed analyses among the Nordic countries (Denmark, Finland, Norway and Sweden) has for many years been a most valuable activity in underpinning the continued development of seed testing.

Denmark has also through its financial contributions from DANIDA and other sources contributed considerably to developing the seed sector in developing countries, through seed projects and training of seed analysts. The laboratory buildings of the Danish Plant Directorate were – due to sufficient space – well suited for training courses. Some of the many courses implemented over the years lasted for one week, others several months. The number of students from developing countries receiving training in such courses varied within a year between 3 and 40.

ISTA Congress and workshops

In 1995, Denmark hosted the ISTA Congress, and in 1973 an ISTA workshop on purity and germination (2).

Annual reports and historical overview

From 1871 to 1990, the Danish State Seed Testing Station published annual reports on its activities. From 1991 the activities are described in the more general annual reports from the Danish Plant Directorate. An overview of the publication of the annual reports can be found in the paper "Statsfrøkontrollen 1871–1971" (1).

Detailed reports on the development of Danish seed testing during its first 25, 50 and 100 years were published by Rostrup (8), Dorph-Petersen, (3) and the staff of the Danish State Seed Testing Station (1).

Consequences of closing the official seed testing laboratory

The examples mentioned above illustrate that experiences obtained through the work in the official seed laboratory have been utilized in the overall function of the ISTA organisation, in several ISTA Committees and in changes to the ISTA Rules. Experiences from laboratory work have, furthermore, been a valuable background for participation in committee work within the European Union and in other international organisations, as well as for seed projects and training of seed analysts.

It is obvious that the number of Danish seed scientists with experience from official laboratory seed testing and quality control will soon decrease, and within 5–10 years such experience will cease to exist in Denmark.

This development is a challenge for the Danish private seed sector. The necessary training through participation in major ISTA working groups and committees of personnel from the private sector must be upgraded and financially supported. Increased economic support from the private sector to research aiming at the development of ISTA testing methods will be necessary.

Without such measures, Denmark will lose international influence, and ISTA will have to continue its important duties in the development of the ISTA Rules without contributions from an important producer of grasses and other seeds.

Conclusion

The aim of this paper was to put into perspective the Danish contributions to the development of ISTA and its Rules for seed testing.

In Denmark, the accreditation of private seed laboratories led to closure of the official Danish seed laboratory. We find it pertinent to suggest that other countries may wish to carefully address the following question: do we want a similar development, where seed testing becomes the exclusive domain of the private sector, except for the official control exercised through outsourcing to other countries, or should we look for a model where private and official laboratories co-exist?

The Danish Plant Directorate will continue as an active member of ISTA, since the Directorate will continue to hold responsibility for official seed sampling in Denmark. Nonetheless, it is clear that the role of the Plant Directorate in contributing to the international development of seed testing methods will be phased out.

Figure 3. The Danish Plant Directorate, Kgs. Lyngby. The building, finished in 1970, was designed for both administration and laboratory seed testing of a high number of samples. It brought together seed testing activities carried out at 3 different locations, and in 1970 was one of the most modern seed laboratories in the world. When the number of samples tested decreased, some of the facilities were utilized for training students from other countries.

Acknowledgements

On behalf of present and former Danish colleagues we wish to warmly thank all our ISTA friends for fruitful cooperation and friendship through many years.

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ISTA membership changes

Status 1 March 2011

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New face at the ISTA Secretariat

Christof Neuhaus

Head of ISTA Technical Development Department

Christof Neuhaus was born in Germany and graduated at the University of Bonn in crop production, horticulture, plant breeding and phytopathology.

He presented an experimental MSc thesis at the Horticultural Institute, Bonn, on dormancy breaking and seed transmissibility of the apple mosaic virus in the raspberry.

He gained a PhD in plant virology at the Plant Disease Institute, Bonn, where he developed plant tissue culture protocols for several forest trees, and focused on interactions between plant viruses and forest decline.

He joined a vegetable breeding and plant production company near Düsseldorf, where he build up a certification system for Elite and mother plant stocks, and established a diagnostic lab for plant disease detection, in particular viruses and bacterial diseases.

He was the lead of an R & D project focusing on the somatic embryogenesis of *Pelargonium*.

From 1996 to 2010, Christof was head of the seed technology and seed quality department of a ornamental seed breeding and seed production company close to Göttingen, Germany. During this time he established a quality management system based on MS Access for several hundred species, introduced a vigour and plug evaluation system for main crops such as pansies, begonias and primroses in cooperation with nurseries, and initiated several R & D projects on seed physiology and seed technology (e.g. upgrading, priming, disinfection, antagonist application) in cooperation with French, Dutch, German and North American universities, research institutes and seed tech service companies.

In the past few years he was also responsible for the small biotech and breeding support lab and the elite stock department, and was a member of the management team of the company.

Christof joined the ISTA Secretariat in January 2011, where his responsibility is to coordinate the Technical Committees of the Association. ■

An obligation from the beginning: comparative, then referee, now proficiency testing

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Rules for seed testing

The basic principles of seed testing were laid down in the 'Statut betreffend die Controle landwirthschaftlicher Saatwaaren' [statute concerning the control of agricultural seeds in trade] of Nobbe (Nobbe 1869, cf. Steiner and Kruse 2007) and in 'Die Technik der Untersuchung von Samenproben' [the technique for testing seed samples] as adopted in Graz 1875 by the 1st Meeting of the Directors of Seed Testing Stations and of other Persons Interested in this Matter (Nobbe 1876). The decades to come were devoted to improving and standardizing the existing methods for seed testing and to elaborating and standardizing new methods, which is now called method validation (Steiner, 1997; Steiner, Kruse and Leist, 2008). In 1906, at the 1st International Conference for Seed Testing in Hamburg, already four seed testing associations presented national rules for seed testing (cf. Steiner and Kruse, 2006). Eventually, the International Seed Testing Association (ISTA) published the first edition of the International Rules for Seed Testing in 1931. The advancement of these Rules has been a steady process to this very day.

Uniformity in seed testing

It is good to have rules for seed testing. However, right from the beginning Nobbe stressed that the ultimate goal of seed testing is the uniform application of approved seed testing methods as prescribed by rules. With view to the rapid increase in national and international seed trade, and the concomitant increase in the number of seed testing laboratories, Nobbe already called for and strongly advocated uniformity in seed testing on a worldwide basis as the ultimate prerequisite for the reliability of seed testing results. The means by which uniformity in seed testing can be monitored is comparative testing. In principle, this works as follows: a seed sample is divided into subsamples. The subsamples are sent to seed testing laboratories, along with a prescription of the testing method to be applied. The results are collected and statistically analysed. Thus, uniformity can be determined by studying the variation of the results.

Comparative testing

In the light of Nobbe's urgent request, the specific motto of the 2nd Meeting of the Directors of Seed Testing Stations in Hamburg in 1876 was 'Einheitlichkeit in der Saatgutprüfung' [uniformity in seed testing] (Eidam 1876), a motto which half a century later became the motto of ISTA. At the 3rd Meeting in Munich in 1877, Nobbe initiated the first comparative test (Eidam 1878). A total of 17 laboratories participated in testing Kentucky bluegrass seed samples (*Poa pratensis* L.) for germination determination. The results were evaluated at the 4th Meeting in Cassel (now Kassel) in 1878 (Kraus 1879).

In those days, in European countries with a relatively small number of seed testing laboratories, comparative tests were used for both monitoring uniformity in testing and method validation. This was later called experimental sample testing. In countries with a larger number of seed testing laboratories, comparative testing was purely for monitoring uniformity; method validation was assigned to working groups. Likewise, after the Association of Official Seed Analysts (AOSA) was founded in the United States in 1908, yearly referees were started (Brown 1922). When the European Seed Testing Association was founded in Copenhagen in 1921, Johannsen (1922) reported on international comparative tests, and an admirable number of international comparative tests were initiated (Dorph-Petersen, 1925). The Constitution of the follow-up association ISTA in 1924 put comparative testing in first place: 'Article 1. *Name and Object.* … for the purpose of advancing all questions connected with the testing and judgment of seeds. The Association seeks to obtain this object through: a) Comparative tests and other researches directed to achieving more accurate and uniform results than hitherto obtained. ...' In the Constitution of 2007, this became: 'Article III: Objects: (a) The primary purpose of the Association is to develop, adopt and publish standard procedures for sampling and testing seeds and to promote uniform application of these procedures for evaluation of seeds moving in international trade.' As a result, comparative testing covered all ISTA member laboratories, while method validation became the task of technical committees (Steiner 1998).

Comparative tests were organized voluntarily by technical committees or even by individual laboratories; the tests were regional or association wide. As an example, the results of five association-wide 'Comparative Tests 1938' of fodder plants are shown, as printed by *Emil Kihlströms Tryckeri* AB, Stockholm, and distributed to the 49 participating stations of 26 countries (Fig. 1). These comparative tests were preceded by similar tests (Sjelby 1938). The stations were grouped into 10 groups according to countries or regions, plus Japan and New Zealand. For the participating stations, the Table presents all information allowing detailed evaluation, but for outsiders it is declared 'Confidentiel *(sic)*! Must not be published!' At that time, in ISTA openness to experience for achieving progress ranked higher than confidentiality (cf. Johannsen, 1922; Dorph-Petersen, 1925). The mutual exchange of ideas, knowledge and skills was thus easily achieved, and open discussion added liberally to advancement in seed testing. Even today we can make a full statistical analysis of the results in the Table, and ponder on the reasons for the differences between regions and stations.

Confidentiel! Must not be published!
Confidentiel! Prière de ne pas publier!
Vertraulich! Darf nicht veröffentlicht werden!

Comparative Tests 1938.
Analyses comparative 1938.
Vergleichende Untersuchungen 1938.

International Seed Testing Association.
Association Internationale d'Essais de semences.
Internationale Vereinigung für Samenkontrolle.

Seed Testing Station	Country	Certificates dated	Germination Results													
			Trifolium pratense No. 1			Trifolium pratense No. 2			Medicago lupulina No. 3			Brassica napobrassica No. 4		Brassica rapa No. 5	
			Germinated Seeds	Hard Seeds	Abnormal & broken Growths	Germinated Seeds	Hard Seeds	Abnormal & broken Growths	Germinated Seeds	Hard Seeds	Abnormal & broken Growths	Germinated Seeds	Abnormal Growths	Germinated Seeds	Abnormal Growths
			%	%	%	%	%	%	%	%	%	%	%	%	%
Stockholm	Sweden	8/6	63	2	33	69	5	14	68	9	14	82	8	84	13
Linköping	»	11/6	65	1	34	68	6	15	65	8	21	83	12	81	18
Skara	»	1/6	62	2	33	69	5	13	61	10	16	81	5	86	12
Orebro	»	7/6	61	1	35	69	5	13	65	13	12	87	3	85	7
Copenhagen	Denmark	31/5	61	2	34	77	5	6	71	10	5	85	5	85	10
As	Norway	1/7	66	2	30	73	6	11	66	12	10	84	6	88	8
Helsingfors	Finland	22/6	72	1	23	74	5	10	69	10	8	85	8	88	9
	Average		64.8	1.6	31.7	71.8	5.8	11.7	66.4	10.2	12.8	83.9	6.7	85.2	11.0
Augustenberg	Germany	15/6	79	1	16	77	3	6	75	9	7	85	5	90	6
Breslau	»	16/7	70	2	20	74	6	1	70	12	8	79	3	87	5
Halle a/S	»	5/6	77	2	18	78	6	6	76	9	6	87	2	93	3
Hamburgh	»	20/6	67	2	29	72	6	17	68	10	16	85	10	85	13
Hohenheim	»	23/6	72	2	24	76	5	3	83	8	2	83	1	91	3
Linz	»	29/6	69	1	27	73	7	6	72	8	8	84	6	88	9
Munich	»	3/6	71	1	26	77	5	6	66	9	7	82	7	89	10
Wiesna	»	5/6	79	2	4	72	5	9	68	7	6	82	5	88	2
	Average		73.0	1.6	20.5	74.9	5.4	6.8	72.3	9.0	7.8	83.4	4.9	88.9	6.4
Leningrad	Russia	22/6	56	1	34	60	3	12	60	3	10	81	11	81	15
Tallinn	Esthonia	14/6	72	2	21	72	6	6	68	10	3	88	3	88	3
Riga I (University)	Lettonia	13/6	60	2	37	72	8	11	67	11	14	81	7	89	5
» II (Seed test. sta.)	»	22/6	70	2	27	73	6	8	71	9	12	81	5	91	7
Dotnuva	Lithuania	24/6	69	2	26	72	6	11	66	10	14	81	9	83	13
	Average		65.4	1.8	29.0	69.8	5.4	9.0	66.4	8.6	10.6	82.4	7.0	86.4	8.6
Danzig	Freestate Danzig	29/5	87	1	10	86	2	3	92	2	4	70	1	90	3
Krakow	Poland	25/6	66	1	31	71	5	9	61	10	25	77	10	76	21
Lwow	»	1/7	71	1	27	76	5	11	67	10	14	76	14	83	15
Poznan	»	28/6	72	0	25	74	11	6	67	9	8	86	6	90	6
Warsow	»	21/6	56	1	41	66	7	11	58	10	14	67	17	72	14
	Average		66.8	0.7	31.0	71.7	7.0	9.3	63.8	9.8	15.2	76.3	11.8	80.3	14.0
Bratislava	Czechoslovakia	25/6	79	2	18	79	6	7	70	12	10	84	6	88	7
Brno	»	29/6	73	2	24	78	5	6	73	8	9	83	9	85	12
Kosice	»	25/6	73	2	23	77	4	7	68	7	7	77	13	87	9
Praha	»	27/6	72	2	23	80	6	6	72	9	12	76	11	86	12
	Average		74.8	2.0	22.0	78.5	5.8	6.5	70.8	9.0	9.5	80.0	9.8	86.5	10.0
Budapest	Hungary	1/6	64	1	34	75	6	9	69	9	15	82	6	81	15
Zagreb	Jugoslavia	10/6	61	1	35	69	5	10	59	10	9	72	12	78	16
Sofia	Bulgaria	29/6	59	2	38	66	6	16	57	10	19	66	5	83	14
Bucarest	Roumania	26/11	89	2	8	64	5	14	66	8	10	70	7	86	10
	Average		68.3	1.5	28.8	68.5	5.5	12.3	62.8	9.3	13.2	72.8	7.5	82.0	13.8
Zurich	Switzerland	30/6	70	0	28	74	4	5	69	3	11	79	6	87	9
Bologna	Italy	19/6	64	1	30	72	7	11	66	9	17	81	5	89	7
Paris	France	1/6	66	1	30	69	3	11	67	7	13	74	13	88	8
Wageningen	The Netherlands	18/6	74	2	20	71	9	8	71	11	14	88	6	85	11
	Average		68.5	1.0	27.0	71.5	5.8	8.8	68.8	7.5	13.8	80.5	7.5	87.2	8.8
Cambridge	Great Britain	30/5	63	2	31	73	7	8	68	10	7	86	6	78	7
Edinburgh	» »	30/5	68	3	25	72	7	9	66	11	9	75	10	87	6
Belfast	» »	7/6	70	3	19	77	5	9	76	7	10	88	0	96	0
Dublin	Ireland	11/6	64	1	32	65	7	17	66	10	10	71	17	81	9
	Average		66.8	2.3	27.0	71.8	6.5	10.8	69.0	9.5	9.0	80.0	8.2	85.2	5.5
Washington, D. C.	U. S. A.	11/7	58	3	32	66	5	12	64	9	12	74	11	78	12
Geneva, N. Y.	»	18/10	61	1	24	67	6	7	62	8	9	83	4	83	5
College Park, Md.	»	28/6	59	1	38	69	7	14	60	11	18	70	11	81	12
Lafayette, Ind.	»	15/6	63	1	30	74	3	11	64	8	13	78	8	81	7
	Average		60.3	1.5	31.0	69.0	5.3	11.0	62.5	9.0	13.0	76.3	8.5	80.3	9.0
Ottawa	Canada	16/6	57	1	41	67	5	24	59	3	28	86	0	87	0
Toronto	»	22/7	49	2	49	71	7	17	62	8	20	57	15	74	18
	Average		53.0	1.5	45.0	69.0	6.0	20.5	60.5	5.5	24.0	71.5	7.5	80.5	9.0
Kurashiki	Japan	12/7	77	1	20	67	5	9	68	10	9	76	6	84	13
Palmerston, North.	New Zeeland	21/7	60	2	36	65	1	16	61	7	17	72	14	75	16
	Average of all 49 stations		67.5	1.6	27.6	72.0	5.5	9.9	67.4	8.8	11.7	79.2	7.0	84.9	9.5

Figure 1. 'Comparative Tests 1938' of fodder plants, as printed by *Emil Kihlströms Tryckeri* AB, Stockholm.

From 1950, comparative testing was the responsibility of the Rules Committee, and after 1953, in parallel to association-wide testing, five regional testing groups were formed, each with a Chair (Justice, 1957, 1962; Koopman, 1962). Independently thereof, technical committees continued to arrange comparative tests.

Referee testing

In 1962, to reinforce the importance of comparative testing, the term comparative test was replaced by the term referee test, and a stand-alone Referee Test Committee was established and chaired by the experienced M.J.F. Koopman. However, there was no change in the nature of the test. Since then, the further development of referee testing can be followed through the triennial Reports of the Referee Test Committee. To mention only three important steps: in 1971 (Washington D.C.), the validity of association-wide testing and regional testing (now in seven regions) was discussed, and the organization of referee tests according to trade channels was considered, but not implemented. In 1974 (Warsaw), association-wide referee testing was adopted. In 1977 (Madrid), participation in referee testing became obligatory for laboratories issuing ISTA International Seed Analysis Certificates. In cases of failure in testing performance, reminders were sent to the laboratories in question, and follow-up assistance was provided.

Proficiency testing

In 1995 (Copenhagen), the ISTA Laboratory Accreditation Standard was approved (Anonymous, 1996) and in 2002 (Santa Cruz, Bolivia), updated referee testing was adopted under the new name proficiency testing, but again without changing the nature of the test (Ashton 2002). Nowadays, the samples to be tested are prepared under the auspices of the Proficiency Test Committee, in consultation with the ISTA Secretariat. This is because since 2004 (Budapest), successful participation in the ISTA Proficiency Test Programme is a prerequisite for the accreditation of laboratories, including authorization for issuing ISTA Certificates (cf. Muschick, 2010). Thus, performance in proficiency testing eventually became decisive. Nowadays, however, each laboratory participating in the Proficiency Test Programme receives its results, including a detailed statistical analysis, individually and confidentially. Tables disclosing full information, as were used in 1938 and before, are no longer published. Only the names and numbers of ISTA Accredited Laboratories indicate successful performance in proficiency testing.

In closing it may be born in mind that the term 'comparative test' is still widely used in technical areas, for example by the International Organization for Standardization (ISO), product testing services, certification agencies and others; it is noteworthy that the ISO uses it in addition to 'proficiency test'. On the other hand, 'proficiency test' is not only used in technical areas such as seed testing, but also in the humanities, for example in languages and education.

Conclusion

Comparative testing for monitoring uniformity in seed testing, renamed 'referee testing' and later 'proficiency testing', is nearly as old as seed testing itself. The first comparative test was organized under the leadership of F. Nobbe in Munich in 1877. The nature of the test did not change; however, the procedure was continually improved over the years, following the state of the art of seed science and technology. Today, successful participation in the ISTA Proficiency Test Programme is one of the prerequisites for ISTA Laboratory Accreditation, including authorization for issuing ISTA Certificates.

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A proposal to revise the pure seed definition for *Arachis hypogaea*

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Seed of *Arachis hypogaea* (peanut) is sold in various parts of the world, and in most markets, the seed is removed from the pod. In a tropical country such as Indonesia it is uncommon for peanut seed to be sold without the pod intact. Under humid conditions leaving the pods intact slows down the seed deterioration process allowing for increase storage time over lots stored as cleaned seed (pod removed). Under this marketing scheme the farmer removes the pod just before the seed is planted in the field.

The problem with the existing rule

The ISTA Rules 2010 state that the pure seed definition (PSD) of *Arachis hypogaea* (peanut) is 11, i.e.:

> Seed, provided a portion of the testa is attached.
> Piece of seed larger than one-half the original size, provided a portion of testa is attached.
> Seed and pieces of seed entirely without testa are regarded as inert matter.
> For *Fabaceae (Leguminosae)*: separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

This definition indicates that we do purity analysis to determine the percentage of clean seed in the sample. The task is simple if the sample comes to the laboratory as clean seed (pod removed), but in Indonesia peanut seed is always marketed in the pod; therefore, sampling is done of pods and the peanut sample arrives to lab in the pod. The problems that we face are:

1. Based on PSD 11, seed analysts must remove the pod during the purity analysis. This is time consuming, and the result depends on the method used. These methods are not always reproducible among seed analysts, because while some analysts are careful to remove the pods without damaging the delicate seed coat (testa), others may damage the testa and possibly the embryo, thus increasing the percentage of inert matter. This can lead to test results that are not truly representative of the seed lot.

2. Once the pod is removed it is classified as inert matter. The amount of inert matter attributed to the removed pods can reach 20–25% of the working sample. In Indonesia, the national regulations state that the maximum allowable level of inert matter is 2%; therefore, if the current ISTA PSD 11 is applied, peanut lots will not meet the requirements for trade in Indonesia.

3. Table 2A of the ISTA Rules states that the submitted and working samples for *Arachis hypogaea* must be 1000 g. Based on the current PSD, it is assumed that this applies to clean seed. If a purity analysis is performed on the pods, should the weight of the working sample be increased?

Discussion

To address these problems, I contacted the ISTA Purity Committee, the Chair of the AOSA Purity Committee, and an expert in the U.S. peanut trade. The following comments were received:

"We test for purity in the pod. The samples always come that way. The seeds are separated from the pod for the germination test only. I do not think that it should be the part of a purity test to separate the seeds as others already pointed out; it would make a big portion of inert matter and a lot of work as well. However, it is the right way according to PSD 11! I think that *Arachis* should be removed from this PSD to either PSD 21 or 24. I prefer PSD 21. I checked *Stylosanthes* pods from the GRIN list, and their beaks can not be seen on the pods of *Arachis*. Also, the referred AOSA description is more PSD 21."

"Seed certification is a tool to control the quality of seed that is traded. In Indonesia, peanut seed is sold in the pod; that is why physical purity information should be from purity analysis in the pod. My suggestion is to use PSD 21 for *Arachis hypogaea*."

"AOSA Rules (2009) describe the pure seed unit of peanut (PSU9) as follows:

> Intact pod, with or without calyx or bracts, whether or not a seed is present.
> Piece of broken pod larger than one-half of the original size, unless no seed is present.
> Seed, with at least a portion of seed coat attached, with or without aril.
> Piece of broken seed larger than one-half the original size, with at least a portion of the seed coat attached, with or without aril.
> Special consideration:
> For *Fabaceae*: Cotyledons that are broken apart but held together by the seed coat shall be classified as pure seed. Cotyledons that have separated and are not held together by the seed coat are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the seed coat may be attached.
> *Chalcid-damaged seeds in *Fabaceae* that are puffy, soft, or dry and crumbly are considered inert matter.
> This definition applies to the following genera: *Arachis, Kummerowia, Lespedeza, Medicago* (*M. lupulina* only), *Melilotus, Onobrychis, Trifolium* (restricted to only *T. alexandrinum, T. campestre, T. dubium, T. fragiferum, T. glomeratum, T. hirtum, T. incarnatum, T. lappaceum, T. pratense, T. resupinatum,* and *T. subterraneum*; basically clovers with one- or two-seeded pods)."

"The purity working weight for peanut is 500 grams. This applies both to pods of peanut or seeds of peanut. Five hundred grams is the maximum purity working weight for large legume crops in the AOSA Rules."

"In the U.S.A., peanut seed are always shelled (removed from the pod) and treated with a fungicide immediately prior to planting.

To summarize, pure seed in peanut must have at least a portion of the seed coat (testa) attached and broken seed must be larger than one-half the original size. Peanut seed entirely lacking attachment of the testa or broken seed less than half the original size would be considered inert matter. Any remaining portion(s) of pod (shell) would be considered inert matter."

"Both the AOSA and FSA allow for the pods of peanut to serve as seed units. Therefore the two sets of rules are in agreement and this would eliminate the need for a laboratory to perform the time-consuming task of removing the pods during a purity analysis should the sample arrive in that condition. There are many more examples in the Rules where the seed unit descriptions include structures that we may not routinely see, but are included in the pure seed description to eliminate the need to remove naturally occurring structures that may occasionally remain attached to a seed unit after conditioning."

"I see that as a serious conflict between "Rules" and meaningful seed analysis as it is universally practiced in the peanut belt of the United States. Indeed, if the intent of the AOSA and FSA seed testing rules is to test unshelled peanut seed, it is not clearly understood or applied in the peanut testing laboratories of the US. From a purity perspective, an analyst could not make an accurate assessment of the peanut seed by examining pods, because it would not necessarily be representative of the seed lot after shelling and conditioning where the seed is sized and there is potential for mechanical damage, etc. By definition, it would be a different seed lot after shelling and conditioning, and the seed testing rules state that seed samples are to be taken in a manner that is representative of the seed lot. Furthermore, peanut seed in the pod could not be utilized to provide a representative germination test for a seed lot. All peanut seed is shelled and treated prior to planting in our region, although I do not know whether this is globally the case. From a practicality perspective for the laboratory as well as representative seed sample for testing, I expect that every commercial and government laboratory in this region would only accept peanut seed samples that have been shelled and treated by the seed conditioner in a manner that is representative of the seed lot. The information that I have provided is not specific to the Georgia laboratory, but rather applies to all seed laboratories in the south-east US that test peanuts. If this discrepancy does exist between what is stated in the respective rules and what is practiced for representative peanut seed sampling and analysis, then the rules should be corrected to reflect how peanut seed is handled commercially. If peanuts are marketed and planted in a different manner in other parts of the world from what I have described, the rules should also provide for that latitude. In either case, the laboratory should not be responsible for in-house shelling, since it would not be a representative seed sample for testing, not to mention that it would place an unreasonable burden on the laboratory."

It should be noted that AOSA is considering adding, through a rule change proposal, the following text to the pure seed unit for peanut and other legumes under AOSA PSU9:

"For seed lots marketed as hulled seed: occasional intact or broken fruits are to be opened and the seeds with at least a portion of the seed coat intact are to be classified as pure seed and the remaining fruit structure classified as inert matter. For seed lots marketed as fruits (burs or pods), the fruits are to be classified as pure seed if they fit the criteria stated above."

The adoption vote will take place at the AOSA/SCST Annual Meeting, June 2011.

Proposal

To facilitate the world seed trade, ISTA should accommodate all conditions in which peanuts are marketed and also consider the impact on marketing restrictions in tropical countries such as Indonesia, where it is uncommon to market peanut seed without pods.

Based on the above discussion, I propose:

1. To change the PSD number for *Arachis hypogaea* from PSD 11 to PSD 21:

Pod, with or without calyx, with seed(s).
Seed, provided a portion of the testa is attached.
Piece of seed larger than one-half the original size, provided a portion of testa is attached.
Seed and pieces of seed without testa are regarded as inert matter.
Separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached).

2. To allow the minimum submitted sample weight for pods or seed to be 1000 g, and the working sample weight for purity analysis for pods and seed to be 1000 grams.

I respectfully ask this issue be considered for discussion at the next ISTA meeting.

Many thanks to Joost van der Burg, Ronald Don, Jane Taylor, Deborah Meyer, Wayne Guerke, Adriel Garay, Ripka Gezane, Gerarda de Boer, Augusto Martinelli and Udin S. Nugraha.

Germination test for *Solanum nigrum*

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Summary

The objective of this validation test is to introduce the species *Solanum nigrum* into Chapter 5 of the ISTA Rules. The genus *Solanum* is already represented in the ISTA Rules by the species *S. melongena* and *S. tuberosum*.

The experiment was carried out by five ISTA-accredited laboratories using three seed lots. The *S. nigrum* seeds were germinated on a top-of-paper medium, using the alternating temperature regime 20<=>30 °C. Light was supplied for 8 h during the high-temperature phase, and germination counts were made at 7 and 14 days, and at 21 days when required.

The validation test showed no significant differences between the results obtained by the five laboratories, and that repeatability and reproducibility requirements were therefore met.

The results of this validation study support the introduction of the TP 20<=>30 °C germination method for *Solanum nigrum*.

Introduction

Solanum nigrum (Black Nightshade) is a common plant utilized as a vegetable and fruit source in Africa, India, Indonesia etc. The plant is cultivated as a food crop both for its fruit and its leaves and has medicinal usage. The unripe fruit may contain high concentration of toxins. In many other countries it is considered as a common garden weed.

Solanum nigrum seeds remain viable for years, depending on the storage conditions and moisture content.

Preliminary experiments conducted in our lab confirmed the findings of laboratories in Kenya, Zambia, Sudan and Botswana that the optimum germination temperature for *Solanum nigrum* is 20<=>30 °C when light is provided during the 30 °C phase.

In the peer validation test, there was a significant lot x lab interaction. Jean-Louis Laffont recommended the performance of additional tests by additional labs to assess the method better. A multi-laboratory validation study was therefore carried out, in order to develop an ISTA germination method for *Solanum nigrum*.

Material and methods

Seed material

Seven lots of untreated *Solanum nigrum* seeds were obtained from Mary Chipili (Zambia) and Joseph Ahenda (Kenya). Three lots were selected (4, 5 and 6) and sent to the participating laboratories.

Participating laboratories

Samples were sent to five ISTA-accredited laboratories in France (FRDL0200), Norway (NODL0100), Scotland (GBDL0400), the Netherlands (NLDL0300) and Israel (ILDL0100).

Germination methods

Three seed lots were tested on top-of-paper (TP) medium, using the alternating temperature regime 20<=>30 °C (Table 1).

For each lot, a total of 400 seeds were tested in replicates of 50–100 seeds. Light was supplied for 8 hours during the high-temperature phase, and germination counts were made at 7 (first counts) and 14 days (final counts). Since the seed lots were produced more than 3 years ago, the seedlings developed more slowly, and final counts were sometimes done after 21 days.

The evaluation of the seedlings was done in accordance with the ISTA seedling evaluation criteria for *Solanum melongena*. Seedling evaluation group: A.2.1.1.1.

Statistical analysis

The germination results were checked to ensure that the sum of the percentages equalled 100%.

Possible outliers were assessed using side-by-side boxplots and using tolerance Table 5B (between replicates). The performance of the method was assessed through the estimation of repeatability and reproducibility parameters in the context of binomial data.

The statistical analysis of the preliminary experiments was done by Waffa Abu-Aklin of the Israeli Seed Testing Laboratory.

The statistical analysis of the validation study was performed by Jean-Louis Laffont, Chair of the ISTA Statistical Committee.

Preliminary experiments

In December 2008, in order to assess the optimal germination temperature, the Israeli laboratory pregerminated two lots at 20, 25 and 20<=>30 °C, with 8 hours' light. Since the results with these lots were similar, only the result of lot No. 4 is given (Figure 1).

Appendices 1 and 2 show germination at 7 and 14 days, respectively. Germination was enhanced, accelerated and increased at the alternating temperature of 20<=>30 °C. Constant temperatures (20 and 25 °C) were found to inhibit and decrease germination.

This preliminary experiment confirmed the findings of the laboratories in Kenya, Zambia, Sudan and Botswana.

Table 1. Germination testing conditions on TP at 20<=>30 °C

Lab No.	Germination apparatus	Type of box/ plate (size)	Type of substrate (size)	Type of water (amount)	No. of seeds per replicate
1	Incubator	Germination box (12 × 18 × 5.5 cm)	Paper, 4 layers (12 × 18 cm)	Deionized water (25 mL per replicate, i.e. 0.9 mL per gram paper)	50
2	Jacobsen tank	Bell jars (Ø 8 cm)	Filter paper, 2 layers (Ø 7.5 cm)	Tap water (3.1 mL per replicate, i.e. 2.6 mL per gram paper)	50
3	Incubator	Germination box (12.5 × 17.5 × 5.5 cm)	Pleated paper, 1 layer (11 × 17.5 × 2 cm) + envelope strip (11 × 75 cm)	Tap water (1.2 mL per gram PP + 1.1 mL/g = 2.3 mL per gram paper)	100
4	Incubator	Germination tray (45 × 54 cm)	Filter paper (Ø 10 cm) on large filter paper (45 × 45 cm)	Deionized water (2.4 mL per gram paper + 1–1.8 mL per gram paper)	100
5	Room germinator	Petri dish (Ø 14 cm)	Anchor brown paper, 2 layers (Ø 14 cm)	Tap water (12.3 mL per replicate, i.e. 3.6 mL per gram paper)	50

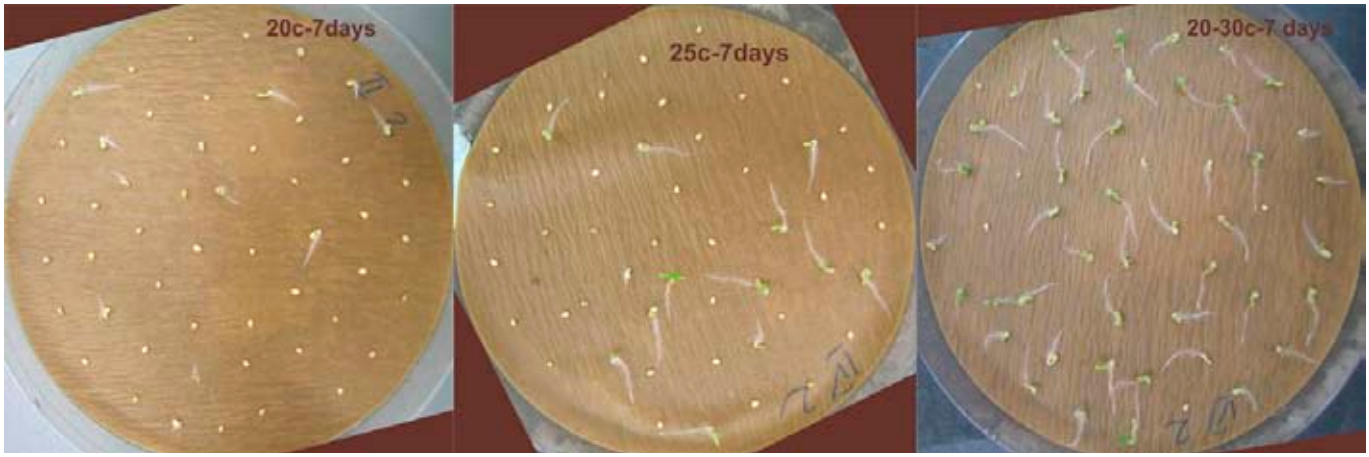


Figure 1. Germination of *Solanum nigrum* at 20, 25 and 20<=>30 °C after 7 days.

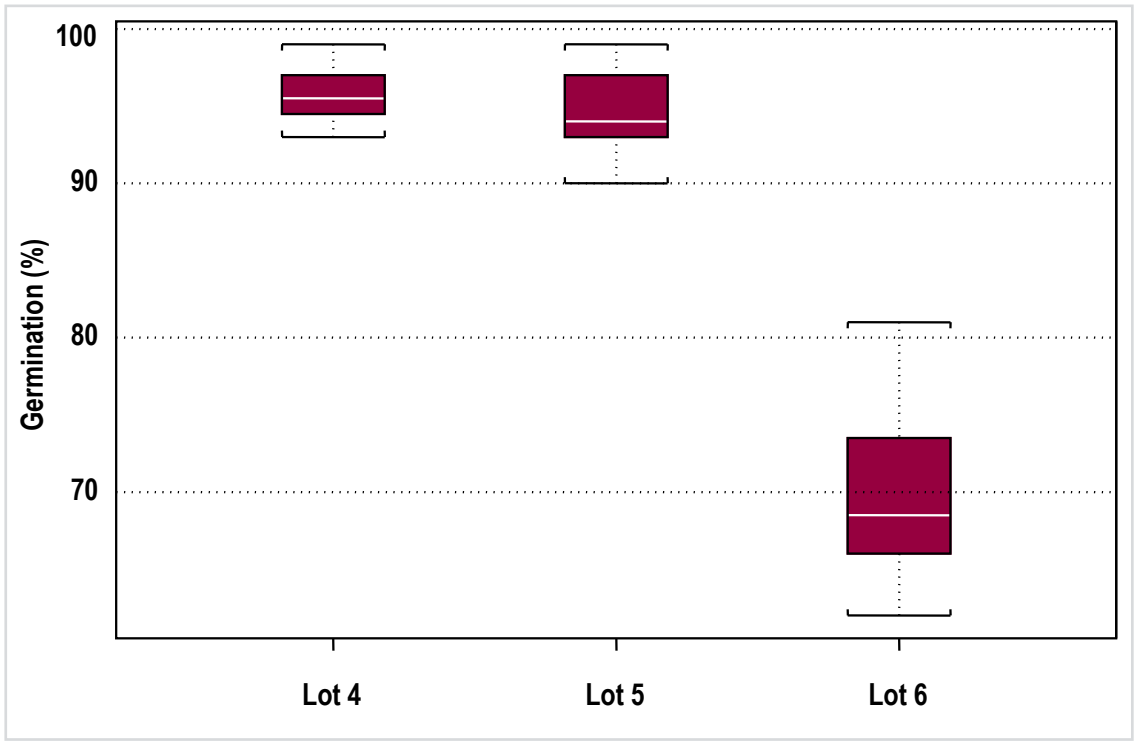


Figure 2. Grouping factor: lot. Germination (percentage of normal seedlings) obtained by the participating labs from the 3 seed lots. The germinations of Lots 4 and 5 was similar, while the germination of Lot 6 was much lower and more dispersed (which is a known feature of binomial data).

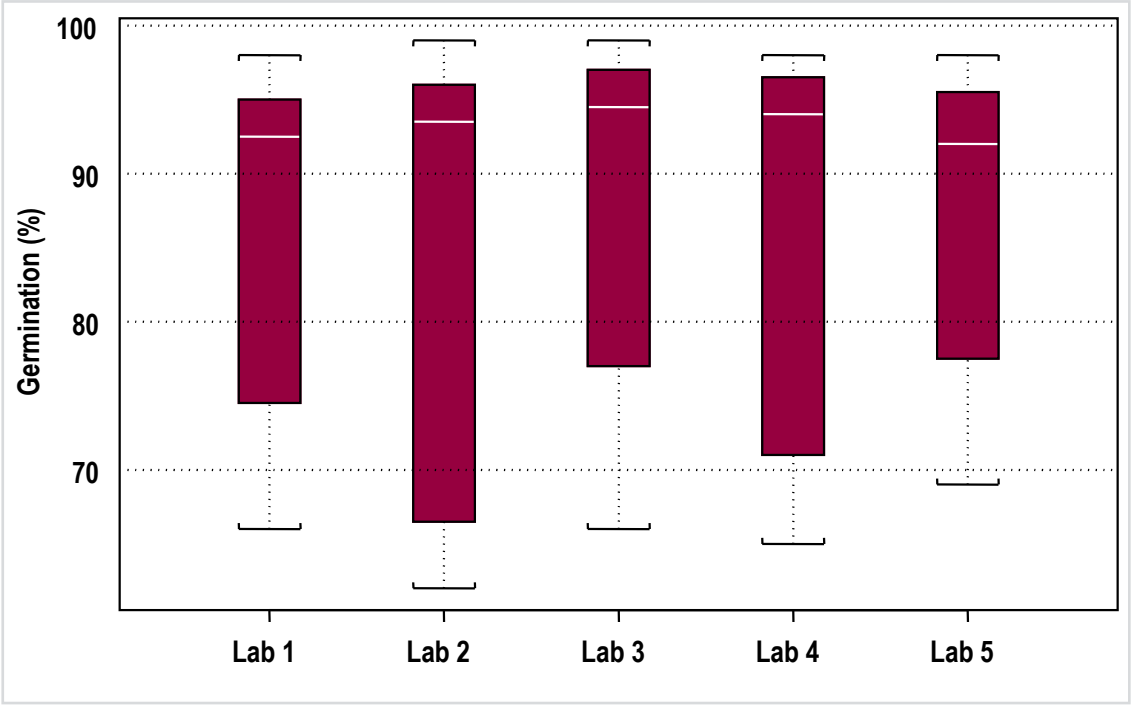


Figure 3. Grouping factor: lab. The germination results (normal seedling percentage) obtained by the participating labs were similar.

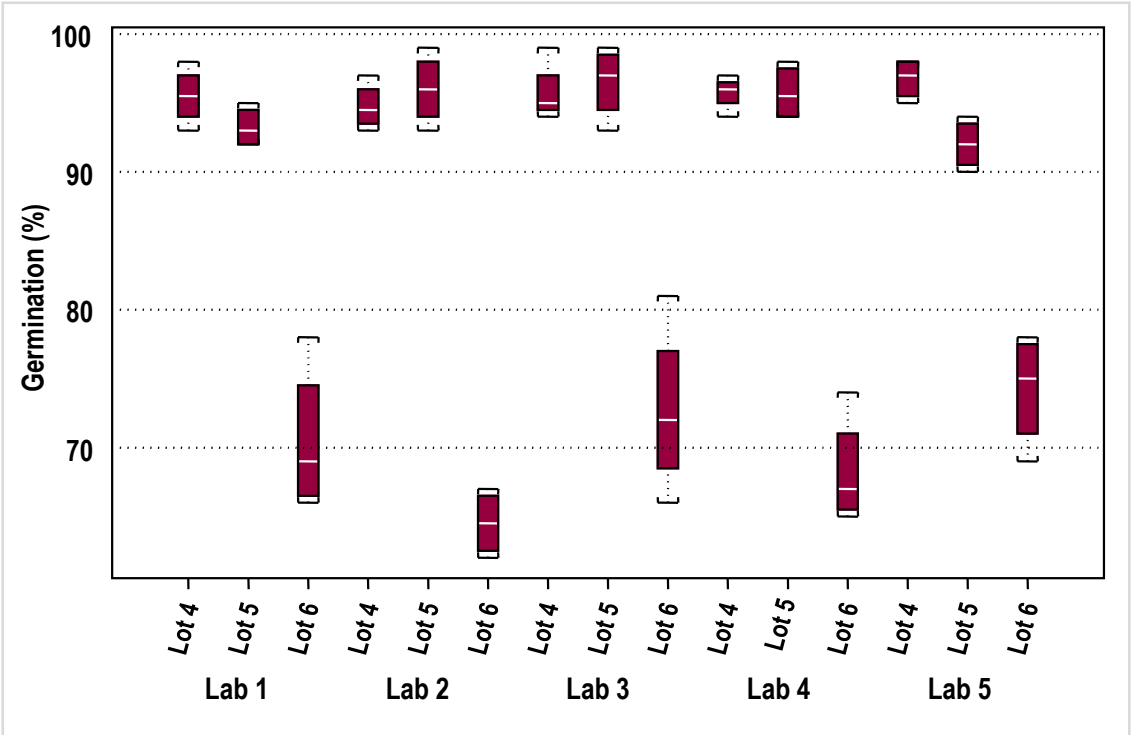


Figure 4. Grouping factor lot × lab. A strong lot effect was shown between lots 4 or 5 and 6. No lot × lab interaction was exhibited in these side-by-side boxplots.

Statistical analysis of the results

1. Data exploration with side-by-side boxplots

See Figures 2–4.

2. Data checking

The data were checked according to the ISTA Rules (ISTA, 2011) by calculating the tolerances for germination test replicates.

All the results were within tolerance (Table 2).

3. Repeatability

Let:

- I be the total number of lots;
- J be the total number of labs;
- K be the number of replicates of m seeds for a given lot in a given lab;
- p_{ijk} be the percentage of germinated seeds for lot i, lab j and replicate k.

The standard deviation of repeatability is computed as:

$$S_r = \sqrt{f_r^2 \frac{\bar{p}_{...}(100-\bar{p}_{...})}{m}}$$

where:

- $\bar{p}_{...}$ is the overall average percentage of germinated seeds;
- f_r^2 is an estimate of the dispersion parameter:

(1) $$f_r^2 = \frac{1}{IJ}\sum_{i,j}\frac{var\_obs_{ij}}{var\_bin_{ij}}$$

where:

$$var\_obs_{ij} = \frac{1}{K-1}\sum_k (p_{ijk}-\bar{p}_{ij.})^2$$

and

$$var\_bin_{ij} = \frac{\bar{p}_{ij.}(100-\bar{p}_{ij.})}{m}$$

with $\bar{p}_{ij.}$ being the average percentage of germinated seeds in lot i and lab j.

If $f_r^2 > 1$, one speaks of overdispersion, because the data have larger variance than expected, if a binomial distribution is assumed.

For this dataset, $I = 3$, $J = 5$, $K = 4$ and $m = 100$.

Results:

$\bar{p}_{...} = 86.82$
$S_r = 3.37$
$f_r = 0.99$
There is no evidence for overdispersion.

Note: consider the following generalized linear model (GLM):

$y_{ijk} \sim \text{Binomial}(m_{ijk}, \pi_{ijk})$

$$\text{logit}(\pi_{ijk}) = \log\left(\frac{\pi_{ijk}}{1-\pi_{ijk}}\right) = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij}$$

where:

- $i = 1, 2, \ldots, I$
- $j = 1, 2, \ldots, J$
- $k = 1, 2, \ldots, K$
- y_{ijk} is the number of germinated seeds out of m_{ijk} in lot i, lab j and replicate k
- μ is the general effect
- α is the fixed effect of lot i
- β is the fixed effect of lab j
- $(\alpha\beta)_{ij}$ is the fixed interaction effect between lot i and lab j.

The φ^2 factor, characterizing overdispersion, can be estimated by dividing the squared sum of the Pearson residuals after fitting the model by the residuals degrees of freedom [here, $IJ(K-1)$]. For this particular GLM, the algebraic expression of this estimate is:

(2) $$f_r^2 = \frac{1}{IJ(K-1)}\sum_{i,j}\sum_k \frac{(y_{ijk}-m_{ijk}\hat{\pi}_{ij})^2}{m_{ijk}\hat{\pi}_{ij}(1-\hat{\pi}_{ij})}$$

where $$\hat{\pi}_{ij} = \frac{\sum_k y_{ijk}}{\sum_k m_{ijk}}$$

When $m_{ijk} = m = 100$, expression (2) simplifies to expression (1).

4. Reproducibility

Consider the following linear mixed-effects model (LMM):

$p_{ij} = \mu + \alpha_i + b_j + e_{ij}$

where:

- $i = 1, 2, \ldots, I$; $j = 1, 2, \ldots, J$;
- $p_{ij.}$ is the percentage of germinated seeds out of n in lot i and lab j;
- μ is the general mean;
- α_i is the fixed effect of lot I;
- b_j is the random effect of lab j. The b_j are iid N(0, σ^2_{Lab});
- e_{ij} are the residuals. The e_{ij} are iid N(0, σ^2).

In the context of an LMM, the reproducibility standard deviation is then defined to be:

$$S_R = \sqrt{\hat{\sigma}^2_{Lab} + \hat{\sigma}^2}$$

where $\hat{\sigma}^2_{Lab}$ and $\hat{\sigma}^2$ are the variance component estimates.

When data are perfectly balanced (no missing lot × lab combination), we have:

$$S_R = \sqrt{\frac{1}{I}\sum_i\sum_j \frac{(p_{ij.}-\bar{p}_{i..})^2}{J-1}}$$

where $$\bar{p}_{i..} = \frac{\sum_j p_{ij.}}{J}$$

Assuming a binomial distribution, the variance of $p_{ij.}$ is:

$$\text{Var}(p_{ij.}) = \frac{p_{ij.}(100-p_{ij.})}{n}$$

Table 2. Germination results and tolerances

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5
Lot 4					
Tolerance range (max.)	8	9	8	8	7
Observed range	5	4	5	3	3
Mean	95.5	94.75	95.75	95.75	96.75
Lot 5					
Tolerance range (max.)	10	8	8	8	11
Observed range	3	6	6	4	4
Mean	93.25	96	96.5	95.75	92
Lot 6					
Tolerance range (max.)	18	19	17	18	17
Observed range	12	5	15	9	9
Mean	70.5	64.5	72.75	68.25	74.25

We then compute the following quantity to characterize overdispersion when lab and lot by lab variations are considered:

$$f_R^2 = \frac{n S_R^2}{\bar{p}_{...}(100 - \bar{p}_{...})}$$

where

$$\bar{p}_{...} = \frac{\sum_{i,j} p_{ij.}}{IJ}$$

The square root of f_R^2 is then compared to the f value defined by Miles (1963) in equation AG4, and which is used to develop ISTA tolerance tables for comparing germination results from different labs.

Results:

For this dataset, n = 400.

$\bar{p}_{...}$: 86.82

S_R: 2.53

f_R: 1.49

f: 1.66

The f_R value is lower than the f values, showing no higher variation among laboratories than the one established by Miles for the development of tolerance tables.

Conclusions

The statistical evaluation of this study shows that repeatability and reproducibility requirements are met.

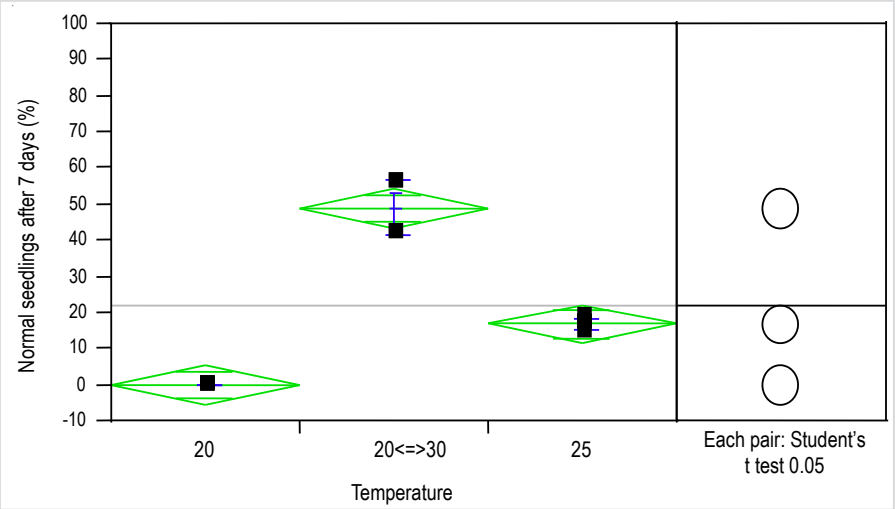
The results obtained in this validation test indicate that TP can be proposed as a suitable substrate, and 20<=>30 °C as an optimum temperature regime, for the germination of *Solanum nigrum*.

Acknowledgements

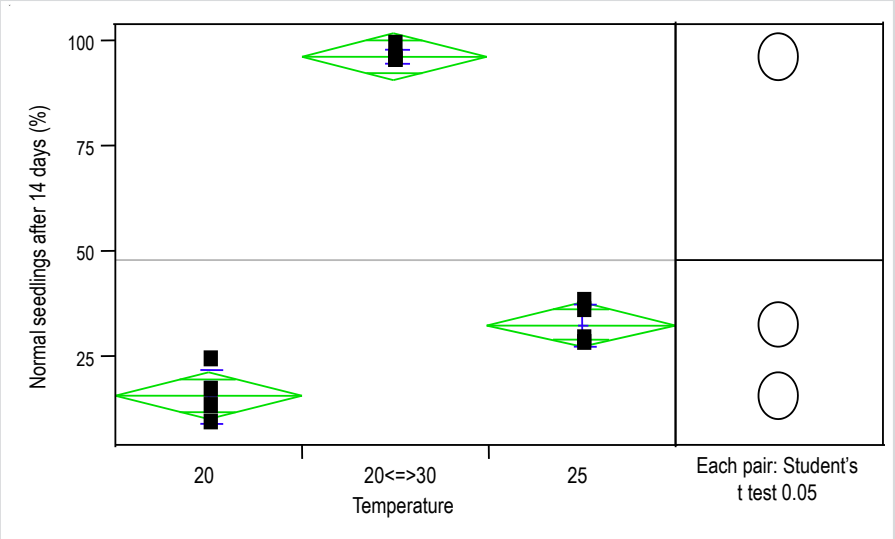
Sincere thanks to Joseph Ahenda (Kenya), who started and promoted the study, and to Mary Chipili (Zambia). Special thanks are due to Jean-Louis Laffont for the statistical analysis, and to Ronald Don and John Hampton for their support.

We are grateful to Sylvie Ducournau, Håkon Tangerås, Gillian McLaren, Gerarda de Boer-Raatgever and Karen Hill for performing the tests, and to Grethe Tarp and Joël Léchappé for their assessment of the test plan and their review of the validation report.

Appendix 1. Normal seedlings (%) at 20, 25 and 20<=>30 °C after 7 days



Appendix 2. Normal seedlings (%) at 20, 25 and 20<=>30 °C after 14 days



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Alternative embryo extraction procedure for detecting *Ustilago* mycelium

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Summary

For forty years, the Nordic laboratories have used a modification of ISTA seed health method 7-013. This Nordic method differs from 7-013 in the embryo extraction technique and the procedure used to clear embryos for examination for the *Ustilago* mycelium. A validation study comparing the two methods was carried out. Three seed lots with infection levels between 1% and 4% were tested by three laboratories using both the current 7-013 and the Nordic method. The validation study showed that the two methods produce equivalent results. Statistical analysis showed that the only significant differences were among samples. There were no differences between methods, laboratories or any interactions. The Nordic method offers an alternative method for laboratories that do not have access to plentiful warm water, nor a fume hood. The alternative embryo-clearing process adds a day to the duration of the test, so may not be suitable where a quicker turnaround is required. It does, however, offer an alternative clearing procedure which could be used in combination with the existing method to provide flexibility of resources within laboratories during busy periods. The data supports the inclusion of the Nordic method in the ISTA Rules as an equivalent method to the existing method 7-013.

Introduction

ISTA method 7-013 describes the current international method for the detection of *Ustilago nuda* in barley *(Hordeum vulgare)* seed. Since the early seventies, the Nordic countries have used a method described by Joelson, 1968. This method differs from 7-013 in the embryo extraction technique and the procedure used to clear embryos for examination for the *Ustilago* mycelium.

Joelson's method includes an additional dehulling step prior to embryo extraction. Following dehulling, the separation of the embryos from the endosperm is similar to 7-013 but with salt added to the sodium hydroxide solution, to help separate the lighter embryos, which float to the top of the solution from the endosperm. The Nordic laboratories adopted this method in preference to 7-013, as they considered it to be a more efficient process for producing good-quality embryos with a high extraction rate, and it did not require an expensive flotation device described in 7-013 (Ref.).

Early comparative tests conducted by the ISTA Plant Disease Committee (PDC) revealed difficulties with broken embryos encountered by some participants when using the Joelson method. Possible reasons included too concentrated a solution of sulfuric acid for dehulling, too long a soaking period, too high a temperature during the process, and mechanical damage (Anon., 1972). It was argued that the method provided consistent results when applied by experienced analysts, and in 1976 a comparative test showed that, as participating laboratories gained experience, there was no difference between results from the Joelson method and 7-013 (Anon., 1976).

Since the extraction method was first developed, various laboratories in Scandinavia have modified the procedure to overcome some of the earlier problems. For example, the temperature and concentration of the sodium hydroxide and sulfuric acid were altered, making the method more suitable for inexperienced laboratories to use successfully. The Nordic laboratories have carried out comparative tests for many years, with good agreement between the results. The ISTA Seed Health Committee's *Ustilago nuda* Working Group agreed that the Nordic method could be included as an alternative embryo extraction procedure. However, the SHC required that the various laboratories agree to procedures where small differences existed between methods before moving to a peer-validated comparative test. The Nordic laboratories agreed on one description and a peer-validated comparative test was agreed upon. Although the comparative test is a separate exercise to a proficiency test, on this occasion the SHC agreed to the use of additional samples from ISTA PT round 08-SH for the comparative test.

The aim of the peer-validated comparative test was to determine whether the two methods are equivalent.

Materials and methods

Seed lots

Three seed lots with infection levels of 4.3%, 1.6% and 1.2% were evaluated. These were the same seed lots used in PT round 08-SH, and were obtained from the test organizer. The organizer provided the Swedish laboratory with additional coded samples for distribution to the laboratories in Norway and Finland, with sample codes known only to the organizer. The participating laboratories each received nine samples of 120 g (three per lot) in addition to their PT samples. Thus, the three laboratories in Norway, Finland and Sweden each examined 18 samples, nine following method 7-013 (as part of the proficiency test) and nine following the Nordic method.

The laboratories were:

- Kimen Såvarelaboratoriet AS, Ås, Norway (contact: Barbro E. Isaksen, Dagny Stave-Larsen)
- Finnish Food Safety, Loimaa, Finland (contact: Hanna Ranta)
- Swedish Board of Agriculture, Seed Division, Svalöv, Sweden (contact: Karin Sperlingsson)

ISTA procedure

Samples were tested according to ISTA Method 7-013. From each sample, 1000 embryos were examined, and the number of infected embryos found per sample was recorded.

Nordic extraction procedure

Dehulling

Each sample was placed in a 600 mL beaker, and sulfuric acid of 25–37% (by weight) (Joelson, no date) was added until the seeds were covered. The seeds were then incubated at 75 °C for 50 minutes or until the seeds turned a medium-brown colour. The sulfuric acid was drained and water added to approximately two thirds of the volume of the beaker. Loosened hulls were removed by stirring the seeds with a glass rod and then being carefully poured away with the water. If not all hulls were removed, new water was added, and either a rod or an electric hand mixer was used to stir for approximately 3 minutes to loosen the remaining hulls. The procedure was repeated until no loose hulls were left.

Embryo removal

The kernels (seeds without hulls) were placed in a container with approximately 1 L of 10–15% sodium hydroxide solution with 130–175 g salt added per litre (NaOH-NaCl). The kernels were then incubated overnight at 22 ± 3 °C. After incubation, the mixture was stirred gently to release the loosened embryos from the dissolved kernels. The released embryos floated to the top of the container and were poured into a new beaker (600 mL). More NaOH-NaCl solution was added to the mixture and left for 5–20 minutes and stirred as before. This process was repeated until no further embryos were released. The required time depends on the variety and how often the mixture is stirred.

To ensure that no embryos remained, the dissolved kernels were placed on top of a sieve with an approximately 2.4 mm mesh (coarse enough to let the embryos pass but retain the remains of the kernels), and washed to allow collection of the remaining embryos in a fine sieve (1 mm mesh) below.

Any large quantity of chaff among the embryos was removed by filling the beaker with water and pouring off the floating chaff. All embryos were then washed in running water for approximately 10 s to rinse off the NaOH-NaCl solution.

Clearing the embryos

The water was drained and the embryos were collected in a fine sieve before being placed in a clean beaker and covered with lactic acid. The beaker was covered with a lid. The embryos were then incubated overnight in an incubator at 75 ± 5 °C.

After incubation, the lactic acid was drained using a fine sieve. Where the embryos were not transparent they were washed in ethanol using a fine sieve or placed in 95% ethanol for a few minutes. The embryos were then covered with a glycerine-ethanol solution (1:3) (Joelson, no date) or with a pure solution of glycerine, ready for examination.

Examination

1000 embryos were examined using a microscope at 16–25× magnification with substage illumination. The number of embryos examined and the number of infected embryos found per sample were recorded.

Statistical analysis

The data was analysed on Genstat Version 8 using a logistic regression (general linear model, binomial data, logistic link).

Results

The total numbers of infected embryos found are summarized in Table 1. Variation was greatest in sample 1.

The accumulated analysis of deviance (Table 2) shows that the sample factor is significant. Method and laboratory are not significant, and neither is the interaction (also Figure 1). The estimated dispersion value 2.27 is greater than that normally expected of binomial data (dispersion = 1). However, this is consistent with the over-dispersion seen in ISTA PT round 08-SH, where variability for U. nuda was

Table 1. Total number of infected embryos found by all laboratories per sample and method

Seed lot	Method 7-013	Nordic method
1	487	391
2	171	179
3	100	120
Grand total	758	690

Table 2. Accumulated analysis of deviance

Change	d.f.	Deviance	Mean deviance	Deviance ratio	Approx. F pr.
+ Sample	2	490.307	245.153	107.98	<0.001
+ Lab	2	7.394	3.697	1.63	0.210
+ Method	1	2.835	2.835	1.25	0.271
+ Sample-Lab	4	12.314	3.079	1.36	0.268
+ Sample-Method	2	10.607	5.304	2.34	0.111
+ Lab-Method	2	2.090	1.045	0.46	0.635
+ Sample-Lab-Method	4	10.804	2.701	1.19	0.332
Residual	36	81.730	2.270		
Total	53	618.081	11.662		

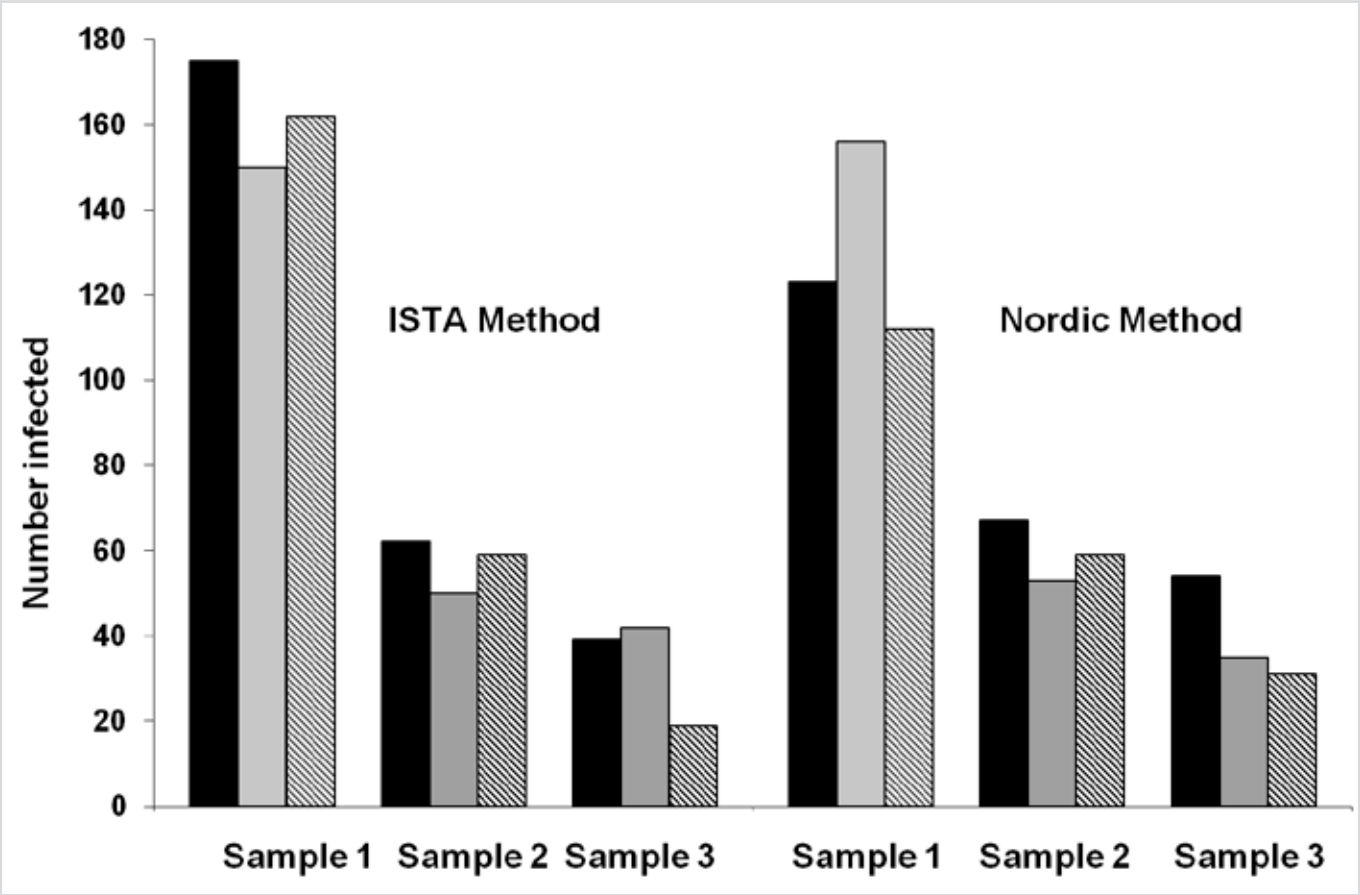

Figure 1. Total number of infected embryos found per sample, laboratory and method.

higher than expected (Cockerell and Roberts, 2010).

Discussion

The results confirm earlier comparative tests showing that the ISTA method and the Nordic method do not differ in the number of infected embryos found. Although the analysis shows an over-dispersion, this is consistent with the over-dispersion seen in the ISTA proficiency test, where the variability for *U. nuda* was higher than expected in the seed lots (Cockerell and Roberts, 2011).

The Nordic method differs from the current method in two main areas. Firstly, the extraction of the embryos involves an additional step to de-hull the seed, followed by separation of the embryos from the endosperm in a solution of sodium hydroxide and salt. The loosened embryos float to the top of the solution and are collected. Secondly, in order to clear the tissues, the embryos are not boiled in a lactic acid solution, but are incubated overnight in lactic acid at 75 °C.

Extraction and cleaning of embryo samples

The Nordic method provides an alternative method for extracting and cleaning the embryo samples by introducing an additional step to remove the hulls prior to soaking in a solution of sodium hydroxide. Compared to method 7-013, the potential advantage of this additional step before the soak is that it does not require warm water and reduces the amount of water needed to aid separation of the embryos. In method 7-013, warm water is an important factor for good extraction. Past data (Rennie, 1984) states that extraction rates can differ from 40 to 80%, depending on the sample, the temperature during soaking and the temperature of the water used to wash the sample. The Nordic method can be useful for laboratories where large amounts of warm water are not readily available.

The amount of time spent cleaning the embryo sample may be reduced, since most of the hulls are removed at an early stage, reducing the problem of hulls among the

extracted embryos. The time spent on cleaning embryo samples will depend on the seed sample and the experience of the analyst; this applies to both methods equally.

Both methods use specific gravity to separate the embryos from the chaff and endosperm. In method 7-013, the extracted embryos are added to a mixture of water and glycerol; the embryos then float to the top and are collected. With the Nordic method, the kernels are incubated in a solution of sodium hydroxide and salt. The free embryos float to the top and are collected.

The size of the embryos that are retrieved can be affected by the salt concentration. When this is low, it is a little more difficult to release the loosened embryos, but the embryos are larger. Higher concentrations make release of the embryos easier, but the embryos shrink somewhat. The salt concentration does not affect the result of the analysis.

With regard to the concentrations of sulfuric acid, sodium hydroxide and salt, during the test both low and high concentrations were used by various laboratories. There was no effect on the result.

Neither is the result affected by the interval between adding further sodium hydroxide and stirring the embryos to loosen them during extraction. Some laboratories do other work in between and leave the embryos during that time.

The Nordic method can achieve 98% embryo recovery (Joelson, 1968). For both methods, embryo release can be affected by the cultivar and the field conditions prior to harvest.

With the Nordic method, less than 1% of the embryos are collected in the sieve. The remainder float to the surface and are poured from the container, rather than being collected in a series of sieves. The Nordic laboratories consider that the risk of embryos being damaged is smaller using this method.

Clearing embryos

In method 7-013, the embryos are cleared by boiling the embryos in a solution of lactic acid and glycerol in a fume hood. In the Nordic method, a fume hood is not required, since embryos are cleared using concentrated lactic acid and overnight incubation in an oven at 75 °C. For laboratories that only need to test few samples, and do not want to go to the expense of purchasing a fume hood, the Nordic method offers a suitable alternative.

The disadvantage of this method is that it extends the duration of the test, which may not suit all customers.

The examination of the embryos requires substage illumination and a magnification of 16–25×. Embryos can be examined in either glycerol or a mixture of glycerol and ethanol (3:1).

Conclusion and recommendation

The Nordic method provides equivalent results to the present ISTA method 7-013. The method of extraction allows laboratories to test for *U. nuda* in barley when a fume hood is not available. It is also an alternative where warm water is not freely available.

It provides for an additional method for clearing the embryos which could also be used with the current ISTA method, when results are not required within 24 hours of receipt of the sample. This would free up staff resources.

It is recommended that an equivalent method for detection of *Ustilago nuda* in *Hordeum vulgare* is introduced to the Annexe to Chapter 7 as Method 7-013b.

Acknowledgements

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I also wish to thank the staff at Kimen Såvarelaboratoriet AS, Norway, the Finnish Food Safety Authority Evira, and the staff at the Swedish Board of Agriculture, Seed Division, Svalöv, Sweden.

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The osmotic method for detection of *Pyrenophora teres* and *P. graminea* on *Hordeum vulgare*

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Summary

The osmotic method has been used for many years in Norway and Sweden as a routine method for detection of *Pyrenophora teres* (anamorph *Drechslera teres*) and *P. graminea* (anamorph *D. graminea*) on barley. The method is based on the ability of *Pyrenophora* spp. to produce red pigments. However, it cannot distinguish between *P. teres* and *P. graminea*, because they produce the same pigment. A validation study was carried out with the aim to provide the necessary documentation for including the method in the International Rules for Seed Testing (ISTA Rules). Seven laboratories participated, and each tested 3 × 300 seeds from three barley seed lots. Analyses of the results demonstrate that the method provides sufficient repeatability, and that there are no particular problems with this test at the laboratory level. Furthermore, in previous studies with the osmotic method, organized by a Nordic working group, it was shown that the osmotic method easily gives reproducible results for *Pyrenophora teres*/*P. graminea* in barley when used by experienced laboratories. Moreover, the osmotic method is well suited for routine analyses, because it is quick and easy to carry out. The study showed that, if used correctly and with proper equipment, the osmotic method for detection of *Pyrenophora teres*/*P. graminea* is easy to perform, and shows good conformity between laboratories.

Introduction

There is no method for detection of *Pyrenophora teres* Drechsler (anamorph: *Drechslera teres* (Sacc.) Shoem.) or *P. graminea* Ito & Kurib., in Ito (anamorph: *D. graminea* (Rabenh. ex Schlecht.) Shoem.) in barley seed in the ISTA Rules (ISTA, 2010). The most common methods used in seed health testing laboratories for detection of these pathogens are agar plate methods, such as potato dextrose agar (PDA) or malt agar (MA), and the freezing blotter method published by ISTA (1964a) in S. 3 No. 6 (barley leaf stripe), revised as Working Sheet No. 6 (2nd ed.) in 1984 (Rennie and Tomlin, 1984), and Working Sheet S. 3. No. 7 (barley net blotch) (ISTA, 1964b). However, these methods are both time-consuming and laborious. A cheap and less laborious method, the osmotic method, was developed by Joelson in Sweden for rapid detection of *Pyrenophora* spp. in cereal seeds (Svensson, 1981; Joelson, 1983). With this method, seeds are incubated on filter paper moistened by a sugar solution. The osmotic pressure from the sugar inhibits the germination of the kernels. The osmotic method is based on the ability of *Pyrenophora* spp. to produce brick-red pigments (anthraquinones) by incubation of seeds under certain conditions (Braverman, 1960; Kietreiber, 1977; Knudsen, 1982). However, the method cannot distinguish between *P. teres* and *P. graminea*, because they produce the same pigment – catenarin (Engström *et al.*, 1993). The pigments turn from brick-red to violet when NaOH is added. Some saprophytes produce pigments, but not of the same colour, and will not react in the same way when NaOH is added. The osmotic method has been used in routine seed health testing of barley in Sweden and Norway for many years (Brodal, 1993).

Previous studies of the osmotic method and comparisons with agar plate and freezing blotter methods

In 1990, 1992 and 1993, a Nordic working group on seed pathology organized meetings and comparative tests to harmonize procedures and performance of the osmotic method for detection of *P. graminea* and *P. teres* in barley seed (Brodal *et al.*, 1994). During these years samples from 57 barley seed lots (range of infection frequencies 0–87% infected seeds) were analyzed in six laboratories in three separate testing series. Correlation coefficients between the results obtained with the osmotic method in the laboratories varied between 0.75 and 0.97 (all significant at the 0.001% level). Results from Swedish and Norwegian laboratories, where the method had been widely used, were best correlated. The same samples were tested with the freezing blotter method. Results from the freezing blotter method correlated well with results from the osmotic method, with correlation coefficients between 0.83 and 0.92 (Brodal, 1995).

Another comparative test of the osmotic method was organized by a sub-working group of the Plant Disease Committee of ISTA in 1994–1995 (Brodal, 1997). Samples from ten barley seed lots (range of infection frequencies 3–90% infected seeds) were tested in 13 laboratories. Four of the participating laboratories had long experience with the osmotic method, whereas other labs were using the method for the first time. The frequencies of *P. teres* and *P. graminea* recorded with the osmotic method were well correlated ($r > 0.90$) between most of the participating laboratories. In addition to the osmotic method, some of the laboratories carried out the freezing blotter and/or the agar plate (PDA) method on the same set of seed samples. Since a high level of agreement was found between the osmotic blotter results from the four experienced laboratories, the average results

obtained in these laboratories were used for comparisons with the other methods. The results from the osmotic method were highly correlated to the results from the freezing blotter method and the agar plate method. However, approximately 50% higher infection frequencies were recorded with the osmotic than with the freezing blotter method, and approximately 20% higher frequencies were recorded with the osmotic method than with the agar plate (PDA) method. This indicates a higher sensitivity of the osmotic method than that of the other methods. The reason might be that with the osmotic method, small pigment spots can be observed from slightly infected seeds, but the low inoculum amount might not be enough to produce any conidia in the freezing blotter method or mycelium on agar, or that slight or superficial inocula might have been removed by pretreatment with NaOCl.

In these studies, the time needed to carry out the various methods was not measured. However, the osmotic method was considered to be less time consuming than the other methods. The main reason was the quick examination based on pigment spots.

From the Nordic and the ISTA-PDC comparative studies, it was concluded that the osmotic method easily gives reproducible results for *Pyrenophora teres* and *P. graminea* in barley when used by experienced laboratories. Moreover, the osmotic method is well suited for routine analyses, because it is quick and easy to carry out with no need for a microscope, preparation of agar media or sterile facilities.

In Sweden and Norway, thousands of barley samples have been tested annually with the osmotic method, to assess the need for seed treatment against *Pyrenophora teres* and *P. graminea*. In order to obtain ISTA accreditation for such tests, it is necessary that this method is included in the ISTA Rules. A final comparative test was organized in 2007 with the aim to provide the necessary documentation (validation) of the method. Seven seed health testing laboratories from Estonia, Finland, Norway and Sweden participated in this test. The repeatability and reproducibility of the osmotic method were evaluated. Results from this validation study are presented below.

Materials and methods

Seed samples

Based on the infection levels determined by two laboratories of the Swedish Board of Agriculture (JV) in Svalöv and Landskrona, three naturally infected lots of barley seed (infection frequencies 6–25% infected seeds) were selected for the validation test. Each seed lot was sampled and divided into three subsamples of 300 seeds. Subsamples were coded randomly and sent to participating laboratories as a blind comparative test.

The osmotic method

The method was, with slight modifications, carried out as described by Joelson (1983). Before plating, seed samples were pretreated by heat treatment in an oven at 90 °C for 2 hours in thin layers in open paper trays or dishes, to reduce the occurrence of saprophytes (Fig. 1). Seeds were placed on filter paper (Munktell quality 1731, 400 g/m^2) moistened by a sugar solution (0.5 M sucrose), made from 170 g sugar per litre of water. The sugar (sucrose) was ordinary table sugar, as used for human consumption. The paper was soaked quickly in the sugar solution and surplus solution drained off. The osmotic pressure from the sugar solution inhibits the germination of the kernels. Development of roots and coleoptiles results in poor contact between seed and filter paper, so to keep the seeds in the correct position and to provide good contact between the seeds and the paper, the seeds were plated in wells or indentations in the paper, which were impressed into the paper after it was soaked in the sugar solution. Samples were incubated in transparent plastic dishes for 7 days with alternating photoperiods of 16 hours at 26 ± 2 °C in strong white light (5000–6000 lux) and 8 hours at 22 ± 2 °C in darkness. These incubation conditions

Figure 1. Seeds prepared for pretreatment at 90 °C for two hours.

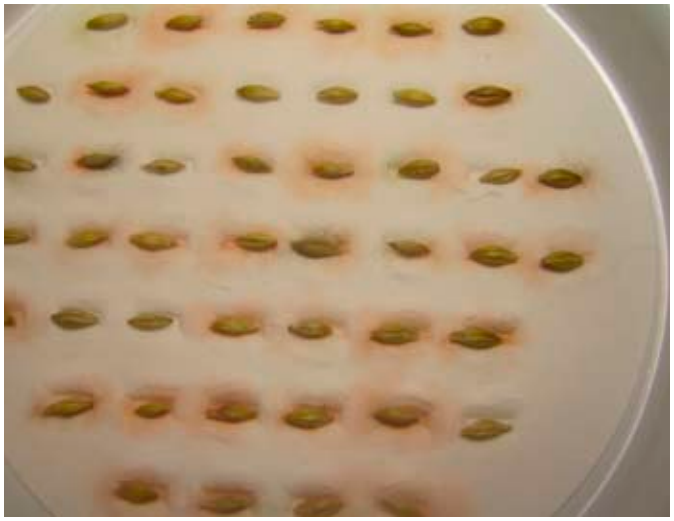

Figure 2. A dish after seed incubation

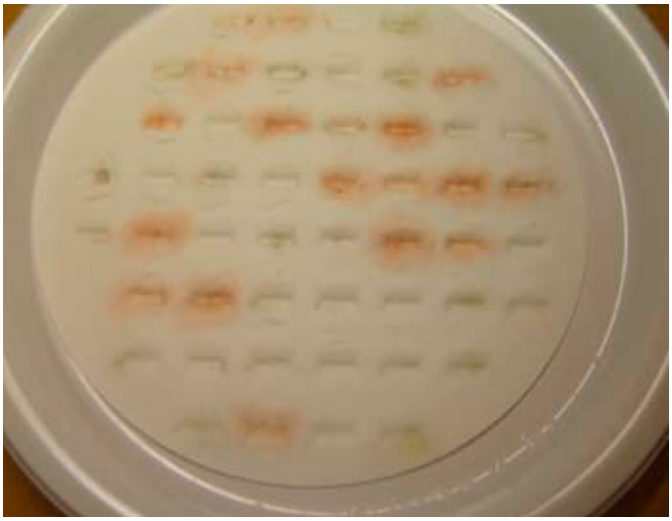

Figure 3. A dish after removal of incubated seeds, before adding the NaOH solution.

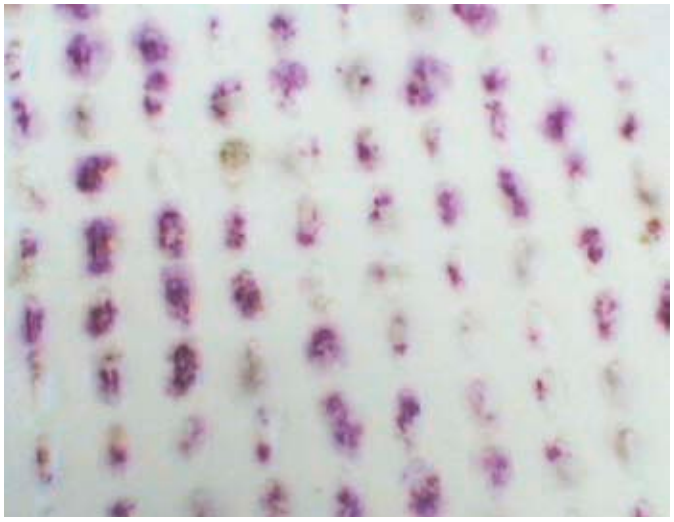

Figure 4. Blotter after addition of the NaOH solution. There are visible differences in spot size.

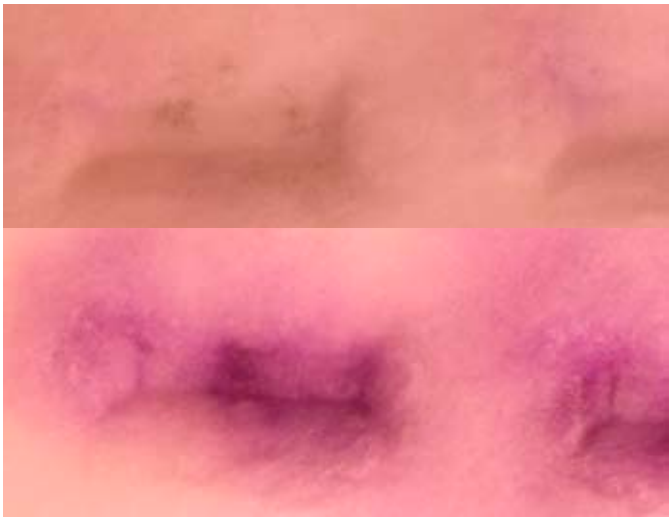

Figure 5. Enlargement of spots visible on the blotters after addition of the NaOH solution. Above: faint spots, not to be recorded; below: normal spots, to be recorded.

inhibit the development of conidia and mycelium, and favour the development of brick-red pigment spots on the paper. These turn violet after a weak solution of NaOH is added.

Two of the participating laboratories did not use the osmotic method routinely, and therefore had no equipment for forming wells in the paper. As it was essential for all laboratories to use filter paper with wells, these two laboratories were sent prepared filter papers together with the seed samples. The filter papers were first dipped in water, the wells were formed and the papers were dried. It only remained for these laboratories to dip the filter papers in the sugar solution. These laboratories were also sent transparent plastic incubation dishes.

For examination, the seeds were removed, and a solution of 1% NaOH was poured onto the paper (Figs. 2 and 3). The colour of the pigment spots then immediately changed to violet, which made the spots easier to discern and count. The sizes of the spots usually varied from 1–2 mm up to 1–2 cm in diameter (Fig. 4). Very faint spots were not recorded (Fig. 5). The wells in the paper made it easy to see exactly where the seeds had been placed.

Statistical analysis

Statistical analysis was conducted by Jean-Louis Laffont of the ISTA Statistics Committee. Generalized Linear Mixed Models (GLMM) were used to assess the effects of the different factors and to estimate repeatability and reproducibility variances. The details of the analyses performed are included in the Appendix.

Results and discussion

The results of the test are shown in Figures 6–8.

All the participating laboratories had some experience in using the osmotic method, and five of the seven labs used the method frequently. Some of the laboratories participating in earlier ring tests had little experience in using the method.

There is a tendency for some laboratories to have higher or lower results than the majority, but in the statistical analysis, no crossover interaction between laboratory

and by was detected, meaning that the lot ranking is consistent across laboratories.

Lab 5 tested under unusual conditions: at the time of the test, it merged with another laboratory, and was closed down. Therefore, in order to participate in the test, a temporary installation was arranged. This could be the reason for their low result.

Lab 4 did not use the osmotic method routinely.

Lab 3 tested under ultraviolet (UV) light, as described by Joelson (unpublished) and Nylund (1990). When UV light is applied, it must have a wavelength of 366 or 254 nm, and the plates must be analysed before being sprayed with the NaOH solution. Since the majority of laboratories do not use UV light, and since it must be used only with proper safety precautions, this will not be recommended in the ISTA method.

Statistical analysis found a strong lot effect, a strong laboratory effect and a significant lot × laboratory interaction effect (Appendix). This interaction effect has limited consequences on the ability of the laboratories in ranking the lots consistently, since no crossover interaction effect was detected. The standard deviations of repeatability, computed for three levels of infection, are close to the binomial standard deviation, thus showing limited over-dispersion within laboratories. The standard-deviations of reproducibility were computed for information only, since there was no reference to which they could be compared.

The use of proper equipment, especially the light source, is critical (Joelson, unpublished). The white light must be at least 4000 lux. The amount of light is most important during the first three to four days of incubation (Joelson, unpublished).

It is essential that filter papers with wells are used. There are some advantages with this procedure compared with placing the seeds on flat paper. First, there is better contact between the moistened filter paper and the seeds; second, the seeds remain in the correct position, and it is easy to ascertain their exact position after incubation (Brodal, 1997; Nylund, 1990).

Water quality is not as important, since the sugar solution is highly concentrated (Joelson, unpublished). There was no advantage in using a buffer solution (Joelson, unpublished).

During incubation, the illumination causes temperature fluctuations; therefore, the incubation room or cabinet must be equipped with a temperature controlling system. During the dark period, the temperature should be 22 °C, and during the light period 25–27 °C (Joelson, unpublished).

The size of the pigment spots on the paper can vary, ranging from a diameter of 10–20 mm to a small spot under the seed (Fig. 4). This variation is probably due to the amount of inoculum on the individual seed.

The main advantage of the osmotic method is that it requires a low input of manpower compared with other methods. The method is less laborious than the freezing blotter and agar plate methods, and is well suited to routine testing.

The osmotic method cannot distinguish between *Pyrenophora teres* and *P. graminea*.

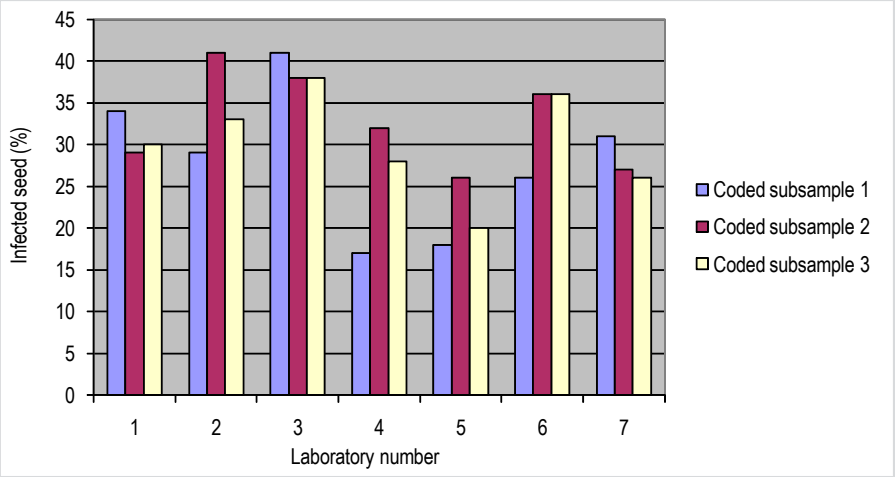

Figure 6. Incidence of *Pyrenophora* spp. in seed lot No. 1 as detected by the osmotic method.

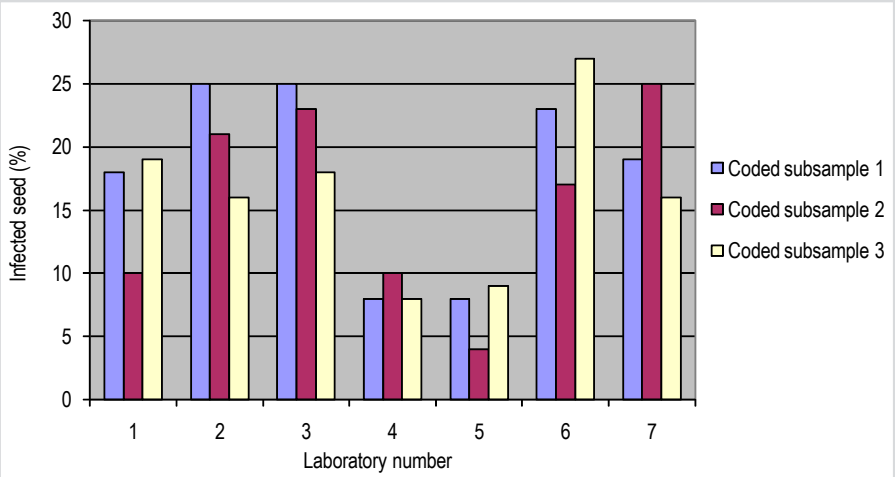

Figure 7. Incidence of *Pyrenophora* spp. in seed lot No. 2 as detected by the osmotic method.

Only pure seed should be used for analysis. Seed not graded before being sent to laboratory must be graded to the same quality as certified seed.

Detection of *Pyrenophora* spp. after seed treatment

The method has not been validated for treated seed. It must only be used for untreated seed.

Conclusions and recommendations

The study showed that when applied correctly and with proper equipment, the osmotic method for detection of *Pyrenophora teres* or *P. graminea* is easy to perform, and shows good conformity between laboratories. It is therefore recommended to include the osmotic method in ISTA Rules as a routine test for *Pyrenophora teres* and *P. graminea*, when it is not necessary to distinguish the two species.

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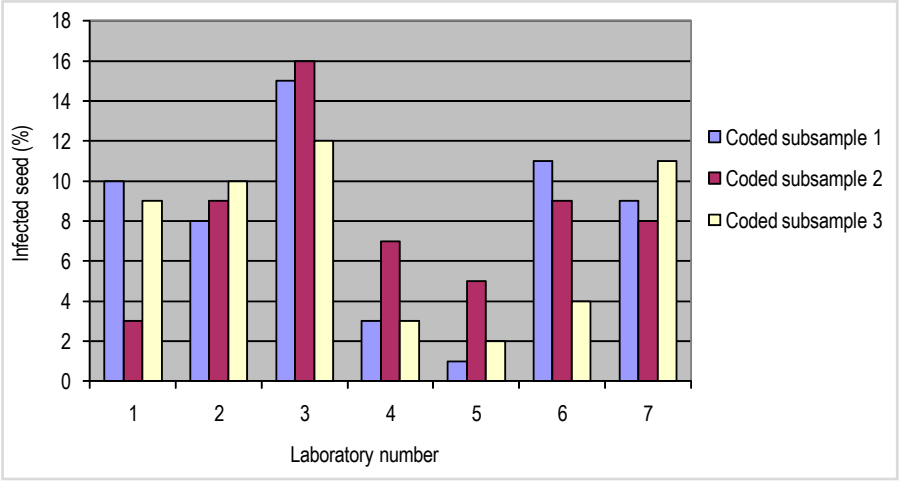

Figure 8. Incidence of *Pyrenophora* spp. in seed lot No. 3 as detected by the osmotic method.

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Early counts of radicle emergence during germination as a repeatable and reproducible vigour test for maize

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Summary

In an international comparison of six commercial seed lots of maize, single counts of radicle emergence (RE; defined as production of a 2 mm radicle) after 6 d at 13 °C (5 laboratories) and 66 h at 20 °C (6 laboratories) consistently ranked the lots in the same order in each of two runs in each laboratory. The two fastest germinating lots gave significantly higher RE counts than the lowest two lots in all but one run in one laboratory over the two temperatures. Absolute RE values showed some variability between laboratories, and although there was an interaction between labs and lots, this did not affect the ranking and differentiation of the lots. The repeatability and reproducibility of the data was comparable with that for previous vigour tests, except for one lot with the lowest vigour, for which the reproducibility between laboratories was higher. The RE counts in Aberdeen at 13 °C and 20 °C for nine lots (including the six for the comparative test) were significantly related to field emergence (%) determined in Iran, slower germinating lots (low RE) giving lower emergence. We argue that early counts of radicle emergence during germination provide a faster, more clearly discerning vigour test than the cold test, and that the method described in this document provides a repeatable and reproducible method.

Introduction

The cold test for maize is the most used of all vigour tests (TeKrony, 2001). There is much evidence, most recently from Italy using commercial seed lots, that counts of normal seedlings after the cold test is highly significantly related to field emergence in cold soils (Lovato and Balboni, 1997; Noli *et al.*, 2008). However, the test is difficult to standardise (Nijenstein and Kruse, 2000).

An alternative vigour test for maize has been suggested (Matthews and Khajeh-Hosseini, 2006, 2007) in which radicle emergence at 13 °C and 20 °C is measured. In the work on rate of germination, two measurements were used: mean germination time (MGT) based on frequent, often daily counts, and single early counts of radicle emergence.

MGT has given consistent rankings of lots in experimental runs in three laboratories that were indicative of field performance (Khajeh-Hosseini *et al.*, 2009). In later work on six commercially available seed lots, comparisons were made in two runs at 13 °C in four laboratories of both MGT and single early counts of radicle emergence (Matthews *et al.*, 2010). Both measurements were consistent in their ranking of the lots (Matthews *et al.*, 2010). A 6 d count of radicle emergence at 13 °C gave the same ranking in two experimental runs in each of the four laboratories, and was significantly related to the cold test results (normal germination) in all experimental runs.

In this paper we present evidence in support of early counts of radicle emergence as a vigour test from a further comparative test at 13 °C and 20 °C on six seed lots of maize in six laboratories.

Materials and methods

Selection of seed lots

Initial assessments were made in late 2007 from stocks of F_1 hybrids held in store at the Pioneer Hi-Bred laboratory in Parndorf, Austria. Nine lots were selected for further tests in Aberdeen and Mashhad, Iran (Table 1). All lots had standard germinations ranging from 90 to 99%, cold test germinations (normal seedlings) from 76 to 96% and similar moisture contents (Table 1).

Further tests on these nine lots of the timing of radicle emergence (RE) at 13 and 20 °C in Aberdeen and on field emergence in Iran were conducted in early 2008 (Appendix I, Table A). On the basis of these

Table 1. Initial assessments of seed quality of the nine seed lots of F_1 hybrid maize from which six were selected (highlighted lots) for further comparative tests. All tests were conducted by the Pioneer Hi-Bred laboratory.

Seed lot code number	Standard germination (%)		Cold test germination (%)		Moisture content (%)
	Normal	Abnormal	Normal	Abnormal	
7	96	3	96	3	13.5
8	96	4	97	3	13.8
1	98	1	90	6	13.9
2	94	5	94	3	13.2
3	92	4	76	10	13.3
9	93	4	88	5	13.9
4	90	7	94	3	12.9
5	96	3	82	10	13.6
6	99	1	96	3	13.5

tests and the initial assessments, six lots were selected having at least 90% germination in the standard germination test, and which exhibited a range of performance in field and radicle emergence (Table1; Table A, Appendix I).

Participating laboratories

Five laboratories conducted comparisons of radicle emergence at 13 °C. These were: University of Aberdeen, Department of Crop Science; Ferdowsi University of Mashhad, Iran; LaRAS, University of Bologna, Italy; SNES, GEVES, Angers, France; Pioneer Hi-Bred Services GmbH, Parndorf, Austria.

Comparisons of the radicle emergence at 20 °C included a further laboratory, namely the Department of Seed Testing, Austrian Agency for Health and Food Safety GmbH, Vienna.

The six selected seed lots, highlighted in Table 1 and numbered from 1 to 6, were sent out to all participating laboratories by the Pioneer Hi-Bred laboratory in Austria in April 2008. Recipients were asked to store the seed in a moisture-proof container at a low temperature prior to use.

Standard germination and cold tests

Standard germination tests were conducted on 400 seeds at 25 °C according to the ISTA Rules (ISTA, 2008) and the cold test was that routinely in use at the Parndorf laboratory of Pioneer Hi-Bred. This test placed 4 replicates of 100 seeds on top of a water-saturated Barden clay/sand mixture held in trays, with the embryo placed down to increase anaerobic stress. Seeds were held in the dark for 4 d at 10 °C, then transferred to 25 °C in the dark for 3 d, after which normal seedlings (ISTA 2009) were counted (Matthews *et al.*, 2010).

Radicle emergence test

Two experimental runs were carried out at each of two temperatures using 4 replicates of 25 seeds in each, i.e.:

At 20 °C for each lot:
4 × 25 seeds run 1 = 100 seeds
4 × 25 seeds run 2 = 100 seeds
At 13 °C for each lot:
4 × 25 seeds run 1 = 100 seeds
4 × 25 seeds run 2 = 100 seeds

Substrate

A rolled towel method was used for germination, accepting that small differences in protocol and/or materials would occur between laboratories.

The maize seeds were placed on the papers with the embryo radicle pointing to the bottom of the paper. Two rows of seeds were suggested to assist counting, one of 12 and one of 13 seeds. Towels were held in moisture-proof bags or containers to prevent them drying out.

Experimental design

A randomised block design of 4 blocks of 6 lots was suggested as the experimental design; otherwise it should be completely randomised.

Temperature

Temperature was stressed as the most important potential variable between laboratories and runs, and within runs. Monitoring of temperature was suggested to be desirable, and daily rotation of blocks was advised. Participant laboratories were also advised to keep blocks close together in the incubation room (e.g. on the same shelf).

Counting germination

The criterion for germination was radicle emergence (RE), which for this test is defined as the production of a 2 mm radicle. If the radicle was not obvious, or if the observer had to examine the seed closely to see the radicle, it was not counted as germinated. A clear and obvious radicle was considered a quicker and more uniform method of germination assessment. A length of 2 mm, judged by eye, was the target for germination. A guide marked on a strip of graph paper was suggested as a useful aid to determine whether the minimum length of 2 mm had been reached.

Timing of radicle emergence counts

Radicle emergence counts were completed after:

- 66 h at 20 °C: As a guide to achieve these counts at a reasonable time of day, participants were advised to set the test up at 16.00 hours for counts at 10.00 hours.
- 6 days at 13 °C: Set up at a time of day convenient for making the subsequent counts.

Statistical analysis

The effect of the various factors (laboratory, seed lot, test run) were analysed by analysis of variance, and the LSD values calculated for $p < 0.05$.

Repeatability and reproducibility were analysed with the statistical tool developed by S. Grégoire according to ISO 5725-2 and available for download at the ISTA website:

http://www.seedtest.org/upload/cms/user/ISO572511.zip

Results

Comparison of runs and laboratories (ANOVA)

13 °C

The ranking of the lots was largely consistent, with only a few instances where the ranking in the experimental runs was different to that of the overall means (Table 2). Lots 3 and 5 had significantly lower RE counts than the top two lots (6 and 1) in both runs of the test in all laboratories.

The ANOVA (appendix II, Table B) showed a highly significant effect of lots and laboratories. There was a significant interaction between lot and laboratory, which can be seen in Table 2, where lab A with the lowest mean overall RE showed the greatest range in RE, while lab D with the highest overall mean had the smallest range.

20 °C

The ranking of the lots was almost perfectly consistent for all runs (Table 3), even though there were differences in the percentages of RE, particularly between laboratories. The RE of seed lot 5 was consistently lower than that for the other lots, and the REs of the top two lots (6 and 1) were significantly higher than the bottom two, with only one exception (run 2, lab D).

The ANOVA (Appendix II, Table C) showed a highly significant effect of lot and laboratory. There was also a highly significant interaction between lot and laboratory. This can be seen in Table 3, where differences in the RE between lots were smaller in laboratories D and E than in the other four laboratories. Nevertheless, the ranking of the lots in these two laboratories remained the same as in the other experimental runs.

Table 2. Seed quality evaluation of six seed lots of maize. Radicle emergence (%) assessed after 6 d at 13 °C in two runs in five laboratories; each mean is derived from 4 replicates of 25 seeds. Cold test assessed in the laboratory of Pioneer Hi-Bred; field emergence assessed in Iran. The order of the lots (1–6) is that of the overall mean, with the highest germinating lot at the top.

Seed lot	Laboratory A		Laboratory B		Laboratory C		Laboratory D		Laboratory E		Overall mean	Cold test (%)	Field emergence (%)
	1	2	1	2	1	2	1	2	1	2			
6	94a	95a	90a	89a	98a	97a	98a	99a	98a	98a	95a	96	92
	1	**1**	**1**	**2=**	**1**	**1**	**1**	**1**	**1**	**1**	**1**		
1	90a	94a	86a	89a	89ab	88ab	92a	91b	90a	97a	91a	90	90
	2	**2**	**2**	**2=**	**3**	**3**	**3=**	**4**	**2**	**2**	**2**		
4	89a	90a	78a	78a	90ab	93a	93a	96ab	91a	92a	89a	94	82
	3	**3**	**4**	**4**	**2**	**2**	**2**	**2**	**3**	**3**	**3**		
2	52b	59b	81a	89a	66bc	78b	92a	94ab	79a	74b	76b	94	78
	5	**4**	**3**	**1**	**4**	**4**	**3=**	**3**	**4**	**4=**	**4**		
3	56b	57b	59b	55b	56c	58c	60b	72c	64a	74b	61c	76	84
	4	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**4=**	**5**		
5	1c	3c	13c	48b	21d	10d	27c	36d	8b	28c	20d	82	78
	6	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**		
Mean	65c		71b		70b		79a		74a				

Any two means without a letter in common are significantly different ($p<0.05$) as determined by LSD following analysis of variance.

Table 3. Radicle emergence (%) of six lots of maize after 66 h at 20 °C in two runs in six laboratories. Each mean is derived from 4 replicates of 25 seeds. The order of the lots (1–6) is that of the overall mean, with the highest germinating lot at the top.

Seed lot	Laboratory A		Laboratory B		Laboratory C		Laboratory D		Laboratory E		Laboratory F		Overall mean
	1	2	1	2	1	2	1	2	1	2	1	2	
6	95a	97a	93a	94a	98a	100a	100a	99a	98a	99a	99a	100a	95a
	1	**1**	**1**	**1=**	**1**	**1**	**1**	**1=**	**1**	**1**	**1**	**1**	**1**
1	92a	96a	90a	94a	90a	94ab	97a	99a	96a	98a	94a	94a	91a
	2	**2**	**2**	**1=**	**2**	**2**	**2=**	**1=**	**2**	**2**	**2**	**2**	**2**
4	90a	94a	88a	90a	89a	88b	97a	94a	93a	92ab	93a	91a	89a
	3	**3**	**3**	**3**	**3**	**3**	**2=**	**3**	**3**	**3**	**3**	**3**	**3**
2	65b	72b	84a	74b	67b	66c	95a	93a	91a	91ab	89a	82b	76b
	4	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
3	62b	64b	–	–	65b	62c	78b	91a	71b	83b	59b	65c	61c
	5	**5**	**–**	**–**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
5	29c	32c	31c	24c	38c	41d	76b	88a	61b	77b	28c	16d	20d
	6	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**
Mean	74c		76c		75c		92a		88b		76c		

Any two means without a letter in common are significantly different ($p<0.05$) as determined by LSD following analysis of variance.

Reproducibility and repeatability analysis

The statistical tool developed by S. Grégoire, based on ISO 5725-2, allows the calculation of h and k values. The h values show the tendency for a laboratory to give overestimations or underestimations compared to the mean of all the results available, whereas the k values give a measure of the variability of the repeats. Higher values indicate greater under- or overestimations (h values) or greater variability between replicates (k values).

13 °C

There was a significant underestimation (h values) of radicle emergence only in laboratory B for two lots (lots 4 and 6) in both runs (Figs. 1a, b).

Significant variability between replicates (k values) was observed in run 1 (Fig. 2a) for single lots in labs A (lot 6) and B (lot

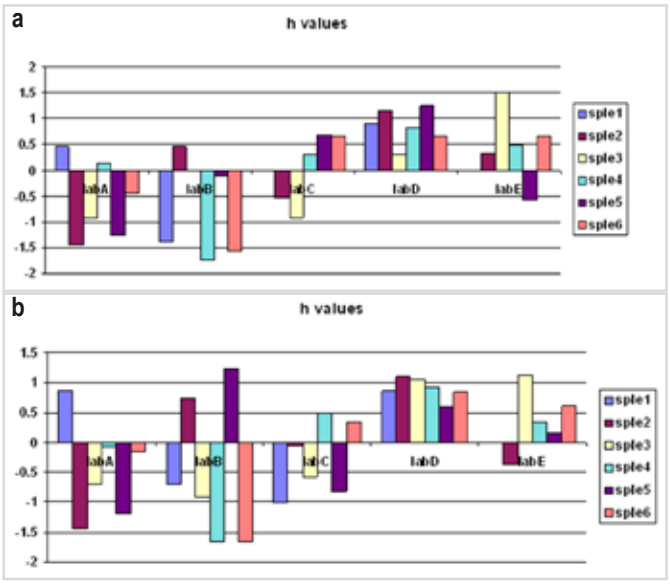

Figure 1a, b. h values for the radicle emergence of six lots of maize assessed at 13 °C in five laboratories in each of two runs.

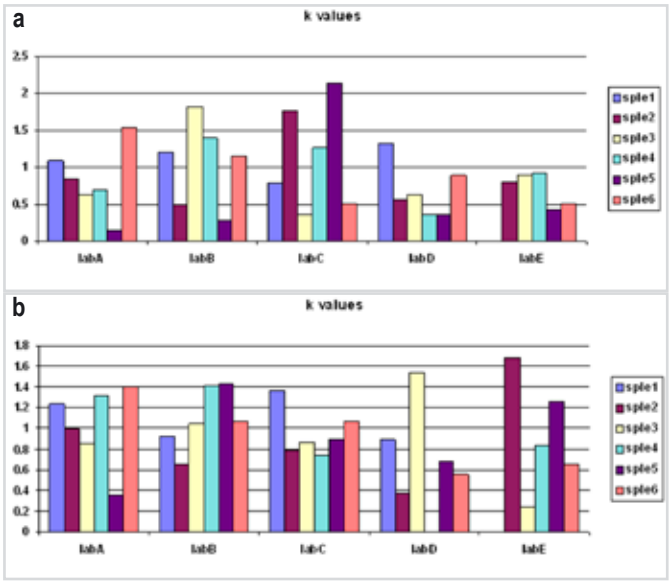

Figure 2a, b. k values for the radicle emergence of six lots of maize assessed at 13 °C in five laboratories in each of two runs.

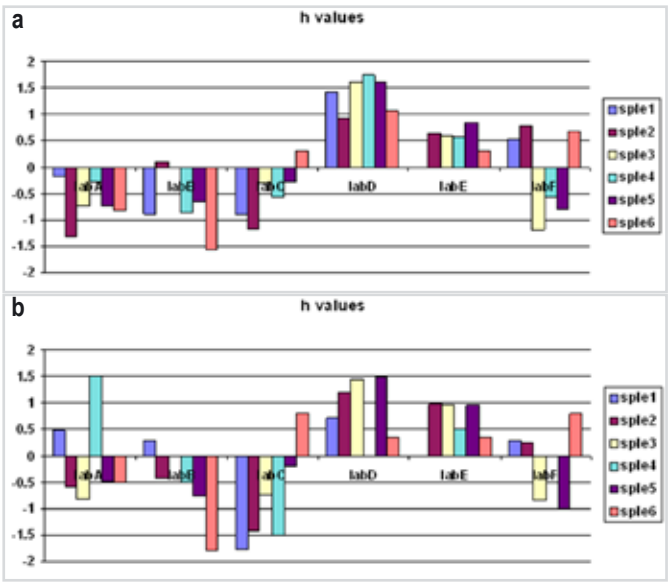

Figure 3a, b. h values for the radicle emergence of six lots of maize assessed at 20 °C in six laboratories in each of two runs.

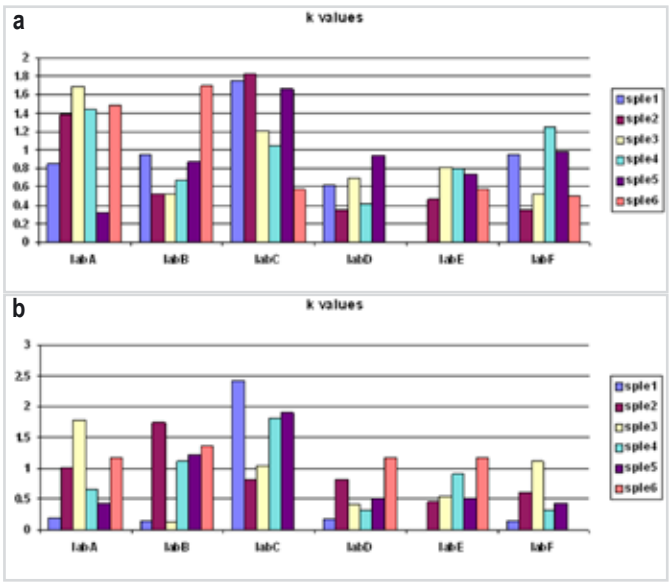

Figure 4a, b. k values for the radicle emergence of six lots of maize assessed at 20 °C in six laboratories in each of two runs.

3) and for two lots in lab C (lots 2 and 5). In run 2 (Fig. 2b) significant k values occurred for single lots in labs D (lot 3) and E (lot 2).

20 °C

The radicle emergence of only one lot (lot 4, lab D) was significantly overestimated in run 1 (Fig. 3a), while in run 2 the radicle emergence of two lots (lot 6, lab B; lot 1, lab C) were underestimated.

Variability between replicates was notable only in lab C, where three lots showed significant variability in both runs. There was also significant variability between replicates in single lots in labs 1 and 2 in both runs (Figs. 4a, b).

Values for repeatability and reproducibility were within similar ranges at both temperatures (Table 4), indicating that neither temperature gave more, or less, consistent results.

Relationships with cold test germination and field emergence

Radicle emergence at both 13 and 20 °C was significantly correlated with final field emergence (%) (Table 5). Radicle emergence at 13 °C was also correlated with germination (normal seedlings) after the cold test. These data originated from those obtained during the preliminary assessment of nine lots before the selection of lots for the comparative test (Appendix I, Table A).

Discussion

Two counts of radicle emergence (RE), 6 d at 13 °C and 66 h at 20 °C, ranked the six lots included in this comparative test in the same order in both runs in all laboratories (Tables 2, 3). This confirmed the findings in previous work on maize at 13 °C in three laboratories (Khajeh-Hosseini *et al.*, 2009) and four laboratories (Matthews *et*

al., 2010). Furthermore, in 21 out of 22 runs of the test (2 runs × 5 labs at 20 °C plus 2 runs × 6 labs at 13 °C), the two lots (6 and 1) with the highest RE (high vigour) were clearly and significantly distinguished from the two (2 and 5) with the lowest RE.

There were, however, highly significant differences between laboratories in absolute percentages of RE, and interactions between laboratories and lots, at both temperatures (Appendix II, Tables A, B). The interactions between labs and lots were seen in the magnitude and significance of some of the differences between the lots in different laboratories. Differences were less, for example, between lots in lab D and greater in lab A, especially at 20 °C. However, these differences were not so great as to change the rank order of the lots.

In terms of statistical significance, the differences in the absolute percentage of RE seen between labs could have arisen as a result of one or both of two reasons: 1) differences in the control of temperature over the whole of the experimental area; (2) differences in the application of the criterion of germination. The fact that lab A had the lowest overall mean and lab D the highest mean at both temperatures suggested that the criterion for germination may have been more important. Thus, lab A may have judged some seeds as not having germinated because they had not reached the 2 mm radicle stage, whereas they were regarded as germinated by lab D. Despite the differences in absolute RE, there were clear significant differences between the top two lots (6 and 1) and the lowest two lots (2 and 5) in all but one run of the test (Tables 2, 3).

Only a few examples of significant under- or overestimation of data were revealed by the h values (Figs. 1, 3). Examples of significant variability between replicates (k values) were seen only in single lots (in one lab, run 2; two labs, run 2) at 13 °C, and at 20 °C only lab C showed significant variability between replicates in single lots in both runs (Figs. 4a, b). With the exception of the reproducibility values for lot 5 (Table 4), all values for repeatability and reproducibility were comparable with the results previously reported for the controlled deterioration test (Powell, 2009) and a standard germination test for sunflower (Ducournau *et al.*, 2007). The low vigour of lot 5 may explain the greater variability observed, as results for low vigour seed lots are typically more variable than those for high vigour lots.

Table 4. Values for repeatability and reproducibility for six seed lots tested in five (13 °C) or six (20 °C) laboratories for radicle emergence in each of two runs

Lot	Repeatability sr2		Reproducibility sR2	
	Run 1	Run 2	Run 1	Run 2
13 °C				
6	4.502	3.578	5.292	5.040
1	7.711	4.033	7.029	5.089
4	5.489	6.733	7.567	9.072
2	10.250	10.558	17.757	16.471
3	9.048	9.587	8.509	12.508
5	13.594	10.721	15.633	20.693
Range	4.502–13.594	3.578–10.721	5.292–17.757	5.040–20.693
20 °C				
6	4.028	1.700	4.374	2.747
1	5.637	16.995	5.727	17.957
4	4.807	6.218	5.379	5.745
2	10.904	8.472	16.330	14.213
3	7.364	11.034	9.309	13.810
5	12.046	7.557	22.552	31.563
Range	4.028–12.046	1.700–11.034	4.374–22.552	2.747–31.563

Table 5. Correlation coefficients between cold test germination, final field emergence (%) and two measures of the rate of germination: radicle emergence (RE) after 66 h at 20 °C and 6 d at 13 °C for nine lots of maize (Table A, Appendix I).

	Cold test germination (%)	Final field emergence (%)
Cold test germination (%)	–	0.415
RE after 66 h at 20 °C (%)	0.605	0.761*
RE after 6 d at 13 °C (%)	0.696*	0.850**

* $p<0.05$; ** $p<0.01$

Comparisons of the data to determine whether replicates, repeat runs of the test in one laboratory and results for the same lots in different laboratories were in tolerance were made using the ISTA Germination Calculator (www.seedtest.org/upload/cms/user/Germinationtolerancescalculator-V0.3.xls). In only 14 of the 132 tests completed were the replicates not in tolerance: 2 out of 60 tests at 13 °C and 12 out of 72 tests at 20 °C. The results from the repeat runs in each laboratory were in tolerance, with the exception of lot 5 in labs B and E at 13 °C and lot 3 (lab D) and lot 5 (lab E) at 20 °C. The results from different laboratories were in tolerance, with the exception of lots 2 and 5 in both runs of the test at 13 °C, lot 2 in run 1 at 20 °C and lot 5 in both runs at 20 °C. Where the data from different laboratories were not in tolerance, this was largely due to over- or underestimations of radicle emergence in one or two laboratories. This could be eliminated by training courses to ensure uniformity for routine testing.

The radicle emergence counts at 13 °C and 20 °C made in preliminary work in Aberdeen for nine lots (Appendix I, Table A) were significantly related to final field emergence determined in Iran (Table 5). The RE after 6 d at 13 °C was also significantly related to the cold test result (% normal seedlings) (Table 5), as previously seen by Matthews *et al.* (2010). The cold test in the present work on nine lots was not significantly related to field emergence, but in comparisons of a larger number of commercial lots, for example by Lovato and Balboni (1997) and Noli *et al.* (2008), it was highly significantly related to field emergence.

It has been suggested (Matthews and Khajeh-Hosseini, 2007; Matthews *et al.*, 2010) that the cold test and radicle emergence assessed at any temperature are both assessments of seed age. Physiologically older seeds need to go through a longer period of metabolic repair before radicle

emergence, thus leading to a longer lag period from the beginning of imbibition to radicle emergence compared to younger seeds. In the cold test, the early cold period and anaerobic conditions might slow down metabolic repair. As a result, in comparison to the warm germination test, a proportion of the older seed would not repair sufficiently at the lower temperature of the cold test to produce normal seedlings.

We argue that laboratories should consider a test of the radicle emergence as an alternative to the cold test for three reasons. Firstly, a radicle emergence test takes less time than the cold test, as little as 2-3 days at 20 °C, compared to up to 7 to 10 days for the cold test. Secondly, a clearer differentiation between low- and high-vigour lots is often achieved by the radicle emergence test than by the cold test, as seen in Table 3. Finally, as the present work suggests, standardisation of the radicle emergence test is possible.

This comparative test has clearly revealed that the radicle emergence test can consistently identify differences in vigour, and that the test is repeatable and reproducible in the ranking of the lots. At the outset of this work, temperature control over the experimental area in the incubator or controlled temperature room was emphasised as a possible source of variation. This may have been the cause of the differences that were observed between laboratories, along with the interpretation of the criterion for radicle emergence. We would recommend the following for routine application of the method, and to ensure uniformity between labs:

1. The temperature over the experimental area should always be monitored. The overall declared temperature control of many of the controlled temperature rooms or incubators used by participating labs ranged from ±0.5 °C to ±2.0 °C. However, data from some laboratories in this current work suggested that in the actual experimental area the temperature control was closer to ±1.0 °C. As a guide to using this

Appendix I: Data used for relationships in Table 4 (main text)

Table A. Counts of radicle emergence* (RE; 2 mm radicle) of 9 lots of F1 hybrid maize after 6 d at 13 °C and 66 h at 20 °C, alongside cold test germination(ex Pioneer Hi-Bred laboratory) and field emergence (%) (Iran). Highlighted lots selected for comparative tests

Seed lot	Radicle emergence (%) after:			Cold test (% normal seedlings)	Field emergence (%)
	48 h, 20 °C	66 h, 20 °C	6 d, 13 °C		
7	60	81	89	96	90
8	14	63	97	97	90
1	53	89	86	90	89
2	5	43	37	94	78
3	24	51	41	76	84
9	12	43	20	88	76
4	66	85	92	94	82
5	0	7	0	82	78
6	73	95	96	96	92

*Determined at 13 and 20 °C in Aberdeen on two replicates of 25 seeds in two runs, i.e. a total of 100 seeds.

Appendix II: ANOVA tables

Table B. Radicle emergence at 13 °C

Source	DF	SS	MS	F	
Model	34	178911.9417	5262.1159	64.15	<0.0001
Error	205	16816.3542	82.0310		
Corrected total	239	195728.2958			
Source	DF	Type 1 SS	MS	F	
Lot	5	163344.5208	32668.9042	398.25	<0.0001
Lab	4	5329.4833	1332.3708	16.24	<0.0001
Run (lab)	5	1261.5208	252.3042	3.08	<0.0106
Lot × lab	20	8976.4167	448.8208	5.47	<0.0001
Source	DF	Type 111 SS	MS	F	
Lot	5	163344.5208	32668.9042	398.25	<0.0001
Lab	4	5329.4833	1332.3708	16.24	<0.0001
Run (lab)	5	1261.5208	252.3042	3.08	<0.0106
Lot × lab	20	8976.4167	448.8208	5.47	<0.0001

Table C. Radicle emergence at 20 °C

Source	DF	SS	MS	F	
Model	40	127896.1429	3197.4036	52.56	<0.0001
Error	239	14539.0000	60.8326		
Corrected total	279	142435.1429			
Source	DF	Type 1 SS	MS	F	
Lot	5	91954.20952	18390.84190	302.32	<0.001
Lab	5	15117.92000	3023.58400	49.70	<0.001
Run (lab)	6	719.0000	113.83333	1.97	<0.0707
Lot × lab	24	20105.01333	837.70899	13.77	<0.001
Source	DF	Type 111 SS	MS	F	
Lot	5	92761.72000	18390.84190	304.97	<0.001
Lab	5	15117.92000	3023.58400	49.70	<0.001
Run (lab)	6	719.0000	113.83333	1.97	<0.0707
Lot × lab	24	20105.01333	837.70899	13.77	<0.001

radicle emergence test method, ±1.0 °C should be the realistic target.

2. Laboratories should consider incorporating an early count of RE (66 h) into a standard germination test carried out at 20 °C in rolled towels. Thus, one test following ISTA Rules would provide a vigour assessment after 2 to 3 days, as well as the standard germination count.

3. A limit should be placed on the number of lots to be tested in any one run, say 15 lots, so that the experimental area covered is not too great and a temperature range is therefore not difficult to avoid.

4. A standard lot of medium vigour (e.g. lot 4 in the comparative test) should be included in all test runs in any one season.

5. Joint training exercises by laboratories in workshops, or collaborative work on the same lots, should be organised to ensure uniformity for routine testing.

Acknowledgements

We thank Pioneer Hi-Bred Services GmbH, Parndorf, Austria for the supply of seed and information. We also thank those who gave technical support to the work. In particular, we acknowledge the contributions of Sandrine Stievenard and Marylène Moron in Angers, Jarad Nezafat and Ali Izadfar in Mashhad, Giovanni Urso and Emma Beltrami in Bologna, and Elisabeth Gorgosilits in Parndorf.

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Matthews, S., El-Khadem, R., Casarini, E., Khajeh-Hosseini, M., Nasehzadeh, M. and Wagner, M.-H. (2010). Rate of physiological germination compared with the cold test and accelerated ageing as a repeatable vigour test for maize. *Seed Science and Technology*, **38**, 379–389.

Nijenstein, J. H. and Kruse, M. (2000). The potential for standardization in cold testing of maize (*Zea mays* L.) *Seed Science and Technology*, **28**, 837–851.

Noli, E., Casarini, E., Urso, G. and Conti, S. (2008). Suitability of three vigour test procedures to predict field emergence of early sown maize. *Seed Science and Technology*, **36**, 168–176.

Powell, A.A. (2009). Evaluation of the controlled deterioration test as a repeatable and reproducible vigour test for *Brassica* species. *ISTA Method Validation Reports*, 2009, 92–123.

Laboratory accreditation changes

Status 15 March 2011

Re-accreditations

Bolivia BODL0100

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New accreditations

Hungary HUML0300

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ISTA-suggested training objectives for seed analysts

At the ISTA seminar on Seed Analyst Training in 2009 it was agreed that ISTA would publish a set of objectives that laboratories could use as guidance when training seed analysts. The following objectives are not exhaustive or what ISTA requires a laboratory to do. They are however meant as a helpful checklist for laboratories that wish to develop a training course. The training objectives are given at two levels: 1) standard and 2) advanced.

Timing of training

Seed analysis is a specialised occupation, and some skills, such as seed identification and seed analytical purity take many years to develop. Therefore even after completing an advanced level course most candidates will need to practise and refine their skills by daily use, proficiency testing etc. The time that is taken to cover the different aspects of training will vary depending on circumstances for the analyst and the laboratory. However many of the skills need repetition in a work environment and a guideline would be that it takes about 1 year to achieve the standard level and a further year to complete the advanced level.

As part of training the most difficult thing to master is the identification of crop and weed species by visual recognition. To help with this, new seed analysts need to create their own reference collections/herbariums. The time needed to develop seed identification skills will depend on the range of crop types being taught. Thus, training required for a laboratory testing only *Zea mays* will be different to a laboratory testing a wide range of species.

Other elements that are not essential to training, but require study to become effective within a national seed testing system include legislation, national certification standards and QA.

Information in support of training

ISTA has a range of publications, including handbooks, which can provide support for training e.g. Seedling Evaluation Handbook, Sampling Handbook, and Moisture Handbook. All handbooks are detailed on the ISTA web site and are available through the ISTA Secretariat. In addition the ISTA web site provides guidance on a number of issues.

Suggested training objectives: standard level

Following training, seed analysts should be able to demonstrate that they (1) have a satisfactory understanding of the theory behind different aspects of seed testing, and (2) can perform the practical skills to a satisfactory standard.

In some cases this might involve participation in ISTA competency test rounds with the aim of achieving an overall 'B' rating in at least three test rounds.

A list of suggested topics for inclusion in a standard level training programme are given below.

1. Seed biology

1.1 Theory: an introduction to the plant kingdom; plant reproduction and seed development; seed anatomy and morphology.

2. Seed quality

2.1 Theory: an introduction to the components of seed quality; significance of seed quality nationally and internationally; an introduction to the International Seed Testing Association.

3. Sampling

3.1 Theory: the importance of accurate seed lot sampling for seed testing; how to sample seed lots correctly; how to reduce the submitted sample to the required working sample size without bias, proper use of mixing and dividing equipment

3.2 Practical: laboratory sample division.

4. Purity analysis

4.1 Theory: what is pure seed; the equipment required for purity testing; internationally agreed methods for purity testing; conducting a purity test; the characteristics of seeds of different plant species; using seed description keys.

4.2 Practical: identification of selected crop and weed seeds; demonstration of mastery of purity testing.

To help with the identification of crop and weed species by visual recognition, new seed analysts need to create their own reference collections of seeds.

The time needed to develop seed identification skills will depend on the range of crop types being taught. Thus, training required for a laboratory only testing one or two species will be different to a laboratory testing all the species listed in Table 2A Part 1 of the ISTA Rules.

5. Determination of Other Seeds by Number

5.1 Theory: internationally agreed methods for determining other seeds by number; conducting an 'other seeds by number' test.

5.2 Practical: determination of other seeds by number

6. The Germination Test

6.1 Theory: the process of seed germination; the equipment required for germination testing including proper use of counting heads, counting boards, germinators; internationally agreed methods for germination testing; conducting germination tests including seedling evaluation.

6.2 Practical: germination testing of selected species.

Suggested Training Objectives: Advanced Level

Following training, seed analysts should be able to demonstrate that they:
(1) have a satisfactory understanding of the theory behind different aspects of seed testing and
(2) can perform the practical skills to a satisfactory standard.

In some cases this might involve participation in ISTA competency test rounds with the aim of achieving an overall 'B' rating in at least three test rounds.

A list of suggested topics for inclusion in an advanced level training programme are given below.

1. Biochemical Test for Viability

1.1 Theory: what is a viable seed; the equipment required for tetrazolium testing; internationally agreed methods for tetrazolium testing; conducting a tetrazolium test.

1.2 Practical: tetrazolium testing of selected species.

2. Determination of Moisture Content

2.1 Theory: the relationship between water and seeds; the effects of seed moisture on seed quality; the equipment required for moisture testing; internationally agreed methods for moisture testing; conducting a moisture test.

2.2 Practical: moisture testing of selected species.

3. Weight Determination

3.1 Theory: why seed weight may differ among and within seed lots; the equipment required for seed weight determination; internationally agreed methods for seed weight determination; conducting a seed weight determination.

3.2 Practical: seed weight determination of selected species.

4. Seed Vigour Testing

4.1 Theory: what is seed vigour; how vigour differences may occur among seed lots; the significance of seed vigour for seed for sowing, storage and transport; the equipment required for seed vigour testing; internationally agreed methods for seed vigour testing; conducting seed vigour tests.

4.2 Practical: seed vigour testing of selected species.

5. Reporting/Certificates

5.1 Theory: the purpose of seed analysis certificates; the ISTA Orange International Seed Lot Certificate and Blue International Seed Sample Certificate; information required for an ISTA Certificate; entering data onto an ISTA Certificate.

5.2 Practical: completion of ISTA seed analysis certificates.

6. Seed Laboratory Quality System

6.1 Theory: what is seed quality assurance; requirements for a seed laboratory quality system; quality assurance for sampling, the purity analysis, determination of other seeds by numbers, the germination test, the tetrazolium test, the moisture test, seed weight determination, seed vigour testing, seed analysis certificates, training tasks for analyst, internal audit process, proficiency testing methodology.

ANNOUNCEMENT

ISTA Workshop on Statistical Aspects of GMO Detection
Mexico City, Mexico, 25–29 July 2011

The National Institute of Ecology (INE), SEMARNAT, Mexico, in collaboration with the Autonomous Metropolitan University (UAM), will host an ISTA Workshop on Statistical Aspects of GMO Detection.

The Workshop will cover sampling principles, testing plan design and uncertainty assessment in GMO testing, and will include practice of specific software such as Seedcalc.

Location

Universidad Autónoma Metropolitana Iztapalapa, México, DF, San Rafael Atlixco N° 186, Col. Vicentina, C.P. 09340, Delegación Iztapalapa

Organizers and lecturers

Martha Rocha Munive, Deputy Director of Genetic Analysis, National Institute of Ecology (INE, SEMARNAT)
Adriana Otero Arnaiz, Coordinator of the Biosafety Program, National Institute of Ecology, (INE, SEMARNAT)
Beatriz Rendón, Autonomous Metropolitan University (UAM)
Berenice Zúñiga, GMO Analysis department chief, National Institute of Ecology, (INE, SEMARNAT)
Jean-Louis Laffont, Pioneer Génétique S.A.R.L.,France, Chair of the ISTA Statistics Committee
Kirk Remund, Monsanto, USA, Vice-Chair of the ISTA Statistics Committee, member of the ISTA GMO Committee
Beni Kaufman, Pioneer (a DuPont Business), USA, member of the ISTA GMO Committee

Preliminary programme (working language is English)

Monday, 25 July: Introduction to GMO testing
Opening and introductions – Qualitative GMO testing methods – Quantitative GMO testing methods – The moving target – The non-statistical aspects of testing plans – The challenges for GMO testing – Assay and process validation – GMO testing rules and activities at ISTA
Tuesday, 26 July: Basic statistical tools used in GMO testing
Probability distributions: binomial, multinomial, hypergeometric, normal – Tools for exploring distributions: box plots, QQ plots – Uncertainties – Linear modeling (a.k.a. ANOVA) – Repeatability/reproducibility
Wednesday, 27 July: Acceptance sampling – Pool testing – Testing plans – Estimation – Stack assessment – Local tour to the floating gardens of Xochimilco
Thursday, 28 July: Quantitative testing
Testing plans – Estimation – Outlier detection – Measurement of uncertainty in GMO testing
Official Dinner (Restaurant Fonda del Recuerdo)
Friday, 29 July: Measurement of uncertainty in GMO testing – LOD/LOQ – Workshop evaluation – Workshop wrap-up

Registration deadline

Send application form to: azuniga@ine.gob.mx
or by fax to +55 5613 3821 (attention: M. en C. Berenice Zúñiga Bustos) by **30 June 2011.**
Places will be considered for registration in order of receipt of application and payments.

Participants

Maximum 25 participants

Sponsorship

For Latin-American applicants, sponsorship will be available for the cost of the course, based on academic background and application of the workshop to their daily work.
Preference will be given to government officials.

Registration fees

ISTA Members: USD 400
Non-members: USD 500
Fee includes didactic material, programs and associated literature, as well as the official dinner, coffee breaks, tour to the floating gardens of Xochimilco, and transport to and from the hotel (Abastos Plaza).

Accommodation

Gran Hotel (granhoteldelaciudaddemexico.com.mx):
single: USD 80, double: USD 100
Plaza Suites (www.plazasuites.com.mx):
single/double: USD 130
Hampton Inn & Suites (hamptoninn.hilton.com/en/hp/hotels):
single/double: USD 94
Room Mate Valentina (www.room-matehotels.com):
single/double: USD 74
Boutique Hotel Cortes (www.boutiquehoteldecortes.com):
single/double: USD 155
Abastos Plaza (www.hotelabastosplaza.com.mx):
single: USD 60, double: USD 70

Additional information

The nearest hotel to the University is Abastos Plaza. Transfers will be arranged.
For questions, please contact M. en C. Berenice Zúñiga Bustos azuniga@ine.gob.mx or tel. +55 5804 6545

Registration

www.seedtest.org

ANNOUNCEMENT

ISTA Workshop on Quality Assurance in Seed Testing
Bangalore, India, 8–12 August 2011

Venue

Hotel "The Chancery", Lavelle Road, Bangalore
Phone: +91 80 22276767, www.chanceryhotel.net,
reservations@chanceryhotel.net

Aim of the workshop

Presentation and discussion of basic principles of quality management, and the needs of laboratories to comply with the ISTA Accreditation Standard and prepare for attaining and maintaining ISTA accreditation. Successful participants will be able to:
- understand the requirements of the Accreditation Standard;
- evaluate the situation of their laboratory with regard to conformity with the ISTA Accreditation Standard;
- document the quality management system for their laboratory in a manual and related documents;
- implement a quality management system;
- implement the checks required for laboratory equipment.

Workshop content

The theoretical background will be given through lectures. Participants will be actively involved through group work, discussions and presentations. The ISTA Accreditation Standard will be used during the group work. Language: English.

Preliminary programme

- Introduction to ISTA and the ISTA Accreditation Scheme
- The quality management system and quality documentation
- Quality management in seed testing, technical aspects: how to check the seed dividing process – how to perform balance checks – checking moisture-proof containers – how to perform germination substrate checks – monitoring of temperature-controlled equipment
- How to use tolerance tables and other statistical tools
- The Internal Audit Programme (establishing the audit check list and performing the audits)
- Corrective and preventive actions: principles and documentation
- The Management Review
- ISTA Certificates and how to issue them
- The ISTA Proficiency Test Programme
- Interpretation of the ISTA Accreditation Standard
- Excursion to an ISTA accredited laboratory
- Workshop dinner

Local organizer

Dr. G.V.Jagadish
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Registration

www.seedtest.org

Lecturers

Rasha El-Khadem (Head of ISTA Accreditation Department)
Ronald Don (ISTA Technical Auditor)

Target group

Quality managers, laboratory managers and seed testing analysts, with or without experience in quality management.

Registration

Number of participants: 25–30
Fees: Members: EUR 400 Non-members: EUR 600
Deadline for registration: **31 May 2011**

The registration fee includes all literature and supporting material for the workshop, lunches and coffee breaks, excursion, workshop dinner and transfer between the workshop venue and hotels (except airport transfer).

Mode of payment

Indo-American Hybrid Seeds (India) Pvt Ltd, Account No. 0408261010631, at Canara Bank, Jayanagar Shopping Complex Branch, 4th Block, Jayanagar, Bangalore 560011 (India), Tel: +91 80 26630880, through Canara Bank, Foreign Department, 44/45, Residency Cross Road, Bangalore 560025; SWIFT code: CNRBINBBLFD through MT-100 Routing No. 021000021 Tel: +91 80 25582520

Indian participants may pay in Indian rupees equivalent to the fee in euros.

Routing details of Telex transfer

By crediting the amount to USD account No. 001-1-395969 with chips UID-107777 with the CHASE MANHATTAN BANK, NEW YORK maintained by Canara Bank, Bangalore for further credit to Canara Bank, Shopping Complex Branch, Bangalore 560011 to current account No. 0408261010631 of Indo-American Hybrid Seeds (India) Pvt. Ltd. Bangalore by Swift CNRBINBBLFD through MT-100 Routing No. 021000021.

Please fax us a copy of the payment details for our follow up. Indian participants may send the Demand Draft in favour of the Indo-American Hybrid Seeds (India) Pvt. Ltd., Bangalore and sent to Dr. G.V. Jagadish, Indo-American Hybrid Seeds (India) Pvt. Ltd., 7th km Banashankari-Kengeri Link Road, Channasandra, Uttarahalli, Subramanyapura Post, Bangalore 560 061.

Accommodation

Hotel The Chancery, Lavelle Road, Bangalore
Single room: EUR 110 per day, incl. breakfast

Hotel Hoysala, S.C. Road, Bangalore 560 020
Single room: EUR 30 per day

For accommodation booking, contact Dr. G.V. Jagadish (jagadish@indamseeds.com).
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ISTA Workshop on Quality Assurance
Saskatoon, Canada, 29 September–1 October 2010

Steve Jones
ISTA Rules Chair, ISTA ECOM Member

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The CFIA's ISTA-accredited laboratory in Saskatoon was very pleased to welcome a total of 28 participants from 6 different countries to the ISTA Workshop in September 2010. Most participants were from Canada or the USA, but we also had visitors from Brazil, Hungary, Syria and the Netherlands. People came from a range of different backgrounds, but all with QA requirements in common. The course was introduced by myself as the local organiser, and delivered by Rasha El-Khadem, the Head of ISTA's Accreditation Department, with help from Joanne Hinke who is an ISTA technical auditor and works at the Saskatoon laboratory. Not everyone on the course was from an ISTA laboratory, so the aims of ISTA, the Orange International Certificate and the requirements for ISTA accreditation were also outlined.

There was an excellent exchange of views on a range of different topics, from monitoring samplers, validating equipment, corrective actions, internal auditing and PT testing. Group work and practical exercises split the days up, and before we knew it the three-day course was over. Experiences were shared, and questions raised for feedback from ISTA's different technical committees.

The need to trend analyst performance, perform internal audits and sampler monitoring were highlighted, and splitting up into smaller groups helped people develop their own solutions. Group ideas were then shared and discussed, with Rasha facilitating the discussion. People from established ISTA laboratories were ready to share their experiences and just as ready to learn new things.

Practical exercises on verifying the riffle divider and performing an internal audit helped break up the classroom sessions, and made good use of the laboratory space available in Saskatoon. A tour of the laboratory and Canada's National Seed Herbarium and imaging equipment, which is part of the Seed Science and Technology Section at Saskatoon, was included in the program.

Even the weather was good to us, as you can see from the group shots around the nearby pond and garden area that we made use of in the lunch breaks. The excellent baking and fresh fruit provided at the

break times helped create a relaxed mood to the meeting, with everyone contributing to discussions and exchanges of ideas.

As the local workshop organiser, it has been a good learning experience for me to know what to do and what to improve on for next time. Judging by the feedback sheets, everyone found the experience both enjoyable and useful. Encouraged by this, we would like to host other ISTA Workshops in the future. I would personally like to thank all the local staff for a great team effort, and Rasha for her excellent presentations.

Editor's note: Dr. Rasha El-Khadem is Head of the ISTA Accreditation Department. The ISTA Accreditation Department is willing to deliver similar courses at locations around the world. Please let them know about your needs (meetings@ista.ch).

ISTA Workshop on Water Activity Measurement
Montargis, France, 13–15 October 2010

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The ISTA Workshop on Water Activity Measurement was the first of its kind, and was held the auspices of the Moisture, Forest Tree and Shrub Seed and Storage Committees at Montargis, France, from 13 to 15 October 2010. It was organized by Cemagref, in connection with the Direction de la Recherche Forestière of Québec, Canada. Cemagref is a French public research organization devoted to agricultural and environmental engineering research. Its facilities at Nogent-sur-Vernisson, which specialize in forest ecology and genetic resources management, is located close to Montargis, one hour south of Paris.

Despite the transportation difficulties which occurred in France at the time, the event attracted 20 participants and 6 lecturers from the USA, Canada, Brazil, Finland, Sweden, Denmark, Netherlands, Germany, Czech Republic, Great Britain, Italy, Croatia, Spain and France.

The aim of the workshop was to present the concept of water activity (a_w), the practical aspects of its measurement, and some results obtained in particular for forest tree seeds. It was also the place to discuss and evaluate the potential role of water activity measurement as a new ISTA method for quantifying seed moisture. Approximately half of the participants were involved with tree seeds and half with agricultural crops; two thirds were either ISTA Members or people working in ISTA-certified seed laboratories.

The three-day workshop comprised lectures, a demonstration and training session, a visit to the Cemagref facilities, and a botanical excursion to the National Arboretum of Les Barres.

There were five sessions of lectures and discussions. The first session dealt with the concepts of water activity and equilibrium with relative humidity. Mr. François Mariette, Senior Research Head of the

Fabio Gorian, Chair of the Forest Tree and Shrub Seeds Committee.

Harry Nijenstein, member and former Chair of the Moisture Committee.

Hugh Pritchard, Chair of the Seed Storage Committee.

IRM Food research team at Cemagref, presented a lecture on the content, distribution and mobility of water in biological matrixes. Patrick Baldet of Cemagref gave the second lecture, on the practical aspects especially of water activity measurement and potential applications to seeds.

The second session concentrated on the needs and expectations of the ISTA Technical Committees. FTS Chair Fabio Gorian gave an overview of tree seed storage; Harry Nijënstein, MOI member and formar Chair, presented an update and addenda to the ISTA Handbook on Moisture Determination, and STO Chair Hugh Pritchard talked about stakes on gene bank management, with special regard to labs without 'moisture' procedures.

The third session was dedicated the operational implementation of water activity measurement. Fabienne Colas of the Direction de la Recherche Forestière of Québec, Canada, and Patrick Baldet gave two presentations, entitled 'Operational implantation of water activity at Berthier Tree Seed Centre (Québec)' and 'Hydric characterization of forest orthodox seeds: building and analyzing sorption isotherms'. These were followed by presentations by Robert Karrfalt (U.S. Department of Agriculture) on '41 month storage data for *Artemisia tridenta* var. *wyomingensis*', and by Markku Nygren (Metla, Finland) on 'Water activity measurements with *Pinus sylvestris* and *Picea abies* seeds'.

The group in front of the 14th century Chateau de Sully.

The fourth session dealt with water activity measurement applied to *ex situ* conservation of genetic resources. Hugh Pritchard spoke about 'Moisture management and genetic resources conservation', and Fabienne Colas about 'Water activity: a tool to optimize *ex situ* genetic diversity conservation in Québec'.

There followed a discussion session on the prospective role of water activity in seed testing and its introduction into the ISTA Rules.

A hands-on session was also organised, to allow each participant to practise using water activity meters on seed and pollen of several forest and agricultural species. Several different types of probes and equipment were presented and demonstrated to show their operational advantages in water activity measurement. The organizers would like to thank the Rotronic company, which sponsored the workshop with most of the water activity meters.

Beside all this interesting and profitable work, on the evening of the first day the participants enjoyed a visit to the Montargis municipal art gallery, followed by an official reception by the 1st Assistant Mayor of Montargis in the historical city hall. The social event and official dinner took place in the Loire valley, and included a trip to the 14th century Chateau de Sully, and the famous Briare aqueduct, built by Gustave Eiffel in 1890.

This workshop was very enjoyable; the organizers express their gratitude to the lecturers, the participants, the Cemagref staff, the municipality of Montargis and ISTA for contributing to the success of this workshop. ■

ISTA Workshop on Germination, Tetrazolium, Sampling and Statistics Nakuru, Kenya, 22–26 November 2010

Jacob Cheptaiwa, Wilson Sitienei and Augustine Mulandi

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The ISTA Workshop on Germination, Tetrazolium, Sampling and Statistics was organized by ISTA in collaboration with the Kenya Plant Health Inspectorate Service (KEPHIS), and was held at the KEPHIS Regional office in Nakuru, Kenya from 22nd to 26th November 2010.

There were a total of 33 participants from South Africa, Tanzania, Zimbabwe, Uganda, Kenya, Somalia and Ghana.

The workshop was opened with an introduction and a welcome note from the hosting regional manager of KEPHIS Nakuru, Mr. Jacob Cheptaiwa. Then the opening remarks were given by the head of the KEPHIS seed certification and plant variety protection, Mr. Simeon Kibet, and the deputy provincial director of Agriculture of the Province of Rift Valley, Mr David Malulu. The workshop was officially opened by Dr. Joseph Ahenda, the General Manager of KEPHIS Inspection Operations, who was also the local workshop organizer. This was followed by a photo shoot of all the participants.

The workshop was begun with a registration of the participants' expectations, led by Anny van Pijlen, after which the first day's training started with a lecture on the germination test, followed by practical work on germination evaluation of *Lycopersicon esculentum* and *Allium cepa*. These were delivered by Anny van Pijlen.

Then, Jean-Louis Laffont gave a lecture on the use of tolerance tables, covering the principles, an overview of the ISTA tolerance tables, and the use of the ISTA germination tolerance calculator. Thereafter, he gave an introduction to pool testing.

On the second day, Anny van Pijlen gave a lecture on the tetrazolium test, after

The tree-planting ceremony.

which she guided the participants through a tetrazolium practical on *Lycopersicon esculentum*. She also covered quality assurance in seed testing, covering calibration of equipment, and ISTA quality assurance and quality management. There was also a lecture on the analysis of bivariate data (application) by Jean-Louis Laffont.

In the evening, there was a colourful official dinner at the Bontana Hotel where the participants were feted with Kenyan dishes and dances.

On the morning of the third day, the participants were taken to a tour of the Kenya Seed company's seed processing plant in Nakuru, followed by a tour of the hosting seed testing laboratory and a commemorative tree-planting ceremony in the laboratory compound. Later in the afternoon, they visited Lake Nakuru National Park.

The fourth and fifth days covered seed sampling theory and a practical on seed sampling, by Anny van Pijlen, and a comparison of the results of the germination and tetrazolium practicals done on the first and second days, run by Jean-Louis Laffont. There were also lectures on the testing of heterogeneity and homogeneity in sampling (Jean-Louis Laffont), monitoring of seed samplers (Dr. Joseph Ahenda), and checking the dividing process and quality checks in germination (Anny van Pijlen).

A workshop evaluation was done where the participants appreciated the training and said that all their expectations were fulfilled. They all thanked ISTA and KEPHIS for organizing the workshop, and indicated that they would make good use of the training in their countries.

The closing remarks were given by Dr. Joseph Ahenda, who congratulated ISTA for holding the workshop in Kenya, thus helping African countries to build capacity in seed testing which in turn would facilitate seed trade. He thanked the lecturers (Anny and Jean-Louis) for giving their time to attend and share their expertise with the participants. Anny van Pijlen said she was happy to come back to Kenya and that she enjoyed the workshop. She added that the participants had been very active and had asked many questions. She also thanked KEPHIS for hosting the workshop and all the people who made the workshop a success. Jean-Louis Laffont thanked the participants for their keen interest during the training, and said that the ISTA tolerance software was almost ready for use. Jacob Cheptaiwa thanked all for their active participation and co-operation to ensure that the workshop programme was fully covered. He wished them a safe journey back home, and encouraged them to use their seed expertise to help provide a better life in their home countries.

After the closing remarks, the participants were presented with certificates of participation, and also gifts to show appreciation for their attendance.

We would also not like to forget the support and advice given by the management of KEPHIS and by the ISTA Secretariat (Nadine Ettel and Jonathan Taylor), and also the donation of photos by Jean-Louis Laffont and Anny van Pijlen for preparing this report. All are acknowledged.

CALENDAR

2011

13–16 June **ISTA Annual Meeting and Germination Seminar, Zurich, Switzerland**
www.seedtest.org

25–29 July **ISTA Workshop on Statistical Aspects of GMO Detection, Mexico City, Mexico**
www.seedtest.org

8–12 August **ISTA Workshop on Quality Assurance in Seed Testing, Bangalore, India**
www.seedtest.org

2012

11–14 June **ISTA Annual Meeting, the Netherlands**
www.seedtest.org

2013

19–25 June **30th ISTA Congress 2013, Turkey**
www.seedtest.org

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