

Seed Testing

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Dear colleagues and friends,

Greetings from the ISTA Secretariat; our team is busy implementing the decisions from the latest Executive Committee (ECOM) meeting.

Early in February, ECOM members, accompanied by representatives of the ISTA Secretariat, gathered for their semi-annual meeting. The meeting took place in Tsukuba, Japan, following an invitation from ECOM member Masatoshi Sato, on behalf of the Japanese National Center of Seeds and Seedlings and the Ministry of Agriculture, Forestry, and Fisheries. The hospitality of our hosts and the organization by Dr. Sato and his team were exemplary, which helped the meeting become very fruitful. Many important decisions were made by the ECOM, of which I would like to mention two that will be a step change for the Association and will impact what and how we operate at the Secretariat.

In the first decision, the ECOM agreed to the principle that contracted system auditors would be employed by the Association. ISTA audits are at the heart of ISTA's Accreditation and Technical Department. At present, ISTA audits comprise two parts, performed by two different auditors: the system audit, performed by a system auditor, who is the lead auditor and an ISTA staff member, and the technical audit, performed by a contracted technical auditor. The most evident limitation of this system is that the number of possible audits depends on the number of available system auditors. This limitation is currently particularly acute, since we are down to only two system auditors. The possibility to employ additional system auditors will add much-needed flexibility to our scheduling options, will moderate the amount of travel we are asking from our full-time system auditors Florina Palada and Branka Opra, and will allow more audits to be performed than at present.

The second decision followed a presentation by our new Marketing Head Pierrick Marcoux, which introduced the general thoughts and scheme of our marketing effort. This effort will eventually be multifaceted, and will include various components of our work, but will start however with a single focused campaign centred on a defined area and targets. The decision urges Pierrick and the Secretariat to identify these targets and finalize the plan for ECOM approval.

These two decisions change how we at the Secretariat fulfil our duties (Accreditation) and define new areas of action and responsibilities (Marketing), and more importantly, both decisions facilitate growth, proactively preparing the Association for the future.

The future is also a topic in this issue of STI that you are about to read. In the near future we will be holding the 2015 Annual Meeting in Montevideo, Uruguay. You are invited to read more about it in Association News, and go ahead and register if you have not yet done so. Steve Jones, Member-at-Large of the ECOM and Chair of the Rules Committee, shares his views of the future in the article 'Future needs in seed testing'. Protecting the future of natural resources is the topic of Costantino Bonomi's article, with the title 'Native seeds for grassland restoration'. I hope you find it all informative and interesting.

Joyous reading,

Beni



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8 NASSTEC: native seeds for grassland restoration



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11 The Palacio Legislativo in Montevideo, seat of the General Assembly, the parliament of Uruguay

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President's Report

Joël Léchappé



I would like to begin this report by thanking the Japanese Designated Authorities, Mr. Takemori, President of the National Center for Seed and Seedlings, and Mr. Sato, ECOM member, for their invitation to host the February ECOM meeting in Japan. It was a great pleasure and honour for the ECOM to hold its meeting in Tsukuba. The main achievements of ISTA in 2014 and the decisions made by the ECOM during this meeting are reported below.

In February 2015, our Association continues to present ongoing and positive developments. There are 218 Member Laboratories, including 135 accredited laboratories in 60 countries or distinct economies, which in 2014 ordered 196 900 International certificates, i.e. 50 000 more than the average of the previous six years. These figures and much more information will be communicated in April in the Activity Reports of the ISTA Committees, which as official documents are sent to the Membership in preparation for the Ordinary General Meeting in Montevideo, Uruguay, in June 2015. I invite you to read these reports, which present the important work done by the Association, its Members and the permanent employees of the ISTA Secretariat.

I would like to acknowledge the recent contributions of Cecilia Jones, Member-at-large of the Executive Committee. Due

to new professional commitments Cecilia Jones can no longer serve as a member of the ECOM and recently informed the ECOM of her resignation. Cecilia was elected to the ECOM at the 30th ISTA Congress in Antalya, Turkey, in 2013. She was involved in ECOM Working Groups (Events WG, Marketing WG), and is the president of the organising committee for the ISTA Annual Meeting in Montevideo. Cecilia was the ECOM representative for South America. The ECOM is thus looking to fill the vacant position with a representative from South America to serve until the next ECOM election in 2016.

The ECOM would also like to thank Rasha El-Khadem, who recently moved to a position with another company. As Head of the Technical and Accreditation Department in the Secretariat, and as ISTA system auditor, Rasha has been a leader in the development of the ISTA accreditation system and the proficiency test programme. She encouraged and led the work to strengthen the harmonisation of conducting audits. This work, carried out together with the system auditors and technical auditors, was aimed at guaranteeing the uniformity of ISTA accreditation worldwide. Harmonisation and uniformity are the key strengths of the ISTA accreditation system, supported by performance in proficiency tests. The ECOM wishes all best success to Rasha in her new activities.

I would now like to report on the main actions undertaken by the ECOM to implement the strategy decided in 2013 in Antalya.

One of the priorities of the ISTA strategy is to increase ISTA membership and active participation in ISTA's work.

Following the opinions expressed by the membership at the last ISTA Ordinary General Meeting (June 2014, Edinburgh), the ECOM has worked on four main items aimed at facilitating participation in ISTA and meeting the needs of the stakeholders:

1. a review of the policy of charging late penalty fees;

2. spreading the accreditation fees more evenly over the three-year audit cycle;
3. not increasing membership fees, and
4. creation of an Industry Advisory Group.

The ECOM decided that the current late penalty fees should be replaced with an incentive to encourage early payment. This will be communicated in the documentation sent before the Ordinary General Meeting in June 2015 and discussed at the meeting.

The Accreditation Review Working Group (led by R. Zecchinelli) proposed to the ECOM to split the audit fee into three smaller amounts, payable annually. This proposal was presented to the Members at the 2014 Annual Meeting, where it received wide acceptance. The fee for the audit performed by ISTA every third year is 13 000 CHF and the payment will be spread over the 3-year interval between audits. An accredited laboratory will pay annually the fees for membership, accreditation and audit. This change does not exclude a more substantial revision of the audit fee structure, which requires further efforts and needs to deal with the different opinions and interest expressed by the laboratories. This audit fee item is related to a broader item, namely the review of the audit process and the accreditation system, which is being handled by the Accreditation Review working group.

With regard to the membership fees, the ECOM will propose at the OGM in June 2015 that they remain at the current level. This is based on the very low level of inflation in Switzerland in 2014, and because the healthy finances of the Association enable any increased costs to be funded from within existing income.

The ECOM and Secretary General implemented the decision, voted in June 2014, to replace the category corporate membership with industry membership. The new industry membership will facilitate the contribution of the seed industry to the Association, and enable the private sector

to deepen its engagement with, and provide support to, the Association. Applications were received from the first Industry Members in January 2015. As a further development, and to facilitate the participation of the industry, an Industry Advisory Group has been established by the ECOM, and the terms of reference defined. The aim of the Industry Advisory Group is to provide a link between the Association and industry needs.

A second key area of the ISTA Strategy is to **maintain and develop the capacity of seed testing worldwide**. This strategy is supported by the international relations policy and the related ECOM Working Group. The policy is aimed at strengthening collaboration with other organisations, including adapting to the priorities in different regions of the world, promoting dissemination of knowledge and contributing to the development of trained seed analysts. As part of this policy, the ECOM considers that it is important for ISTA to participate in meetings of other organisations, workshops, training sessions and seminars. The ECOM wishes to build a network that will include ECOM members, the Secretariat staff (Secretary General, Marketing, Accreditation, TCOM coordinator) and the Technical Committees to represent ISTA at international fora. An "International relations policy regarding involvement of Technical Committees" was agreed by the ECOM and presented to the TCOM chairs at the ECOM/TCOM meeting during the Annual Meeting in Edinburgh. The representation of ISTA and the role of the ISTA representative is determined as part of the discussion of the goals of attendance and needs of the inviting organisations.

As part of the strategy to promote harmonised Rules, in 2014 the ISTA Rules became available in electronic format only. There are several benefits of electronic publication, such as the ease with which a complete new edition of the Rules can be produced each year, and that Members can access as many copies of the Rules as needed, and no longer have to pay for additional copies or amendments. Electronic versions of Chapter 1 and Chapter 2 of the Rules are now also provided free to Members and non-members. However, one consequence is that separate amendments are no longer produced, which means that non-members need to purchase a complete new copy each year rather than simply purchasing

amendments. This is more expensive. The ECOM Working Group on Publications (Leader Craig McGill) prepared a discussion document on the cost of the Rules to non-members, and with cost information from two years of producing the electronic Rules the Executive Committee will make a decision on the cost of electronic Rules to non-members when it meets during the 2015 Annual Meeting.

The Working Group on Publications is, with the Secretariat, now focusing on increasing the number of different language publications of both the ISTA Rules and handbooks. The immediate aim is to have the Seedling Evaluation Handbook in Spanish available and translation of the Rules into Spanish started in 2015. There is also work being undertaken on a general policy for translation of ISTA publications, including whether ISTA should be charging royalties for translations. This and the question of the cost of the ISTA Rules to non-members are important components in achieving the ISTA strategy of promoting harmonised Rules and meeting the needs of stakeholders.

Also as part of meeting the needs of stakeholders the Working Group on Publications, in close liaison with the Secretariat, has been working on the use of electronic ISTA Certificates. A strategy to promote electronic certificates was discussed at the ECOM meeting in February 2015.

A fourth key area of the strategy is to strengthen the scientific and technical work in ISTA.

I would like first to acknowledge the very important work done by the Technical Committees, which have been very active since the Annual Meeting in Edinburgh, Scotland in June 2014. The main part of this work is to propose new Rules or to simplify and clarify the existing Rules. In addition to this technical work, the core activity of the Association, the Technical Committees have organised and participated in many workshops and seminars, where the dissemination of expert knowledge takes place in a friendly atmosphere. 2014 was a very fruitful year. I invite you to read the reports of the workshops and seminars in this issue of STI, and to consider hosting workshops and seminars; it is an unforgettable and rewarding experience.

As part of the support that the ECOM wants to give to the Technical Committees, when there is a surplus in the final account

of a workshop or seminar, this is shared between the organising laboratory to compensate for indirect costs, and is also made available to the Technical Committees involved in the workshop or seminar. These funds are in addition to the 3000 CHF allocated to each Technical Committee, aimed at encouraging technical work for ISTA. Any expenditure of the funds is subject to approval by the ECOM beforehand and a report afterwards.

I am convinced that it is essential for ISTA's future developments to strengthen the links between scientific developments and applications for seed testing. I am happy that the ECOM decided to set up and approve a Seed Science Advisory Group, based on the proposal of ISTA Members Stan Matthews and Honorary Life Member Alison Powell. The goals of the Group are to provide a link between fundamental research and the use of that research to meet the needs of ISTA Members. This will include identifying fundamental research results that apply to ISTA, and facilitating collaboration between the researcher and the end user. The Group will work in collaboration with any appropriate ISTA TCOMs, and will report to the ECOM and the Membership.

In addition to the ECOM Working Groups ongoing work, the main topics under development within the ECOM for the coming months will be:

- Continue to strengthen the technical and scientific developments, including the financial support to Technical Committees for travelling and representing ISTA, and the start of the Seed Science Advisory group.
- Make progress on ISTA electronic certificates.
- Develop the marketing and promotion of ISTA services such as increasing the Membership in under-represented regions, and facilitating official Rules translations of Rules into other languages.
- Examine the needs for training to maintain and develop the capacity of seed testing worldwide. As a background, I invite you to read the article by Steve Jones, a personal view, in this issue of STI on the future of seed testing.
- Analyse the EU conditions for the recognition of ISTA accreditation of laboratories in the EU.

I would like to conclude by inviting you to the ISTA Annual Meeting in Montevideo, Uruguay in June, which will start with the Seminar on “Molecular tools for seed quality & seed health”. The Annual Meeting will be preceded by workshops on “Seed Sampling and Quality Assurance in Seed Sampling”, and on “Tetrazolium Testing for Viability and its use as a Vigour Test for *Glycine max*”, organised by La Estanzuela Experimental Station of the National Agricultural Research Institute (INIA), Colonia, and followed by one on “Moisture

determination”, organised by the National Seed Institute (INASE).

Please also remember that the next 31st ISTA Congress will be in Tallinn, Estonia, in June 2016. The triennial Congress is the opportunity to catch up on scientific development in seed testing, review and redefine the ISTA strategy and elect a new Executive Committee. In particular I would like to invite you to start thinking about the renewal of the ISTA Executive Committee, make suggestions for candidates, and propose new strategic ideas for the period

2016–2019 under the Presidency of Craig McGill, the current Vice President.

Thank you for your contribution to our Association.

Your President and ISTA colleague
Joël Léchappé

(Prepared from the work and reports provided by ECOM Working Groups, with contributions from Craig McGill, Steve Jones, Rita Zecchinelli)



**31st ISTA
CONGRES**

Tallinn | Estonia 2016



Where next?

ISTA is seeking Member Countries to host the Annual Meetings in 2017 and 2018, and the 32nd ISTA Congress in 2019.

Applications are welcome from any ISTA Designated Authority of a Member Country.

For more information about requirements, application procedure etc., please see the guidelines available on the ISTA web site at:

www.seedtest.org/hosting

or contact the Secretariat at ista.office@ista.ch

Future needs in seed testing – a personal view

Steve Jones

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Disclaimer

The views expressed in this article are the personal views of the author and have not been endorsed by the CFIA, AOSA or ISTA.

In 2013 I was asked to write an article for the journal of the Association of Official Seed Analysts (AOSA) *Seed Technology* with my thoughts on the future needs for seed testing. After discussing the article with colleagues in ISTA I have updated it and present my ideas here. The original article was published in *Seed Technology* in 2014, and was titled “Future Seed Testing Needs for Seed Analysts and Researchers – A Personal View.” (Jones, 2013). It is reprinted and modified here with kind permission of AOSA/SCST.

Introduction

I have worked in seed testing for over 25 years, and as the ISTA Rules Chair have presented the ISTA Rules changes at every ISTA meeting since 2001. I hope the personal views I present here help stimulate debate and take seed testing forward in a positive way.

Although I have been involved with seed testing for most of my career, I am not a seed analyst. I worked in both research and seed testing for 30 years in the UK and now in Canada for 7 years. My career has been in government-funded laboratories, as a researcher, tree seed tester, sampler, sampler trainer, laboratory auditor, manager, and as part of the ISTA, AOSA and Canadian seed testing community. In 2008, I moved to work in the Canadian Food Inspection Agency’s (CFIA) Seed Science

and Technology Section, Saskatoon, Canada. In Canada, I have continued my role as ISTA Rules Chair and served on ISTA Technical Committees and the ISTA ECOM (2010–present).

As a manager within seed testing, I see a number of requirements to ensure that seed testing continues to progress and maintain reliable standards that facilitate international trade. These requirements involve seed analysts, seed researchers, testing rules, the regional seed analysts associations (e.g. Commercial Seed Analysts Association of Canada, British Association of Seed Analysts) and the wider seed trade.

Analysts and researchers

Seed testing often involves both seed analysts and researchers. The seed analysts I have met over the years have been well trained, competent and motivated to learn on the job. As part of their training they become aware that the aims of seed testing are met by a combined effort of samplers and their own work as analysts, managers and researchers. The relationship between seed analysts and seed researchers is very important, as it is one way for seed testing to progress by using advanced technologies and modern methods. Analysts use well-established and validated methods in their daily work, so they are often the best people to identify gaps in existing methods, to suggest improvements, and to identify methods that lack repeatability.

The applied researchers active in seed science are often based in seed testing laboratories, so know what the end-users of their work need and that the ISTA and AOSA Rules change every year. Other innovative changes will probably come from outside the world of seed testing but be applied to seed testing, such as advances in molecular testing or image analysis. Close links between researchers and seed

analysts can provide guidance regarding what is useful within seed testing, and can also help to turn novel research ideas into practical tests that are fit for purpose. Seed researchers need to be aware that during the development and validation of new methods, the seed material used should include seed lots from the major worldwide production areas.

Standardised internationally accepted methods

The existing international seed sampling and testing methods of ISTA (ISTA, 2015) and AOSA (AOSA, 2014) provide a multi-lateral standardised approach. Countries worldwide and organisations such as the International Plant Protection Convention (IPPC) and the Organisation for Economic Cooperation and Development (OECD) make use of the international rules to apply seed quality standards for international trade, and to prevent the movement of invasive species and diseases. With standardised methods, laboratories do not need to spend valuable time and resources developing their own methods. To help facilitate the use of standardised sampling methods, Chapter 2: Sampling of the ISTA Rules is available free to both members and non-members from the ISTA web site.

In my opinion, the continued collaboration between ISTA and AOSA to provide harmonised methods is essential. AOSA is already surveying laboratories with the aim of removing some of the multiple options in the germination methods and increase uniformity. If the use of in-house germination methods that are not detailed in the rules is to be allowed, then just having one standard reference method in the rules would help facilitate the validation of in-house methods ‘as good as’ the standard reference method. In addition, I believe that ISTA and AOSA should use the same pure seed definitions and definitions

for inert matter. In the future, should we also use just one shared set of rules that includes both sampling and testing? This would not happen quickly, since to achieve this, national and European legislation would require changes. For example, in North America maximum seed lot sizes and sampling methodologies would need to be included in the shared Rules.

Seed testing associations such as AOSA and ISTA have an important role to maintain and promote seed testing, as they currently do; in the future, perhaps with closer collaboration. A main aim should be to promote the use of established methods and applicable new technology, and train analysts and laboratories to help achieve uniformity and harmonisation in seed testing. To ensure this, I believe that expertise in government laboratories needs to be sustained to help maintain seed quality assurance systems and protect countries from invasive weed species. However, the delivery of this work should be a private-public partnership using the expertise of trained seed samplers and analysts.

There is also the need to fully utilise the limited number of researchers active in seed science, in order to develop new technologies relevant to seed testing. Perhaps the associations can find better ways to provide financial support to encourage new members from both research and testing laboratories; indeed, ISTA, AOSA and the Society of Commercial Seed Technologists (SCST) are already trying to do this by providing financial support to technical committees and specific research projects. This is becoming even more important as finances become even tighter worldwide, but especially in government laboratories. For the successful continuance of seed quality assurance systems, the associations need to ensure continued support for robust accreditation systems for analysts and laboratories supported by independent proficiency test (PT) provisions.

The international seed trade (e.g. trade organisations and private companies) and private seed laboratories have a role to play by their continued support of the associations. By allowing their staff to actively participate in the work of the different associations around the world, providing seed lots for research, and sponsoring

workshops and other initiatives, the seed industry already plays a role, hopefully that will continue. Indeed, ISTA wants stakeholder input and now has an Industry Membership category, and has recently established both Seed Science and Industry Advisory Groups to help provide input into ISTA strategic developments. The seed trade could consider more direct funding of research projects associated with traditional seed testing and provide financial support to training seed analysts. Perhaps the industry could fund university programmes in seed technology and testing. Industry could also help raise the profile and compensation/rewards for seed analysts. AOSA/SCST have a Consolidated Examination system for seed analysis in the USA, many other countries also have their own seed analyst examination systems/programmes, but international standardisation or recognition is lacking in seed analyst training.

Future needs

I had excellent on-the-job training when I started tree seed testing in the UK, making use of existing staff with many years of experience. We have all heard about the demographic shift, and the declining numbers of new people coming into seed testing is a concern. Seed analysts have always been at the lower end of the pay scale and rewards. In addition, people often move away from routine seed testing into other roles.

The ability to visually recognise, identify and name the seeds of 200 to 400 crop and weed species from memory is a unique skill. It takes about three years or more of on-the-job training to acquire such a skill in an established laboratory. Development of computer aids for purity and seed identification, such as Lucid keys and image libraries, is already helping analysts do their job more effectively. It is not only the purity analysts that need to develop their skills. Samplers need to follow standardised methods to provide representative samples. Germination analysts are required to decide which seedlings are normal and which are abnormal. Then there are the other tests for diseases, viability, vigour, moisture and other attributes that all need to be mastered. Seed analysis is an acquired

practical skill in addition to the educational background which people have.

I estimate that there are no more than 10 000 seed analysts worldwide. Although this is not the most common occupation, it is essential to the seed industry and current system of seed certification. The numbers of researchers active in seed science is an even smaller number. (Note: an exact figure for seed analysts and researchers worldwide is difficult to establish; perhaps that is something that seed associations could facilitate in the future).

A problem for seed researchers is that funding is very closely focused on molecular-based research, which does not often have a clear link to applied work such as seed testing. Research and technological advances may one day make it possible to load a seed sample into a single machine, and flip a switch to obtain complete germination and purity results within minutes. My background in seed physiology and training in seed testing predispose me to want to see and feel the results. However, if future researchers can provide me with valid results that correspond to 'real life,' I will use those, and so will others in the seed industry. But how far off in the future is this? In the meantime, I believe that the seed industry will still need people, and the proper training and education is the key to survival for seed testing. I hope others will still want to be a part of this work in the future. The continued use of competent people along with regular PT and accreditation of laboratories will be essential for uniformity and reliability in seed testing.

Governments currently have a clear role in regulating the seed industry. Some governments choose to regulate via alternative service delivery mechanisms, and use the government laboratories to provide conformity verification by monitoring private laboratories and the seed industry. I believe that alternative service delivery by the private testing laboratories is essential for the seed industry, but I also believe that there is still an important role for government laboratories. However, some European countries have closed their government (official) seed testing laboratories. If seed testing in governmental laboratories is abandoned, who will provide the independent audit of abilities in laboratories, PT and

enforcement monitoring of seed at the point of sale? This could be an expanded role for the seed testing associations. ISTA already provides a laboratory accreditation and PT service for members, while AOSA and SCST certify and accredit analysts. Perhaps third-party independent laboratories could also provide the enforcement testing and/or PT provision?

Another question is who will pay for the work of monitoring and training of analysts and laboratories if the government laboratories are no longer involved? Perhaps it is the users who should pay, but how can this money be made available? An industry levy could work, but at what level? Should the seed breeders, growers, exporters, seed buyers, bread makers, brewers and other end-users all contribute to maintaining the seed certification system? Would industry be prepared to pay a small amount more today for something it gets for 'free', but that could ensure the industry's future? Should royalties paid to plant breeders be 'shared' with seed testing associations to provide future seed analyst training?

What next?

- How to fund the education, training and long term provision of seed analysts?
- Could a joint initiative with the seed associations, seed trade organisations and governments provide a way to ensure the continued survival of seed analysts and scientists in a professional career?
- How to raise the profile of all those involved in the whole cycle of breeding, growing, testing and certifying seed and grain?
- Those involved in seed science and testing know how important seed and seed testing are, but do others?
- What can we all do to change the profile of seed testing?
- Could regular Tweets and blogs help spread the seed message?

I certainly do not have answers to all the questions I pose, but I do hope to have given some ideas to help start a continued debate. I encourage others to get involved with the work of ISTA and AOSA and help provide some future solutions. Feel free to e-mail me at steve.jones@inspection.gc.ca.

Acknowledgments

I thank all the people who have helped me over the years while working in the UK and Canada, and for ISTA. Special thanks to Alison Powell (University of Aberdeen, UK) and my CFIA colleagues Janine Maruschak, Willy Drost and Michael Scheffel for commenting on the original article. I also thank Riad Baalbaki and Susan Alvarez for suggesting the original article, and then providing very useful comments as editors of Seed Technology.

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NASSTEC: a European project to promote native seeds for grassland restoration

Costantino Bonomi

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What is NASSTEC?

The Native Seed Science, Technology and Conservation Initial Training Network (NASSTEC) is a Marie Curie Initial Training Network of the European Union, running for four years from April 2014 to March 2018, with funding of 3.4 million Euros. The project will train 11 PhD students at the initial stage of their research careers in native seed science, conservation and use. Like all Marie Curie Actions, NASSTEC is designed to promote the training of researchers and their

transnational mobility, making research an attractive career for people in the early stage of their academic training. There are currently 11 NASSTEC researchers from seven countries: Canada, Croatia, Italy, Sri Lanka, Spain, Portugal and the United States. According to the Marie Curie mobility rules, they are based in a country other than the one where they spent the last three years of their activity. One more person will join the network in December 2015 as an experienced researcher. This position will be advertised in mid-2015.

NASSTEC – the partnership

NASSTEC involves seven full partners, where the various researchers in the network are based. These include four academic institutions: the Trento Science

Museum MUSE, Trento, Italy as coordinator, the Royal Botanic Gardens, Kew, England, Pavia University, Italy, and the James Hutton Institute, Scotland. Furthermore, three producers of native seed are included: two small companies, Scotia Seeds in Scotland and Semillas Silvestres in Spain, and Syngenta Seeds in the Netherlands. A further seven institutions are associated with the project, and contribute to the training and research of the students. These include Kings Park, Perth, Australia, the National Trust for Scotland, the European Research Agency, Rome, a local public administration in Trento, two small companies of tourist and research services in Spain and in Scotland, and the Jardín Botánico Atlántico, Gijón, Spain.



Alpine meadow in the Dolomites showing native species.



The PhD students participating in the NASSTEC programme with their organizations and projects (see text for details).

Top row, left to right:

Stephanie Frischie (US), Semillas Silvestres, 2A
 Antonio da Costa Teiveira (Portugal), RBG Kew, 7B
 Cristina Blandino (Italy), RBG Kew, 9C
 Emma Ladouceur (Canada), MUSE, 1A
 Maria Tudela Isanta (Spain), Pavia University, 4B
 Matias Hernandez Gonzalez (Spain), Semillas Silvestres, 5B

Bottom row, left to right:

Erica Della Jacovo (Italy), James Hutton Institute, 3A
 Holly Abbandonato (Canada), MUSE, 11C
 Laura Lopez del Egido (Spain), Syngenta, 10C
 Malaka Madhuranga Wijayasinghe (Sri Lanka), Pavia University, 6B
 Maria Marin (Croatia), Scotia Seeds, 8C

NASSTEC – the need

Habitat loss and degradation caused by human activity has led to an increased demand of native seeds for restoration purposes that in many countries is not met by an adequate supply. Large-scale native seed production is now a significant challenge for native seed companies, and one of the main constraints for effective habitat restoration. In Europe, native seeds are highly needed for a wide range of grassland restoration activities such as those involved in roadworks, ski slopes, new building sites and quarries. In addition, the use of native seeds is mandatory in all Natura 2000 sites according to the habitat directive, yet native seeds are not widely available in Europe. The market is underdeveloped, and only small-scale operations are active, detached from the academic sector, very often lacking baseline knowledge of key species and their biology and seed ecology. Restoration is commonly carried out using non-native plant material in the absence of seed quality protocols, policies and adequate training for restoration practitioners, thus introducing potentially

invasive species and mixing up the ecotypes of widely distributed species.

NASSTEC – the aim

NASSTEC will focus its efforts in the next four years in promoting the use of native seeds for grassland restoration, building the capacity in local companies for large-scale native seed production and lobbying the relevant stakeholders to widely promote the use of native seeds in land restoration and reclamation activities, both in the public and private sector. NASSTEC's ambitious plan wants to create the conditions for a win-win situation, providing ecosystem services, fighting soil erosion, generating income and conserving biodiversity with native seeds.

NASSTEC plans to meet these needs by delivering well-trained human resources to support industries and develop new companies, to bridge academia and industry by delivering key information where needed with project manuals, guidelines and toolkits, and to link developed markets in the USA and Australia with Europe, in order

to stimulate the largely unexpressed potential of the European market.

Three specific pilot projects for grassland restoration will be carried out in four EU biogeographical regions (Alpine, Atlantic, Continental and Mediterranean) to demonstrate the potential for grassland restoration, e.g. in ski slopes in the Alps, in major roadwork development in the Scottish highlands, and on arable fields in the Mediterranean.

NASSTEC – the science

NASSTEC plans to interconnect the public and private sector through the establishment of a multidisciplinary European doctoral school, with the aim of integrating knowledge in plant ecology, molecular biology, taxonomy, conservation, seed biology, breeding and horticulture. The scientific programme of NASSTEC is articulated in three subprogrammes mimicking the plant reintroduction cycles and the relevant steps necessary for successful habitat restoration.

Subprogramme A covers *in situ* seed sampling, and includes the following three PhD projects:

- 1A (based at MUSE): A biogeographical approach to species selection for the Alpine and Atlantic region;
- 2A (based at Semillas Silvestres): Selection of high-quality grasses for the Mediterranean and Continental bioregion;
- 3A (based at the James Hutton Institute): Methods for seed and seedling phenomics.

Subprogramme B covers seed biology characterization, and includes four PhD projects:

- 4B (based at Pavia University): Biogeographical aspects of seed dormancy;
- 5B (based at Semillas Silvestres): Propagation protocols for the restoration of grassland habitat in Europe;
- 6B (based at Pavia University): Seed longevity in storage;
- 7B (based at RBG Kew): Life history traits in contrasting environments – intra-species variation in stress tolerance.

Subprogramme C covers production and deployment of seed, and includes four PhD projects and one post-doc:

- 8C (based at Scotia Seeds): Improving seed quality in large-scale production;
- 9C (based at RBG Kew): Propagation and seed multiplication protocols for herbaceous flora;
- 10C: (based at Syngenta): Seed pretreatments of native species for optimal establishment, for use in *in-situ* restoration;
- 11C (based at MUSE): Certification of seed quality and provenance;
- 12C (the post-doc, based at Scotia Seeds): Transfer of NASSTEC knowledge to European seed producers.

NASSTEC – the training

From an academic point of view, all students are registered in a cross-cutting doctoral programme managed by the University of Pavia, and upon successful completion of the training programme they will each be awarded a PhD qualification in Earth and Environmental Science. The training programme includes both host-based training and network training, delivering a balanced scheme of exchange visits and secondments, a rich programme of events, news of network achievements and

research information. The network training events include two summer schools, providing training in seed collecting (in Asturias, Spain) and in seed germination and processing. Three specialist workshops will deliver training in molecular diversity at the James Hutton Institute in Dundee, Scotland; in intellectual property rights, patenting and grant writing at Syngenta, the Netherlands; and in education and outreach at MUSE in Trento, Italy. The outputs of the project will be presented in the final conference to be held at Kew in summer 2017, provisionally entitled “Native seeds for environmental mitigation”. The training is completed by three one-month secondments to other network partners, three annual network meetings, various exchange visits and an education and outreach programme.

NASSTEC – the outreach programme

- It is particularly important:
- to reach out to the wider public and society in general;
 - to raise awareness of the importance of native seeds;
 - to grant appropriate ecosystem services and biodiversity conservation and
 - gain support of the civil society of the research being carried out in NASSTEC,
 - demonstrating it is highly relevant for human wellbeing and environmental conservation,
 - and that meet the request for Responsible Research and Innovation (RRI).

Contact with local schools and community organizations, newsletters, press releases and the use of social media will encourage practical activities based around native seeds, such as seed collecting days, seed sowing events and display gardens of native plants. Globally, NASSTEC researchers will carry out a selection of the following outreach activities:

- Designing Inquiry Based Science Education (IBSE) activities for schools, building on the INQUIRE project, selecting, adapting and using established resources for environmental education in schools and botanic gardens relevant for native seeds and plants, both in formal and informal settings.
- Taking part in the local editions of the Researcher’s Night, usually held in

September, contributing with a stand to illustrate the benefits of grassland restoration using native seeds.

- Playing the role of Marie Curie Ambassadors, carrying out day visits to local schools, introducing the project and demonstrating its benefit to society, using one of the IBSE activities designed earlier. These visits might include seed collecting days and seed sowing events that will also involve pupils’ parents, to raise awareness of the importance of native seeds and of the research being carried out in NASSTEC.
- Taking part in the Fame lab contest (www.famelab.org) to communicate NASSTEC research to the wider public. This contest aims to select the brightest science communicator in each partner country, eventually reaching the European finals.
- Hosting two-week schoolteacher placements in each partner lab during the summer break, offering local teachers the opportunity to get to know the research being carried out in partner institutions, raising awareness of the use of native flora among teachers and educators.
- Developing native flower-bed displays in five key cities of the partner countries, celebrating NASSTEC with native seeds and wildflower grassland displays, showcasing the relevance of native biodiverse grassland and its garden value.

NASSTEC – the long-term impact

NASSTEC ambitiously plans to make a long-term impact on plant conservation in Europe, increasing the competitiveness of the human capital, ensuring that it is directed towards the development of a sustainable and dynamic European native seed industry capable of supplying the native seeds required for sustainable grassland restoration. The ultimate goal of NASSTEC is to stimulate a wider use of native seeds in grassland restoration at the European scale.

For further news and updates on the development of the project and its outputs, check out www.nasstec.eu.

NASSTEC is funded by the European Union under FP7. ■

ISTA Annual Meeting 2015 Montevideo, Uruguay 15–18 June 2015

Overview

Tuesday–Friday 9–12 June	ISTA Workshop on Seed Sampling and Quality Assurance in Seed Sampling
Tuesday–Friday 9–12 June	ISTA Workshop in Tetrazolium Testing and Vigour by Tetrazolium in <i>Glycine max</i>
Monday 15 June	Opening ceremony
Monday 15 June	ISTA Seminar: Molecular tools for seed quality and seed health
Tuesday 16 June	City tour of Montevideo
Tuesday–Wednesday 16–17 June	Presentation of ISTA's technical work
Wednesday 17 June	Official Dinner
Thursday 18 June	ISTA Ordinary General Meeting
Friday 19 June	Post-meeting tours
Friday–Saturday 19–20 June	ISTA Workshop on Moisture Determination



Invitation by Ing. Agr. Tabaré Aguerre Minister for Livestock, Agriculture and Fisheries of the República Oriental del Uruguay

It is an honor to invite you to the International Seed Testing Association's 2015 Annual Meeting to be held in Montevideo, Uruguay.

Located at the heart of the southern cone of America, Uruguay is located in the middle of one of the most fertile regions of the world. Agriculture, forestry and cattle raising promote the economic growth of the country. Innovation and sustainable intensification of agriculture are supported

by public policies for soil use and environmental preservation.

The National Seeds Institute (INASE) is the host of the 2015 ISTA Annual Meeting. The Institute was founded in 1997 to facilitate the trade of best quality seed in Uruguay. The INASE oversees more than 40 seed laboratories in Uruguay. The National Agricultural Research Institute (INIA) will host the workshops that complement the Annual Meeting, providing

opportunities for practical experience of ISTA methods.

Seed scientists, specialists and officials from all over the world are invited to come to the 2015 ISTA Annual Meeting in Montevideo, Uruguay. It will be an excellent opportunity for exchange of knowledge and information related to the progress of seed science.

Hope to see you there.

Ing. Agr. Tabaré Aguerre

Invitation by the Organizing Committee of the ISTA Annual Meeting 2015

The International Seed Testing Association (ISTA) invites you to its Annual Meeting to be held in Montevideo, Uruguay from 15–18 June 2015.

The National Seed Institute (INASE) is delighted to be hosting the next ISTA Annual Meeting and would like to cordially invite you to Montevideo.

The ISTA Annual Meeting provides an excellent 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.

The Annual Meeting also provides a chance for in-depth discussions about topics of interest to the ISTA community. For the 2015 meeting, a one-and-a-half day

Seminar will be held on “Molecular tools applied to seed quality and seed health”. This seminar will be a opportunity to receive the latest research and development.

Moreover, we are delighted to announce that two ISTA workshops will take place in Colonia: a Workshop on Seed Sampling and Quality Assurance in Seed Sampling and a ISTA Workshop in Tetrazolium Testing and Vigour by Tetrazolium in *Glycine max*. ■

ISTA Annual Meeting 2015
Montevideo, Uruguay, 15–18 June 2015
Online registration now open: www.seedtest.org/AM15

Programme

Venue: Radisson Montevideo Victoria Plaza,
Plaza Independencia, Montevideo

Sunday, 14 June 2015

12:00–18:00 Registration of participants at conference venue

19:00 **Welcome reception**

Monday, 15 June 2015

08:00–18:00 Registration of participants at conference venue

08:30–18:00 **ISTA Seminar: Molecular tools for seed quality and seed health**

08:30–08:45 **Opening**

08:45–10:00 **1. Varietal identification**

To be announced Ana Laura Vicario, INASE, Argentina

Molecular techniques for certification of genetic purity and varietal identification Elisa Vieriria, Embrapa, Brazil

10:00–10:15 Coffee

10:15–12:45 **2. GMO**

New Rules Chapter and Handbook René Mathis, GEVES, France

Tentative title: Detection, identification and Quantification of OGM? Nilson Castanheira, Fiscal Federal Agropecuário na Ministerio da Agricultura, Brazil

Tentative title: The European Network of GMO Laboratories (ENGL) – experience in GMO detection Marco Mazzara, European Commission Joint Research Centre

Future challenges: Evolution of GM crop development – from the 1990s to today; pipeline products; next generation GMOs and impacts on seed testing Raymond Shillito, Bayer Crop-Science, USA

12.45–13:45 Lunch

13:45–14:30 **3. Statistics**

A tour of statistical tools used in GMO testing: Seedcalc, tools for computing Measurement Uncertainty, tools using Bayesian Statistics Jean-Louis Laffont, Dupont Pioneer, France

14:30–15:30 **4. Seed health**

Pests and seed health testing. General introduction on detection of bacteria, viruses, fungi and nematodes in seeds Valerie Grimault, GEVES, France

15:30–15:45 Coffee

15:45–17:30 **DNA technologies to combat seed pathogens** Marcel Toonen, Naktuinbouw, Netherlands

Production of healthy seed, seed health approach Siham Assad, ICARDA, Lebanon

Major seed-borne diseases of wheat in Northern Europe: impact and control Valerie Cockerell, SASA, United Kingdom

What makes *Fusarium* accumulating more or less mycotoxins in cereals grains? Christian Barreau, INRA, France

17:30–18:00 **Summary of Seminar**

Tuesday, 16 June 2015

08:00–18:00 Registration of participants at conference venue

08:30–18:30 **Presentations of ISTA's technical work and meetings of ISTA Technical Committees**

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

08:30–10:00 Purity Committee (Chair: Jane Taylor)
Germination Committee (Chair: Sylvie Ducournau)
Moisture Committee (Chair: Jette Nydam)

10:00–10:30 Coffee break

10:30–12:30 Tetrazolium Committee (Chair: Stefanie Krämer)
Seed Vigour Committee (Chair: Alison Powell)
Seed Health Committee (Chair: Valérie Grimault)
Variety Committee (Chair: Ana Laura Vicario)

12:30–13:30 Lunch break

13:30–14:00 GMO Committee (Chair: Cheryl Dollard)

14:00–15:00 Flower Seed Committee (Chair: Rita Zecchinelli)
Forest Tree & Shrub Seed Committee (Chair: Fabio Gorian)

15:00–15:30 SST Editorial Board (Chair: Fiona Hay)

15:30–16:00 Coffee break and official photo session

16:00–18:30 Individual ISTA Technical Committee meetings

Wednesday, 17 June 2015

08:00–17:00 Presentations of ISTA's technical work (cont.)

- 08:30 Opening by the ISTA President, Joël Léchappé
- 08:30–10:00 Bulking and Sampling Committee (Chair: Eddie Goldschagg)
Statistics Committee (Chair: Jean-Louis Laffont)
Nomenclature Committee (Chair: John Wiersema)

10:00–10:30 Coffee break

10:30–11:30 Seed Storage Committee (Chair: Hugh Pritchard)
Advanced Technologies Committee (Chair: Bert van Duijn)

11:30–12:30 Proficiency Test Committee (Chair: Günter Müller)
Laboratory Accreditation and Quality Assurance Programme (Chair: Rita Zecchinelli)

12:40–13:30 Lunch break

13:30–15:30 Rules Committee (Chair: Steve Jones)

15:30–16:00 Coffee break

16:00–18:00 Rules Committee (cont.)(Chair: Steve Jones)

19:00 Official Dinner

Thursday, 18 June 2015

09:00–17:30 ISTA Ordinary General Meeting

- 09:00–10:00 Welcome by the ISTA President, Joël Léchappé
- Presentation by Ing. Agr. Tabaré Aguerre, Minister for Livestock, Agriculture and Fisheries of the República Oriental del Uruguay**
- Presentation by Paul Civetta, director of the National Association of Seed Producers of Uruguay (ANAPROSE), on the Uruguayan seed business**

10:00–10:30 Coffee break

Agenda

- 10:30–12:30
1. Call to order
 2. President's address
 3. Roll call of Designated Members entitled to vote
 4. Comments about the minutes of the previous General Meeting
 5. Report of the Executive Committee
 6. Report of the Secretary General
- 12:30–13:30 Lunch break
- 13:30–15:00
7. Fixation of annual subscriptions
 8. Changes to the Articles
 9. Consideration and adoption of the proposed Rules changes 2015
- 15:00–15:30 Coffee break
- 15:30–17:30
10. Consideration and adoption of reports
 11. Announcement of the places and dates of the next Ordinary General Meetings
 12. Any other business raised by a Member, of which notice in writing has been received by the Secretary General at least two months prior to the date of the General Meeting
 13. Any other business raised by consent of the Executive Committee
 14. President's closing address
 15. Adjournment

Preparatory documents for Ordinary General Meeting: see page 18

Venue

On central Independence Square, the Radisson Montevideo Victoria Plaza offers stunning views of Harbor Bay.

The pleasant rooms at the hotel include air conditioning, Wi-Fi, a bathtub and coffee maker. Every spacious suite features a separate lounge area and stunning views of the Rio de la Plata.

Guests at the Radisson Victoria can benefit from a variety of dining options, including a restaurant serving Uruguayan specialties, and Bar del Puente with its selection of cocktails. Additional facilities include gym, casino, pool and spa.

The hotel offers over 2500 m² of space for meetings and receptions with capacity for 2500 guests, equipped with advanced technology (audio, video, lighting, IT). Sixteen meeting rooms are available, with capacity

for up to 1100 persons and spacious foyers for services like coffee breaks and cocktails, as well as exhibitions and displays set up concurrently with conferences.

About Montevideo

Montevideo, the capital of Uruguay, harmoniously combines modernity and tradition. Ancient architectural treasures standing alongside Art Nouveau and Art Deco give the city a unique identity.

The capital's Rambla (waterfront promenade) provides access to over 30 kilometers of coastline. It is one of the main attractions of Montevideo and an unforgettable stroll for its inhabitants who frequently visit it.

Montevideo also has an extensive cultural scene. Its theatrical productions are

remarkably extensive and varied, and include classic, modern and alternative shows.

Climate

The climate in Uruguay is temperate and humid, with warm summers, moderate temperature in winter (10–15 °C) and fairly uniform rainfall throughout the year.

Flight information

Flights arrive at Montevideo through connections from South and Central America, the USA and Europe. The new Carrasco International Airport was opened in 2009 and is considered one of the most modern and attractive in the world. The taxi fare from the airport to Montevideo city centre will cost approximately USD 30.

Registration fees (online registration at www.seedtest.org/AM15)

Periods	Events	Registration (1 March–15 May 2015)
ISTA Members		
15–18 June	Annual Meeting incl. Seminar	USD 1290
15–16 June	Seminar only	USD 380
Non-members		
15–18 June	Annual Meeting incl. Seminar	USD 1930
15–16 June	Seminar only	USD 570
Students		
15–16 June	Seminar only	USD 100
Accompanying persons*		
15–18 June	Social events, lunches etc. only	USD 450

*This category is applicable only for the spouse, companion and/or children of a delegate or for one additional person at the exhibition booth. 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, cocktails and Official Dinner.



Local time

Local time in Montevideo is UTC (GMT) –3 hours.

Visas

Certain foreign visitors need to follow some special immigration procedures. Citizens of bordering countries can enter with their national ID document. Nevertheless, in all cases it is recommended to consult diplomatic representatives.

For more information visit the National Immigration Department web site:

<http://www.dnm.minterior.gub.uy/visas.php>

If an official invitation letter is required, please contact Vanesa Sosa at:
vsosa@inase.org.uy

VAT and service charges

Value-added tax (VAT) is 22%. VAT is usually included in quoted prices. Certain shops are authorized to refund the tax.

Credit cards

International credit cards are widely used in Uruguay. Major credit cards (Visa, MasterCard, American Express etc.) are accepted in most establishments. Most ATMs accept international credit/bank cards.

Accommodation

Hotels	Class	Single room	Double room	Distance to venue by taxi	Taxi fare	Website
Radisson (venue)	★★★★★	USD 155*	USD 175 *	–	–	http://www.radisson.com/montevideo-hotel-uy-11100/urumont To make your reservation at Hotel Radisson please write to reservas@radisson.com.uy When booking at the hotel refer to the ISTA 2015 event to access preferential prices
Sheraton	★★★★★	USD 185*	USD 195 *	15 minutes	USD 10*	http://deals.sheraton.com/Sheraton-Montevideo-Hotel-1238/special-offers?PS=LGEN_AA_DNAD_CGGL_TPRP
Four Points	★★★★	USD 115*	USD 125 *	10 minutes	USD 10*	http://deals.fourpoints.com/Four-Points-Montevideo-Hotel-1576/special-offers?PS=LGEN_AA_DNAD_CGGL_TPRP
NH Columbia	★★★★	USD 100*	USD 100 *	10 minutes	USD 10*	http://www.nh-hotels.es/hotel/nh-montevideo-columbia
Tryp Montevideo	★★★	USD 108*	USD 108 *	15 minutes	USD 10*	http://www.tryphotels.com/en/montevideo-hotels-city-centre-4-star-tryp-montevideo.html
Regency Golf	★★★★	USD 90*		15 minutes	USD 10*	http://www.regencygolf.com.uy/

*Estimated

Currency and exchange

The currency in Uruguay is the Uruguayan peso (UY\$) and the exchange rate is approximately € 1 to UY\$ 28, or USD 1 to UY\$ 25.

Exhibitors

Reach seed professionals from laboratories and organisations worldwide. Only a limited number of exhibition stands are available.

The exhibitor registration fee includes one exhibitor for the duration of the Annual Meeting (15–18 June, 2015) as well as welcome reception, coffees, lunches and official dinner.

Sponsors

There are also possibilities to sponsor the ISTA Annual Meeting 2015 with a variety of sponsoring packages to choose from.

For detailed information about sponsorship, please contact Andrea Puppi:
ista2015@personas.com.uy



Hereford beef cattle.

Social programme

City tour of Montevideo

A unique historical-cultural tour for accompanying persons, round the old part of Montevideo, visiting its emblematic buildings. You will be able to visit the Independencia and Zabala squares, the elegant neighbourhoods of Pocitos and Carrasco and the Rambla, the waterfront promenade that provides access to over 30 km of coastline. It is one of the main attractions

of Uruguay's capital, and an unforgettable stroll for its inhabitants who frequently visit it.

Date: June 16

Duration: 3 hours (8:30–11:30,
 14:00–17:00)

Languages: English, Spanish and Portuguese

Lunch: not included

Cost: USD 35

Minimum: 25 people



Mate, the popular Uruguayan beverage, in a traditional calabash gourd. (Photo: Jorge Alfonso Hernández)



A yerba mate shrub (*Ilex paraguariensis*), the source of mate leaves.



Rice fields in Eastern Uruguay. (Photo: Neil Palmer, CIAT)

Post-meeting tour: Colonia del Sacramento

Declared a World Heritage Site by UNESCO in 1995, Colonia del Sacramento invites you to travel back in time and arrive at a Lusitanian town. Founded by the Portuguese and disputed for years by the crowns of both Spain and Portugal, its magical history remains intact. It has charming cobbled streets lit by its traditional and characteristic yellow lanterns, on which there are countless historical and cultural attractions to discover and enjoy.

The Calle de los Suspiros (Street of Sighs), the Casa del Virrey, the Iglesia Matriz, the Plaza de Toros, the Puerta de la Ciudadela (City Gate) are just some of the beautiful examples of architecture in this spectacular city. Just 2 hours from Montevideo or 6 km from the Laguna de los Patos International Airport, Colonia is ideally located, whatever the place of departure.

Date: June 19

Duration: 10 hours (8:30–18:30)

Languages: English, Spanish and Portuguese

Lunch: not included

Cost: USD 82

Minimum: 25 people

Post-meeting tour: Punta del Este

Punta del Este is internationally recognized as one of the top resorts in the Americas and the most exclusive in the region. It is located in the department of Maldonado, just an hour and a half from Montevideo.

With over 20 kilometers of coastline and high hills overlooking the sea, the resort also offers charming places and landscapes for those who come in search of absolute peace and tranquility.

The meeting point of its two most famous beaches, Playa Mansa and Playa Brava, marks the end of the Río de la Plata and the start of the Atlantic Ocean. The resort has grown westward forming Punta Ballena and eastward creating La Barra and José Ignacio.

Date: June 19

Duration: 10 hours (8:30–18:30)

Languages: English, Spanish and Portuguese

Lunch: not included

Cost: USD 54

Minimum: 25 people

ISTA Annual Meeting 2015 Montevideo, Uruguay, 15–18 June
Online registration now open: www.seedtest.org/AM15

Preparatory documents for the Ordinary General Meeting

The following documents are submitted to the ISTA Ordinary General Meeting 2015 for information and discussion and/or acceptance by the nominated ISTA Designated Members voting on behalf of their respective Governments:

- OGM15-01 Agenda for the Ordinary Meeting 2015 [information document]
- OGM15-02 Minutes of the Ordinary Meeting 2014 [information document]
- OGM15-03 Activity Report of the ISTA Committees 2014 [voting document]
- OGM15-04 Proposal for the Membership Fees 2016 [voting document]
- OGM15-05 Rules Proposals for the International Rules for Seed Testing 2016 Edition [voting document]
- OGM15-06 Method Validation Reports on Rules Proposals for the International Rules for Seed Testing 2016 Edition [supporting document to voting document OGM15-05]
- OGM15-08 Proposals for Changes to the ISTA Rules of Order [voting document]

Please note that no 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/OGM15 ■

Proposal to change the Rules of Order

A change to the Rules of Order of the International Seed Testing Association (ISTA) is being proposed for voting at the 2015 Ordinary General Meeting.

The procedure for voting within the Association is outlined in the Rules of Order for Ordinary General Meetings. This states that voting is 'by a show of voting cards.' The change to electronic voting therefore requires a change to the Rules of Order. The ECOM is proposing a wording change to the Rules of Order that will enable both electronic keypad voting and cards to be used for voting at the ISTA Ordinary General Meeting. The reason for retaining the option of cards is to ensure that voting is still possible in the event of the unavailability of the electronic keypads.

Proposal

The following change to the Rules of Order is proposed to the membership (new text in blue and underlined):

Proposed

- 4) The voting delegate will vote on the motion:
The vote on the motion is taken by using an electronic keypad or in the event of the unavailability of keypads by a show of the voting cards. Electronic keypads are identified to the user (country). In putting ...

The document has been posted on the ISTA web site at:
www.seedtest.org/OGM15 ■

ISTA Annual Meeting 2015 Montevideo, Uruguay, 15–18 June
Online registration now open: www.seedtest.org/AM15

Proposed changes to the *International Rules for Seed Testing* 2016 Edition

Jonathan Taylor
 ISTA Publications Unit

ISTA Secretariat
 8303 Bassersdorf, Switzerland
 jonathan.taylor@ista.ch

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. See also the Method Validation Reports on pp. 26 and 31, and on the ISTA web site at

www.seedtest.org/OGM15.

Among the proposed changes are the following:

Chapter 1: Certificates

- Clarification about issuance of certificates and other details related to duplicate and provisional ISTA Certificates (1.2, 1.3, 1.4)
- Clarification what should be entered under 'Seal of lot' (1.4.2)

Chapter 2: Sampling

- Changes in 2.8 Tables for lot size and sample sizes
- Removal of 'herbage', where differentiation between 'herbage' and 'amenity' (turf) is no longer appropriate (2.5.4.1, 17.6, 17.7)

Chapter 3: The purity analysis

- Revision to Pure Seed Definition 4 re: *Helianthus* seeds with fused pericarps
- Revision to 3.5.2 on retention of separated components (other seeds only)

Chapter 4: Determination of other seeds by number

- Amendment of definitions for Other seeds determinations (4.2, 4.5.1)

Chapter 5: The germination test

- Germination tests allowed with only 200 seeds, but only for Blue International Seed Sample Certificates (5.6.1)
- Clarifications on required actions when counting errors occur (5.6.1)
- Clarification on reporting germination when disinfection is applied (5.6.3.4)
- Omitting the first count when germination tests are carried out in Organic Growing Media (5.6.4)
- Amendment to the process for retesting (5.7, 5.9)

Chapter 7: Seed health testing

- Correction to text in existing seed health method 7-007
- Modification to existing seed health method 7-022 with replacement of images
- Additional grow-out method for existing seed health method 7-026
- Replacement and/or addition of photographs in seed health testing methods 7-001a, b; 7-004; 7-010; 7-011; 7-012

Chapter 9: Moisture determination

- Changes to methods for cutting seeds (9.1.5.2, 9.1.5.5)
- Changes to methods for cutting large tree seeds for moisture testing (9.1.5.5)

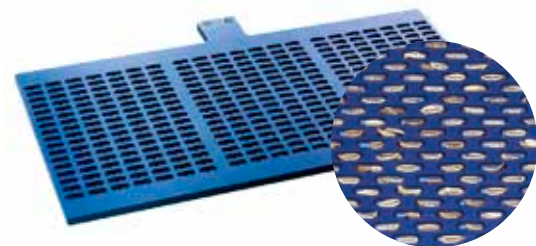
Chapter 10: Weight determination

- Changes to improve the clarity of text, including consequential changes to Chapters 1 and 18.

Chapter 11: Testing coated seeds

- Clarification of procedures for seed pellets (11.5)

Image based seed analysis & sorting equipment



Technologies

- Single seed precise
- X-Ray
- Fluorescence
- Hyper-spectral
- Automatic sowing
- Advanced image analysis



Applications

- Seed quality enhancement
- Selection for breeding
- Phenotype selection
- Seed purity
- Capacity: 1.. 200 seeds/sec
- High throughput screening

31st ISTA Congress Seed Symposium

Tallinn, Estonia, 15–17 June 2016

Dr Laura Bowden

Symposium Convenor; Member, ISTA Moisture Committee; Member, Seed Science and Technology Editorial Board

Official Seed Testing Station for Scotland
Science and Advice for Scottish Agriculture
(SASA)
Edinburgh, EH12 9FJ, UK
Laura.Bowden@sasa.gsi.gov.uk

1st call for papers

This is the first invitation to people interested in presenting a paper during the Seed Symposium of the 31st ISTA Congress under the theme:

Progress in seed testing and seed quality improvement through science and technology

The symposium will be made up of five oral sessions and two poster sessions, covering the same topics. Each oral session will be chaired by a lead speaker who is well known in the field of seed science and technology.

The symposium will bring together seed analysts, technologists, researchers and managers from universities, research institutes, government and the seed trade to discuss all aspects of seed quality. The symposium will provide a forum for the discussion of recent advances in seed science and technology as well as an opportunity for the exchange of ideas and information.

Intending participants are encouraged to submit oral and poster papers topics under the above theme. The research reported in offered papers can cover both the scientific basis of aspects of seed quality and its technological application in seed testing. In all sessions we welcome papers on established and novel seed testing methods, and on tropical and temperate crop species, wild species, flowers, trees and shrubs, including species with potential for use in plant breeding and in habitat regeneration.

A book of abstracts containing invited and selected (oral and poster) presentations will be available to all Symposium participants.

Submission of papers

Offers of papers should be submitted **online only** in the form of an abstract in English, of 400 words maximum. Papers will be presented orally and in poster form, both forms having equal status. Papers will be selected for presentation by the Symposium convenor and a small scientific committee. As the number of oral presentations will be limited by time constraints, oral presentation of your paper may not be possible and you may be asked to present your paper as a poster.

Timeline

23 October 2015

Deadline for submission of all papers. Authors of papers considered for oral presentation will be contacted for further information on experimental results, additional to the abstract.

18 December 2015

Authors informed whether papers have been accepted for oral or poster presentation.

Authors of papers not accepted for oral presentation may be invited to present their work as a poster.

12 February 2016

Deadline for payment of registration fee by authors of accepted oral papers. **If the presenter of an oral paper has not registered, the paper will be replaced in the programme.**

11 March 2016

Deadline for payment of registration fee by authors of accepted poster papers. **If none of the poster authors has registered by this time, it will not be possible to present the poster and the abstract will not be published.**

Funding

Authors of proposed papers are encouraged to explore possible sources of funding for their attendance at the Symposium

as early as possible. **ISTA cannot offer any financial support to authors of papers.** However, a letter of acceptance of a paper for presentation (subject to funding) can be provided to assist in funding applications from early January 2016.

Session topics

Session 1: Applications of germination and dormancy testing

Lead speaker: Fernando Silveira, Federal University of Minas Gerais, Brazil

Topics:

- Advances in the understanding of seed germination and dormancy
- Characterisation of germination
- Physiology of germination and dormancy
- Seed development and maturation
- Dormancy breaking treatments
- Image analysis

Session 2: Advances in seed health testing for ensuring quality during seed production and storage

Lead speaker: Gary Munkvold, Iowa State University, USA

Topics:

- Seed treatments
- Epidemiology
- Novel methods for seed health testing
- Emerging diseases
- Phytosanitary issues

Session 3: Environmental effects on seed quality

Lead speaker: Ilse Kranner, University of Innsbruck, Austria

Topics:

- Stress tolerance
- Seed vigour
- Effect of the environment on seed development and production
- Organic seed production
- Maternal effects
- Epigenetics
- Climate change

**Session 4 (ISSS Collaborative Session):
Cellular and molecular methods and new
approaches to seed quality determination**

Lead speaker: Julia Buitink, INRA,
France

Topics:

- Genetics of seed quality
- QTL markers
- Variety and GM identification

- New approaches to vigour and viability testing
- Phenotyping
- Imaging

**Session 5: Conservation and use of
genetic resources in crop, forest and wild
species**

Lead speaker: Mati Koppel, Estonian
Crop Research Institute, Estonia

Topics:

- Collection of plant genetic resources
- Seed storage and longevity
- Desiccation tolerance
- Plant breeding
- Habitat restoration
- Genetic resources in forestry



Rasha El-Khadem leaves ISTA

Florina Palada

ISTA Accreditation and Technical Department

Rasha El-Khadem joined the ISTA Secretariat in October 2009. In her position as Head of the Technical and Accreditation Department, she was responsible for all accreditation activities within ISTA, including auditing, organizing quality assurance workshops and the Proficiency Test Programme.

Rasha was always active in whatever ISTA work she was involved in; the performance of the audited laboratories has improved. A professional approach of the training programmes and workshops should be highlighted.

In December 2014, Rasha decided to leave the ISTA Secretariat and to leave

Switzerland too. The most important achievement for her is her daughter Lotte, born when she was employed at ISTA.

We must thank Rasha for her support of the development of ISTA, and wish her good luck in her professional and personal challenges.

Join ISTA now!

- Contribute to seed quality worldwide
- Be part of a unique network and vibrant community
- 320 ISTA Members in 80 countries
- In 2014 alone, 250 seed analysts were trained in 10 ISTA workshops!

Benefits

- Improve the performance of your laboratory
- Receive ISTA Rules free of charge each year
- Share knowledge with the scientists in ISTA's 18 Technical Committees
- Receive free copy of each new ISTA publication
- Priority at ISTA events

More information at: www.seedtest.org/membership
or e-mail to: cannice.gubser@ista.ch

A tribute to Karen Ann Hill († 25 August 2014)

Murray Hill



Many of you will know that Karen Hill, the Laboratory Manager of the Queensland Seed Technology Laboratory in Australia died on 25 August 2014 aged 59. Her death was tragic, sudden and unexpected and has brought major sorrow to her many friends and colleagues in the Australia and New Zealand seed industries and to her ISTA friends across the world.

I know many people had great respect for Karen. This was seen in the 117 e-mails and messages I received in the seven days after her death — from people in 14 different countries. The grapevine certainly ran hot! Very flattering e-mails from Dr Joël Léchappé, President of ISTA, and from Dr Rasha El-Khadem, Head of the ISTA Accreditation Department, were particular highlights, as were the messages from many of the seed technology students who studied with Karen in New Zealand and her many ISTA friends and colleagues.

Karen began her seed testing career at the Department of Agriculture Seed

Testing Station in Palmerston North, New Zealand, in the early 1970s. At that time that seed laboratory was the second largest in the world, employing 65 seed analysts and testing 85 000 samples each year. How times have changed!

Karen subsequently worked as an academic staff member at Massey and Lincoln Universities in New Zealand and at the University of Queensland in Australia. She established three ISTA-accredited seed labs, two of which are still operating. She was a brilliant teacher, and we ran many seed testing workshops, both in Australasia and overseas, for organisations such as ISTA, the Asia & Pacific Seed Association, the World Bank, AVRDC–The World Vegetable Center, the Australian International Development Assistance Bureau and the New Zealand Ministry of Foreign Affairs and Trade. From 1975, when she joined the staff of the new Massey University Seed Technology Centre, until this year, Karen had been closely involved in teaching seed testing to more than 1200 people from 64 different countries. She was also involved in ISTA workshops in China, Thailand and the Philippines.

Until the time of her death, Karen had been a Director of Seed Technology Institute Australia and Laboratory Manager of its trading entity, the Queensland

Seed Technology Laboratory, established in 2002. She had successfully developed QSTL's reputation for testing accuracy and service, and it is still the only ISTA-accredited seed testing station in the Northern Territory, Queensland and New South Wales (ADL02).

Karen also had a major technical involvement in ISTA. In fact, she was the only seed analyst in Australia currently involved on any of ISTA's Technical Committees. She was a Personal Member of ISTA and served on the Purity, Germination and Tetrazolium Committees. She was Vice-Chair of the Tetrazolium Committee and was an invited speaker at the ISTA Germination Seminar in 2011.

Perhaps the pinnacle of Karen's career occurred in 2013, when she was appointed as a Technical Auditor by ISTA – a great honour and one she was very proud of. She carried out technical audits for ISTA in Korea and Japan last year and had been scheduled to carry out audits in India and Nepal this year. Unfortunately, her two heart operations and her loss of health prevented her from fulfilling this year's commitments.

So that is the Karen that was. We say farewell with a real sense of loss to a very special lady. ■



In memory of Prof. Patricia Berjak († 21 January 2015)

David Mycock

Vice-Chair, ISTA Seed Storage Committee



School of Animal, Plant & Environmental Sciences
University of the Witwatersrand
Johannesburg, South Africa

Professor Patricia (Pat) Berjak, a long-time ISTA member, died on 21st January 2015. Professor Berjak was a world-renowned botanical scientist who achieved remarkable breakthroughs in the understanding of recalcitrant seeds.

Patricia obtained her B.Sc. (Hons) from the University of the Witwatersrand, Johannesburg, and her Ph.D. from the University of Natal, Durban, South Africa. After three years at the University of Leeds in the UK, she returned to South Africa in 1972 and worked at the University of KwaZulu-Natal in Durban for the rest of her career, during which she held various academic titles, including head of department.

Professor Berjak's association with ISTA was substantial – she was a member of the Seed Storage Working Group of the Moisture and Storage Committee from 1977–1980, and was thus a founding member of the Storage Committee from 1980. She was the Vice-Chair of the Storage Committee between 1992 and 1995, Chair between 1995 and 2001 and remained a member until her death. Her major contributions to seed biology and ISTA were the development of the fundamental understanding of

the difference between orthodox and recalcitrant seeds. By better understanding the underlying biology, Berjak's research unravelled the response of recalcitrant seeds to storage and had significant impact on long-term preservation of such germplasm via cryopreservation. In this regard, she participated in numerous workshops and contributed to ISTA handbooks.

It was her personal drive, exemplary work ethic and desire to make a difference that established her research group as one of the most respected in the field of recalcitrant seed biology worldwide. Patricia also understood the necessity for cross-disciplinary science and she consistently, and enthusiastically, collaborated with other people. She always maintained that her achievements resulted from the contributions of those scientists; most especially her life partner, Professor Norman Pammenter, with whom she shared many other passions including aerobatics, ballroom dancing, classic cars and cooking.

Professor Berjak's innovative research was recognised globally, and her numerous accolades include being elected a member of the Academy of Science of South Africa (she was Vice-President at the time of her death), a member of the Third World Academy of Sciences, Fellow of the Royal Society of South Africa and a Fellow of the University of Natal. During her career she was also president of the International Society for Seed Science. Professor Berjak also received the Order of Mapungubwe (silver), the highest honour granted by the President of South Africa.

Professor Berjak held a South African National Research Foundation (NRF) 'A' rating, signifying world leadership in her

field and received the NRF's President's Award for Lifetime Achievement.

On occasion Pat was called "The Iron Lady", a title which she coveted. Many undergraduate students would agree with the statement and even go as far as to say "move over, Margaret Thatcher". However, beneath the steely demeanour and highly focused eyes was a person who had an unwavering belief in the uniqueness of other human beings and their ability to achieve. Over the years she helped many people in their quest for personal development, and this was generally at the expense of her own private time and resources. Using her exemplary supervisory skills, Pat gave South Africa and indeed the international community numerous academics, entrepreneurs and scientists. At whatever level of post-graduate training, students under Pat's guidance were stimulated towards self-improvement and deep understanding of their research subject.

Pat was a socially aware scientist and was involved in numerous urban projects, such as the rehabilitation of the mangroves in the Durban area and the maintenance of the Hawan coastal forest north of Durban. In those arenas she was often a key player, and it was her ability to bring her science and solid common sense together that led to fruition.

Associated with this generous nature was a delightful and often wicked sense of humour. At tense times, Pat could be counted on for her dry observation and sense of the ridiculous of any situation.

The seed biology world has lost a dear friend, colleague and driving force. ■

News from Seed Science and Technology

Fiona Hay¹ and Jonathan Taylor²

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Online submission of papers

We are now using an online system, Editorial Manager, for submitting papers for publication in Seed Science and Technology. The website for this system is: <http://www.edmgr.com/sst/>. Authors are required to register before answering questions relating to the submission and then uploading the manuscript files. The system creates a PDF from the submitted files which has to be approved by the author before the manuscript is considered submitted. The paper is then in the hands of the editorial team. Authors can log-in to the system at any time to check on the status of their paper (for example, whether it's under review) and will receive correspondence about the paper by e-mail. From the editorial side, using the Editorial Manager system should make the work flows very much simpler.

Associated with this change, please see the updated instructions to authors at: <http://www.seedtest.org/en/content---1--1089.html>.

Online publishing ahead of print

Seed Science and Technology now uses the Ingentaconnect Fasttrack service. This will allow finalized papers to be published online ahead of print, before the issue of SST has been completed. ■

Online publishing ahead of print

Seed Science and Technology now uses the Ingentaconnect Fasttrack service. This will allow finalized papers to be published online ahead of print, before the issue of SST has been completed. ■

New face at the ISTA Secretariat

Pierrick Marcoux

Head of Marketing



Pierrick Marcoux was born in France and speaks English and Spanish in addition to his mother tongue. He enjoys living in Zurich (Switzerland) for more than three years now – in particular the vicinity of mountains in winter and swimming into the lake during summer time.

He graduated from the EM Strasbourg School of Management in 2004, obtaining a Master's Degree in Marketing, Sales and Communications. He also studied in 2002/2003 at the University of Glasgow (Scotland) as part of the EU's Erasmus programme.

In the last ten years, Pierrick Marcoux worked in a variety of retail industries, including home furnishing, cosmetics and jewellery. He worked for several leading companies holding different positions in the field of Marketing – always with global scope.

He started as a marketing analyst at IKEA where he provided several recommendations to increase the number of visitors. He then moved to a product manager role within the multinational group Sara Lee. There, he worked for two different house & body care brands with the objective of maximising market share in a very competitive environment.

Pierrick then joined Swarovski Consumer Goods Business. At first, he worked in the Paris office (France), before he moved in 2012 to the international headquarters in Zurich (Switzerland). He successively acted as international public relations manager and global marketing manager.

He positively contributed to the enhancement of the brand positioning and launch of new collections across different regions of the world.

The diversity of roles and industries he worked in enable Pierrick to create effective marketing and communications campaigns – always with a strategic mindset and consumer focus.

Pierrick Marcoux joined the ISTA Secretariat in January 2015 as the Head of Marketing.

The opening of this new position is a direct consequence of the implementation of the strategy of the Association, voted on by the Membership in 2013 at the ISTA Congress in Antalya, Turkey.

Pierrick Marcoux fully embraces this strategy and will be actively working on promoting ISTA and increasing membership. ■

ISTA membership changes

Status 11 March 2015

New Member Laboratories

Bangladesh BDML0300/BDML0301

Laboratory representative: Shyamol Kumar Sen
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BDML0400/BDML0401

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Turkey TRML0200/ TRML0201

Laboratory representative:
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Proposal for a confirmation method of seed-transmitted *Squash mosaic virus* (SqMV) on DAS-ELISA positive cucurbit seeds

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Summary

A method for the confirmation of seed-transmitted SqMV on cucurbit seed was developed by ISHI-Veg, ISF and validated in an international study between four laboratories. The method includes a greenhouse grow-out test – with seed of a lot which had screened SqMV-positive according to ISTA method 7-026 – and a DAS-ELISA confirmation of symptomatic plants at the 3–4 true-leaf stage. All participants detected and confirmed the seed-transmitted SqMV in the positive seed samples supplied. It was shown that an SqMV infection can be symptomless, as pools of symptomless plants were SqMV-positive in the DAS-ELISA test. In the case of negative confirmation or non-appearance of symptoms, the symptomless plants are confirmed with DAS-ELISA, as SqMV can be transmitted without expression of symptoms. This method is an extension of the ISTA method 7-026, which verifies whether an SqMV-positive cucurbit lot according to ISTA method 7-026 will result in diseased seedlings. In this study, melon seeds were used. However, all cucurbits could be evaluated in a greenhouse grow-out. However, the ELISA confirmation test on plant tissue samples should be evaluated and verified for the various cucurbits being evaluated in the grow-out test before results are accepted.

Introduction

Squash mosaic virus (SqMV) belongs to the *Comovirus* genus (Bruening, 1978). It is pathogenic on several species of the *Cucurbitaceae* family, with melon being the principal host (Bruening, 1978; Freitag, 1956). It is a seed-borne virus, transmitted by seed, some species of beetles and mechanical inoculation (Freitag, 1956; Campbell, 1971; Alvarez and Campbell 1978; Nolan and Campbell, 1984). Seed transmission of SqMV and its influencing factors have been studied on seed of various cucurbit species (Powell and Schegel, 1970; Alvarez and Campbell, 1978).

SqMV is located in the seed coat, in the papery layer and in the embryo of a seed. However, only the embryonic infection leads to virus transmission from seed to seedling (Alvarez and Campbell, 1978; Nolan and Campbell, 1984).

The actual reference method for the detection of SqMV in cucurbit seed is described in ISTA method 7-026, which demonstrates the possibility of the simultaneous detection of *Cucumber Green Mottle Mosaic Virus* (CGMMV), *Melon Necrotic Spot Virus* (MNSV) and *Squash Mosaic Virus* (SqMV) from a single extract of a ground seed sample (Koenraad and Remeus, 2009). The principle of this detection method is based on an ELISA test developed for plant viruses (Clark and Adams, 1977). The precision of a DAS-ELISA test in detecting SqMV-infected melon seed in a given sample size has been demonstrated by Franken et al. (1990).

However, it is known that the DAS-ELISA test detects both infectious and non-infectious virus particles in a test sample, and so it can lead to a false positive result (Nolan and Campbell 1984) and to an overestimation of the actual seed transmission level of the seed lot. In addition, when ground seeds are used, the DAS-ELISA test cannot give any information on the location of the virus in the seed, i.e. embryonic versus seed coat. Thus, it cannot be known whether

an ELISA-positive seed sample would result in diseased plants (Maury *et al.*, 1987; Koenraad and Remeus, 2009), and subsequently the virus transmission rate in the sample can be overestimated.

Nevertheless, the DAS-ELISA test can serve as a virus prescreening step (Hamilton and Nichols, 1978) of melon seed lots, and positive results can be further confirmed by a grow-out test with melon seeds from the same lot. Results of the grow-out will show whether the virus that is present in the lot is alive, infectious and able to cause seed transmission (virus location in the embryo of the infected seeds) or not.

In the grow-out test, melon seeds are sown in a suitable substrate and incubated in greenhouse conditions until emerged plants reach the growth stage of 3-4 true leaves, when they are visually evaluated for SqMV symptoms (Powell and Shlegel 1970; Alvarez and Campbell, 1978; Nolan and Campbell, 1984). Symptoms are compared to symptoms developed on mechanically inoculated control plants following the inoculation method described by Alvarez and Campbell (1978). Tissue of plants showing typical and atypical SqMV symptoms is collected individually and tested by DAS-ELISA for confirmation of visual findings. The seed lot would be considered SqMV-positive as long as there is at least one positive result in the DAS-ELISA test. Preliminary tests have shown that SqMV can be transmitted to plants without expressing any symptoms (H. Lybeert, HM-Clause SA, France, personal communication). Therefore, if no SqMV symptoms are observed on the plants in the grow-out, the tissue of symptomless plants is confirmed by a DAS-ELISA test in pools of a maximum of 20 plant-tissue samples. The seed lot in this case would be considered SqMV-positive as long as there is at least one positive result in the DAS-ELISA test. The summarized procedure of the grow-out and DAS-ELISA confirmation tests application is presented in Appendix I.

Sensitivity of the grow-out method

The ability to detect SqMV could be influenced by variations in environmental conditions in the greenhouse. Therefore the recommended temperature and light should be respected, and the grow-out test should not be performed during the winter period, unless artificial light and heating can compensate for the lack of natural light and temperatures. If plants are damped off or other disease symptoms are present, then the SqMV test should be considered invalid, and should be redone.

The grow-out test method is suitable for untreated seed. It is also considered suitable for seed that has been treated using chemicals or physical processes with the aim of disinfestation/disinfection, as well as seed treated with protective chemicals or biological substances.

Aim and objective of the peer validation study

The aim of this ISHI-Veg peer validation study was to determine whether the grow-out method is suitable and can be used by other laboratories to estimate the level of seed-transmitted SqMV in a seed lot.

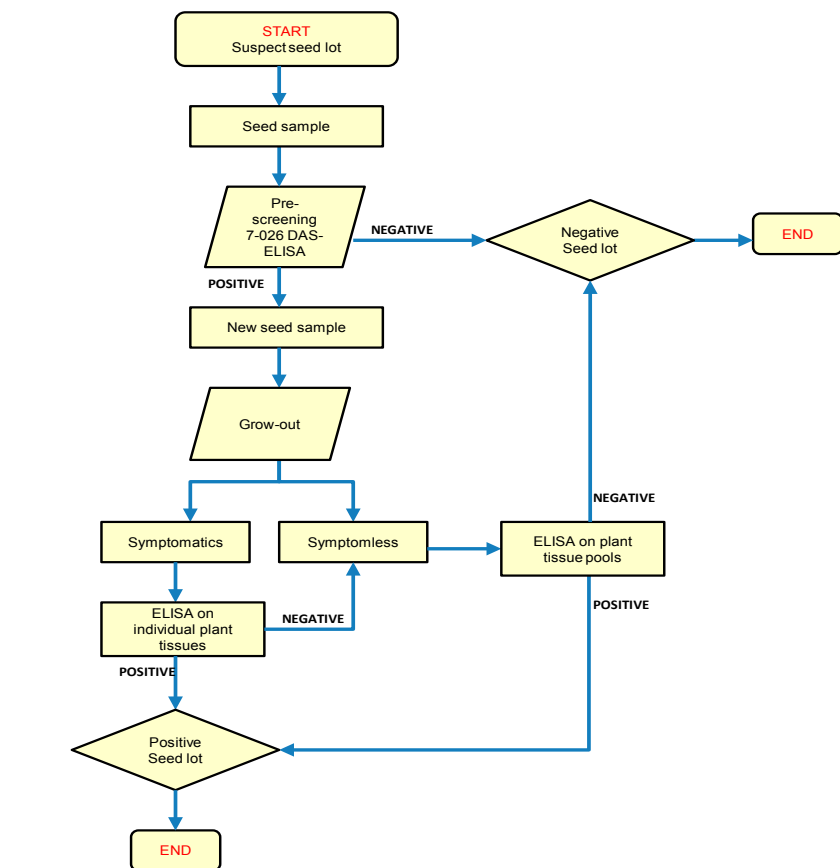
The objective of this ISHI-Veg peer validation study is to provide an extension of the ISTA method 7-026 to verify whether a seed lot that has been found positive with the prescreening ELISA test on ground seed samples will result in diseased seedlings. This finding will allow for the determination of the actual seed transmission rate of such a seed lot.

In this peer validation study, four seed health laboratories from France, USA, the Netherlands and Israel participated.

Materials and methods

Characterization of seed lots

Two lots of melon (*Cucumis melo*) seed, with different levels of natural contamination with SqMV, and one non-contaminated seed lot were selected by the laboratory of HM Clause in France. Prior to the peer validation study, the seed lots were characterized based on the results of a DAS-ELISA prescreening and a grow-out test. The DAS-ELISA prescreening test was performed on 20 subsamples of 100 seeds



Appendix I. Flow chart showing the grow-out and DAS-ELISA confirmation tests in practice.

Table 1. Characterization of the three melon seed lots used in the peer validation study

Seed lots	Contamination level	DAS-ELISA on seeds (ISTA method 7-026)	DAS-ELISA on grow-out collected plants	Indicative seed transmission rate (STR)
1	Healthy	0+/20 ¹	0+/0 symptomatics ² and 0+/50 pools ³	0%
2	Low	20+/20	2+/8 symptomatics and 2+/50 pools	0.43%
3	Medium	20+/20	5+/5 symptomatics and 3+/50 pools	0.91%

¹ X+/Y tested seed subsamples in DAS-ELISA

² X+/Y tested plants with typical and plants with atypical SqMV symptoms in DAS-ELISA

³ X+/Y tested pools of symptomless plants in DAS-ELISA

following the method described in the ISTA method 7-026. The grow-out test was performed on a sample of 1000 seeds following the proposed method, which determined the seed transmission rate (STR) of the virus and provided the final characterization in terms of the contamination level in each seed lot. The details of the characterization of these 3 seed lots are given in Table 1.

The percentage of the actual seed transmission rate (%STR) for the initial characterization of seed lots as well as in the grow-out test was calculated using the following formula, which combines results of the grow-out and DAS-ELISA tests:

$$\%STR = \frac{[\text{No. ELISA-positive, symptomatic} + \text{No. ELISA-positive symptomless}] / \text{total No. emerged plants} \cdot 100}{100}$$

where ‘symptomless’ are the plants with typical and the plants with atypical SqMV symptoms, and ‘symptomless’ are the plants that did not express any SqMV symptoms on their leaves.

The number of ELISA-positive symptomless plants in the ELISA-positive pools was calculated from the contamination rate that was obtained from the “Quality Impurity Estimation” sheet of SeedCalc8 (http://www.seedtest.org/en/statistical-tools-for-seed-testing-_content---1--1143--279.html). In order to obtain this rate, the number of pools that were tested by ELISA, their size (number of pooled plants) and the number of the pools that were found positive were entered in this sheet. If needed, a second calculation of the contamination rate was performed with a different size of pools to approach the actual total number of symptomless plants that were tested positive. The contamination rate corresponding to the actual number of tested symptomless plants was then revealed through intrapolation in an x/y graph showing (x = contamination rate (%), y = No. of symptomless tested plants). The number of ELISA-positive symptomless plants out of the total tested in pools was finally calculated in the ‘rule of three’. The overall characterization results of the three seed lots are given in Table 1. The ‘healthy’ and ‘medium’-contaminated seed lots were untreated, whereas the ‘low’-contaminated seed lot was treated with Thiram fungicide.

Seed samples and subsamples

Participating laboratories received 1000 seeds in total from each of the low and medium lots and 500 seeds from the healthy lot. Each of these samples was broken down to subsamples of 20 seeds, resulting in 125 subsamples in total for each laboratory. This practice aimed to ensure the clear identity of the pools of the symptomless plants.

The subsamples were prepared in the SNES sampling department with the use of the rotary divider machine based on the thousand-seed weight of the corresponding lot. Subsamples were coded and their correspondence to seed lots was known only to the test coordinator. However, the codes to subsamples within the same seed

lot were given in continuous numbers and not randomly. This aimed to reduce the chances of cross contamination between melon plants of different lots as they can grow tall enough to bend and touch each other.

In addition, each laboratory received 2 extra subsamples of approximately 50 seeds from the healthy seed lot. The emerged seedlings of one subsample were mechanically inoculated and served as the positive control plants, and the emerged seedlings of the second subsample remained untreated and served as negative control plants. The identity of these two subsamples was known to laboratories, as they required special manipulation. The peer validation study was performed during June to August to ensure the recommended environmental conditions in the greenhouse, although the participants were located in very different geographical locations.

Grow-out method description

1. Seed sowing and greenhouse incubation

125 plastic trays were filled with well-watered potting soil. Each tray was labeled with the number of one of the received seed subsamples. Seeds of each subsample were then sown in approximately 2 cm depth into the corresponding tray and were covered with a thin layer of vermiculite. The seeds of the 50-seed subsamples that came from the healthy lot and were designated to serve as negative and positive control subsamples were sown first, followed by the rest of the subsamples. During sowing, gloves were changed between each subsample.

Trays were placed in an insect-proof greenhouse to avoid transmission of virus by beetles. Adequate space was kept between the trays to reduce the chances of cross-contamination between subsample plants. The greenhouse temperature was maintained at 24–30 °C during the day and 16–22 °C during the night until seedling emergence. After this time and until the final reading the temperature was maintained at 24–35 °C. After emergence the plants were inspected every 3 to 5 days without handling.

2. Mechanical inoculation of positive control plants

When the 1st true leaf of each melon plant began to emerge (approximately 10 days after sowing), the virus inoculum was prepared as follows: the dehydrated SqMV-infected melon leaves (~ 1 g fresh weight), provided by the test organizer, were ground into 4 ml of virus extraction buffer (0.53 g Na₂HPO₄ 2H₂O and 0.2 g (C₂H₅)₂NCSSNa 3H₂O in 100 ml distilled water) in a mortar with a pestle. Then, 0.075g of carborundum powder were added and all ingredients were mixed well. The virus inoculum was placed on ice and the inoculation procedure was performed in a short time.

A drop of the virus inoculum was placed on the cotyledon surface of all plants of the positive-control labeled tray and was smeared with fingers. For this purpose, plastic gloves and/or finger tip gloves were used. Light pressure was applied while smearing to avoid damaging the leaf tissue. Cotyledons were rinsed with tap water 5 min after the inoculation and plants continued their greenhouse incubation with the rest.

3. Collection of plant-tissue samples and DAS-ELISA confirmation

When the majority of plants reached the stage of 3–4 true leaves (approximately 18–24 days after sowing), plants were evaluated individually. Typical SqMV symptoms are the systemic mosaic or vein banding in leaves and sometimes the leaf deformation (ICTVdB Management, 2006). However, it is possible to observe atypical SqMV symptoms such as discoloration of leaves and development of spots on them (Lecoq *et al.*, 1998).

Collection of plant tissue samples started from the negative-control plants, continued with the rest of the subsample plants and ended with the positive-control plants. Aseptic materials (e.g. scalpel, forceps, cork borer) and disposable gloves that were changed after a collection of each plant tissue sample were used to avoid cross-contamination between the collected plant tissue samples.

For the negative-control plants, a 1 cm² piece was cut from one of the youngest leaves of each plant. The pieces were placed

in suitable containers (e.g. plastic extraction bags from BioReba) with a maximum of 10 pieces.

Within each subsample and tray, the collection started with the symptomless plants. As previous, a 1 cm² piece was cut from one of the youngest leaves of each plant and all pieces were pooled together in one suitable container (maximum of 20 symptomless plants). The collection continued with the atypical SqMV symptomatic plants, if present. These were collected individually, therefore each 1 cm² piece that was cut from the leaf of each plant was placed separately in a suitable container. Finally, typical SqMV symptomatic plants were collected individually, if present.

Plant-tissue samples from the positive-control plants were collected following the same procedure for the negative-control plants, i.e. a maximum of 10 plant tissues was pooled in one container.

Plant-tissue samples were then ground in DAS-ELISA extraction buffer (described in ISTA method 7-026) at a ratio of 1 g of plant tissue in a 10 ml extraction buffer with the use of a suitable grinding device. Samples were stored at 4 °C and confirmed by DAS-ELISA (described in ISTA method 7-026) the next day. Alternatively, laboratories stored the unground plant tissue samples at 4 °C and continued with the grinding, buffer addition and the DAS-ELISA confirmation step the following day.

Due to the large number of plants for evaluation, the collection of plant tissue samples and DAS-ELISA confirmation was done in three consecutive time periods. On day 24, tissue was collected from the samples coded 1–42, on day 26 from those coded 43–48, and on day 28 from those coded 85–125. Each collection day, a fresh plant tissue sample of approximately 10 plants from each of the negative and positive controls was collected.

Data analysis

For each subsample and tray, laboratories recorded the results of the grow-out and DAS-ELISA confirmation tests. The grow-out test results were the number of observed plants with typical SqMV symptoms and the number of observed plants with atypical SqMV symptoms out of the

Table 2. DAS-ELISA results per contamination level in each laboratory

Seed lots	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4
	DAS-ELISA on grow-out collected plants	DAS-ELISA on grow-out collected plants	DAS-ELISA on grow-out collected plants	DAS-ELISA on grow-out collected plants
Healthy	0+/0 typical ¹	0+/7 typical	0+/1 typical	0+/0 typical
	0+/1 atypical ²	0+/44 atypical	1+/112 atypical	0+/9 atypical
	0+/25 pools ³	0+/25 pools	0+/25 pools	0+/25 pools
Low	7+/7 typical	0+/2 typical	4+/6 typical	3+/3 typical
	1+/6 atypical	0+/14 atypical	1+/225 atypical	0+/41 atypical
	10+/50 pools	1+/50 pools	1+/50 pools	1+/50 pools
Medium	6+/6 typical	13+/14 typical	5+/6 typical	5+/6 typical
	1+/1 atypical	0+/16 atypical	2+/293 atypical	5+/61 atypical
	14+/50 pools	4+/50 pools	3+/50 pools	1+/50 pools

¹ X+/Y tested plants with SqMV typical symptoms in DAS-ELISA

² X+/Y tested plants with SqMV atypical symptoms in DAS-ELISA

³ X+/Y tested pools of symptomless plants in DAS-ELISA

total which had emerged. The DAS-ELISA confirmation test results were:

- i) the numbers of SqMV-positive plants with typical and atypical SqMV symptoms out of the total number tested, and
- ii) the DAS-ELISA result from the pool of the symptomless plants.

The subsample was considered SqMV-positive when there was at least one positive DAS-ELISA result, either from the symptomatic plants or the pools of the symptomless plants.

The final result of the thousand-seed tested sample per level was determined as follows: the sample was considered SqMV-positive if there was at least one positive result in the DAS-ELISA test (either from symptomatic or symptomless plant tissue samples). If there was no positive result in DAS-ELISA test, the seed sample was considered negative.

The norm NF EN ISO 16140 (AFNOR, 2003) was followed to evaluate the performance criteria (sensitivity, specificity and accuracy) for each contamination level, using the final result of the thousand-seed tested samples. This evaluation was performed by comparing the expected results of all laboratories with those obtained. The results were in the form of positive and negative agreements and deviations.

For each contamination level, concordance (reproducibility of qualitative data) was evaluated using the final result of the thousand-seed tested samples in the method developed by Langton *et al.* (2002). For this evaluation, the definitions developed by Josefsen *et al.* (2004) were followed, i.e: ‘the percentages of finding the same result positive or negative from two similar

samples analysed in the same or different laboratories respectively and under standard repeatability conditions’.

Results and discussion

All participating laboratories submitted their generated results in the data record sheet provided. The results of each laboratory are presented in Table 2 per contamination level.

In the samples of the healthy seed lot, laboratories 1 and 4 recorded zero plants with typical observed SqMV symptoms in the grow-out test, while laboratories 2 and 3 recorded 7 and 1 plants from this category, respectively. However, all plants from the healthy lot that were observed with SqMV typical symptoms were confirmed as SqMV-negative by the DAS-ELISA test. This probably reflects variation between laboratories in the interpretation of what is considered an SqMV-typical symptom. Regarding the plants with atypical SqMV symptoms in the same lot, a variable number was recorded in all laboratories. All of these plants except one in laboratory 3 were confirmed as SqMV-negative by the DAS-ELISA test. However, the ELISA positive result in the healthy seed lot could be also attributed either to a cross-contamination while processing the ELISA sample, or in the grow-out, or a very low SqMV infection level that was not detected in the characterization test. Finally, the pools of symptomless plants in the healthy seed lot in all laboratories were confirmed as SqMV-negative by the DAS-ELISA test.

Laboratory 1 recorded one positive DAS-ELISA result on a negative-control

Table 3. Statistical evaluation of final results

Contamination level	Sensitivity (%)	Specificity (%)	Accuracy (%)	Concordance (%)*
Healthy	N/A	75	75	50
Low	100	N/A	100	100
Medium	100	N/A	100	100

sample (raw data not shown), which was attributed by the laboratory to a cross-contamination while processing the ELISA sample, as its well was near to the well of the positive control.

Regarding the low- and medium-contamination level lots, all laboratories recorded a variable number of plants with typical and atypical SqMV symptoms, with laboratory 3 having the highest number. This laboratory did not have previous experience with the grow-out method. Moreover, nutrient deficiency of plants was reported, which made the observation of SqMV symptoms difficult. Laboratory 4 also reported nutrient deficiency of plants and plants with etiolating symptoms, which implies that there was insufficient light in the greenhouse.

The highest number of SqMV-positive pools of symptomless plants was recorded by Laboratory 1 in both low- and medium-contaminated levels compared to the other laboratories. All of the participating laboratories were well versed in ELISA testing. Therefore, the variations seen are a result of ELISA methods between the laboratories. The most obvious and logical source of their variation is the different antisera used in each laboratory. Antisera against viruses are available from various suppliers, and it is known that their quality may differ. Differences are not so evident in the basic detection of the virus in medium and high levels of the virus, but more in the background levels. The differences in background levels could affect the cut-off levels and the ability to detect low levels of the virus. Laboratory 1 used antisera provided by PRI, laboratory 2 by Envirologix and laboratories 3 and 4 by Prime Diagnostics. In addition, the quality of microtiter plates, incubation conditions and handling are factors that can have an impact on ELISA results. The interpretation of the DAS-ELISA response as positive or negative was done by each laboratory based on

their equipment, software and threshold values instructed by the antisera supplier. Finally, since each laboratory used its own available grinding tools to grind the plant tissue samples, variation in tissue maceration might have been a factor.

Specificity and accuracy values of the healthy level were less than 100 %, demonstrating that there was a false-positive result (Table 3). Nevertheless, for the low and medium levels, sensitivity and accuracy values were 100 %, demonstrating that there was no false-negative result (Table 3). Regarding the concordance value, this was 100 % for the two positive levels, but only 50 % for the healthy lot, due to the false-positive result (Table 3).

Conclusions and recommendations

All laboratories found the SqMV that was present in the positive seed lots, showing that the proposed method is able to detect and confirm infectious SqMV in the seed lot.

In this study, it was shown that SqMV infection can be symptomless, as pools of symptomless plants were SqMV-positive in the DAS-ELISA test.

Confirmation by a DAS-ELISA test of the visually observed symptoms on the plants is a necessary step, as SqMV can express atypical or no symptoms. It is also necessary because it is possible to have plants infected with other seed-transmitted viruses, whose symptoms may be confused with SqMV symptoms, or plants with symptoms of nutrient deficiency.

Performing the grow-out in the recommended environmental conditions will ensure the SqMV multiplication and thereafter its detection in the plant tissue samples.

Use of appropriate precautions throughout the grow-out and DAS-ELISA tests will minimize or prevent the potential for cross-contamination. Moreover, the use of

negative controls in the grow-out and ELISA tests is important to identify possible cross-contaminations.

In order to harmonize with the sample size of the prescreening method described in ISTA method 7-026, a sample of 2000 seeds of a lot should be tested in the grow-out.

In this study, melon seeds were used. However, all cucurbits could be evaluated in a greenhouse grow-out and symptoms evaluated with no problem. However, the ELISA confirmation test on plant tissue samples should be evaluated and verified for the various cucurbits being evaluated in the grow-out test before results are accepted.

The grow-out and DAS-ELISA confirmation of SqMV in a seed lot that was previously found to be SqMV-positive in the prescreening ISTA method 7-026 allows seed health laboratories to assess whether the seed lot will result in diseased plants.

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Validation study on moisture determination in forest tree seed

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Summary

The International Rules for Seed Testing currently require that seed of *Pinus* spp. with a thousand seed weight of greater than 200 g is cut before moisture determination; and that for tree seeds with a thousand seed weight of greater than 200 g, two replicates of five seeds are used for moisture determination. This validation study was undertaken with the aim of determining whether cutting was needed for seed of *Pinus* spp. with a thousand seed weight of greater than 200 g (scope 1) and whether for tree seeds with a thousand seed weight of greater than 200 g 5 g of seed could be used for each working sample rather than five intact seeds (scope 2). The species used for scope 1 was *Pinus cembra* and for scope 2 *Quercus ilex*. For each scope two seed lots and three moisture levels were used.

For scope 1 moisture was determined in whole and cut seed and for scope 2 moisture was determined using an amount of seed equivalent to 5 intact seeds for each replicate and 5 g of seed for each working sample. Six laboratories participated in the validation study.

The results of the validation study showed that the methods for Scopes 1 and 2 are yielding comparable moisture results in terms of the moisture determined and the repeatability and reproducibility of the determination. This validation study therefore supports the proposal that for *Pinus* spp. where the TSW is greater than 200 g there is no need to cut the seed prior to moisture determination and that whole seed therefore be used for the moisture determination. And that for tree seeds where the TSW is greater than 200 g two working samples of 5 g be used rather than two replicates of an amount of seed equivalent to 5 intact seeds

Aim

The aims of this validation are to verify whether:

1. cutting is compulsory for the seeds of *Pinus* spp. when the TSW is > 200 g (scope 1);
2. for tree seeds where TSW is > 200 g, the two working samples of 5 g (or 10 g) rather than 2 replicates of 5 seeds can be tested (scope 2).

Materials and methods

1. Species used

Seed of two species for which cutting is obligatory because the TSW is > 200 g will be used for this validation: *Pinus cembra* and *Quercus ilex*.

Scope 1

Pinus cembra will be used as the representative species for the *Pinus* genus to verify whether cutting is compulsory for the seeds of this genus when the TSW is > 200 g.

Scope 2

Quercus ilex will be used to determine whether it is possible to test two working samples of 5 g (or 10 g) of seeds instead of 2 replicates of 5 seeds.

2. Participating laboratories

Six laboratories from six countries participated in the validation
 ITML0600 – contact Sergio Pasquini
 CZDL0200 – contact Marta Dohnalová
 GBDL01 – contact Jane Taylor
 DEDL0400 – contact Andrea Jonitz
 FRDL0200 – contact Céline Herbert
 BEDL0200 – contact Anja Ritserveld

In this report to maintain anonymity each participating laboratory has been randomly assigned a number between 1 and 6.

3. Preparation of samples

Sample preparation for both scope 1 (*Pinus cembra*) and scope 2 (*Quercus ilex*) was the same. For each species two seed lots were used and for each seed lot three moisture levels were assessed; low moisture (*Pinus cembra*: 5 and 4%; *Quercus ilex*: 20 and 14%), the natural moisture of the seed (*Pinus cembra*: both 7%; *Quercus ilex*: 40 and 43%) and high moisture *Pinus cembra*: 19 and 10%; *Quercus ilex*: 40 and 48%). For each seed lot a total of 900 seeds was used. The 900 seed lot was split into three 300 seed sub lots, one for each moisture level. At the end of the moisture adjustment these sub lots were further subsamples to give a total of 60 smaller lots for each species to be distributed to the participating laboratories and for verification of moisture homogeneity:

- a. 2 seed lots x 6 laboratories x 3 moistures = 36 lots for distribution to the laboratories
- b. 2 seed lots x 3 moistures x 4 lots = 24 lots for verification of moisture homogeneity.

The homogeneity of the seed lots was assessed by determining the moisture of four randomly selected packets for each seed lot at each moisture level. For both scopes both the current pre-drying sample preparation method and the proposed method was used to determine the moisture content.

The low and high moisture contents were achieved as follows:

- a. On receipt the moisture content of each lot was determined using the low temperature oven method (17 hours at 103°C). The weight that the seed needed to reach either after drying or hydration to be at the required moisture was then calculated.
- b. The seed lot was split into three sub lots of 300. The moisture content of one subplot was reduced to the required lower moisture content by drying the seeds in an oven operating at 40°C until the weight calculated to be equivalent to the required moisture content of was reached. When the required weight was reached the seeds were mixed thoroughly, a subsample taken to verify the moisture content had reached the predetermined percentage. The sub lot was then subsampled into lots of 30 seeds which were placed in 116 x 188 mm 12/20/50 micron laminated polyester/aluminium foil/polythene packets which were then heat sealed. The moisture content was verified using the low temperature oven method.
- c. The second 300 seed subplot was placed in a room germinator at 20°C and 85-95% RH until the weight calculated to be equivalent to the required moisture content was reached. When the required weight was reached the seeds were mixed and subsampled as described for the low moisture treatment.
- d. The moisture content of the third sub lot of 300 seeds was not adjusted. This is the “natural” moisture seed lot.

At the end of the sample preparation there were 60 packets of seed in total, comprising the following samples:

- a. Ten packets of each seed lot to give a total of 20 packets at **low moisture content**.
- b. Ten packets of each seed lot to give a total of 20 packets at **natural moisture content**.
- c. Ten packets of each seed lot to give a total of 20 packets at **high moisture content**.

4. Scope 1 testing protocol

The species used was *Pinus cembra*. Each laboratory determined the moisture

content on the seed for each moisture level using two methods:

- a. Applying the low temperature oven method, as described in Chapter 9 of the ISTA Rules (2012) with **whole seeds** used for the moisture determination and
- b. Applying the low temperature oven method, as described in Chapter 9 of the ISTA Rules (2012) with **cut seeds** (cut in 4 pieces with scissors) used for the moisture determination.

Participating laboratories completed a results sheet sent by the organising laboratory. The detailed SOP for the validation study was included in the results sheet. Each laboratory was also asked to provide the SOP they followed for the validation and to enter their moisture data into the results sheets. The specific protocol followed by the laboratories is in Appendix One.

5. Scope 2 testing protocol

The species used was *Quercus ilex*. Each laboratory determined the moisture content on the seed for each moisture level using two methods:

- a. Applying the low temperature oven method, as described in Chapter 9 of the ISTA Rules (2012) using the current method of taking ten intact seeds, cutting them, then mixing and subsampling two working samples for moisture determination approximately equal in weight to 5 intact seeds and
- b. Applying the low temperature oven method, as described in Chapter 9 of the ISTA Rules (2012) but cutting more than 10 g of seed and **then taking two working samples of 4.5g (± 0.5g)** for the moisture determination. The specific protocol followed by the laboratories is in Appendix Two.

6. Statistical analysis

This section of the validation report was prepared with Kirk Remund, Vice-Chair of the ISTA Statistics Committee

The moisture data submitted by the participating laboratories was analysed to assess the reproducibility/repeatability of the methods used in Scopes 1 and 2 and to assess if the moisture method means are statistically different at a 0.05 significance level for Scope 1 and 2.

Results

1. Missing laboratory

Laboratory 1 was removed from the statistical analysis for Scope 1 due to differences from the other laboratories.

2. Moisture determined

Scope 1: *Pinus cembra*

a. Moisture determined for confirmation of homogeneity

The seed lots were considered to be sufficiently homogeneous for the validation to proceed (Tables 1 and 2).

b. Moisture determined by the participating laboratories

There was no significant difference (t-test) in the moisture determined using cut seed versus whole seed for any moisture for either seed lot (Tables 3 and 4), except, the low moisture treatment for seed lot 2 (Table 4) where the moisture determined in cut seed was higher than that determined in whole seed.

Table 1. Moisture per cent determined for four lots (packets) of seed lot 1 for each moisture using both whole and cut seed. Individual data averaged over the duplicate working samples and the individual data averaged over the four samples lots is presented. Standard errors of the mean are given in brackets

Moisture (%)	Pre-drying seed treatment	<i>Pinus cembra</i> Seed lot 1				
		Sample 1	Sample 2	Sample 3	Sample 4	Average
Low	Whole seed	5.3	5.5	5.3	5.6	5.4 (± 0.08)
	Cut seed	5.4	5.4	5.2	5.2	5.5 (± 0.05)
High	Whole seed	19.2	20.3	19.7	19.3	19.6 (± 0.26)
	Cut seed	18.9	19.0	18.0	19.3	18.8 (± 0.28)
Natural	Whole seed	7.0	7.1	7.1	7.0	7.0 (± 0.01)
	Cut seed	6.8	7.0	6.9	7.1	7.0 (± 0.08)

Table 2. Moisture per cent determined for four lots (packets) of seed lot 2 for each moisture using both whole and cut seed. Individual data averaged over the duplicate working samples and the individual data averaged over the four samples lots is presented. Standard errors of the mean are given in brackets

Moisture (%)	Pre-drying seed treatment	<i>Pinus cembra</i> Seed lot 2				
		Sample 1	Sample 2	Sample 3	Sample 4	Average
Low	Whole seed	4.0	3.8	3.8	3.9	3.9 (± 0.04)
	Cut seed	4.0	4.1	4.0	3.9	4.0 (± 0.05)
High	Whole seed	10.0	9.9	10.1	10.1	10.0 (± 0.06)
	Cut seed	9.7	9.6	9.5	9.7	9.7 (± 0.05)
Natural	Whole seed	7.0	7.3	7.0	7.3	7.1 (± 0.08)
	Cut seed	7.0	7.2	6.7	7.6	7.1 (± 0.18)

Table 3. Moisture determined by the six laboratories for *Pinus cembra* seed lot 1 for each moisture using whole and cut seed. Data presented is the average of the moisture determined for the duplicate working samples. The average moisture determined by the six laboratories and with laboratory 1 removed is given

Moisture (%)	Pre-drying seed treatment	<i>Pinus cembra</i> Seed lot 1						Average (6 laboratories)	Average (5 laboratories)
		Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5	Laboratory 6		
Low	Whole seed	7.5	5.6	5.6	5.6	5.8	5.7	6.0 (± 0.31)	5.7 (± 0.03)
	Cut seed	7.6	5.5	5.9	5.7	4.9	5.6	5.9 (± 0.37)	5.5 (± 0.17)
High	Whole seed	18.2	19.3	23,2	18.2	19.6	19.5	19.7 (± 0.75)	20.0 (± 0.85)
	Cut seed	17.2	17.8	21,3	19.5	19.2	19.0	19.0 (± 0.58)	19.4 (± 0.57)
Natural	Whole seed	8.0	7.2	7,1	6.9	7.0	7.3	7.2 (± 0.16)	7.1 (± 0.08)
	Cut seed	7.9	6.8	7,1	6.9	7.9	7.1	7.3 (± 0.19)	7.2 (± 0.19)

Table 4. Moisture determined by the six laboratories for *Pinus cembra* seed lot 2 for each moisture using whole and cut seed. Data presented is the average of the moisture determined for the duplicate working samples. The average moisture determined by the six laboratories and with laboratory 1 removed is given

Moisture (%)	Pre-drying seed treatment	<i>Pinus cembra</i> Seed lot 2						Average (6 Laboratories)	Average (5 Laboratories)
		Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5	Laboratory 6		
Low	Whole seed	6.8	3.9	4,0	3,9	3.7	4.1	4.4 (± 0.49)	3.9 (± 0.05)
	Cut seed	6.7	4.1	4,2	4,2	4.3	4.3	4.6 (± 0.41)	4.2 (± 0.03)
High	Whole seed	10.2	10.0	10,2	9.9	10.3	9.9	10.1 (± 0.07)	10.1 (± 0.09)
	Cut seed	9.7	9.4	10,0	11,6	9.9	9.5	10.0 (± 0.33)	10.1 (± 0.40)
Natural	Whole seed	8.9	7.2	7,2	7,2	7.8	7.3	7.6 (± 0.28)	7.3 (± 0.12)
	Cut seed	8.6	7.2	7,4	7,3	7.2	7.0	7.4 (± 0.23)	7.2 (± 0.07)

Table 5. Moisture per cent determined for four lots (packets) of seed lot 1, at each moisture level, using both weighed and counted seed. Individual data averaged over the duplicate working samples and the individual data averaged over the four samples lots is presented. Standard errors of the mean are given in brackets

Moisture (%)	Amount of seed used determined by	<i>Quercus ilex</i> Seed lot 1				
		Sample 1	Sample 2	Sample 3	Sample 4	Average
Low	seed weight	23.3	18.4	21.0	22.0	21.2 (± 1.03)
	seed number	21.0	19.8	21.5	21.8	21.0 (± 0.44)
High	seed weight	38.9	40.6	39.6	41.5	40.1 (± 0.57)
	seed number	37.6	40.2	38.2	37.8	38.4 (± 0.60)
Natural	seed weight	42.2	40.3	40.0	45.2	41.9 (± 1.18)
	seed number	40.8	41.2	41.6	41.5	41.3 (± 0.17)

Table 6. Moisture per cent determined for four lots (packets) of seed lot 2, at each moisture level, using both weighed and counted seed. Individual data averaged over the duplicate working samples and the individual data averaged over the four samples lots is presented. Standard errors of the mean are given in brackets

Moisture (%)	Amount of seed used determined by	<i>Quercus ilex</i> Seed lot 1				
		Sample 1	Sample 2	Sample 3	Sample 4	Average
Low	seed weight	15.3	14.1	13.0	14.9	14.3 (± 0.50)
	seed number	15.2	13.9	13.4	14.3	14.2 (± 0.40)
High	seed weight	51.6	43.4	46.4	43.1	46.1 (± 1.97)
	seed number	56.0	43.2	46.4	43.1	47.4 (± 2.93)
Natural	seed weight	44.2	47.9	45.7	45.3	45.8 (± 0.77)
	seed number	41.7	47.9	45.7	45.3	44.2 (± 1.21)

Table 7. Moisture determined by the six laboratories for *Quercus ilex* seed lot 1 for each moisture using weighed and counted seed. Data presented is the average of the moisture determined for the duplicate working samples. The average moisture determined by the six laboratories and with laboratory 1 removed is given

Moisture (%)	Pre-drying seed treatment	<i>Quercus ilex</i> Seed lot 1						Average (6 laboratories)	Average (5 laboratories)
		Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5	Laboratory 6		
Low	seed weight	40.8	42.5	40.5	43.3	39.6	41.3	41.3 (± 0.55)	41.4 (± 0.66)
	seed number	40.5	44.2	42.4	42.0	41.9	40.2	41.9 (± 0.59)	42.2 (± 0.64)
High	seed weight	22.8	22.2	20.4	21.1	22.2	22.8	21.9 (± 0.39)	21.7 (± 0.43)
	seed number	22.5	22.0	23.0	20.1	22.0	21.7	21.9 (± 0.40)	21.8 (± 0.47)
Natural	seed weight	38.4	39.6	41.7	39.5	39.4	39.4	39.7 (± 0.44)	39.9 (± 0.45)
	seed number	39.3	40.2	41.4	38.9	39.8	39.8	39.8 (± 0.37)	39.9 (± 0.44)

Table 8. Moisture determined by the six laboratories for *Quercus ilex* seed lot 2 for each moisture using weighed and counted seed. Data presented is the average of the moisture determined for the duplicate working samples. The average moisture determined by the six laboratories and with laboratory 1 removed is given

Moisture (%)	Pre-drying seed treatment	<i>Quercus ilex</i> Seed lot 2						Average (6 laboratories)	Average (5 laboratories)
		Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5	Laboratory 6		
Low	seed weight	46.8	47.1	43.9	42.4	46.0	46.3	45.4 (± 0.75)	45.1 (± 0.87)
	seed number	43.3	45.1	44.4	44.8	43.1	42.8	43.9 (± 0.39)	44.0 (± 0.45)
High	seed weight	15.2	14.6	15.7	15.1	13.1	14.0	14.6 (± 0.38)	14.5 (± 0.45)
	seed number	15.2	14.5	15.5	14.8	12.6	14.3	14.5 (± 0.41)	14.3 (± 0.47)
Natural	seed weight	47.0	43.3	44.9	42.5	47.2	42.7	44.6 (± 0.87)	44.1 (± 0.88)
	seed number	38.9	41.9	41.1	43.3	44.6	38.9	41.4 (± 0.94)	42.0 (± 0.97)

Scope 2: *Quercus ilex*

a. Moisture determined for confirmation of homogeneity

The seed lots were considered to be sufficiently homogeneous for the validation to proceed (Tables 5 and 6).

b. Moisture determined by the participating laboratories.

There was no significant difference (t-test) in the moisture determined when the amount of seed used was determined by weight rather than by number (Tables 7 and 8).

3. Box plots

The box plots for Scope 1 and 2 show visual agreement between the methods used.

4. Analysis of repeatability and reproducibility of the methods

Table 9 contains the estimates of repeatability and reproducibility for each method within each scope. These estimates are functions of the mixed model that was fit to the data. While a formal test of differences in repeatability and reproducibility between methods is not presently available for ISTA calculations the observed differences between methods repeatability and reproducibility in the table below are judged to be acceptable for identifying whether differences exist.

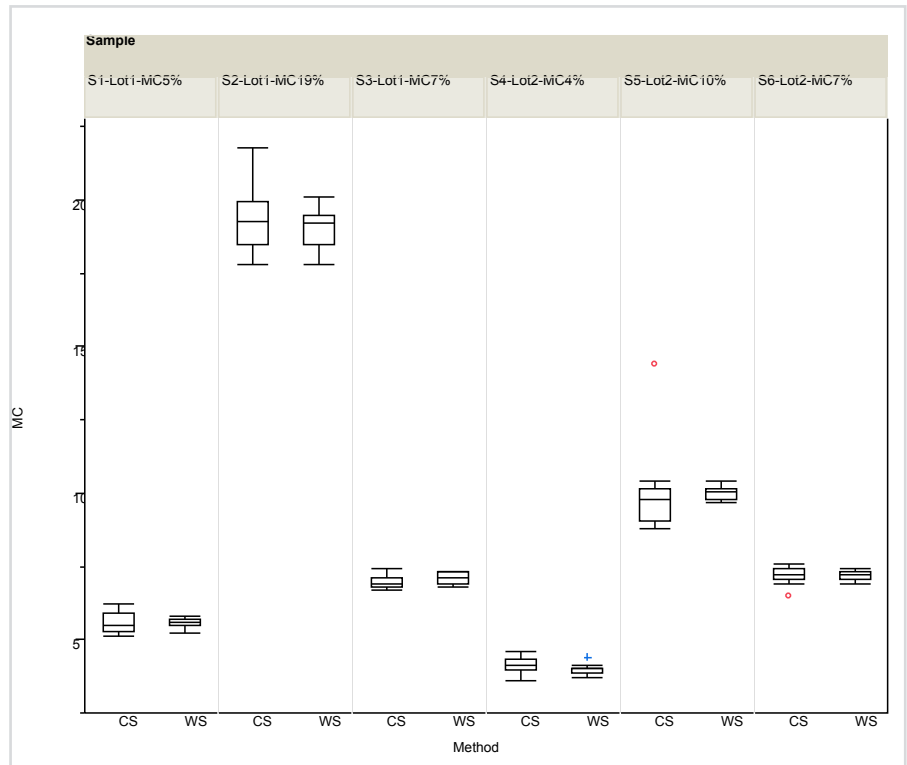
Table 10 gives the repeatability estimates for each laboratory (for Scope 1 laboratory 1 is included for the calculations).

A model with a fixed method effect was used to assess whether the moisture determined by the two methods within each scope differ statistically. The experimental unit for these comparisons are the samples by laboratory (i.e. each sample bag). For Scope 1, there was no significant difference between method means (p-value=0.543) with an estimated 0.9% difference in between method moisture percentages. For Scope 2 there was also no significant difference between method means (p-value=0.081) with an estimated 2% difference between moisture percentages.

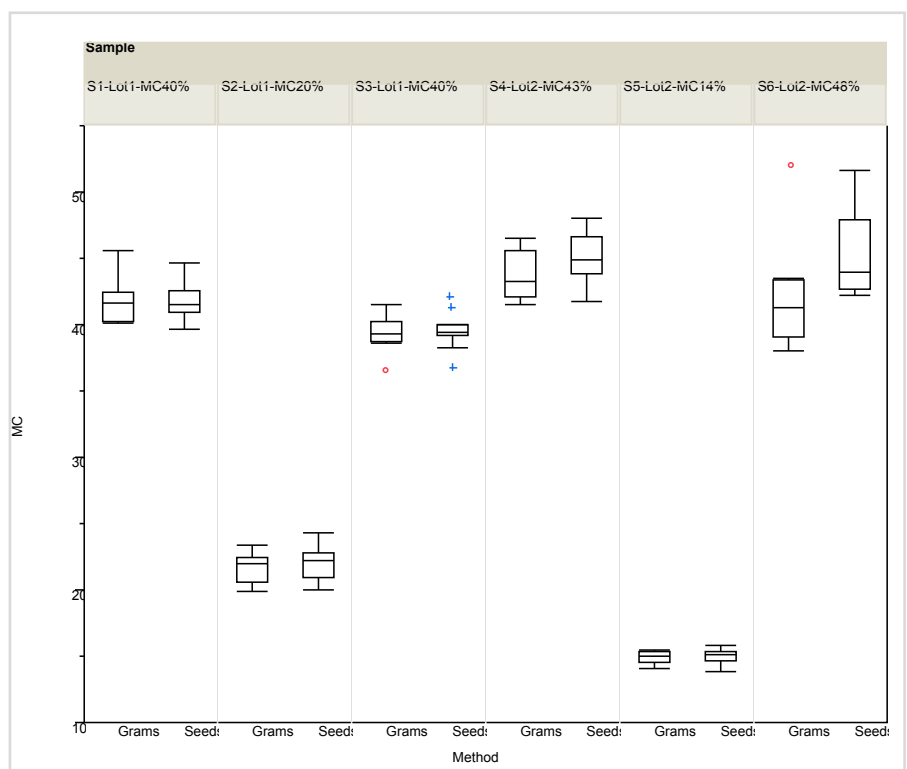
Based on these validation data and associated statistical analysis results, the methods for Scopes 1 and 2 are yielding comparable moisture results in terms of mean and variance (repeatability/reproducibility).

Discussion and recommendations

The results of this validation study after statistical analysis have demonstrated that for both scopes there is no significant difference between the current method of sample preparation (i.e. cutting for seed of *Pinus* spp. with a TSW of > 200 g for scope 1 and using two replicates of five seeds for scope 2) and the proposed methods of testing whole seed of *Pinus* spp. if the TSW is > 200 g and for any species where the TSW is > 200 g using 5 g of seed rather than 5 seeds for each replicate. The statistical analysis also showed that the results



Scope 1. Box-plots by sample and method (Laboratory 1 removed)



Scope 2. Box-plots by sample and method. Note that the samples 1 through 6 for each scope are three samples selected from each of two lots at differing moisture levels.

RULES DEVELOPMENT

Validation study on moisture determination in forest tree seed

Table 9. Estimates of the repeatability and reproducibility between using cut seed and whole seed for Scope 1 (*Pinus cembra*) and determining the amount of seed used for the moisture determination by weight and by number for Scope 2 (*Quercus ilex*)

Method	Scope	Random Effect	Variance Comp.	% of Total	Repeatability	Reproducibility
cut seed	1	Lab	0.07	17	0.15	0.40
		Lab*Sample	0.18	45		
		Residual	0.15	38		
whole seed	1	Lab	0.01	10	0.07	0.09
		Lab*Sample	0.02	18		
		Residual	0.07	72		
by weight	2	Lab	0.51	23	1.10	2.24
		Lab*Sample	0.64	28		
		Residual	1.10	49		
by number	2	Lab	0.00	0	0.83	3.01
		Lab*Sample	3.01	78		
		Residual	0.83	22		

Table 10. Estimate of the repeatability for each laboratory for both scopes (1 - *Pinus cembra* and 2 - *Quercus ilex*)

Scope	Laboratory	Method	Repeatability
1	1	cut seed	0.09
	2	cut seed	0.21
	3	cut seed	0.18
	4	cut seed	0.10
	5	cut seed	0.09
	6	cut seed	0.17
	1	whole seed	0.55
	2	whole seed	0.04
	3	whole seed	0.20
	4	whole seed	0.01
	5	whole seed	0.01
	6	whole seed	0.07
2	1	by weight	0.78
	2	by weight	0.95
	3	by weight	0.47
	4	by weight	2.32
	5	by weight	1.38
	6	by weight	0.67
	1	by number	2.51
	2	by number	0.84
	3	by number	0.16
	4	by number	0.16
	5	by number	0.45
	6	by number	0.86

obtained had comparable variance i.e. the same repeatability and reproducibility.

As a result the recommendation for Scope 1 is that for *Pinus* spp. where the TSW is > 200 g there is no need to cut the seed prior to moisture determination and that whole seed therefore be used for the moisture determination. While the *Pinus cembra* seeds are easy to cut with a scalpel on a board and the mixing and weighing was accomplished within the three minute time limit permitted removed of the cutting step will speed up the moisture determination and reduce a potential source of error (the cutting step) from the moisture determination process.

Similarly the recommendation for scope 2 is that for tree seeds where the TSW is greater than 200 g two working samples of

5 g be used rather than two replicates of an amount of seed equivalent to 5 intact seeds. For very large seeds such as *Quercus ilex* using 5 g of seed has several advantages including that this seed weight will fit into a moisture container with a diameter of greater than 5 cm and less than 8 cm, whereas 5 seeds of *Quercus ilex* equates to 10-15 grams of seed and is therefore outside the amount permitted for containers with a diameter of greater than 5cm and less than 8cm, and in some cases also for the largest weight of seed permitted of 10.0 (± 1.0) g (in containers greater than 8 cm in diameter). Moreover judging five seed fractions from the cut seed can be time-consuming, and lengthens the time the seed is exposed to the atmosphere before moisture determination. The proposed change will

speed up the moisture determination in the laboratory.

Acknowledgements

This work was supported by the NCFB (National Centre for the Study and the Conservation of the Forestry Biodiversity), Peri (Italy), who provided the seed used in this validation and allowed Sergio Pasquini (the working group leader) undertake the validation as part of the study and the conservation of forest biodiversity by the NCFB. The participating laboratories, technical and statistical reviewers and Technical Committee Coordinator (ISTA Secretariat) study are thanked for their contribution to this validation. ■

Seed Health Committee Official Method Review 2014

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Table 1. The two ISTA Official Seed Health Testing Methods reviewed in 2014.

Method No.	Pathogen	Host
7-019	<i>Xanthomonas campestris</i> pv. <i>campestris</i>	<i>Brassica</i> spp.
7-026	<i>Squash Mosaic Virus</i> (SqMV), <i>Cucumber Green Mottle Mosaic Virus</i> (CGMMV), <i>Melon Necrotic Spot Virus</i> (MNSV)	Cucurbits

Table 2. Number of respondent laboratories using the method and whether they consider it fit for purpose

Method No.	Pathogen/host	Method used		Fit for purpose?	
		Yes	No	Yes	No
7-019	<i>Xanthomonas campestris</i> pv. <i>campestris</i> / <i>Brassica</i> spp.	4	2	5	1
7-026	<i>Squash Mosaic Virus</i> (SqMV), <i>Cucumber Green Mottle Mosaic Virus</i> (CGMMV), <i>Melon Necrotic Spot Virus</i> (MNSV)	2	4	5	1

The Seed Health Method Validation Programme requires that methods approved as Official Methods should be reviewed every 5 years to ensure their continuing effectiveness and suitability. Official Seed Health Testing Methods are found in the International Rules for Seed Testing 2014, Annexe to Chapter 7, Seed Health Testing Methods. There are two ISTA Official Seed Health Testing Methods reviewed in 2014 (Table 1). This is the first review for Method 7-026 and the second for Method 7-019. As part of the review process, a questionnaire was sent to all ISTA Member Laboratories and a copy was posted on the ISTA web site for other interested bodies to respond.

Report on the 2014 review questionnaire

Table 1 reports the two Seed Health Methods reviewed in 2014.

Replies to the 2014 method review questionnaire

Only six laboratories from six countries answered the 2014 review questionnaire (Table 2).

7-019 *Xanthomonas campestris* pv. *campestris* / *Brassica* spp.

Of the 4 laboratories that used the method, all agreed that the method was fit for purpose. One laboratory that did not use the method found the method not fit for purpose as they did not use it and could not judge.

To the question: ‘Do you think further improvement is necessary?’, three responded ‘No’ and three laboratories (using the method) ‘Yes’. The following comments were made by these laboratories:

1. ‘Alternative method which can be applied for treated seed has already been in the ISTA Rules, and modification of recipe of two different semi-selective media has been accepted in the ISTA OGM 2014. I think that further improvement is not necessary.’ (Low priority)
2. ‘On pathogenicity test, further instructions are required for DNA extraction’. (High priority)
3. ‘Alternative reagents required.’ (Low priority)

7-026 *Squash Mosaic Virus* (SqMV), *Cucumber Green Mottle Mosaic Virus* (CGMMV), *Melon Necrotic Spot Virus* (MNSV) / *Cucurbits*

Of the 2 laboratories that used the method, both agreed that the method was fit for purpose. One laboratory that did not use the method found the method not fit for purpose as they did not use it and could not judge.

To the question: ‘Do you think further improvement is necessary?’, three responded ‘No’ and three laboratories (using the method) ‘Yes’. The following comments were made by these laboratories:

1. ‘Confirmation of positive results (RT-PCR).’ (High priority)

2. ‘We have not used this method for these viruses. But we use this method for kyuri green mottle mosaic virus.’ (Low priority)
3. ‘More clarification is required on the ELISA plates to be prepared since the numbers tend to increase after addition of the seed extract. The specific amount of antisera to be added in the microtitre plates needs to be clarified too.’ (High priority)

To the question: ‘Is there a need to respond to a technological change in the method? If yes explain in a few words’, five responded ‘No’ and one laboratory made the following comment:

1. ‘Explore the addition of molecular confirmation assays after a positive.’

To the question: ‘Most methods are not validated for use on treated seed. Do you have a need to test treated seeds for this pathogen? If yes please give brief explanation’, three laboratories responded ‘No’. Comments from the other three laboratories:

2. ‘Yes for treated seeds with chemical for viruses.’
3. ‘We have some requests to test Xcc in treated seeds from seed industries. When we issue domestic certificate, we do test Xcc in treated seeds. In that case, we should confirm that chemical which is used for seed treatment does not affect the growth of Xcc prior to the test.’
4. ‘Yes, need arises from the consumption brought by a client, if he wants to have treated seed to be tested. In that case the method to test the seed will need to be used.’

SHC proposals

As a result of the review and in particular comments received via the questionnaires, the SHC makes the following proposals for the two methods.

7-019 *Xanthomonas campestris* pv. *campestris* / *Brassica* spp.

Accept method with a new review date (2019). In the last review the following changes were made to the method: the method changed to 7-019a; new method in the Rules 2014: ‘7-019b Detection of *Xanthomonas campestris* pv. *campestris* on *Brassica* spp. disinfested/disinfested seed’ and the addition of PCR as an alternative to the pathogenicity test.

7-026 *Squash Mosaic Virus* (SqMV), *Cucumber Green Mottle Mosaic Virus* (CGMMV), *Melon Necrotic Spot Virus* (MNSV) / *Cucurbits*

Accept method with new review date (2019).

General editing

The methods will be subject to editing of errors, correction of taxonomy and updated formatting where necessary.

In conclusion, no formal requests for changes or withdrawal of either method under review were submitted to the ISTA Secretariat or the ISTA-SHC since their adoption in 2004 and 2009, respectively. It appears that the methods are thus considered fit for purpose.

The Seed Health Committee wishes to thank all the laboratories for taking the time to complete the questionnaires. Their inputs are greatly appreciated. ■

ISTA Annual Meeting 2015 Montevideo, Uruguay, 15–18 June
Online registration now open: www.seedtest.org/AM15

Accreditation of the Spirou Quality Control Laboratory, Athens, Greece

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Spirou House of Agriculture AEBE is a Greek company active in the fields of agricultural technology, that is research and development, production and distribution of advanced technology varieties and hybrids of seeds and seedlings, offering professional farmers a wide range of vegetable and field crop seeds. The company's operations are spread all over the world, to create products for a wide range of climatic requirements and to support sales all over the Mediterranean region and the Middle East.

Aiming to cover its increasing needs for advanced seed quality, in 1998 the company decided to establish a seed testing laboratory. We started working on germination tests, moisture estimation and thousand-seed weight. Step by step, our needs led us to a) understand those tests in depth, and b) to perform additional tests such as purity, other seed determination and vigour tests. As a result, year after year, the level of our knowledge and premises improved. For this improvement, our route map comprised the ISTA Rules and Handbooks, and the journal *Seed Science and Technology*.

In order to participate in proficiency tests and be informed about all ISTA Rules updates, in 2009 we decided to initiate the procedure for becoming an ISTA Member, and for our quality assurance department to become an ISTA-accredited laboratory. However, while working on our first Proficiency Tests, we found out that we had to work more on some of them, and especially on those related to other seed determination. We also discovered the difficulty of establishing a reliable quality system while not obstructing other activities of our seed testing lab.

We therefore focused on the following points:

- working in depth with Proficiency Tests;
- establishing training procedures for all staff members, e.g. ISTA workshops or in collaboration with the ISTA-accredited Seed Testing Station of the Ministry;
- improving our premises;
- organizing laboratory material and equipment better;
- establishing a standard methodology to calibrate our testing devices.

As a result, we first developed an initial quality system. Later on, we applied this system to our work and tested its effectiveness. In 2013, we submitted the formal application for accreditation to ISTA.

The whole procedure of our laboratory audit was an experience that added value to our knowledge and understanding of the proper route of testing operations. Our methodology was much improved by the pre-audit recommendations and by those of the auditors on site. However, for us, the most valuable knowledge was the behaviour and the way of thinking of the experienced ISTA auditors. Their approach was

quite an eye-opener for us in revealing the best practice to evaluate, correct and improve our activities.

Since the end of 2014, our laboratory is ISTA accredited. The staff consists of six persons: Anna Kontoravdi, Vasiliki Tsioubri, Manolis Papagiannakis, Christos Kotoulas, Anastasios Spyrou and Panayiotis Terzopoulos. The scope of accreditation includes sampling, purity testing, germination testing, other seed determination, moisture content estimation, determination of the thousand-seed weight and biochemical viability test, for grasses, cereals, small legumes, pulses, other agricultural crops and vegetables. The laboratory is also accredited for the conductivity test for *Pisum sativum*, *Phaseolus vulgaris* and *Glycine max*. It is our intention to expand our scope to all vigour tests.

The aim of our laboratory is to continuously improve our knowledge on seed science and technology. For that purpose, we strongly encourage teamwork and cooperation with other laboratories and with institutes and universities. On this basis we already have established a close cooperation with the Agriculture University



The staff of the laboratory. From left to right: Vasiliki Tsioubri, Anastasios Spyrou, Manolis Papagiannakis, Panayiotis Terzopoulos, Anna Kontoravdi and Christos Kotoulas.



of Athens, the University of Thessaly and the Benaki Phytopathological Institute on various topics, including:

- selection of sunflower genotypes for drought resistance;
- development of a protocol for the creation of double haploids in maize;
- development of protocols to select maize and sunflower genotypes for resistance to *Orobanche* and imidazoles;
- development of techniques to select maize genotypes for resistance to

Fusarium verticillioides and *Fusarium proliferatum*;

- development, application and standardization of existing and novel seed vigour tests;
- improvement of seed viability and vigour by innovative chemical and biological treatments;
- optimization of the seed treatments currently employed;
- establishment of a new section devoted to screening genetic purity with molecular techniques.

Our participation in the ISTA family of accredited laboratories gives us the opportunity to establish more co-operation than previously, and become a part of ISTA's effort to achieve its vision of 'Uniformity in seed quality evaluation worldwide'. Since our lab is located in the Eastern Mediterranean region and close to the Middle East, with only few other accredited labs, our contribution could prove to be very helpful for the whole region. ■

Automation of processing of PT results

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We are pleased to inform readers and participants of the ISTA Proficiency Test Programme that recording and reporting of the Proficiency Test results within the Secretariat has been automated.

Participants of the latest PT rounds, PT14-2 on *Callistephus chinensis* and PT14-3 on *Poa pratensis*, received reporting forms in PDF format. Participants were asked to complete the forms electronically, save them without changing the file format or name, and submit the results by e-mail to the ISTA Secretariat.

Adjustments to the calculation programme have been made to enable it to accept an automatic import of the results from the completed PDF reporting form. In addition, the accreditation database has been programmed to facilitate electronic reporting of results to participants. With this step in place, the mailing of paper copies of results by surface post will be discontinued.

During this first trial in automatic reporting, several challenges and improvement points were identified:

1. In some cases, completed report forms could not be saved or were not saved correctly, with the file name and format being changed.
2. In the fields for reporting the weights of pure seed, inert matter and other seed, results were automatically rounded from four to three decimal places.
3. If the percentages of inert matter or other seed were less than 0.05 %, this could not be imported into the database as '<0.05 %' (see example below).

Pure Seed	Other Seed	Inert Matter
0.974	0.000	0.014
98.6	<0.05	1.4

In other cases, the fields were left without any entry (see example below).

Pure Seed	Other Seed	Inert Matter
1.041		1.200
98.8		1.2

4. Data transcription errors when reporting Purity components: percentages calculated by laboratories did not correspond to the reported weights of the components.
5. There was insufficient space for reporting the numbers of other seed.

For the next PT round these points will be addressed as follows:

1. Instruction will be given on the correct procedure for saving the results form.
2. The fields for reporting the weights of pure seed, inert matter and other seed have been adjusted to prevent the rounding of the results entered, and will allow results to be entered to up to four decimal places.
3. Laboratories will be instructed to enter '0.0' when the percentages of inert matter and other seed are less than 0.05 %. Only numbers, not characters, can be entered in the reporting field, and laboratories must complete all fields, leaving none of them blank.
4. Each section of the purity form will contain a reminder to check that the results were transcribed correctly.
5. The forms for purity and other seed will be divided into two separate forms, to create more space for reporting the numbers of other seeds.

We would like to thank all Proficiency Test participants who contributed to this automation process by providing their observation and comments for improvement. ■

Laboratory accreditation changes

Status 15 March 2014

Re-accreditations

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ISTA Workshop on Seed Sampling and Quality Assurance in Seed Sampling

Colonia, Uruguay, 9–12 June 2015

www.seedtest.org/ws201506bsc

The ISTA Bulking and Sampling Committee and the National Seed Institute, Uruguay, invite you to a Workshop on Seed Sampling and Quality Assurance in Seed Sampling in June 2015. The workshop will be made up of lectures, interactive sessions and practical experience in sampling of seed lots and evaluation of samplers. It will also offer the opportunity for general discussion of seed sampling and provide time for participants to ask specific questions regarding automatic samplers and different sampling methods and procedures.

Workshop content

The workshop will consist of lectures and practical exercises including evaluation and examination of candidates. It will offer the opportunity for general discussion on automatic and manual seed sampling and dividing, as well as quality assurance in seed sampling and monitoring of seed samplers. In connection with the lectures, there will be practical session on seed sampling and sample division. The session on quality assurance will focus on control, calibration and maintenance of automatic sampling and manual sampling equipment, as well as audit sampling and monitoring of seed samplers. The use of the ISTA Rules and the ISTA Handbook on Seed sampling will be discussed during the workshop, as well as the ISTA accreditation standard in connection to seed sampling. The language of the workshop will be English (translation to Spanish).

Overview programme

- Introduction to ISTA
- General principles of seed sampling and sample dividing
- Automatic seed sampler (installation, operation, approval and monitory check)
- Marking, labelling and sealing of seed lots and samples
- Sampling in relation to the ISTA Accreditation standard
- Quality Assurance in Seed Sampling

- Control, calibration and maintenance of automatic and manual sampling and dividing equipment
- Internal quality control in seed sampling
- Non-conformities and corrective actions
- Training and authorisation of seed samplers
- Monitoring of seed samplers
- Evaluations and examinations
- Future developments and plans in the Bulking and Sampling Committee

Lecturers

Eddie Goldschagg: Chair of the ISTA Bulking and Sampling Committee (South Africa)

Gerry Hall: Member of the ISTA Bulking and Sampling Committee (United Kingdom)

Max Soepboer: Former Vice-Chair of the ISTA Bulking and Sampling Committee (Netherlands; TBC)

Leena Pietilä: Vice-Chair of the ISTA Bulking and Sampling Committee (Finland; TBC)

Organiser

National Seed Institute, Uruguay
Cno. Bertolotti s/n y Ruta 8, km 29
Barros Blancos, Canelones - 91001
Uruguay

Vanessa Sosa Ph.D. (Manager of Seed Quality Laboratory)

E-mail: vsosa@inase.org.uy

www.inase.org.uy

Telephone: (+598) 2288 70 99 ext. 124

Mobile: (+598) 98 571 000

Sponsor

National Agricultural Research Institute (INIA)

Location

The theory and practical sessions of workshop will take place in the La Estanzuela Experiment Station of the National Agricultural Research Institute (INIA), Colonia (Uruguay). INIA is responsible

for developing science and innovation that meet the needs of the agricultural sector. INIA main experience in field crops is related to: Seed Production, Cultivation and processing, Quality Control, Plant Breeding, Biotechnology Developments and Applications. More information about INIA can be found at inia.uy.

Accommodation

Participants are asked to book accommodation directly with the hotels. Please indicate 'ISTA Workshop' when booking and inform the local organiser

Real Colonia Hotel & Suites
González Moreno 001, Colonia del Sacramento Colonia, Uruguay
Tel: (+598) 4522 1395
www.realcolonia.com
Single room including breakfast: USD 120 (estimated)

Posada Don Antonio
Ituzaingó 232, Colonia del Sacramento Colonia, Uruguay
Tel: (+598) 4522 5344
www.posadadonantonio.com
Single room including breakfast: USD 115 (estimated)

Days Inn Casa del Sol Hotel
Ruta 1 km 170, Colonia del Sacramento Colonia, Uruguay
Tel: (+598) 4522 6383
www.daysinncasadelisol.com
Single room including breakfast: USD 115 (estimated)

Posada del Virrey
Calle de España 217, Colonia del Sacramento Colonia, Uruguay
Tel: (+598) 4522 2223
www.posadadelvirrey.com
Single room including breakfast: USD 100 (estimated)

Participation fee

ISTA members: USD 600

Non-ISTA members: USD 900

The participation fee includes all literature and supporting material for the workshop, lunches and coffee breaks, official dinner and transfer between Montevideo-Colonia-Montevideo and the workshop venue and hotels (except airport transfer). It does not include accommodation or meals other than those specified.

The number of participants is for a minimum of 20 and restricted to a maximum of 25 participants.

If you would like to attend the workshop please fill in the registration form. An invoice will be sent to you, which has to be paid before the participation confirmation will be generated.

Payment by credit card is possible upon request to the ISTA Secretariat. Registration and payment deadline is 30 April 2015.

Please note: For cancellations made before 30 April 2015, registration fees are refundable less USD 50.00 administration fee. For cancellations made after 30 April 2015, registration fees are non-refundable.

General information about Uruguay and Colonia

See page 14. ■

ISTA Workshop on Tetrazolium Testing for Viability and its use as a vigour test for *Glycine max*

Colonia, Uruguay, 9–12 June 2015

www.seedtest.org/ws201506tez

The ISTA Tetrazolium Committee, the Vigour Committee and the National Seed Institute, Uruguay, invite you to a Workshop on Tetrazolium Testing for Viability and its use as a vigour test for *Glycine max*. The workshop will be made up of lectures, interactive sessions and practical experience. It will also offer the opportunity for general discussion of these techniques and provide time for participants to ask specific questions regarding tetrazolium and vigour test methods and procedures.

Workshop content

The workshop will consist of the presentation of several issues covering the different quality problems on soybean seeds, and their identification by using the technique of tetrazolium staining. The knowledge provided to participants will enable them to apply evaluation criteria to classify the seeds in different viability and vigour levels, and finally, to predict the quality of seed lots of *Glycine max* based on their viability and vigour. The language of the workshop will be Spanish and Portuguese.

Overview of programme

- Principles of the tetrazolium test
- Concept of vigour
- Viability and vigour by tetrazolium test in *Glycine max* seeds
- Significance of staining levels
- Viability and vigour pattern
- Samples of working sheets

- Samples of recording sheets
- How to fill in the analysis certificate
- Statistical tolerance tables

Practical work:

- Preconditioning samples cares
- Materials, equipment, concentration and incubation time
- Share diagnostic workshop
- Analysis of quality assurance
- Comparative analysis with germination and vigour
- Green seed samples analysis
- Fractured seed samples analysis
- Mechanically damaged seed samples analysis
- Weathered seed samples analysis
- Stink-bug-damaged seed samples analysis
- genetic damaged seed samples analysis
- Optimum quality seed lot workshop
- Self-correction technique
- Questions and answer sessions: these will consider questions on all aspects of seed viability testing in *Glycine max*.

Lecturers

Jose B. França-Neto: Member of ISTA Tetrazolium Committee, Research Scientist of the Brazilian Corporation for Agricultural Research (Embrapa Soybean) and President of ABRATES- Brazilian Association of Seed Technology (Brazil).

Carina Gallo: Member of the ISTA Vigour Committee, Research Scientist of the National Agricultural Technology Institute (INTA)(Argentina).

Roque Craviotto: Co-Director of the Laboratory for Seed Testing – Oliveros Experimental Station of the National Agricultural Technology Institute (INTA)(Argentina)

Miriam Arango: Research Scientist of the National Agricultural Technology Institute (INTA)(Argentina).

Silvana González: Member of National Agricultural Research Institute (INIA) (Uruguay).

Organiser

National Seed Institute
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Mobile: (+598) 98 571 000

Sponsor

National Agricultural Research Institute (INIA)

Location

The theory and practical sessions of workshop will take place in the La Estanzuela Experiment Station of the National Agricultural Research Institute (INIA), Colonia (Uruguay). INIA is responsible for developing science and innovation that meet the needs of the agricultural sector. INIA main experience in field crops is related to: Seed Production, Cultivation and processing, Quality Control, Plant Breeding, Biotechnology Developments and Applications. More information about INIA can be found at inia.uy

Accommodation

Participants are asked to book accommodation directly with the hotels. Please indicate 'ISTA Workshop' when booking and inform the local organiser:

Real Colonia Hotel & Suites
González Moreno 001, Colonia del Sacramento Colonia, Uruguay
Tel: (+598) 4522 1395
www.realcolonia.com
Single room including breakfast: USD 120 (estimated)

Posada Don Antonio
Ituzaingó 232, Colonia del Sacramento Colonia, Uruguay
Tel: (+598) 4522 5344
www.posadadonantonio.com
Single room including breakfast: USD 115 (estimated)

Days Inn Casa del Sol Hotel
Ruta 1 km 170, Colonia del Sacramento Colonia, Uruguay
Tel: (+598) 4522 6383
www.daysinncasadelsol.com
Single room including breakfast: USD 115 (estimated)

Posada del Virrey
Calle de España 217, Colonia del Sacramento Colonia, Uruguay
Tel: (+598) 4522 2223
www.posadadelvirrey.com
Single room including breakfast: USD 100 (estimated)

Participation fee

ISTA members: USD 700
Non-ISTA members: USD 1050

The participation fee includes all literature and supporting material for the workshop, lunches and coffee breaks, official dinner and transfer between Montevideo-Colonia-Montevideo and the workshop venue and hotels (except airport transfer). It does not include accommodation or meals other than those specified.

The number of participants is for a minimum of 20 and restricted to a maximum of 25 participants.

If you would like to attend the workshop please fill in the registration form. An invoice will be sent to you, which has to be paid before the participation confirmation will be generated.

Payment by credit card is possible upon request to the ISTA Secretariat. Registration and payment deadline is 30 April 2015.

Please note: For cancellations made before 30 April 2015, registration fees are refundable less USD 50.00 administration fee. For cancellations made after 30 April 2015 registration fees are non-refundable.

General information about Uruguay and Colonia

See page 14. ■

ISTA Workshop on Moisture Determination

Canelones, Uruguay, 19–20 June 2015

www.seedtest.org/ws201506moi

Aim of the workshop

This workshop aims to improve the knowledge and technical skills of seed analysts performing moisture determination as part of their daily work. Participants should be able to:

- Know about ISTA rules for moisture determination;
- How to take working samples for moisture;
- Quality assurance in a moisture determination laboratory.

The workshop will be held in Spanish.

Workshop content

The workshop will focus on moisture determination in different species, using

the different ISTA methods as well as give introduction to different quality assurance aspects in a moisture determination laboratory.

Local organiser

National Seed Institute (Uruguay)
Cno. Bertolotti s/n y Ruta 8, km 29 Barros Blancos
Canelones – 91001, Uruguay
Vanessa Sosa Ph.D. (Manager of Seed Quality Laboratory)
E-Mail: vsosa@inase.org.uy
www.inase.org.uy
Telephone: (+598) 2288 70 99 ext. 124
Mobile: (+598) 98 571 000

Lecturers

Jette Nydam: Chair of ISTA Moisture Committee (Sweden)
Sergio Pasquini: Vice-Chair of ISTA Forest Tree and Shrub Seed Committee and Tetrazolium Committee; Member of Moisture and Proficiency Test Committees (Italy)
Teresita Farrás: Seed Quality Laboratory, INASE (Uruguay)

Workshop organiser

ISTA Moisture Committee

Preliminary programme

- Moisture test: theoretical aspects of basic reference method for determination

- of moisture content and practical exercises
- Sampling moisture content determination
- Testing containers for moisture proofness (practical work)
- Taking working sample for moisture and weighing – three different species (practical work)
- Quality assurance in moisture
- Moisture testing in Uruguay: practical aspects (local organizer) and discussion
- Calculation and reporting results
- Measurement of water activity: a non-destructive test
- ISTA Moisture Committee (future)

Participation fee

ISTA members: USD 550
 Non-ISTA members: USD 825

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

It does not include accommodation or meals other than those specified. The number of participants is for a minimum of 12 and restricted to a maximum of 15 participants. If you would like to attend the workshop please fill in the registration form. An invoice will be sent to you, which has to be paid before the participation confirmation will be generated.

You can pay by credit card upon individual request to the ISTA Secretariat. Registration and payment deadline is 11 May 2015.

Please note: For cancellations made before 15 May 2015 registration fees are refundable less USD 50.00 administration fee. For cancellations made after 15 May 2015 registration fees are non-refundable.

General information about Uruguay

See page 14.

Accommodation

Dazzler Hotel
 21 de Setiembre 2752, Montevideo, Uruguay
 Tel: (+598) 2716 00 00
 reservas@dazzlermontevideo.com
 Web: www.dazzlermontevideo.com
 Single room including breakfast: USD 99

Regency Golf
 Francisco Solano García 2473, Montevideo, Uruguay
 Tel: (+598) 2710 44 44
 ventas@regencygolf.com.uy
 Web: www.regencygolf.com.uy
 Single room including breakfast: USD 90

Armon Suites Hotel
 21 de Setiembre 2885, Montevideo, Uruguay
 Tel.: (+598) 2712 41 20
 ventas@armonsuites.com.uy
 Web: www.armonsuites.com.uy
 Single room including breakfast: USD 90

International Society for Seed Science Workshop on Seed Longevity: ‘Seeds For Future Generations – Determinants of Longevity’

IPK Gatersleben, Germany, 5–8 July, 2015

Plant genetic resources play a major role for global food security. Worldwide, 7.4 million accessions are stored in about 1750 ex situ gene banks. Since the majority of gene bank holdings globally are stored as seed, seed longevity is of exceptional importance for germplasm conservation. Great differences between plant species were recognised. In addition a huge variation within a species is present. However, there is a deficit in understanding the biology behind long and short seed life. The seed longevity workshop will focus on all aspects of seed ageing/conservation. It will concentrate on molecular mechanisms of biochemistry, physiology, biophysics and genetics of seed survival. Hence, it will bring together scientists involved in seed science and seed banking.

With a total inventory of 150 000 accessions of 3212 plant species and 776 genera, the gene bank at IPK in Gatersleben, Germany, holds one of the most comprehensive collections worldwide. Therefore, it will be a predestined host for the workshop. Early July will be an appropriate time for the workshop. It will allow the participants to visit the gene bank facilities, including the extensive seed regeneration activities (~ 8000 accessions) in the fields and glass-houses of IPK.

The following topics will be discussed:

- Seed banking – state of the art
- Role of pre- and post-harvest environmental factors on seed longevity
- Genetics of inter- and intra-specific variation of seed survival

- Physiology and biochemistry behind seed ageing - deleterious effects vs. repair mechanisms

Detailed information about registration, accommodation and travel details you will find under:

http://meetings.ipk-gatersleben.de/ISSS_Longevity_2015/

Registration fee

ISSS Members: EUR 410
 Non-ISS members: EUR 450
 Students: EUR 380
 Accompanying persons: EUR 250

Deadline for final registration and hotel booking: 31 May 2015

ISTA Workshop on Quality Assurance for advanced laboratories

Álvares Machado, Brazil, 9–12 September 2014

Rasha El-Khadem

ISTA Accreditation and Technical Department

ISTA Secretariat
8303 Bassersdorf, Switzerland

In September 2014, the first Quality Assurance Workshop to be organized in South America took place in Álvares Machado, Brazil. The interest from our Brazilian seed testing colleagues was overwhelming. Pedro Henrique Lorenconi from the accredited Matsuda Seed Testing Laboratory and Miriam Alvisi from the government seed testing station Laboratório Oficial de Análise de Sementes Supervisor em Minas Gerais were both involved in the preparation of the workshop, and helped to channel the needs of the participants to draft a workshop programme. The Ministry of Agriculture in Brasília also co-operated, providing the simultaneous interpretation service. The workshop was attended by 23 participants, 22 from Brazil and one

from Bolivia. The participants were from the Ministry of Agriculture, government seed testing stations and the seed industry.

Rita Zecchinelli, ISTA Technical Auditor and member of the ISTA Executive Committee, and Rasha El-Khadem, head of the ISTA Accreditation Department, delivered the lectures.

Matsuda sponsored the workshop by renting a well-equipped lecture room in a hotel. The Brazilian Ministry of Agriculture sponsored the event by organizing simultaneous interpretation by two professionals. It was the first time for both lecturers to work with simultaneous interpretation, and it was a very positive experience for participants and lecturers. Questions and discussions could be addressed quickly and with no delay, offering non-English speaking participants and the non-Portuguese speaking lecturers a great tool of communication.

Lecturers were provided for the quality management system as well as for the technical part of a seed testing laboratory. Rita gave a brief introduction about the International Seed Testing Association. The following topics were covered regarding the technical part:

- Management of equipment in general including equipment: balances, divider check
- Sampling
- Equipment: balance check
- Divider check
- Equipment: temperature measurement and control
- Substrate check
- Evaluation of a phytotoxicity test using ANOVA (using laptop with Excel)
- Statistical aspects of seed testing
- Storage of samples





The lectures dealing with the quality management system were the following:

- Internal audits and how to prepare a checklist
- Document control
- Internal Quality Control and Monitoring of laboratory staff
- Corrective actions and non-conforming work
- General Management
- Management Review

After each day, the lecturers offered a ‘Question and Answer’ session where they asked the participants about the content of the day to see if all topics have been clear and if all aspects were understood.

During the group work sessions, the participants were given the possibility to work on specific topics where they could exchange experience and come up with solutions to defined tasks. The groups could present their conclusion to all participants and explain how and why they came to the solution they suggested.

During the group work related to the management of equipment, the participants reviewed forms related to equipment registration, maintenance and calibration that they were handed over to see if the ISTA requirements related to equipment and calibration are met.

After the lecture on internal audit, the participants had to develop in their groups a checklist to be used to perform an internal audit on the verification of riffle dividers.

The participants suggested non-conformities that they would be interested in to be used to set up corrective actions. From the seven non-conformities, the participants chose the highest ranked ones to define corrections, investigate possible root causes and to define corrective actions and a plan on how to measure effectiveness thereof. Both lecturers provided the participants with their experience from auditing and on how to deal with non-conformities when they occur in seed testing.

The group work on the monitoring of staff dealt with setting up a monitoring plan for a laboratory for either sampling or a specific test. The groups had to define which monitoring tools they would use, how and in which frequency they would record data and how they would check for possible trends.

We visited the cultural center in Presidente Prudente. An old factory building used for processing cotton has been



reconstructed to present a diverse cultural offer for visitors. The center hosts courses in different areas such as painting, music, dance and literature. Libraries, cinema and theatre have also been integrated and are available for the public.

The official dinner took place in the same hotel and it has been a pleasure to meet Jorge Matsuda and his wife in person.

The group visited the ISTA accredited seed testing laboratory of Matsuda and

had a guided tour through the laboratory facilities. The participants had the opportunity to practice and check their competency in reducing a sample by applying the hand halving method.

We would like to thank the staff of Matsuda for their hospitality, the great opportunity to meet them and visit their laboratory as well as the smooth and continuous support throughout all phases of the workshop. The Ministry of Agriculture

must be thanked for organising and financing the simultaneous translation as well as the printing material.

It has been a pleasure to meet all participants and to exchange experience among each other. We sincerely hope that this is just the beginning of more ISTA workshops in Brazil and the region. ■

ISTA Workshop on Seed Sampling and Quality Assurance in Seed Sampling Saskatoon, Canada, 30 Sep.–3 Oct. 2014

Steve Jones

Member, ISTA Executive Committee, Chair, ISTA Rules Committee, Member, ISTA Bulking and Sampling Committee

Canadian Food Inspection Agency
Saskatoon Laboratory
Seed Science & Technology Section (SSTS)
Saskatoon, Sask. S7N 4L8, Canada
steve.jones@inspection.gc.ca

In September 2014, the Canadian Food Inspection Agency's (CFIA) Saskatoon Laboratory, Seed Science & Technology Section (SSTS) hosted its fourth ISTA workshop in five years. There were 17 people attending, mainly from North America, but also from Japan and South Korea. All of Canada's ISTA-accredited and Member Laboratories were represented, and people from government, private labs and seed companies were present.

SSTS is an ISO 17025- and ISTA-accredited laboratory working on behalf of the Government of Canada to monitor the quality of seed, test for export, both train and examine seed analysts and monitor private laboratories in Canada. Janine Maruschak (Section Head, Seed Science & Technology Section) and Steve Jones (Chief of Germination and Purity) welcomed the participants. Lectures were presented by Eddie Goldschagg (Chair of ISTA Bulking and and Sampling Committee), Gerry Hall (Member of the ISTA Bulking and Sampling Committee) and Steve Jones. The topics covered included an overview of ISTA, the theory behind sampling, automatic samplers, manual



Gerry Hall demonstrating bag sampling

sampling, mixing and dividing techniques, and quality assurance linked to sampling, equipment and monitoring of samplers.

Marc Sabourin (Director, Saskatoon Laboratory) joined the participants on the visits to a local seed producer and seed merchant. CFIA Inspectors demonstrated sampling techniques used in Canada to sample from both on-farm metal storage bins and bagged seed for marketplace monitoring of seed. The challenges of a national sampling program in a country

the size of Canada and with large seed lots were discussed.

The group also discussed and exchanged experiences in seed sampling and best practices for training and monitoring samplers. During the workshop all participants had the opportunity to practise sampling, mixing and dividing techniques using a range of equipment.

During the visit to CFIA's ISTA-accredited laboratory in Saskatoon, participants were given a tour of the laboratory, which included a visit to Canada's only National



Bin sampling



Visit to Wanuskewin Heritage Park

Seed Herbarium (NSH). Ruoqing Wang (NSH Curator) gave the background to this unique and essential reference collection of seeds which are regularly used in the identification of crop seeds and weeds during seed certification and phytosanitary testing in SSTS.

It was not all work, and the official dinner was held at Wanuskewin Heritage Park, a national historic site and Northern Plains Indians cultural interpretive centre located 5 km north of Saskatoon. Participants enjoyed a walk, during which a guide

described the role that various plants and the buffalo played in the life of the aboriginal people who lived in this area. This was an opportunity to sample Saskatchewan history, culture and food, with buffalo burger on the menu.

On the final day of the workshop, as well as a question and answer session, Eddie Goldshagg summarised the workshop and outlined the future work of the ISTA Bulk-ing and Sampling Committee.

Special thanks go to colleagues at SSTS and CFIA Operations, especially Tanya

Staffen, who helped arrange the workshop and venues. The workshop feedback showed that everyone found the experience enjoyable, interesting and useful. As hosts we thank all the participants, lecturers and CFIA staff, as well as the ISTA Secretariat, who helped to make the workshop a success. Special thanks are due to SANSOR, South Africa and SASA, Scotland, UK for allowing both Eddie and Gerry the time to lecture at the workshops. ■



ISTA Workshop on Variety Identification Using Molecular Markers

Ottawa, Canada, 6–9 October 2014

Trevor Mazutinec, B.Sc.

Assistant Laboratory Manager

BioVision Seed Labs
 Sherwood Park, Alberta, Canada T8H 2R6
 E-mail: trevor.mazutinec@biovision.ca

The ISTA Workshop on Variety Identification using Molecular Markers was held from 6–9 October 2014 at the Fallowfield Laboratory of the Canadian Food Inspection Agency. The workshop was attended by participants from Canada, Nigeria, Turkey and Zimbabwe. Lecturers for the workshop included Dr. Daniel Perry from the Canadian Grain Commission, Dr. Marie-Jose Cote, Ms. Cheryl Dollard and Ms. Willy Drost of the Canadian Food Inspection Agency.

The workshop began on Monday morning with a quick outline of the workshop and a general introduction of ISTA. We then heard a short presentation on Variety Identification from a Canadian perspective. The participants were split into four groups and were then driven out to a

few plots of wheat that had been planted earlier in the summer specifically for the workshop. We walked through the different plots and looked for contaminating varieties by observing different phenotypic traits. The week before the workshop, the hosts selected two known plants (Normal-Bulk) and six suspected off-type plants for each group. These selected plants were then used by the groups for the remainder of the workshop.

Monday afternoon started with a short presentation on the theory of DNA Extraction. This was followed by everyone heading to the lab to extract our DNA from the selected plants. We followed the procedure for DNA Extraction from a kit that was designed specifically for that purpose. There was a lot of pipetting involved with using the kit and some of the participants hadn't used a pipette in a long time. It took the remainder of the afternoon to complete the DNA Extraction procedures.

Tuesday was Microsatellite Day and began with a lecture on the basics of DNA structure and PCR. From there, we moved back to the lab and quickly discussed a different DNA Extraction method for extracting the DNA from seed. After a quick break, there was a short lecture on the theory of using Microsatellites for Variety Identification. Back in the lab, we received the quantification data from our DNA extractions the day before. Using these values, we were able to calculate how much DNA we needed to use for the different methods of Variety Identification.

Next, we prepared and set up our samples for two different PCR reactions. The first PCR reaction was prepared for the DNA to be loaded on a Li-COR 4300 DNA Analyser. The second PCR reaction was prepared for the DNA to be loaded on an ABI 3730xl Genetic Analyser. Both PCR reactions took about 2 hours to run. After the PCR was complete, the participants each loaded their PCR products on the Li-COR gel; which was prepared in advance by the workshop organizers. The products from the second PCR reaction were run through the ABI machine by the organizers, with the participants observing how the ABI 3730xl operates.

Wednesday was SNP day and began with a lecture on the theory of using SNPs for Variety Identification. There were six SNPs that were chosen for the workshop by the organizers and each group used the same six SNPs for their analysis. We prepared two more PCR reactions, one for Allele specific PCR and one for a Real-Time TaqMan Assay. The Allele specific PCR reaction took about 2 hours to finish. Once it was complete, we loaded the PCR products onto an Agarose gel. The Agarose gel was prepared for us by the organizers. The TaqMan Assay PCR is a Real-Time reaction, which means that you can get results immediately after the reaction is complete. There is no need to load a gel or have any secondary analysis. The reaction took about 2 hours and was looked after by the workshop organizers.





Wednesday evening was set aside for the group dinner which was held at a restaurant called The Black Tomato. The dinner was attended by both the workshop participants and organizers. The food was very good and we had a lot of fun and good conversation.

Thursday was the final day of the Workshop and was the day that we interpreted all of our data. We had an image of a gel from the Li-COR 4300 DNA Analyser, chromatograms from the ABI 3730xl, a gel image from our Allele Specific Agarose Gel and Allelic Discrimination Plots from our Real-Time TaqMan Assay. We also were given some SNP data from an ABI OpenArray system; which was provided to us by the workshop organizers. They used our extracted DNA and sent it to Dr. Daniel Perry's Lab at the Canadian Grain Commission in Winnipeg for analysis on the OpenArray.

Once the data had been collected and interpreted, we sat down and discussed what each group found. We were able to determine that two groups each had two different contaminating varieties in their plots, while one of the other groups only had one contaminating variety in their plot. Interestingly, the fourth group determined that all of the samples taken from their plot (Normal-Bulk and Suspected Off-Types) did not match the variety that the plot was supposed to be. It was revealed by the organizers that the wheat variety that they wanted to use, would not germinate properly, so they used a different mix of wheat varieties, while still keeping the original label on the plot.

The workshop was concluded with a short presentation on the work of the ISTA DNA Working Group. Each participant was then presented with a certificate showing recognition of participation in the workshop. The lecturers and organizers

were also presented with gifts in recognition of their hard work in preparation for the workshop.

A very big Thank You to Ms. Lisa Leduc and Mr. Adam Colville for all of their hard work and preparation for the practical portions of the workshop. Thank you to Ms. Eliane Guillemette for her hard work and preparation of the field plots of wheat. Thank you to Dr. Marie-Jose Cote, Dr. Daniel Perry and Ms. Cheryl Dollard for their hard work and organization of the workshop. Thank you to Ms. Willy Drost for taking her time to be a lecturer for the workshop. Thank you to the Canadian Food Inspection Agency (CFIA) for hosting the workshop and allowing their employees to participate. Thank you to the ISTA Secretariat for their support during the organization of the workshop, especially Ms. Nadine Ettel. ■

ISTA Hands-on Seminar on Seed Image Analysis

Angers, France, 14–17 October 2014

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From 14–17 October, 2014, the ISTA Advanced Technologies Committee (ATC) and GEVES (Angers, France) organized the first ISTA Hands-on Seminar on Seed Image Analysis. The venue of the event was the GEVES laboratories in Angers, France, a true seed science inspiring environment, and a Valhalla for the seed enthusiasts. The interest to participate in the Hands-on Seminar was too large to accommodate. However, the organisers managed to accept a few more participants

than anticipated and finally a total of 45 participants from 14 countries all over the world joined.

The Hands-on Seminar was a balanced mixture of active working with imaging and image analysis systems, demonstrations, lectures and discussions. Participants in small groups visited dedicated practical sessions, data analysis workshops and demonstrations.

After the introduction to the Hands-on Seminar and the GEVES/SNES laboratories by Joël Léchappé (director of GEVES/SNES and ISTA President), a general introduction to technologies applicable for seed imaging was presented (Bert van Duijn, ATC Chair). Technologies based on electromagnetic radiation (from gamma rays via X-rays to visible light to terahertz radiation), acoustic waves, mechanical

sensing, NMR etc. were illustrated with examples from seed science. To be able to link these technologies to the current and future requirements of the ISTA Technical Committees, Sylvie Ducournau (Chair, ISTA Germination Committee) gave an extensive overview of the needs, current status and requirements of seed imaging in seed testing practice based on feedback of the ISTA Committees.

Follow-up lectures were more detailed on the technical aspects, and provided basic introductions to the workshops. Fabio Gorian (Chair, ISTA Forest Tree and Shrub Seed Committee) introduced NMR imaging of seeds as a promising tool for seed analysis. As an introduction to the workshops, lectures on data analysis of multispectral images (Birte Boelt, ATC member), and the basics of X-ray imaging



At the wine tasting

(Bert van Duijn) were presented. Later in the week there were various lectures on instrumentation and image processing for multimodality imaging (Landry Benoit, Angers University, France), multispectral data analysis (Benoît Jaillais, INRA, Nantes, France) and near-infrared (NIR) imaging (Eloise Lancelot, INRA Nantes, France), which were and lively discussed in the frame of the hands-on experience already acquired by the participants.

The lectures and introductions were appreciated by the participants but, of course, all were eager to work with equipment and experience the possibilities and pitfalls, and produce and analyse seed images themselves. In a revolving scheme of workshops, demonstrations and visits, small groups worked hard to learn and discuss the various imaging technologies that are applied in seed analysis. Workshop and practical sessions were held on multispectral image acquisition and multispectral

image data analysis. Participants worked with selected seed lots illustrating various seed properties that can be distinguished in multispectral images. In the workshops on 2D X-ray image acquisition and 3D X-ray image analysis, the participants followed a step-by-step approach using different types of seeds to learn about all aspects of X-ray seed imaging and image analysis. In the chlorophyll fluorescence (CF) workshop, the participants studied the relationship between seed maturity and CF for different seeds, as well as the influence of e.g. seed moisture content on the CF readings. The demonstration of the automated seed germination and seed imaging tables was followed by a germination image analysis workshop. Demonstrations of X-ray computed tomography and visits to the germination laboratories, physical analysis laboratories, sampling laboratories and phytopathology laboratories completed the programme.

Despite the very intensive programme and long days in the laboratories, time was reserved for wine tasting and an inspiring visit to Brissac Castle and Angers old town. During the joint dinner at the first evening everybody got an opportunity to get acquainted with each other which facilitated the interactions in the rest of the week.

The Hands-on Seminar was finalized by three discussion groups (based on working background; industry, testing station and research) on the needs and hurdles in application of imaging technologies in seeds. Each group reported their findings and opinions.

The Hands-on Seminar on Seed Image Analysis was a great success, with enthusiastic participants, strong interaction and steep learning and experience curves. This success was only possible because of the enormous amount of work, expertise and hospitality of the people of GEVES. ■



ISTA Workshop on Seed Vigour

Bengaluru, India, 10–13 November 2014

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An ISTA Vigour Workshop was held in Bengaluru, India, on 10–13 November, 2014. The workshop was hosted by Indo-American Hybrid Seeds, Bengaluru, India as part of their Golden Jubilee celebration of the founding of their establishment in 1965. The workshop was presented by Alison Powell, Chair of the ISTA Vigour Committee, and Vigour Committee member Stan Matthews. A full participation of 30 persons came from 8 countries: Australia, Bangladesh, Brazil, Japan, Philippines, South Africa and Sudan, with a large representation from India. The majority of the participants were from seed companies, as well as from the Directorate of Seed Research of the Government of India, and from the State Agricultural Universities in Bengaluru and Dharwad.

The workshop began with a welcome session with the special invitees Dr. Malavika Dadlani, former member of the ISTA Vigour Committee and Joint Director, Research, IARI, New Delhi, and Dr. V. Sankaran, Director TQM, Krishidhan Seeds, representing public and private seed organizations in India. We were warmly welcomed by Mrs Rashmi Attavar, Joint Managing Director, Indo-American Hybrid Seeds, and also by Dr G.V. Jagadish, Head of the Seed Laboratory and local organiser of the workshop.

The workshop started with an Introduction to ISTA, illustrating the work of ISTA and the Technical Committees, followed by an Introduction to Seed Vigour. The aim of the workshop was to present the validated vigour tests and new and future developments in vigour testing. The lectures were held at the Hotel Chancery in Bangalore, which provided very good facilities. There were lectures on the validated vigour tests, viz. the conductivity, controlled deterioration, accelerated

ageing and radicle emergence tests, and also on other commonly used tests, the cold, cool and seedling growth tests. These were followed a lecture emphasizing the importance of precision in vigour testing. An integrating hypothesis, seed ageing and its repair, was presented in another lecture, illustrating how these two processes and their interaction explain both the causes

of vigour differences and the basis of all vigour tests. Finally, the workshop lectures considered the development of new vigour tests, test validation and use of vigour tests. We also held a data-based practical session in which participants used example test results to consider how they would develop current tests for new species.



Dr G.V. Jagadish and staff from IN07.



Interactive seminar.



Discussing data to develop tests for a new species.



Evaluating the radicle emergence test for maize.

Our practical work took place at the Head Office of Indo-American Hybrid Seeds, where the ISTA-accredited seed laboratory IN07 is based. Dr. Jagadish and his colleagues had worked meticulously to provide 10 groups of participants with test material and results. The participants were able to complete the conductivity test and assess results from the radicle emergence

and cold tests for corn; controlled deterioration test and radicle emergence tests for cauliflower; electrical conductivity test for soybean and chick pea, and the accelerated aging test for cauliflower. It was encouraging for the participants to see that different tests identify the same differences in seed vigour. Comparison of the vigour test results with the standard germination test

clearly showed that the vigour tests provide additional information about seed quality.

Highlights of the workshop were the two interactive seminars led by Alison Powell and Stan Matthews. In each seminar, six groups discussed aspects of vigour testing and its application, including the characteristics of the validated vigour tests; application of vigour tests to new species;



Workshop participants and organisers.

constraints in implementing vigour tests; how vigour test results could be used to classify seed lots for seed trading; and research and development priorities for seed vigour. The ideas from the discussions were subsequently shared with the whole group. These interactive sessions opened up many ideas and discussions. The experiences shared by participants from different countries and working on different crops were most illuminating.

The lecturers at the workshop particularly appreciated the very active role of

the participants from the first minutes of the workshop. Their views and experiences often support and enhance information in the lectures and also add to the lecturer's knowledge of the seed sector in different countries. In addition to working, the participants relaxed during a visit to the Lalbagh Botanical Garden in the city, and attended the workshop dinner, held thirteen floors above the city, which offered a beautiful view over the evening city skyline. We enjoyed a delightful meal and excellent company.

We are very grateful to Indo-American Hybrid Seeds for inviting us to Bengaluru and for their warm and generous hospitality. We particularly thank Dr. Jagadish for the care and attention he gave to every detail through the planning to the execution of the workshop. He was ably assisted by many people, most of whom are shown in the photo; we cannot thank individuals in case someone is missed out, but we are grateful to everyone for their contribution and welcome to Bangalore. ■

ISTA Workshop on Seed Health Testing Depok, Indonesia, 10–14 November 2014

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The ISTA Workshop on Seed Health Testing was organized by Balai Besar PPM-TPH, Ministry of Agriculture, Indonesia. The seed testing laboratory of Balai Besar-PPMB-TPH is an ISTA Member Laboratory involved in seed health testing for rice fungus and nematodes.

This workshop aimed to improve the understanding and practical ability in seed health testing based on the ISTA Rules.

There were 15 participants from nine countries: Malaysia (1), Thailand (2),

Sudan (1), the Philippines (2), Indonesia (4), South Africa (2), Bangladesh (1), Pakistan (2) and India (1). The majority of participants were from seed companies or research institutes, or were governmental officers.

The instructors were Valerie Grimault (Chair, ISTA Seed Health Committee), Mark Buimer (member, ISTA Seed Health Committee), Masatoshi Sato (member of the ISTA Seed Health Committee and Executive Committee), Corinne Sarniguet (nematode specialist of the Laboratory of Plant Health ANSES-LSV), and Evi Todong Tondok (lecturer of the Plant Protection Department of Bogor Agriculture University).

The workshop included lectures and practical work, and it was designed as an introduction to seed health testing for fungi, bacteria, viruses and nematodes. The topics included good laboratory practice and critical control points in seed health testing, quality assurance, sampling, blotter test for rice fungus, molecular techniques and serological techniques in seed health testing, and especially for nematodes. The lecture was given by web presentation.

There was a very tight schedule during the workshop, and all the participants worked very hard at the practical sessions, switching between mycology, bacteriology and virology with productive discussions during the course. The practical work





was done in laboratories and in the screen house.

On the last day of the workshop, all participants had the chance to visit the Agriculture Quarantine Laboratory. Here the participants were given an overview of plant and animal quarantine in Indonesia, the regulations and also the facilities.

The next visit was to Indonesia Miniature Park. This park represents all local culture from 33 provinces in Indonesia, and includes some museums dedicated to the due to the local culture. The participants

visited places such as the Indonesia Museum, a traditional house of West Sumatra and a traditional house of Papua. The evening before, the participants and local organizers had dinner at the Kintamani restaurant, where there was some traditional dancing from West Java called Jaipong, and some Indonesian food. Some participants and lectures tried to join in with the dancers.

There was new knowledge, new experiences and also many new colleagues from many countries during the workshop. Thanks to all participants for their full attention given to the lectures and practical work, thanks to all lecturers who had share their knowledge, thanks also to Mr. Tri Susetyo, Mrs Dewi Taliroso and all staff for their support of this training, and special thanks to all laboratory staff who helped to prepare the tremendous amount of work to make this workshop a success.



ISTA Workshop on Seed Sampling & Quality Assurance in Seed Sampling Bengaluru, India, 17–20 November 2014

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The ISTA Workshop on Seed Sampling and Quality Assurance in Seed Sampling was hosted by Indo-American Hybrid Seeds (India) Pvt. Ltd., Bengaluru, India (IAHS) and organised by Dr. G.V. Jagadish. The workshop was conducted by Eddie Goldschagg, Chair of the ISTA Bulking and Sampling Committee (BSC); Gerry Hall, member of the BSC and Head of Seed Certification at Science and Advice for Scottish Agriculture; and Max Soepboer, former Vice-Chair of the BSC and consultant in seed sampling and seed inspection systems, from the Netherlands. All three are long-standing members of the BSC and have lectured at previous sampling workshops.

The workshop focused on two topics: seed sampling and quality assurance. Both were dealt with in theory and in practice. There were 26 participants from Bangladesh, India, Indonesia, Sudan and Thailand. The participants came from ministries of agriculture, seed testing

laboratories (both official and private), research centres, certification agencies and the seed industry.

The theoretical lessons were held in the Hotel Chancery in Bengaluru, and the practicals in the seed processing unit at IAHS. At this seed processing unit, seed lots in various types of container (hessian bags, big bags, bulk, tins, pouches and small packages) were made available for sampling training and examination purposes by the IAHS sampling staff.

With regard to seed sampling, the following aspects were covered:

- General principles of seed sampling
- Introduction to sampling methods
- Seed sampling: sealing and labelling of seed lots
- General principles and types of automatic seed samplers
- ISTA protocol for approval of automatic samplers
- Testing and approval of automatic seed samplers
- Methods for reduction of the composite sample
- Training, examination and authorization of seed samplers

Concerning quality assurance, the following items were dealt with:

- The ISTA Accreditation Standard
- Quality assurance in seed sampling
- Internal quality control in seed sampling
- Monitoring of seed samplers (internal audits and assessments)
- Non-conformities and corrective actions
- Check and calibration of sample dividers

The item of blending of seed and seed lots was also dealt with. Although there are no ISTA rules nor other international regulations for blending, good practices of blending were discussed on request and examples of blending were shown.

Each item was covered by a presentation for which the participants received hand-outs. In the interactive session, participants were divided into groups for lively discussions and enriching knowledge on the topics covered during the workshop.

During the practicals, participants practiced various ways of sampling such as sampling of bags with the Nobbe trier, sampling of boxes and pouches with the sampling stick, sampling of big bags with the extended Nobbe trier and sampling of





small containers such as tins, small packages etc. Besides this participants carried out various sample homogenization and reduction methods.

The practicals on quality assurance in seed sampling consisted of:

- drafting a programme for the calibration of various sample reduction methods (soil divider, centrifugal divider, hand halving method);
- defining the root cause of a non-conformity and drafting a plan to prevent the same non-conformity in the future;

- drafting a checklist for the assessment of seed samplers with the aim to detect possible trends;
- classifying conformities into 'substantial' (major) and 'non-substantial' (minor).

For these assignments the group was divided into small subgroups that each had to work out the assignment. The results of the work of the subgroups were subsequently discussed in the plenary group.

The social events included the workshop dinner on the second day at the company's farm. IAHS also organized a safari programme at Bannerghatta National Park, Butterfly Park and Biological Park on the evening of the third day, which was an impressive trip and experience.

The feedback provided by the workshop participants showed that their expectations were met and that they found the lectures and the group work very useful. ■



2015	25–27 May	ISF World Seed Congress 2015	Kraków, Poland	www.worldseedcongress2015.com
	30 May–5 June	AOSA/SCST 2015 Annual Meeting	Tampa, Florida, USA	www.aosaseed.com/annual_meeting_2015_tampa
	9–12 June	ISTA Workshop on Seed Sampling and Quality Assurance in Seed Sampling	Colonia, Uruguay	www.seedtest.org/ws201506bsc (see p. 43)
	9–12 June	ISTA Workshop on Tetrazolium Testing for Viability and its use as a vigour test for <i>Glycine max</i>	Colonia, Uruguay	www.seedtest.org/ws201506tez (see p. 44)
	15–18 June	ISTA Annual Meeting	Montevideo, Uruguay	www.seedtest.org/am15 (see p. 11)
	19–20 June	ISTA Workshop on Moisture Determination	Canelones, Uruguay	www.seedtest.org/ws201506moi (see p. 45)
	5–8 July	ISSS Workshop on Seed Longevity	Gatersleben, Germany	http://meetings.ipk-gatersleben.de/ISSS_Longevity_2015/index.php (see p. 46)
	11–13 October	European Seed Association Annual Meeting/European Seed Trade Meeting	Vienna, Austria	http://esa.conceptum.eu
16–19 November	Asian Seed Congress	Goa, India	http://apsaseed.org	
2016	June	31st ISTA Congress	Tallinn, Estonia	

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