Seed Testing
INTERNATIONAL

ISTA News Bulletin No. 143 April 2012

4 Monitoring ISTA seed samplers

12, 16 The ISTA Annual Meeting at the Floriade 2012, Venlo, the Netherlands

FLORIADE HORTICULTURAL WORLD EXPO
VENLO 2012 HOLLAND

EDITORIAL

Dear Reader,

You have in your hand the latest issue of ISTA Newsletter Seed Testing International. As every year, the spring issue is published around six weeks before the ISTA Annual Meeting, and contains important information and documents in preparation of the Annual Meeting, with a focal point on the Ordinary Meeting.

This year's ISTA Annual Meeting is indeed special, and especially exciting. Not only is it in combination with the Floriade, an extraordinary horticultural exposition, but also important discussions and decisions are on the agenda of the Ordinary Meeting. The whole ISTA constitution needs to be worked through, with a focus on the clarification of legal questions.

In this issue of STI you will find all relevant information for your participation at the ISTA Annual Meeting, and it will be my special pleasure and honour to welcome you personally at this important meeting.

Sampling of seed lots is a highly important topic. The whole reliability of the final testing result will depend on the homogeneity of the sampled seed lot, the sampling techniques of the lot and the submission of the sample to the laboratory. Starting with this issue, Seed Testing International will present a series about the importance of seed sampling for seed testing. We would also be happy to receive your comments on the individual articles which will also be published.

In closing, I wish you a lot of fun in reading this issue of Seed Testing International, and I sincerely hope to meet you in person at the ISTA Annual Meeting 2012.

Sincerely yours,

Michael Muschick

Seed Testing International
No. 143 April 2012
ISSN 1999-5229

Produced on behalf of the
ISTA Executive Committee

International Seed Testing Association (ISTA)
ISTA Secretariat
Zürichstrasse 50
CH-8303 Bassersdorf, Switzerland

Phone: +41 44 838 60 00
Fax: +41 44 838 60 01
E-mail: ista.office@ista.ch
Internet: www.seedtest.org

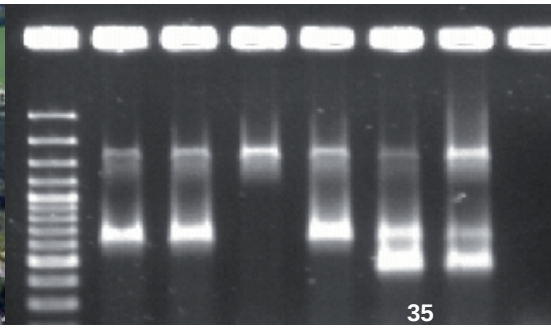
Seed Testing International No. 144
Deadline for article submission: 15 August 2012
Publication date: October 2012
Circulation: 1500
No. of copies printed: 2000
Instructions to contributors: www.seedtest.org/STI

Other ISTA publications

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

Photo credits
Front cover, p. 1 centre, pp. 12–15: ©Floriade 2012

CONTENTS



Dear Subscriber,

Seed Testing International is currently available as a hard copy and as a download from the ISTA website (www.seedtest.org/STI). Consideration is also being given to distributing Seed Testing International directly to subscribers as an electronic copy.

In preparation for this possibility we would be grateful if you could provide a current email address to which Seed Testing International could be sent. Any other comments you have on electronic distribution of Seed Testing International would be welcome.

President's Report

Joël Léchappé

Since the Ordinary meeting in Zurich June 2011, our Association has made significant progress on a number of issues, which will be presented during the next ordinary meeting in June in Venlo (Netherlands). I would particularly like to highlight the work of the Technical Committees. They have worked out important Rules proposals that you can read in the preparatory documents for the Annual Meeting. As one example, after six years of experiments, a Rules proposal to increase herbage seed lot size is submitted for a vote, preparing the way for a successful completion of the experiment which ends in 2013. Other ideas from the Technical Committees, such as flexibility in germination tests (see STI 142, p. 3), will be presented for discussion only by the Membership at the June meeting.

This report also gives me the opportunity to draw your attention to a new major item, the legal status of ISTA. Although Members and Designated Authorities already view ISTA either as a non-profit Association or as an association under Swiss law, there is no signed treaty by governments or any other official documents. Legal advice received by the Executive Committee (ECOM) at the end of 2011 was that the ISTA Constitution does not fully meet the Swiss Civil Code requirements for ISTA to be an association in Switzerland. Therefore, action has been taken as a top priority by the ECOM to ensure that the Status of ISTA is clarified. The proposal from the ECOM is for ISTA to be considered as an association under Swiss law. Advice from a Swiss lawyer appointed by the Secretariat confirmed that ISTA is currently very close to fulfilling the requirements of the Swiss law as an association. Clarifying this under Swiss law will protect the Secretariat and ECOM members from legal actions as individuals, and will allow the Association to more easily comply with Swiss tax laws and be recognized as a legal entity for business and insurance purposes. In addition, ISTA would still be able to apply for the status of an inter-governmental organization in the future.

This revision of the status will require a revision of the constitution (now to be referred to as Articles). Please read carefully the proposed amendments to the constitution when you receive them, to facilitate the vote in June and to regularize the status of our Association.

Alongside the question of Status, I would like to focus on the main actions and working items of the Executive Committee during the last months, to provide an inside view of the functioning of the ECOM and our Association.

1 Support of the Technical Committees

Support of the Technical Committees is carried out via the ECOM Liaison Officers linked to each Committee. In 2011 their role was developed to strengthen the links with the technical work within ISTA, as defined in the ISTA strategy.

On a day-to-day basis, there is two-way communication between the ECOM Liaison Officer and the Chair of the Technical Committee. As examples, the GMO committee together with its Liaison Officer alerted the ECOM to difficulties in organizing GMO proficiency tests. This important topic is under careful examination for possible solutions. In mid-2011, the Purity Committee and the Secretariat were informed by the laboratory in charge of preparing the reference samples for blowers that it was no longer able to provide this service for ISTA. In close collaboration, the ECOM Liaison Officer, the Purity and Rules committees, the Secretariat and a voluntary accredited ISTA laboratory worked out a solution in a short time. Reference samples for calibration of blowers are again available from the ISTA Secretariat.

On a longer-term basis, Liaison Officers contribute and support the committees on defined topics: in 2006, the herbage seed lot size experiment was started. Thanks to an active collaboration between the Bulking and Sampling Committee, the Liaison Officer and the Secretariat, a Rules proposal will be submitted for a vote in June 2012 at the ordinary meeting.

Monitoring of samplers, a topic raised by the ISTA auditors, combines policy and technical aspects. This demands a close collaboration between the ECOM via the Liaison Officer, the Bulking and Sampling Committee (BSC) and the ISTA Accreditation Department. After consultation with the Membership via a questionnaire and a BSC workshop in autumn 2011, BSC produced an outline of the requirements for monitoring of samplers. Nevertheless, there is still a lot of work to do to find a balance between accuracy and reliability of sampling and the cost for accredited laboratories.

Flexibility in germination tests: this topic, based on technical aspects for simplification of tests or shortening the tests, has policy consequences, and may influence uniformity in the seed trade. Discussions within the Germination Committee, with other committees such as the Statistics Committee, are ongoing. A close coordination with the Executive Committee is in place to prepare the discussions with the Membership in Venlo in June 2012.

2 International relations

The international activity is mainly based on the representation at meetings of other organizations. This representation is mostly done by the Secretary General. The main aim of the representation is to provide information about ISTA and get feedback on the needs of the regions with respect to seed testing, such as methods, training and workshops. The goal of the ECOM Working Group on International Relations is to co-ordinate the contributions of the ECOM in relation to the international activities of ISTA and to support the actions of the Secretary General. Specific items being studied are the contribution to the World Seed Project, together with the FAO, OECD, UPOV and ISF, and relations with NAL in the Netherlands. International relations is an area that the ECOM intends to give more focus to, once the issues surrounding the legal status of ISTA, the management and the internal financial management of the Association have been achieved.

3 Seed science

The activity in seed science is supported by a joint ECOM and Technical Committee Working Group. Significant developments were made in 2011, strengthening the collaborations with scientific organizations, and disseminating scientific knowledge. The arrangements for the 29th ISTA Seed Symposium began in spring 2011, and the theme for the Symposium: 'Evaluation of seed quality: a key step in exploiting the benefits of plant breeding and genetic conservation' along with the topics of the sessions, the lead speakers and session chairs and the timetable for submission of papers were announced in STI and on the ISTA website in October.

The position of Chief Editor of Seed Science and Technology was handed over from Alison Powell (UK) to Fiona Hay (Philippines) at the beginning of October 2011. Alison has returned to her previous role as one of the Associate Editors.

Discussions with the Royal Botanic Gardens (RBG), Kew, UK, towards the signing of a Memorandum of Collaboration (MoC) between ISTA and the RBG, Kew were completed late in the year, and an agreement should be signed in spring 2012. This MoC will encourage further the links between members of the two organizations that have existed informally for some years.

The first ISTA session at an International Society for Seed Science meeting was organized by Alison Powell as part of the 10th Conference of the ISSS, which was held in Costa do Sauípe, Bahia, Brazil. The ISTA session on the theme 'Application of seed science to the evaluation and improvement of seed quality' was chaired by Alison Powell and involved five presentations from the invited speakers, Françoise Corbineau (France), José Marcio Rocha Faria (Brazil), Stan Matthews (UK), Alan Taylor (USA) and Marie-Hélène Wagner (France).

4 Management and finances

These topics are dealt with by an ECOM working group made up of the whole ECOM. A sub-working group was set up to prepare the work, formulate plans and discuss issues with the Secretariat and the whole ECOM. The main task of the subgroup was to review:

- The issue of the legal status of the Association, in close collaboration with the Constitution working group.
- The financial accounting processes of the Secretariat, and linkages with a time sheet recording system.
- The management regulations of the Secretariat.

Very good progress was made on all three topics. The revision of the status will be accompanied by first a revision of the constitution and subsequently, by a revision of the linked regulations and documents for the management of the Secretariat, such as management regulations, job profiles, standard operation procedures for the organization of meetings etc.

A simple and accurate draft financial model has been worked out by the Secretariat, and analysed and tested. Further important and extensive work is needed for the validation of this draft financial model and its implementation with experimental data before it is used. Validation and routine use of the model, with one to two years of data to cover the annual variability, will allow us to get an accurate picture of the finances and set up business plans for specific work projects.

5 Constitution

In addition to the constitution changes linked to the revision of the legal status of ISTA and the motion of Australia and New Zealand on audit fees, the Working Group on the ISTA Constitution and By-laws has the aim of ensuring that when motions are voted on, any subsequent constitutional or by-law changes that are needed are also provided and voted on at the same time. Constitution changes other than those related to the legal status are being delayed until the ordinary meeting in June 2013, to facilitate concentration on the legal status issue in 2012.

6 Electronic publishing

The ISTA strategy on publications and products includes investigating ways to increase sales and reduce cost. Electronic publishing is likely to be a way of achieving this. There are some demands from within the ISTA membership for electronic publishing of ISTA material. The ECOM Working Group considers the candidates for electronic publishing, including the ISTA Rules and handbooks. Among several important questions, the Working Group is considering which publications should be published electronically and in which priority order. It is also looking at the options for electronic publishing and potential costs, and whether electronic certificates should be included as a separate project.

The impact on ISTA development in all areas of the world and the level of security required by electronic publication are also being carefully studied.

Over the last ten years, our Association has constantly developed, as witnessed by the increase in the number of accredited laboratories, the extensive programme of proficiency tests provided to members, the yearly evolution of ISTA Rules, scientific projects, and a regularly renewed programme of workshops. In parallel, the needs of the seed sector evolve faster every year, demanding more diversity of methods. In addition, the current world economic environment creates more constraints for many of the ISTA Member countries. In this context, the ECOM, together with the Secretariat, has made it a priority to work

(Continued on page 15)

Monitoring ISTA seed samplers

Ronald Don[1], Leena Pietilä[2] and Rasha El Khadem[3]
[1]Honorary ISTA Member; [2]Chair, ISTA Bulking and Sampling Committee; [3]Head, ISTA Accreditation Dept.

[3]ISTA Secretariat
8303 Bassersdorf, Switzerland
auditor@ista.ch

Sampling is the cornerstone for seed testing and has therefore its major importance within the ISTA accreditation system. Sampling distinguishes the ISTA accreditation system from ISO 17025 where accreditation only relates to the quality tests conducted on samples submitted for testing. For ISTA accreditation, the results of the quality tests conducted are attributed to the seed lot from which the seed sample was obtained. For ISTA the confirmation that warehouse sampling is accurate and controlled is as important as the accuracy and control of laboratory quality testing. The control of warehouse sampling and the rules contained in the sampling chapter of the ISTA Rules give rise to the definitive difference between ISTA Orange Seed Lot Certificates and AOSA certificates. Whereas, certification systems such as of the ISO 9000 series are based on the control of systems and processes, the ISTA Accreditation Standard requires, in addition to systems and process control, a check on the output of the systems and processes.

Whereas ISTA has a system of proficiency tests that monitor the performance of laboratories in terms of the seed quality tests they perform, there is no proficiency testing associated with seed sampling and it could therefore be argued that internal checking of seed sampler performance is of greater importance than the checking of seed analyst performance.

ISTA has to safeguard the status and standard of its Orange Seed Lot Certificate in order to maintain its relevance to the international seed trade. To do this controls on the quality of the samples drawn in the warehouse by authorised seed samplers are as important, (if not more important since there are no sampling proficiency tests) as controls on the quality of tests conducted in the laboratory.

At the present time laboratories do not need to be accredited for sampling and laboratories can choose to be accredited only for seed testing. If laboratories find the requirements that need to be fulfilled too restrictive they can decide not to issue ISTA Orange Seed Lot Certificate and only issue ISTA Blue Seed Sample Certificates. It is laboratories that have to make the decisions regarding whether it is of economic and/or political benefit to be accredited for sampling or not.

ISTA currently accredits for either:
– Laboratory seed quality testing;
or
– Laboratory seed quality testing and sampling.

Equal level of monitoring and checking should be applied to warehouse sampling and laboratory seed quality testing if a laboratory is accredited for both elements. The ISTA Accreditation Standard outlines the system and process control procedures for warehouse sampling as well as the output checks in terms of monitoring that must be carried out. There are relevant paragraphs covering: sampling sites and facilities; the training skills and expertise of seed samplers; the use of appropriate sampling equipment; the use, maintenance, calibration and monitoring of sampling equipment; the monitoring of seed samplers; and the yearly internal audit of sampling.

The ISTA Accreditation Standard is not specific in its requirements, but gives freedom to the laboratories to develop their own solutions:

"Adequate procedures and practices exist to monitor the performance of individual samplers with respect to adherence to the ISTA Rules […]" (current ISTA Accreditation Standard 6.1.4).

It could be asked what is adequate?

The answer is that it is up to the laboratory to demonstrate that their procedures and practices are adequate.

"The quality system must define and document quality control procedures specific to seed lot identification and sampling arrangements, and laboratory testing procedures. These may include check sampling, check testing and other monitoring programmes. The resulting data must be recorded in such a way that trends are detectable and, where practicable, statistical techniques must be applied to the reviewing of the results. […]" (current ISTA Accreditation Standard 10.3.1).

A laboratory must define the monitoring programme it is using for seed sampling and it must be able to technically justify it and demonstrate that it is able to detect trends. In addition to the monitoring programme laboratories are required to carry out an internal audit of sampling at least every year:

"At least yearly, the laboratory must perform internal audits of its activities in accordance with a predetermined schedule and procedure. Audits must be performed in such a way that they verify the laboratory's continuous compliance with this Standard and its quality system. The internal audit programme must address all elements of the quality system, including the testing and sampling activities. […]" (current ISTA Accreditation Standard 10.8.1).

Meeting the ISTA Accreditation Standard with respect to Sampling

There are no standards, whether it is ISO or ISTA that gives details on how the standard can be satisfied. It is up to those seeking accreditation, or the maintenance of accreditation, to demonstrate that they meet the criteria that have to be fulfilled in order to obtain and maintain their status.

In seed sampling the critical and perhaps the most laborious point is monitoring of the seed samplers. Monitoring may involve many different methods including audits of individual seed samplers, check sampling and examinations.

Why do seed samplers need to be monitored?

In addition to the requirements in the ISTA Accreditation Standard, experience from ISTA audits would suggest that sampling is one of the areas where there are more non-conformities than other technical areas. Indeed it is the opinion of most auditors that warehouse sampling is an area where there is the greatest need for ISTA workshops.

Experience from monitoring seed samplers (using check samples, refresher course, practical exams and audits) would suggest that seed samplers develop bad habits over time and errors are made. The reasons for that are various and can be frequently found in the nature of the sampling environment. Samplers as apposed to seed analysts are often working alone and have for longer durations of limited contact with other colleagues. Monitoring is required to detect trends and provide corrective actions before errors become significant, samples are compromised and not representative of the lot from which they have been obtained.

Check sampling

Most laboratories have adopted check sampling as part of their monitoring programme. In setting up such a programme laboratories have consulted the ISTA Accreditation Department for advice on levels of checking required. Historically advice from the ISTA Accreditation Department has been that the rate strongly depends on the number of seed lots sampled and the number of samplers but that a 5% checking rate will frequently satisfy the standard. Others have suggested that, to provide any degree of confidence in the performance of individual seed samplers, the check sampling programme for each seed sampler should be, at the very least, one sample per month during the sampling season.

However, ISTA laboratories are all different and a "one size fits all" approach is not appropriate. In suggesting a 5% check with one check sample per month a situation where seed samplers were drawing hundreds of ISTA samples each season was anticipated. Moreover, a reduction in the level of checking could be justified by demonstrating that the monitoring was spread over the seed-testing season and that a sensitive technique was used to identify possible trends. The suggestion of checking at least one sample per month during the sampling season is of course not applicable to situations where a laboratory has for example 40 or more seed samplers, each of which manually samples less than 10 seed lots per season for ISTA purposes. In such circumstances a different approach to monitoring is required.

For example:

If you have 3 authorised seed samplers and each only draws 7 manual samples per year:

A 5% check would be 1 check sample. Every year you would have 1 check sample drawn by one seed sampler.

Do you have control of seed sampler performance?

No, you have no performance data for 2 of the seed samplers over 2 years. Moreover you also have no idea how good your seed sampler who participated in the check testing is, because you just have one data point. What would happen if you have the suspicion from the data that one of your seed samplers draws systematically different samples. Will you reject the test results related to samples drawn by this seed sampler over the last 2 years? How will you explain this to your customers especially as most likely the seed has been already reached its final customer and probably has been planted?

In such a situation the idea of the internal auditor and seed sampler drawing a sample from a seed lot at the time of the annual audit of each seed sampler would seem to be the most sensible approach to monitoring. In such a situation the detection of trends is difficult and will require a novel approach.

If you have 3 authorised seed samplers who manually draw 500 ISTA samples per seed testing season of say 9 months:

A 5% check would be 25 check samples and you divide them by the 3 seed samplers. Let us assume you take 27 check samples with each seed sampler drawing 9. So every month each seed sampler would draw a check sample.

Do you have control of your staff performance?

Yes, a very good one and if you have any doubts regarding the performance of one of the seed samplers, only the results of one month would need to be investigated.

With experience and data to prove that there is no trend towards discrepancies it would be possible to reduce the check sampling to perhaps 2.5% and checking could be maintained at this low level provided there was an absence of any tendency towards discrepancy. Of course checking levels for newly qualified and authorised seed samplers would be initially at the higher level.

These two examples illustrate that the definition of a checking threshold is tricky and depends on the number of samples drawn by each seed sampler, the experience of the seed samplers and the performance of seed samplers (in terms of trends).

Novel ways of obtaining Check Sample data

Check sampling need not be a standalone process. Many laboratories obtain check sample data from processes already in place and without encountering any additional costs. The check sampling results do not need to come only from ISTA samples but can include any samples drawn by seed samplers provided the laboratory can demonstrate that ISTA sampling procedures have been followed.

Examples of situations where check sample data can be obtained without additional inputs include:

- **Situations where seed is sampled separately for Certification purposes.** The results of tests on the certification sample can be compared with the results obtained on the ISTA sample.
- **Situations where a sample is taken for Regulation Enforcement purposes on a lot sampled by an ISTA seed sampler.** The results obtained on the two samples can be compared. In parts of Europe where 5% of all certified seed is tested for enforcement and certification control purposes it is possible to have a comprehensive check of seed samplers and monitor their performance without the necessity of drawing any additional check samples using the results of enforcement/certification control samples.
- **Situations where designated authorities perform checks on the performance of seed samplers for legislative purposes.** In EU countries where their Seed Marketing Regulations require a 5% check on the performance of private sector seed samplers licensed to take

samples for legislative purposes, this performance data can be used to satisfy the ISTA accreditation requirements without the necessity of drawing any additional check samples.

– **Situations where seed lots are re-sampled.** Re-sampling of seed lots is common when the results of seed quality tests indicate that the seed lot sampled is below a pre-determined quality standard but within limits of the standard (this could be a company or legislative standard). The results of tests on the original and the re-sample can be compared.

Check sampling versus re-examination

Most sampling courses held by ISTA Accredited laboratories will involve practical sampling of seed. However in many cases the sizes of lots used are smaller than those that the seed sampler will encounter in the warehouse. They suit the training purpose but it is difficult to elaborate performance in such training courses to performance in the warehouse. In addition, performance in an examination does not always equate to routine performance. To overcome this problem some laboratories supplement practical exams on courses with a practical exam on-site, which takes place during an on-site annual audit of seed samplers. Although this inevitably gives a better measure of performance it could still be argued that routine performance can only be properly assessed through blind check sampling where the seed sampler has no prior knowledge that he/she is being checked. Implementation of blind checks (both check testing and check sampling) might be very difficult or in some cases even impossible to achieve within some organizations. This can be the case, e.g. for laboratories with very few staff or for laboratories covering a very large geographical area for seed sampling. Finding the "adequate procedures and practices for individual samplers" as required by the current ISTA Accreditation Standard is the challenge that seed testing laboratories face and which must be solved.

Suitable quality tests for checks

Experience of crops and seed quality attributes are required in order to determine the most suitable and relevant tests that should be applied in a check sampling monitoring programme. If we have hybrid maize or sunflower seed lots with analytical purities of 99.9% and germinations of 99% it is worthless to base any comparison on test results obtained for purity, other seed content or germination. For such seed lots it is more relevant to look at other attributes such as thousand seed weight, size grading, or numbers of broken seed. On the other hand, for herbage seed lots the number of other seeds may be most appropriate.

In addition, the time difference between initial sampling and check sampling could result in differences in germination and moisture content. It is extremely unlikely that germination or moisture content would ever unequivocally indicate or give a measure of errors due to sampling. These tests should not be used for monitoring purposes.

Use of automatic seed samplers to check manual sampling performance

The use of automatic seed samplers requires an approval of such equipment. The basis of the approval is a comparison of test results from samples obtained with the automatic seed sampler with test results from samples obtained manually. This is the case even although correctly installed and operating automatic seed samplers are considered by many to provide more representative samples than those obtained by manual sampling. That being the case it is possible that checks on seed sampler performance could be easily (and economically) obtained by getting seed samplers to manually sample lots that have been automatically sampled. Should there be a significant difference in results between the samples obtained by the automatic seed sampler and those obtained manually then investigations would be required to ascertain if the differences were due to a fault in the automatic seed sampler or errors in the manual sampling.

If a seed sampler never draws samples manually, there is no need for check sampling. Whereas a seed sampler who is engaged in manual sampling on a daily basis gains experience and competence, one who is only intermittently engaged in manual sampling can loose competence. It could therefore be argued that check sampling is even more important for those who are infrequently required to sample manually.

More information on the approval of automatic seed samplers and regular checking of their function can be found on the website of ISTA.

Assessing trends

Tolerances are a "crude" statistical tool that will only detect non-conformities and not trends or even errors in some cases.

If for example we have a seed lot of *Spinacia oleracea* that has a contamination level of 5 *Brassica* sp. seed per 250 grams. These *Brassica* seeds are evenly distributed throughout the lot, which is homogenous at the time of packaging. During the process of packaging and transport of the packed seed the *Brassica* seed will be redistributed within each package due to difference in their size, shape and density in comparison with the *Spinacia* seed. The *Brassica* seed will tend to migrate to the bottom of the packages.

We now have 2 seed samplers who are asked to manually sample the seed lot. Seed sampler A takes a representative sample ensuring that primary samples are taken from the top, middle and bottom of packages. The technique of seed sampler B is erroneous and the majority of primary samples are taken from the top of packages. We now analyse the samples submitted by seed sampler A and seed sampler B and find that in 250g working samples 5 *Brassica* seed are found in the sample drawn by seed sampler A and 1 *Brassica* seed is found in the sample from seed sampler B. Using the tolerance table F1b we find that there is no significant difference in the number of *Brassica* seed found in the samples (Average of the two results =3, difference between the two results = 4, tolerance = 5).

Tolerance tables have not been able to identify the poor performance of seed sampler B. If however we looked at trends we would more likely be able to identify the problem (Fig. 1).

In Figure 1 we examine the trends when the results of 9 seed lots that were sampled by seed sampler A and seed sampler B are plotted. In no case are the results out of tolerance but there is a clear trend in that when seed sampler B samples lots, fewer other seed are found. The reason for the difference should become apparent when

witnessing the sampling techniques of seed samplers A and B during an audit.

Another example of how trends can be monitored using the results of check samples is to plot results of individual seed samplers over a period of time against the results obtained when lots are check sampled (Fig. 2).

In the example illustrated we have 3 seed samplers who have each had 10 check samples taken from lots they originally sampled. The difference between the purity results on the samples they submitted and the check sample results are plotted. This plot indicates that the performance of seed sampler 1 is appropriate whereas with seed sampler 2 there is a trend towards obtaining higher purity results on the check samples and for seed sampler 3 a trend towards obtaining lower purity results on the check samples. With this information corrective action can be taken to bring the performance of seed samplers 2 and 3 into line before out of tolerance results are obtained. (It could be that seed sampler 2 is taking too many samples from the top of containers where purity tends to be lower and seed sampler 3 takes too many samples from the bottom of containers where purity tends to be higher).

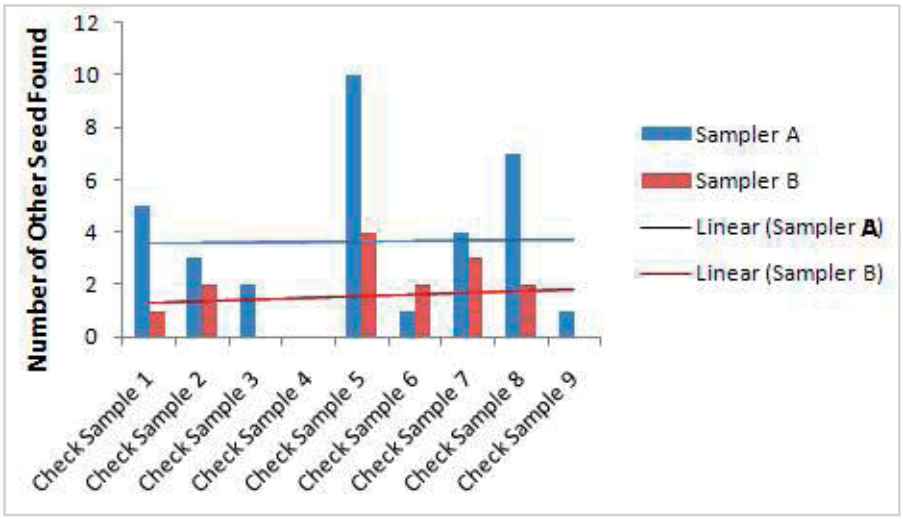


Figure 1. Identifying trends in the performance of two samplers

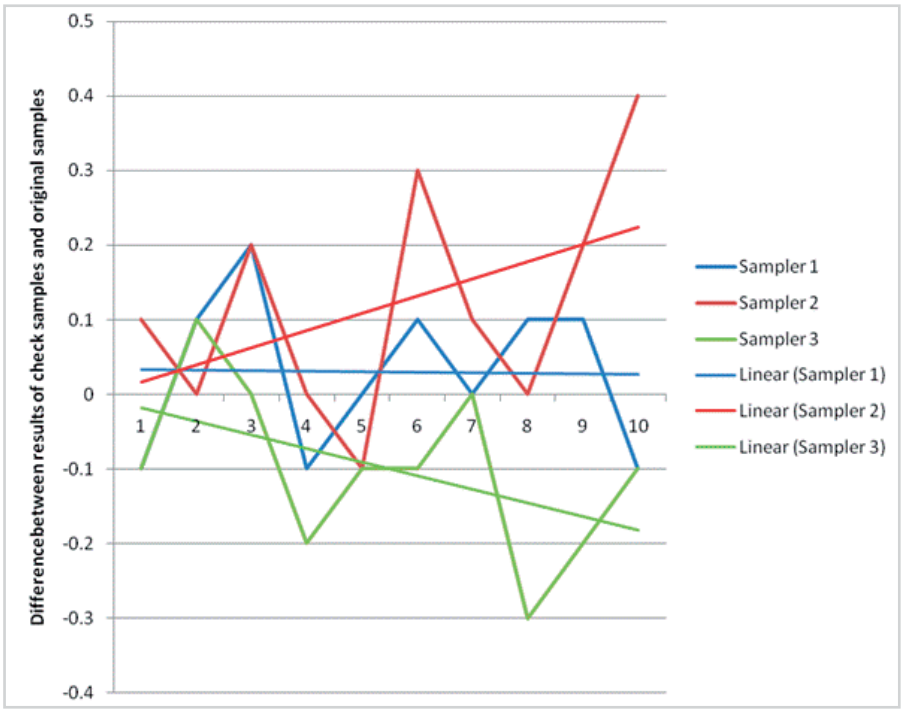


Figure 2. Assessment of performance when results are compared with the result on the original sample

Uniqueness of ISTA Orange Seed Lot Certificates and its difference from other Certificates

For ISTA Certificates the customer is the seed trade and the use of ISTA Certificates is associated with international trade rather than national certification purposes. ISTA's stakeholders have not questioned the need for ISTA Certificates and the ISTA Orange Seed Lot Certificate is considered as an essential pre-requisite for international trade by many countries.

The process of obtaining a relatively small portion of seed that is representative for the entire seed lot is so important since all test results depend on this seed sample. The uniqueness of the ISTA Orange Seed Lot Certificate is that sampling has been assigned its importance by defined sampling rules and sampling requirements. Proper implementation is a key aspect during ISTA (re-)accreditation audits and is given the needed attention.

Without adequate control of sampling the ISTA Orange Seed Lot Certificate would lose its major competitive advantage over other seed quality certificates. ISTA does offer the option of an ISTA Blue Seed Sample Certificate where there is no control over sampling but for 95% of customers this control and the link between the test results on the certificate and the seed lot is considered essential.

Sampling is unique in terms of ISTA accreditation as there are no proficiency tests associated with this activity. Quality control procedures are as important, if not more important than checks on seed analysis and we must be careful to maintain this control and not jeopardize the value of ISTA accreditation and the ISTA Orange Seed Lot Certificate. The guaranteed link between seed lot and test results offered by the ISTA Orange Seed Lot Certificate is viewed as a major advantage. ■

Monitoring ISTA seed samplers: results of questionnaire

Ronald Don[1], Rasha El Khadem[2] and Leena Pietilä[3]
[1]Honorary ISTA Member; [2]Head, ISTA Accreditation Dept.; [3]Chair, ISTA Bulking and Sampling Committee

[2]ISTA Secretariat
8303 Bassersdorf, Switzerland
auditor@ista.ch

The ISTA Accreditation Standard requires quality control procedures specific to sampling and that the resulting data must be recorded in a manner that enables the detection of trends. The quality control procedures may include check sampling, audits and other monitoring programmes. As a result of questions regarding appropriate procedures the ISTA Executive Committee asked for a survey to be undertaken to get a better view on the current monitoring programmes used for manual sampling.

A questionnaire was developed by the ISTA Accreditation Department in conjunction with the Bulking and Sampling Committee and Ronald Don, and was sent to the 107 ISTA laboratories accredited for manual sampling (as of April 2010). Sixty-six laboratories submitted completed questionnaires, which represented a response rate of 62%, and this article gives an overview of the responses and conclusions that can be drawn from them.

Monitoring programmes implemented by laboratories

Sixty-five of the 66 laboratories that responded had a monitoring programme in place. The one laboratory that did not had not issued any ISTA Certificates, and was working on the implementation of the monitoring programme involving a combination of check sampling, refresher training and audits.

Laboratories were asked to categorise their monitoring programme using one or more of the three categories: "Check sampling", "Auditing of samplers" and "Other means".

Most laboratories employed a combination of different monitoring procedures and of the 65 laboratories 50 (77%) have implemented a check sampling process; 58 (89%) have an auditing process in place and 22 (34%) indicated "other means" as a monitoring tool for their sampler (Fig. 1). Other means included training, exams, checking incoming samples and documentation, and unannounced audits.

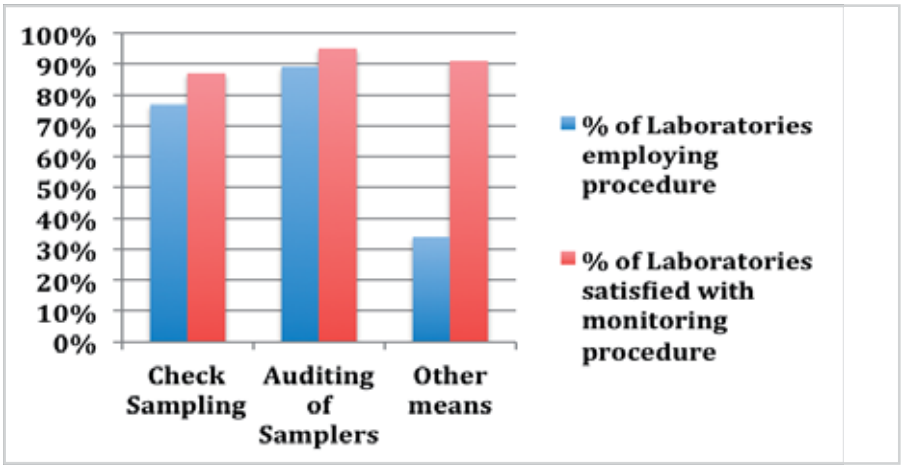


Figure 1. Proportions of laboratories that use different means for monitoring of seed samplers in percent based on the 65 laboratories that provided answers to these questions. Proportion of laboratories that are satisfied with the monitoring system they use based on the number of laboratories per category. (E.g. 33.8 % (or 22 laboratories) of the 65 responding laboratories uses "other means" as monitoring programme. 91% of these 22 laboratories were satisfied with their monitoring procedure)

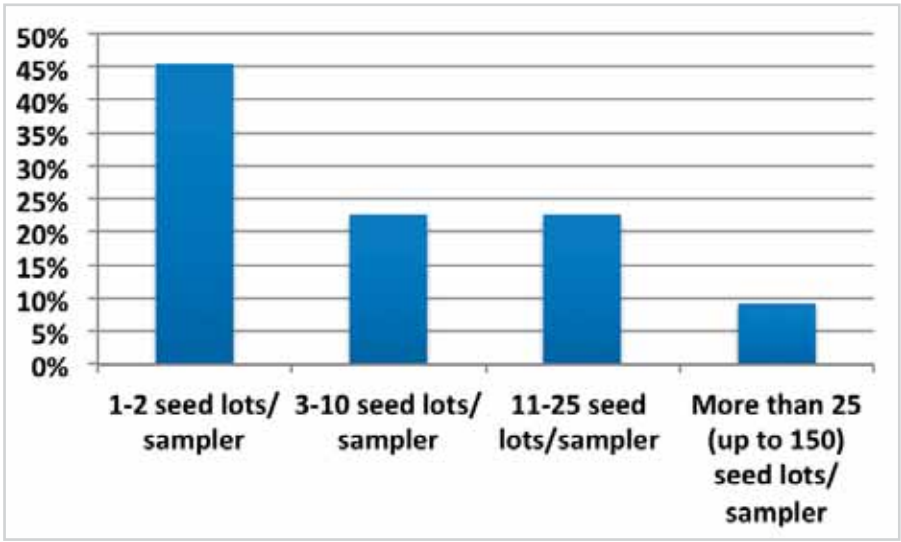


Figure 2. Proportions of laboratories employing different check sampling rates. (Only those 44 laboratories that took check samples from each Seed Sampler every year are included).

Check sampling

Of the 50 laboratories that employed check sampling, 44 used the procedure on each sampler every year. Of these, the number of check samples taken for each sampler per year varied from between 1 and 2 to 150 (Fig. 2).

Ninety per cent of laboratories used the same check sampling procedures for the different crop groups they tested with only a few laboratories indicating that they selected specific species (grasses and vegetables) specifically for check sampling. When sampling for "check sampling" purposes, 45% of seed samplers were aware that they were drawing a "check sample" with 55% of check sample requests being processed as if they were a regular sample request (a blind process with the seed sampler being unaware that the sample is being requested as part of the check sampling programme).

The majority of laboratories tested the check sample for the determinations that were requested for the initial sample (Fig. 3). Only 56% of laboratories performed any analysis to identify trends in the results of check samples, and of these this analysis was limited to a check of tolerances in 60% of cases.

Eighty eight per cent of laboratories that adopted a programme of check sampling stated that they had confidence in their programme as being an appropriate monitoring tool for ISTA seed samplers.

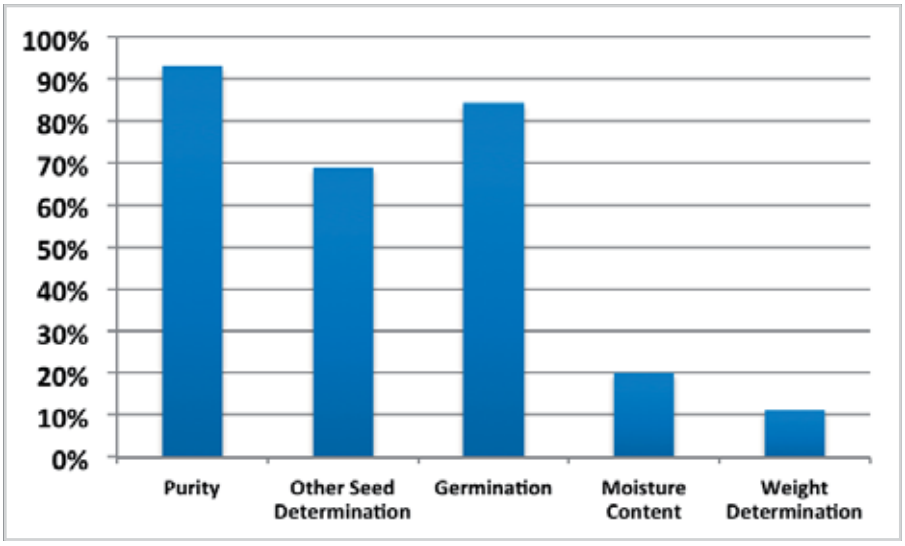


Figure 3. Types of seed analysis carried out on check samples.

Auditing of seed samplers

Of the 58 laboratories that used auditing as a monitoring tool, 88% of laboratories carried out audits at least every year, but only 48% of laboratories monitored all of their ISTA seed samplers at least once every year (Fig. 4).

During the audit process, over 91% of these 58 laboratories audited documents, equipment used by the seed samplers, and the practical work of the seed samplers (Fig. 5).

Only 33% of laboratories performed any analysis of trends in the results of audits, and of these, 63% used evaluations of non-conformities, and 32% used results from check samples drawn during the audit.

Ninety-five per cent of the 58 laboratories which used auditing to monitor samplers, stated that they had confidence in auditing as being an appropriate monitoring tool for ISTA seed samplers.

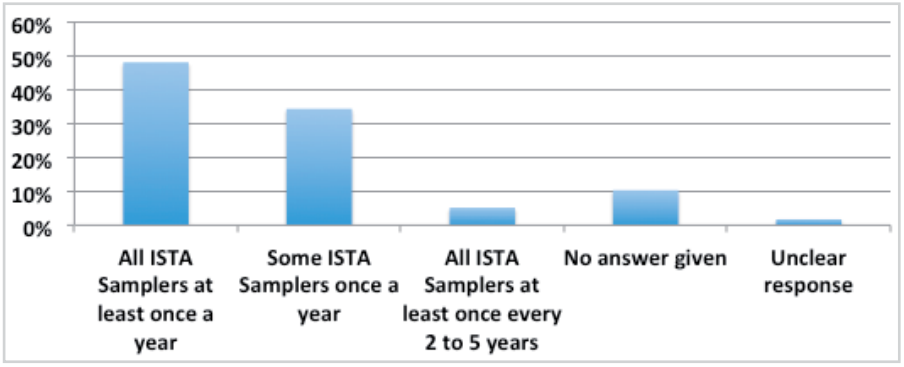


Figure 4. Frequencies of audits of individual seed samplers (n= 58)

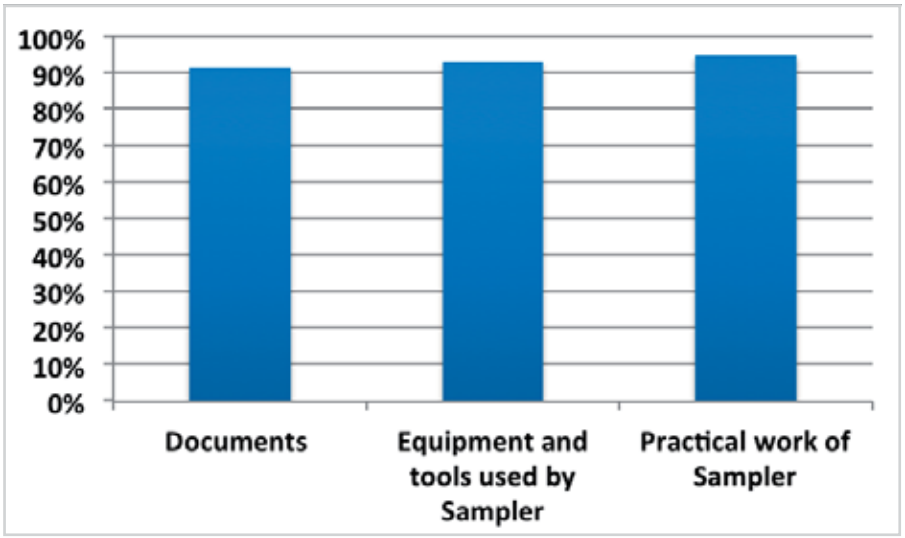


Figure 5. Proportions of laboratories that audited different aspects of the sampling process. (Proportions based on the 58 laboratories that used audits of seed samplers as a monitoring tool).

Methods of monitoring other than check sampling or auditing

Of the 22 laboratories that indicated the use of other means of monitoring (Fig. 6), 13 listed the provision of training as a

monitoring procedure. Since ISTA laboratories accredited for sampling must provide training to its entire staff either by internal or external training, it is clear that only a few of the laboratories completing the questionnaire (19%) consider training as a monitoring tool.

Similarly, all accredited laboratories check incoming documents and samples, and whilst this may be a good check on the administrative aspects of a seed sampler's work, it is difficult to imagine how such a check can monitor the practical aspects of drawing representative samples.

Whilst training followed by an examination or stand-alone examinations could be used as means of checking the practical aspects of a seed sampler's work, performance in exam situations does not always equate to routine performance. Moreover, unless the practical examination takes place in the workplace using actual seed lots, artificial and unrealistic situations can detract from the value of data obtained.

Twenty-one of the laboratories (95%) using other means of monitoring covered all ISTA seed samplers every year, but only 7 laboratories (32%) stated that they analysed trends. Twenty of the laboratories (91%) that used other means to monitor samplers stated that they had confidence that their method of monitoring was an appropriate monitoring tool for ISTA seed samplers.

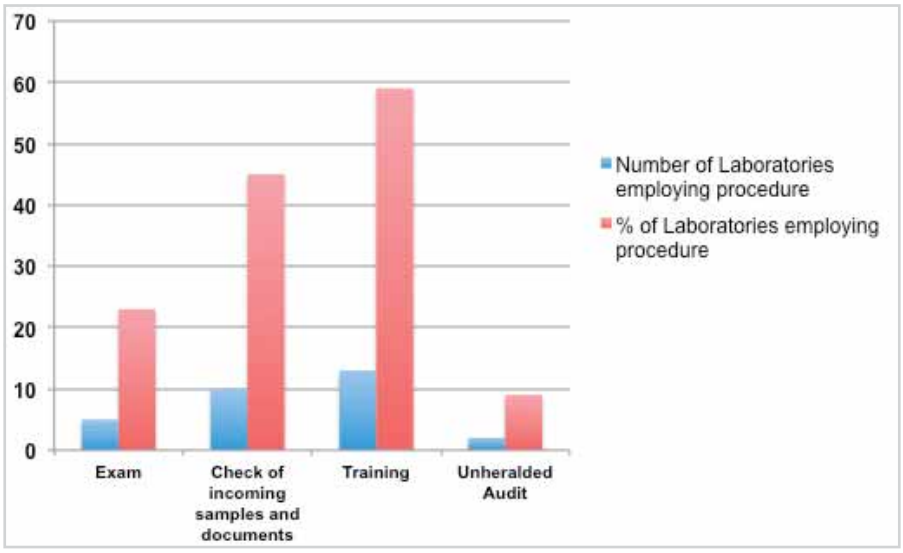


Figure 6. Number and proportion of laboratories that use methods of monitoring other than check sampling or auditing.

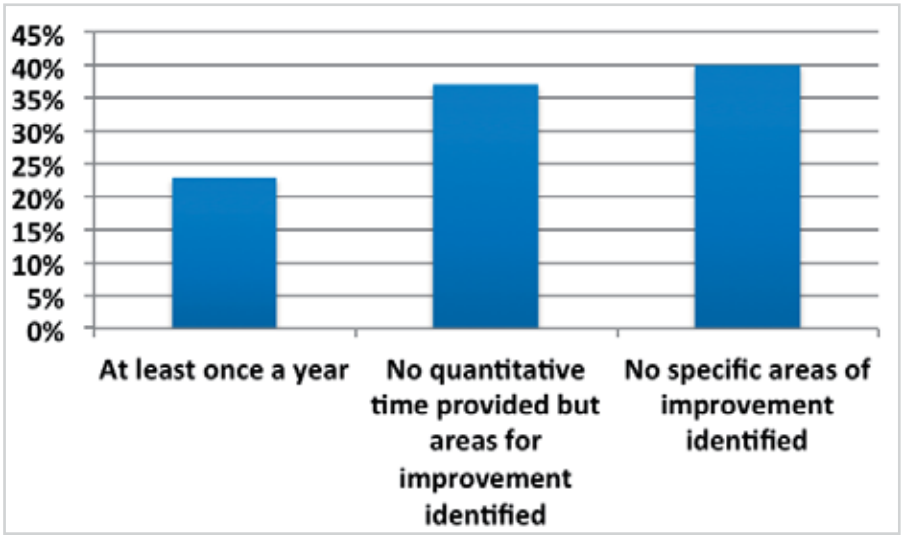


Figure 7. Effectiveness of monitoring: proportions of laboratories that have identified areas of improvement. (Only those 60 laboratories that gave an answer that could be classified are included.)

Effectiveness of monitoring

The goal of this question was to quantify the occasions where the laboratory was able to improve its sampling process using the monitoring programme (Fig. 7). Some would consider that the effectiveness of monitoring systems is the ability of the systems to identify areas of non-conformity or requiring improvement both in the performance of the seed sampler and also in the instructions, training and guidance given by the laboratory.

In addition, the detection of findings gives feedback to those carrying out the monitoring.

The monitoring tools were at least sometimes responsible for the identification of specific areas of improvement in 60% of cases but only 23% of the laboratories identified areas of improvement at least once a year (Fig. 7).

Comments from questionnaire respondents

The questionnaire gave respondents the opportunity to provide additional comments related to the monitoring of seed samplers. None of the respondent questioned the need of a monitoring programme in general however, some critically questioned certain aspects of and others asked for greater guidance.

– Two laboratories questioned the cost effectiveness of monitoring procedures and requested the development of simplified, more economic procedures that were fit for purpose.
– Three laboratories asked for feedback from ISTA on different monitoring possibilities.
– One laboratory debated the timing of monitoring activities, arguing that it should be carried out regularly during the seed testing season rather than being limited to a one-off event.
– One laboratory questioned the need for trend analysis.

With regard to check sampling:
- One laboratory questioned the effectiveness when seed lots were of high quality and differences between samples were never found.
- One laboratory highlighted difficulties in obtaining check samples when dealing with small seed lots of expensive seed.
- One laboratory questioned the conclusions that could be made when discrepancies were obtained due to: differences in the time that samples were drawn, and difficulties in attributing whether the differences were due to a particular sampler or differences due to errors/variations in the laboratory analysis of samples.
- Two laboratories requested guidance on whether stand-alone check sampling procedures were required, or whether results from other procedures could be used.

With regard to auditing:
- Two laboratories questioned paragraph 10.8.1 of the ISTA Accreditation Standard ('At least yearly, the laboratory must perform internal audits of its activities in accordance with a predetermined schedule and procedure.'). One asked whether this actually implies that all samplers need to be individually audited annually, or whether it is the sampling process that needs to be audited annually. The other asked whether the internal audit requirement could be dispensed with if an external body conducted an annual audit of the sampling process.

Conclusion

Monitoring programmes for seed samplers seem to be widely used and accepted in the laboratories that returned completed questionnaires, and laboratories have put a lot of effort in monitoring programmes. That being said, it is clear that laboratories require additional guidance on monitoring procedures to ensure that maximum benefits are derived from the resources used in monitoring, and that the requirements of ISTA Accreditation are satisfied.

With respect to check sampling, it is surprising that many laboratories appear to use a stand-alone procedure. Only one laboratory used the results of supervision samples drawn by an official laboratory, and one laboratory mentioned using a comparison of results from manual and automatic samplers. Check sampling need not be a stand-alone process. Many laboratories could obtain check sample data from processes already in place and without encountering any additional costs. Moreover, the check sampling results need not come only from ISTA samples, but can include any samples drawn by samplers, provided that the laboratory can demonstrate that ISTA sampling procedures have been followed. Examples of situations where check sample data can be obtained without additional inputs include the following situations:
- where seed is sampled separately for certification purposes;
- where a sample is taken for regulation enforcement purposes on a lot sampled by an ISTA sampler;
- where private sector sampling is supervised and monitored by the countries' official laboratories;
- where seed lots are re-sampled;
- where the performance of automatic samplers is checked by comparing results obtained on samples drawn manually.

It is also surprising that there is no targeting of tests carried out on check samples or seed lots selected for check sampling. Germination and moisture content are dynamic quality attributes, and the time difference between initial sampling and check sampling could result in significant differences in both. It is unlikely that germination or moisture content would unequivocally indicate or give a measure of errors due to sampling. These tests should not be used for monitoring purposes. If only seed lots of high purity and germination are available, it is pointless to carry out comparisons of purity, other seed determinations and germinations. For such seed it is more relevant to look at, say, thousand-seed weight, size grading, or numbers of broken seed. The tests carried out should be related to the species sampled, so for herbage seed lots the number of other seeds may be the most appropriate comparison, whilst for many other species thousand-seed weight, broken seed and inert matter components may be the most relevant.

For all monitoring procedures there is a need for guidance on how analysis of trends should be undertaken. Although trend analysis is a requirement of the ISTA Accreditation Standard, only one half of laboratories conducted analysis of trends with respect to check sampling, and one third or fewer did so when auditing or other means were used to monitor seed samplers. Even when trend analysis was carried out using the results of check tests, the analysis was limited to the use of tolerances in many cases. Tolerances are a "crude" statistical tool that will only detect non-conformities, and not trends or even errors in some cases. It is clear that laboratories need guidance on methods of trend analysis that could be very simple, and that will enable them to identify non-conformities, and to take corrective actions before deviations in performance result in non-conformities.

For a minority of laboratories, training and checking documentation accompanying submitted samples was considered to be monitoring procedures. Though they are important, there may be a limited possibility to use them for monitoring purposes. It is difficult to envisage how these, taken on a stand-alone basis, can give an assessment on the performance of a sampler with respect to practical aspects of obtaining representative seed samples from a seed lot.

A detailed result summary of the questionnaire was provided to the ISTA Executive Committee after the annual meeting in June 2011. The ISTA Executive Committee has evaluated the results and has come to the conclusion that more investigations are needed to be able to provide ISTA laboratories with more guidance about feasible monitoring programmes. The results of the survey will be used to develop guidance and update the Handbook of Seed Sampling on the monitoring of seed samplers. The Executive Committee has moreover confirmed the compulsory requirements for a monitoring system for samplers as stated in the current ISTA Accreditation Standard. It has stated that check sampling is strongly recommended, and that the monitoring must include audits.

We would like to thank all the laboratories that participated in this survey.

Floriade Venlo 2012: “Be part of the theatre in nature, get closer to the quality of life!”

The Floriade is the name given to the World Horticulture Expo, which is organised in the Netherlands every ten years. The sixth Floriade will be held in the region of Venlo, in Northern Limburg, from 5 April until 7 October 2012. With an expected two million visitors, the Floriade is the largest event to be staged in the Netherlands in 2012.

Northern Limburg is the first region outside of the Randstad conurbation to be asked by the Dutch Horticulture Council to organize the Floriade. The region around Venlo ranks as one of the major concentrations of horticulture in the Netherlands. If the neighbouring German region of the Lower Rhine is also included, the Floriade park is situated in one of the largest contiguous horticultural areas of Western Europe. Thanks to the important agribusiness and its favourable, central location, Venlo has developed into a logistics hotspot. For these reasons, the Dutch government has designated the Venlo region to be one of the five centres of the horticultural sector in the Netherlands, known as ‘Greenports’.

This year’s Floriade is far more than just a fascinating day out for two million visitors. It marks a strategic moment in the permanent development of an area covering 5000 hectares to the north west of Venlo. After the Floriade, the site will become the Venlo GreenPark, a sustainable business park in leafy, green surroundings. Knowledge and expertise in the domain of agribusiness converge here.

This Floriade will also serve to drive the development of knowledge relating to sustainability. The various sustainable buildings constructed on the Floriade site and surroundings are witness to this.

Visitors to the Floriade will be introduced to the many facets of the horticultural sector in an inspirational and interactive manner. The principal starting point is the central theme: Be part of the theatre in nature, get closer to the quality of life! This theme has been translated into five themed zones or worlds, where visitors can use all their senses to experience the influence of horticulture on their daily lives.

Overview over the Floriade, with the prominent “Innovatoren” forming the gateway to the exposition park

FLORIADE HORTICULTURAL WORLD EXPO
VENLO 2012 HOLLAND

The Floriade park will host more than one hundred exhibits in the form of gardens or pavilions representing participants from the horticultural sector and business community. In addition, each day the Floriade will feature a cultural programme of music, dance, theatre and visual art from all over the world. Each themed zone has its own play area and hospitality areas, where guests can enjoy an array of national and international culinary dishes. They can also savour a picnic surrounded by flowers and trees, lounge on the banks of the water or sit on the theatre hill and delight in the countless shows and performances.

For a panoramic view of the entire park, take a ride in the Floriade Cable Car. With a length of 1.1 kilometres and a height of more than 30 metres, the Floriade Cable Car is a unique attraction in the Netherlands, whisking visitors from one side of the park to the other in five minutes.

The Floriade 2012 is expecting at least two million paying visitors, 40% from the Netherlands, 40% from Germany and 20% from other countries, including Great Britain, France, Italy and China. The Floriade is being promoted in these countries by the Netherlands Board of Tourism and Conventions.

The Floriade expects 85% of the visitors to be consumers, with trade professionals making up the remaining 15%. Floriade 2012 places a high emphasis on families

with children. A special programme – Floriade Kids – has been set up for the 4–12 years age group.

The plants

Flowers, plants, shrubs and trees play the starring role during the Floriade. So expect to see these beauties in abundance. In between the original wooded areas, some 1.8 million bulbs, 190 000 perennials, 18 000 shrubs, 15 000 hedge plants, 5000 rose bushes and 3000 trees have been planted.

The planting schemes reflect the five theme zones: Relax & Heal, Green Engine, Education & Innovation, Environment and World Show Stage. The plants bring a unique atmosphere to each of the different theme worlds.

The Floriade is home to a huge number of exceptional plants and plant collections. For instance, the Friends' Woodland houses a large collection of fruit trees and fruit hedges, comprising heirloom varieties and modern varieties. The avenue of trees is formed by 120 different and unusual species, including *Ginkgo biloba*, also known as the Japanese nut tree or Chinese temple tree. This tree was introduced to Europe in 1730, being first planted in the Botanic Gardens in Leiden.

Other amazing attractions are the pleached limes with ten (!) layers of branches in the Education & Innovation zone, and the variety of office gardens in Environment, many of which are the result of a competition among garden and landscape designers and professional gardeners in the Netherlands and Belgium.

Participants

The participants at the Floriade fall into a number of categories.

To start with, there are national and international participants with exhibits at the park. The Floriade expects to display around forty exhibits from nations all over the world.

The national participants partly represent the five main sectors of horticulture: flower bulbs, fruit and vegetables, arboriculture, ornamental plants and flowers and professional landscape gardeners. In addition, countless Dutch companies are also contributing, either with their own exhibits or by helping to form the basic structure of the park. A number of public and semi-public bodies will also be present.

Artistic and cultural programme

More than ever before, the Floriade will be devoted to music, theatre, graphic art and entertainment from all corners of the earth. The basic programme on its own offers over 2000 performances during the 185 days of the Floriade. On weekends, the Floriade park will host numerous events.

Daily programme

Six days a week, the spectacular stilt walkers of the Close Act theatre group will be giving the public a warm welcome. A mobile pedal-powered stage will convey musicians through the park. Every day there will be a wide ranging programme packed with fascinating world music. Troupes and bands from countries as diverse as Indonesia, Hungary, Togo, Turkey and Romania will be appearing in the park. The Floriade is working on this event with, among others, Tilburg-based Mundial Productions, well-known for their annual Festival Mundial.

Every day the Floriade Show will be staged in the Floriade Theatre to round off a day of experiences. This show will last about 30 minutes, and is an impressive combination of acrobatics, world music and traditional and modern dance. A fantastic showpiece for all ages. The show will be performed by young musicians, acrobats and dancers from developing countries in Africa, Asia and Latin America.

Every Floriade day ends with the Floriade Goodbye: no fewer than 180 wind and brass bands from Limburg will be taking part.

Evening programme

The Environment and World Show Stage theme worlds will be open until midnight on weekends from mid-June to August. The pavilions, gardens, restaurants, shops and all other facilities in the two theme worlds will be open as normal during the evening. A diverse evening programme entitled “Gardens by Night” is sure to make your summer evening at Floriade one to be remembered. For live music simply head to the Floriade Beach Club. The open air cinema will be screening documentaries and films. Flowers, plants, trees, gardens and buildings play the leading role in a unique spectacular light show being produced especially for the Floriade by a well-known team of light artists.

Major national and international artistes will be treading the boards of the Floriade Theatre during the summer evenings of 2012.

The full calendar of cultural events can be found on the web site www.floriade.nl/evenementen-en-cultuur.

Organization

The Floriade is an initiative of the Netherlands Horticulture Council (NTR). Patron is Her Majesty Queen Beatrix of the Netherlands. The Bureau International des Expositions (BIE) in Paris and the Association Internationale des Producteurs de l’Horticulture (AIPH) have recognised the Floriade as a World Horticultural Expo.

All the countries affiliated to the BIE have been invited by the Dutch government to participate at this year's Floriade.

Floriade park with a green heritage

The Floriade park covers an area of 66 hectares, of which 40 hectares are designated exhibition grounds with 7500 square metres of indoor exhibition space.

The park encompasses five unique themed worlds, designed by landscape architect John Boon, linked by wooded areas. It is this 'green heritage' that is already in place that makes this Floriade unique and so exceptional. The five themes are Relax & Heal, Green Engine, Education & Innovation, Environment and World Show Stage.

Each world is characterised by its own planting schemes, programme and activities. In addition, each zone has its own thematic restaurant, one or more kiosks and play areas for children, making each individual theme world a complete exhibition in itself.

During the landscaping of the park, the existing natural features were disturbed as little as possible, and incorporated to their best advantage. The historical and archaeological heritage, such as a centuries-old chapel, was also gratefully used in the design blueprint.

The five theme worlds can be viewed as five examples of how to design public spaces. More than 3000 trees of 250 different species have been planted.

In the design and construction of the Floriade its legacy to the region in the form of Venlo GreenPark was emphatically taken into account: a sustainable business park in leafy, green surroundings. Knowledge and expertise in the domain of agribusiness converge here. Venlo GreenPark is the working landscape of the future, an area where work and recreation in verdant surroundings unite in harmony.

Two imposing structures are iconic for the region: the Innovatoren (Innovation Tower) and the Villa Flora. During the Floriade, the 70 metre high Innovatoren will form the gateway to the park. The building houses various rooms for business meetings, and also the Limburg pavilion. The Villa Flora will be the venue for an indoor exhibition and the World Pavilion during the Floriade season.

After the Floriade closes its gates, the Innovatoren will be developed into a centre for innovation in the agricultural sector. The Villa Flora will become a sustainable office building and exhibition venue. ■

Text and photos: ©Floriade 2012

President's report (continued from page 3)

on adapting the organization and tools required to run the Association efficiently, to take in account these developments. The new organization, new and better adapted financial tools and management regulations, and a decision on the legal status are all aimed to build for the future. Progress has been fast on a number of issues, thanks to the teamwork within the ECOM and collaboration with the Secretariat and the Technical Committees.

You are welcome to join the Technical Committees' work via working groups, where your experience, thoughts and comments will be welcome. Perhaps this report will inspire you to become more involved in your Association on a Technical Committee or as a future ECOM member. We hope to publish an article in the next edition of STI about how to become elected onto the ECOM in June 2013 to serve our Association for the period 2013 to 2016.

To conclude, I have the pleasure to invite you to participate in the annual ordinary meeting in June in Venlo, the Netherlands. In addition to the meetings of the Association, we will have the opportunity to enjoy the Floriade, an exceptional event that occurs only once every ten years.

This report was prepared from the work and reports provided by the ECOM Working Groups, with contributions from Alison Powell, Steve Jones, Craig McGill, Rita Zecchinelli and the Secretariat. ■

Calibration samples

Calibration samples for general seed blowers are now again available from the ISTA Secretariat. To order online, please go to www.seedtest.org/bookstore and click on 3.6 Laboratory equipment.

Prices are:

Dactylis glomerata 3.0 g: CHF 1050.00

Poa pratensis 1.0g: CHF 1250.00

We regret that for calibration samples, there is no price reduction for ISTA Members. ■

ISTA Annual Meeting 2012

11–14 June 2012, Venlo, Netherlands

The International Seed Testing Association (ISTA) takes pleasure in inviting you to its Annual Meeting to be held in Venlo, Netherlands, from 11 to 14 June 2012. The National ISTA Designated Authority of the Netherlands is delighted to be hosting the next ISTA Annual Meeting and would like to cordially invite you to the Floriade 2012 in conjunction with the meeting.

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 of 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.

Meeting venue

The meeting will take place at the Congress Pavilion of the World Horticultural Expo, Floriade 2012 (http://www.floriade.com). This is a large event which is organised every 10 years. The exhibition covers 66 hectares, and it would take a few days to see everything! Countries from all over the world create country pavilions to show what their horticultural sector has to offer: vegetables, flowers and trees of course, but also landscaping for various purposes. The city of Venlo has prepared itself to welcome you for moments of relaxation. There are brand new meeting facilities and we are glad to have the unique possibility of combining our meeting with visits to the exquisite country pavilions. A cable car of 1.1 km length connects you to the far corners of the garden and to the various meeting places. (see page 12 for further details.

Registration

Registration for the full Annual Meeting includes the ISTA Seminar on 11 June, the technical presentations on 12 and 13 June, and the Ordinary Meeting on 14 June.

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

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

All the fees include free admission to the Floriade during the meeting days. The tickets will be distributed upon registration on the spot.

Online registration is now possible at www.seedtest.org/AM12.

Registration will close on 15 May 2012.

Accompanying persons

The category ‘accompanying persons’ is applicable only for the spouse, companion and/or children of a delegate. Registration as an accompanying person does NOT

Registration fees (online registration at www.seedtest.org/AM12)

Periods	Events	Registration
ISTA Members		
11–14 June	Annual Meeting incl. Seminar	660 €
11 June	Seminar only	200 €
Non-members		
11–14 June	Annual Meeting incl. Seminar	1200 €
11 June	Seminar only	250 €
Students		
11 June	Seminar only	40 €
Accompanying persons		
11–14 June	Social events, lunches etc. only	200 €
Exhibitors (Members)		
11–14 June	Exhibition booth	2000 €
Exhibitors (non-members)		
11–14 June	Exhibition booth	3000 €

The Netherlands, showing the location of Venlo.

include participation in any of the meetings or sessions, but only to social events, lunches and coffee breaks, the Welcome Reception and the Official Dinner.

Climate

Holland's climate is rather mild because of the proximity of the sea and the large river system. The weather in June is rather unpredictable. Sunny spells usually alternate with short showers. The temperature is generally around 20 °C (15–25 °C) during the day and 10 °C (5–15 °C) during the night. So bring a sweater, raincoat and/or umbrella. There is a chance that you won't need them, but please don't take that risk.

Travel information

Venlo is located in the east of the Netherlands. It is surrounded by the international airports of Düsseldorf, Maastricht, Eindhoven and Weeze. The main airport for overseas travellers is Amsterdam Schiphol airport (AMS). Trains depart from Schiphol airport to Eindhoven and from there to Venlo.

A shuttle bus is available from Venlo railway station to the Floriade, where the meeting registration and all meetings will take place. There will also be shuttle buses between the Floriade and the hotels on Saturday, 9 June, 13:00–19:00, and on Sunday, 10 June, 10:00–13:00 and 16:00–23:00. If you arrive outside these times, please go straight to your hotel by taxi, or by public transport (Van der Valk only).

During meeting days a regular bus service will be provided. Details will be announced upon your arrival.

For train tickets please use the online reservation system (www.ns.nl), as it is most convenient to buy an e-ticket. At the airport there is also a ticket office and ticket vending machines (Maestro, Visa and MasterCard are accepted).

The currency used in the Netherlands is the euro. Cash dispensers (ATMs) are available at all banks, including the airport, and exchange rates are the same everywhere.

Accommodation

Three hotels in the Venlo area are offering special rates for participants at the Annual Meeting: the Van der Valk Hotel, Venlo (EUR 104), the Parkhotel, Horst (EUR 155.60) and the Hotel Asteria, Venray (EUR 94). are available Accommodation can be booked online via the ISTA web page www.seedtest.org/AM12.

Pre-meeting workshops

See pages 46 and 47 for further information.

Exhibitors

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

The exhibitor registration fee includes one booth and one exhibitor for the duration of the Annual Meeting (11–14 June 2012) as well as cocktails, coffees, lunches and official dinner. An additional person to man the booth can register as an accompanying person, with the same benefits.

For detailed information about exhibition spaces, please contact Mr. Marcel Toonen (m.toonen@naktuinbouw.nl).

Sponsors

There are also possibilities to sponsor the ISTA Annual Meeting 2012, with a variety of sponsoring packages to choose from.

For detailed information about sponsoring, please contact Mr. Marcel Toonen (m.toonen@naktuinbouw.nl). ■

Final Programme ISTA Annual Meeting 2012

Sunday, 10 June 2012

12:00–18:00	Registration of participants at conference venue
19:00	Welcome Reception

Monday, 11 June 2012

08:00–18:00	Registration of participants at conference venue
08:30–18:00	ISTA Seminar "New developments and technologies in seed testing" Chair: Bert van Duijn (Netherlands) (see page 19)

Tuesday, 12 June 2012

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	Session 1: a. Report of the Purity Committee b. Report of the Tetrazolium Committee c. Report of the Moisture Committee d. Report of the Vigour Committee
10:00–10:30	Coffee break
10:30–12:30	Session 2: a. Report of the Variety Committee b. Report of the Seed Health Committee c. Report of the Germination Committee
12:30–13:30	Lunch break

13:30–15:30 Session 3:
- a. Report of the GMO Committee
- b. Report of the Flower Seed Committee
- c. Report of the Forest Tree and Shrub Seed Committee
- d. Report of the Editorial Board of Seed Science & Technology

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

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

16:00–18:30 Advanced Technologies Committee Meeting

16:00–17:30 Germination Committee

16:00–17:00 Variety Committee Meeting

17:00–18:00 GMO Committee Meeting

Wednesday, 13 June 2012

08:30–18:30 Presentation of ISTA's Technical Work (continued)

08:30 Opening by the ISTA President

08:30–10:00 Session 4:
- a. Report of the Bulking and Sampling Committee
- b. Report of the Statistics Committee
- c. Report of the Nomenclature Committee

10:00–10:30 Coffee break

10:30–12:40 Session 5:
- a. Report of the Seed Storage Committee
- b. Report of the Committee on Advanced Technologies
- c. Report of the Proficiency Test Committee
- d. Report of the Accreditation Department
- e. Signing of the memorandum of the collaboration between the International Seed Testing Association and the Royal Botanic Gardens, Kew

12:40–13:30 Lunch break

13:30–15:30 Session 6:
- a. Meeting of the Rules Committee

15:30–16:00 Coffee break

16:00–18:00 Session 6 (continued):
- a. Meeting of the Rules Committee
- b. Discussion on in-house germination methods

19:00 Official Dinner

Thursday, 14 June 2012

08:30–17:30 ISTA Ordinary Meeting

08:30–09:30 Welcome by the ISTA President

Presentation on the development of the seed industry in the Netherlands

09:30–10:00 Presentation on facilitating the international seed trade – essential components of a credible and transparent accreditation quality assurance system

10:00–10:30 Coffee break

10:30–12:30
1. Call to order
2. President's address
3. Roll call of Designated Members entitled to vote
4. Comments about the minutes of the previous meeting
5. Report of the Executive Committee
   - 5.1 Executive Committee Report
   - 5.2 Legal Status of the Association
   - 5.3 Internal finance tool
6. Report of the Secretary General

12:30–13:30 Lunch break

13:30–15:40
7. Constitution changes
8. Fixation of annual subscriptions
   - 8.1 Discussion and decision on the ISTA membership fee and annual fee for an ISTA accredited laboratory
   - 8.2 Presentation on the prices of the ISTA Certificates by the ISTA Executive Committee
9. Consideration and adoption of the proposed Rules Changes
10. Consideration and adoption of reports
11. Announcement of the place and date for the next ordinary meeting of the Association
    - 11.1 Congress 2013
    - 11.2 Annual Meeting 2014

15:40–16:00 Coffee break

16:00–17:30
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 meeting
    - 12.1 Discussion: Executive Committee motion on the procedure for setting the accreditation audit fee
    - 12.2 Vote: Executive Committee Motion
    - 12.3 Discussion: Constitution Change proposal from Australia and New Zealand Designated Authorities
    - 12.4 Vote: Constitution Change proposal from Australia and New Zealand Designated Authorities
    - 12.5 Discussion: Notice of motion jointly by Australia and New Zealand to request Executive Committee to review the audit process and report to members
    - 12.6 Vote: Notice of motion jointly by Australia and New Zealand to request Executive Committee to review the audit process and report to members
13. Any other business raised by consent of the Executive Committee
14. President's closing address
15. Adjournment

ISTA Seminar "New developments and technologies in seed testing"

Bert van Duijn
Chair, ISTA Advanced Technologies Committee

The next Annual Meeting of the International Seed Testing Association (ISTA) will take place in Venlo, the Netherlands from 10–14 June, 2012. Traditionally, a seminar is part of the Annual Meeting. The 2012 seminar is organized by the ISTA Advanced Technologies Committee and is entitled "New Developments and Technologies in Seed Testing". The seminar will be held on Monday, 11 June 2012, and will give a broad overview of the newest developments of applicable technologies in seed testing, ranging from vision and image analysis, to sensor applications and molecular technologies.

The organizers are very pleased to invite you to join the seminar. For more information on the Annual Meeting 2012 and the seminar please refer to the ISTA website: www.seedtest.org.

Looking forward to welcoming you in Venlo.

Monday, 11 June 2012

08:30	Opening by the ISTA President, Joël Léchappé (France) Introduction to the Seminar by the Chair, Bert van Duijn (Netherlands): Future technologies from the past: do we use them? And what about future technologies of today?
08:45–11:35	Session 1: Vision and image analysis
08:45	Multispectral cameras and imaging in seed quality parameters (Birte Boelt, Aarhus University, Denmark)
09:10	X-ray based seed analysis and sorting (to be confirmed)
09:35	Automatic primed seed analysis (Stephen Harper, GTG/Germains, United Kingdom)
10:00	Coffee break
10:20	Non-invasive computer vision tools to monitor seedling elongation (Étienne Belin, SNES/GEVES, France)
10:45	Magnetic resonance imaging (Fabio Gorian, Forestry Research and Conservation Institute, Italy)
11:10	Application of vision systems in horticulture and tissue culture (John Bijl and Cees Visser, ViVi, Netherlands)
11:35–14:20	Session 2: (New) Sensor applications in seed testing and developments in seed technology
11:35	Oxygen measurements in seed testing (Kent Bradford, UC Davis, USA)
12:00	Ethanol breath analysis in seed testing (Steven Groot, WUR, Netherlands)
12:25	Lunch
13:30	Water activity measurements in seed testing (Craig McGill, Massey University, New Zealand)
13:55	Smart seed coatings and pellets (Frans Tettero, Incotec, Netherlands)
14:20–16:00	Session 3: Molecular technologies in seed testing
14:20	Systems Biology approach to seed testing, all-in-one: gene-protein-metabolite-structure analysis (Steven Penfield, Exeter University, United Kingdom)
14:45	Applied molecular genetics testing for quality control in seed testing (Patrik Stolt, ScanBi Diagnostics, Sweden)
15:10	Coffee break
15:30	DNA markers in purity testing (Beni Kaufman, Pioneer a DuPont Business, USA)
16:00–17:00	Session 4: Discussion/panel session: New technologies and ISTA Rules, needs and implementation barriers
17:00	Concluding remarks

New Advanced Technology Committee

Brigitte Hamman
Vice-Chair, ISTA Advanced Technologies Committee

Syngenta Crop Protection Münchwilen AG
4332 Stein, Switzerland
brigitte.hamman@syngenta.com

The Advanced Technologies Committee (ATC) was formed in 2007. Since then, considerable experience has been gained, leading to an increase in value for ISTA and a considerable evolution. Along the way, the aims of the ATC were redefined, and so too was the working programme. Currently, the aims of the committee are as follows:

- form a bridge between the research and development world and other TCOMs;
- collect and interpret data and opinions on "advanced" technologies;
- provide the ISTA community with information on "advanced" technologies;
- chair or participate in research or tests of "advanced" technologies in cooperation with other TCOMs, and/or act as reviewers;
- formulate views on ongoing or future technological developments (e.g. nanotechnology, microelectronics) from a seed-testing perspective to aid the ISTA community.

It is important to note that the ATC does *not* aim to develop "ISTA-approved" or "ISTA-advised" technologies, and will also *not* aim to produce Rules proposals. The committee is a provider of services to the other TCOMs, each of which has nominated a member to act as contact person to the ATC (see list below). There will be a greater focus on dissemination of information (e.g. web site, where details of the working programme will be posted and regularly updated), and the relevant committees will be informed about the ATC programme in advance, since they will play a role in setting priorities.

New tasks will be added to the working programme, such as the organization of workshops or sessions focusing on ATC topics at ISTA congresses, and the support of workshops and sessions that include ATC-related topics. In addition, when a particular technology or development warrants it, a specific meeting or workshop will be organized.

Finding new technologies will be the responsibility of all ATC members, and anyone within ISTA is invited, and welcome, to do the same!

Current committee

Bert van Duijn (Chair)
Brigitte Hamman (Vice-Chair)
Birte Boelt
Kent J. Bradford
Rukui Huang
Bob Legro
Craig McGill (ECOM contact)
Harry Nijenstein
Graeme Smith
Joost van der Burg
Zhujun Zhu

Contact persons in other TCOMs

Statistics: Tim Perez
Purity: Adriel Garay
Variety: Berta Killermann
GMO: Ana Laura Vicario
Germination: Harry Nijënstein
Nomenclature: John Wiersema
Moisture: Craig McGill
Vigour: Alison Powell
SST: Fiona Hay
Proficiency: Günter Müller
Flower Seed: Rita Zecchinelli
Seed Health: Karin Sperlingsson

Some of the members of the Advanced Technologies Committee (left to right): Bert van Duijn (Chair), Joost van der Burg, Craig McGill (ECOM contact), Birte Boelt, Zhujun Zhu, Brigitte Hamman (Vice-Chair), Rukui Huang.

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

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

Among the changes are the following:

Chapter 1: Certificates

- issuing of Original Orange International Seed Lot Certificates for partial seed lots

Chapter 2: Sampling

- Addition of *Solanum nigrum*
- Addition of *Prunus* spp., for species that are difficult to distinguish
- Submission of large herbage seed lots (see page 22)
- Clarification of taking the container-sample for heterogeneity testing

Chapter 3: The Purity Analysis

- Transferring *Arachis* to PSD 21
- PSDs 11 and 20–24: broken seed or separated cotyledons contained within testa must be considered to be pure seed
- PSD 36: removal of pedicel no longer compulsory

Chapter 5: The Germination Test

- Amendment of seedling abnormalities 11/05 and 11/06
- Ending a germination test at a predetermined germination level

Annex to Chapter 7: Seed Health Testing Methods

- New method 7-028: Detection of infectious tobamoviruses on *Lycopersicon esculentum* by the local lesion assay on *Nicotiana tabacum* plants
- Modification to method 7-019 by adding a PCR testing option
- Minimum recommended seed sample sizes for seed health methods (Amendments to methods 7-001a, 7-001b, 7-002a, 7-002b, 7-016, 7-017 and 7-018)
- Updates to text of method 7-024

Chapter 9: Moisture Content

- Removal from Table 9A Part 1 of the low-temperature method for those species where it has not been individually validated

Chapter 13: Testing Seeds by Weighed Replicates

- Clarification of purity testing for weighed replicates test

Preparatory documents for the Ordinary Meeting

The following documents are submitted to the ISTA Ordinary Meeting 2012 for information and discussion and/or acceptance by the nominated ISTA Designated Members voting on behalf of their respective Governments:

- OM12-01 Agenda for the Ordinary Meeting 2012 [information document]
- OM12-02 Minutes of the Ordinary Meeting 2011 [information document]
- OM12-03 Activity Report of the ISTA Committees 2011 [voting document]
- OM12-04 Proposal for the Membership Fees 2013 [voting document]
- OM12-05 Rules Proposals for the International Rules for Seed Testing 2013 Edition [voting document]
- OM12-06 Method Validation Reports on Rules Proposals for the International Rules for Seed Testing 2013 Edition [supporting document to voting document OM12-05]
- OM12-07 Constitution Change Proposals 2012 [voting document]
- OM12-08 Proposal to increase the price for blank ISTA Certificates [voting document]
- OM12-09 Executive Committee motion on the procedure for setting the accreditation audit fee [voting document]
- OM12-10 Submission from members: Constitution Change proposal from Australia and New Zealand [voting document]
- OM12-11 Submission from members: Notice of Motion from Australia and New Zealand [voting document]

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

The documents have been posted on the ISTA website at www.seedtest.org/OM12.

Update on the ISTA/ISF experiment on herbage seed lot size

Max Soepboer
Vice-Chair, ISTA Bulking and Sampling Committee and Coordinator, ISTA/ISF Experiment

Th. de Bockweg 19
6871 EG Renkum
Netherlands

Introduction

The ISTA/ISF experiment on herbage seed lot size was begun in 1995, to assess whether sufficiently homogeneous herbage seed lots with a maximum weight of 25 t could be made. The complete set of conditions for the experiment are given in the document 'ISTA/ISF Experiment On Herbage Seed Lot Size: Technical Protocol', which can be found on the ISTA web site.

This article starts with a brief outline of the technical conditions, followed by an overview of the results obtained so far.

Conditions and species groups

A company plant must show that it is able to produce large herbage seed lots that are sufficiently homogeneous. It is then approved to produce seed lots up to 25 tonnes for which ISTA Certificates can be issued. The company plants that have fulfilled the requirements and those at the stage of testing are published on the ISTA web site (http://www.seedtest.org/en/ista_isf_experiment).

The focus of the experiment is on two groups of species:

I. *Lolium perenne, Lolium multiflorum, Lolium × boucheanum, Festuca pratensis, Festuca arundinacea* and *Phleum pratense*;
II. *Festuca rubra, Festuca lemanii, Dactylis glomerata, Poa pratensis* and *Poa trivialis.*

The main technical conditions are as follows:

a) Companies taking part in the experiment must have a quality manual focusing on the quality level of individual lots and the blending of cleaned lots.
b) At least six lots of the relevant species group must be tested per company plant.
c) Twenty independent container samples must be taken.
d) Each independent sample must be tested for purity (1000 seeds), germination (100 seeds) and other seeds by number (10 000 seeds).
e) Statistical analysis of the data must be carried out according to the ISTA Rules, Chapter 2.
f) At least five of the six seed lots tested must be homogeneous.

Once a company plant has been approved to produce larger seed lots and issue ISTA Certificates on these, there is a 10 % heterogeneity testing rate for the first 100 seed lots (including the six initial tests). After that the monitoring rate is 5 %.

Results

To date (February 2012) a total of 20 plants (eight in Denmark, four in Germany, one in the Netherlands and seven in the USA) have participated in the experiment. Some plants have applied for both species groups. In total, 144 large seed lots (24 species groups with six lots each) have been produced, and homogeneity testing demonstrated that all 20 plants have met the requirements. A total of 133 seed lots have been found to be sufficiently homogeneous for all quality traits.

Eleven seed lots showed statistically significant heterogeneity: five failed on purity, and a further six on other seeds by number.

The following species have so far been tested:

– *Lolium perenne* (Group I): 51 lots
– *Festuca arundinacea* (Group I): 31 lots
– *Lolium multiflorum* (Group I): 26 lots
– *Festuca rubra* (Group II): 19 lots
– *Dactylis glomerata* (Group II): 10 lots
– *Poa pratensis* (Group II): 7 lots

To date, three plants that have been authorized to make larger seed lots have submitted the results of 13 seed lots in the framework of monitoring. Eleven seed lots proved to be sufficiently homogeneous. One lot was heterogeneous for purity and one for other seeds by number.

Summary of the results

All 20 participating plants have met the requirements of the experiment.

Eleven larger seed lots were not sufficiently homogeneous; five lots failed on purity and six on other seeds by number.

All tested seed lots were sufficiently homogeneous on germination.

So far, three approved company plants have provided testing data in the framework of monitoring (two seed lots each for two company plants and nine for a third company plant). Of these 13 seed lots, one failed on purity and another failed on other seeds by number.

Very little information has been received concerning the quality handbooks or procedures that are applied for making homogeneous larger seed lots.

Future permanent regime

The Bulking and Sampling Committee (BSC) recently discussed the results of the ISTA/ISF experiment on larger herbage seed lot size. Since all participating company plants have proved that they are technically able to make larger seed lots, the BSC concluded that the experiment has been successful. Consequently the BSC proposes that in 2013 the experiment be replaced by a permanent regime allowing individual company plants to be approved for making larger herbage seed lots. It is proposed that the conditions for such an approval should be the same as those of the present experiment. The other conditions for such a regime (monitoring, level of monitoring, responsible body for approval of individual company plants etc) are being discussed within ISTA and will be presented at the Annual Meeting in Venlo. ■

Constitution change proposal: to establish ISTA as an Association under Swiss Law

Craig McGill
ISTA Executive Committee member; Chair, ISTA Moisture Committee; Vice-Chair, ISTA Rules Committee

The changes to the ISTA Constitution presented for a vote are required to establish ISTA as an Association under Swiss Law. Also numbering has been changed from Roman numbering of articles, e.g. XIV, to Arabic numbers, e.g. 14.

For ISTA to be considered to be an Association under Swiss Law **all** the changes proposed must be approved. For this reason all the changes are proposed as a single document for voting.

The changes fall into the following categories:

Articles or parts thereof mandatory under Swiss Law.
Article 6 – required under Article 75 of the Swiss Civil Code;
Article 7 – required under Article 72 (1–3) of the Swiss Civil Code;
Article 11 – number of Designated Members needed to request an extraordinary meeting reduced from two-thirds to one-fifth as required by Article 64 (3) of the Swiss Civil Code;
Article 12 – new clause (d) as required by Article 68 of the Swiss Civil Code;
Article 17 – new as required by Article 69b (1–4) of the Swiss Civil Code;
Article 21 – two new clauses added (b) and (c) as required by Articles 77 and 78 of the Swiss Civil Code.

To maintain the hierarchy of the bodies in the Associations as provided under Swiss Law and define their capacity to act.
Articles 8 and 9

To define the Powers of the General Meeting as the highest body in the Association, including the right to dismiss a body if good grounds exist (Article 65(3) of the Swiss Civil Code).
Article 10

To define the liabilities of the Association and its Members (Article 75a of the Swiss Civil Code). The Article limits liability to that contained within the Associations Assets.
Article 19

Establishing ISTA as an Association under Swiss Law will limit the liability of the Association to its assets and mean that the Members will not be personally liable, give legal protection to the Secretariat and ECOM members as individuals, allow the Association to more easily comply with Swiss tax laws and be recognised as a legal entity for business and insurance purposes.

Current	Proposed
	The Articles of The International Seed Testing Association (ISTA)
~~ARTICLE I~~ ~~Name~~	I. Name and Seat Article 1 Name
The Association shall be known as The International Seed Testing Association, hereinafter referred to as 'the Association'.	The Association shall be known according to the Swiss Civil Code articles 60 ff. as The International Seed Testing Association, hereinafter referred to as "the Association".
~~ARTICLE II~~ ~~Seat~~	Article 2 Seat
The ~~headquarters~~ of the Association shall be at the office of the Secretary General. ~~The change of the place of the seat of the headquarters must be approved by a majority vote of the Executive Committee.~~	The seat of the Association shall be at the office of the Secretary General in Switzerland.
~~ARTICLE III~~ ~~Objects~~	II. Objects Article 3 Primary Purpose and Secondary Purposes
(a) The primary purpose of the …	(a) The primary purpose of the …
~~ARTICLE IV~~	III. Membership Article 4 Governments, Authorities and Members
~~Government~~ (a) The word Government shall mean ...	(a) Government The word Government shall mean …
~~Designated Authority~~ (b) A Designated Authority is an …	(b) Designated Authority A Designated Authority is an …
~~Designated member~~ ~~(c)~~ A Designated Member is a Personal Member designated by their Designated Authority and, subject to the provisions of Article ~~IX, entitled to vote in meetings of the Association~~.	(c) Designated Member A Designated Member is a Personal Member designated by their Designated Authority and entitled to vote, subject to the provisions of Article 12.
~~Membership~~ ~~(d)~~ A Member Laboratory is a …	(d) Member Laboratory A Member Laboratory is a …
~~(e)~~ A Personal Member is a person …	(e) Personal Member A Personal Member is a person …
~~(f)~~ An Associate Member is a person …	(f) Associate Member An Associate Member is a person …
~~(g)~~ A Corporate Member is any …	(g) Corporate Member A Corporate Member is any …
~~(h)~~ An Honorary Life Member …	(h) Honorary Life Member An Honorary Life Member …

Current	Proposed
~~Accredited Laboratory~~ ~~(i)~~ An Accredited Laboratory is a member laboratory accredited by the Executive Committee according to the Accreditation Standards approved under Article ~~VII~~(c)(15) of the Constitution.	(i) Accredited Laboratory An Accredited Laboratory is a member laboratory accredited by the Executive Committee according to the Accreditation Standards approved under Article 15(c)(15) of the Constitution.
~~ARTICLE XV~~ ~~Withdrawal~~	Article 5 Withdrawal
(a) Any Government may withdraw …	(a) Any Government may withdraw …
(b) If a Government states, in its notice, that its withdrawal is because it cannot comply with an amendment adopted under Article ~~XII~~, such withdrawal shall be effective on the date of the entry into force of such amendment, provided the Secretary General has received the notice not more than 30 days after the entry into force of the amendment. Withdrawal under any other circumstances shall become effective at the end of the calendar year in which the notice for that purpose is given.	(b) If a Government states, in its notice, that its withdrawal is because it cannot comply with an amendment adopted under Article 20, such withdrawal shall be effective on the date of the entry into force of such amendment, provided the Secretary General has received the notice not more than 30 days after the entry into force of the amendment. Withdrawal under any other circumstances shall become effective at the end of the calendar year in which the notice for that purpose is given.
(c) The financial obligation to the ...	(c) The financial obligation to the …
(d) Any Member may withdraw …	(d) Any Member may withdraw …
(e) If a Member states, in their notice, that the withdrawal is because they cannot comply with an amendment adopted under Article ~~XII~~, such withdrawal shall be effective on the date of the entry into force of such amendment, provided the Secretary General has received the notice not more than 30 days after the entry into force of the amendment. Withdrawal under any other circumstances shall become effective at the end of the calendar year in which the notice for that purpose is given.	(e) If a Member states, in their notice, that the withdrawal is because they cannot comply with an amendment adopted under Article 20, such withdrawal shall be effective on the date of the entry into force of such amendment, provided the Secretary General has received the notice not more than 30 days after the entry into force of the amendment. Withdrawal under any other circumstances shall become effective at the end of the calendar year in which the notice for that purpose is given.
(f) The financial obligation to the …	(f) The financial obligation to the …
	Article 6 Protection of Members
	Any Member who has not consented to a resolution which infringes the law or the Articles of the Association is entitled by law to challenge such resolution in court within one month of learning thereof.
	Article 7 Exclusion
	Any exclusion of Members requires a resolution by the Members and good cause.

Current	Proposed
	IV. Bodies and Capacity to Act Article 8 Bodies
	(a) General Meeting of Members (b) Executive Committee (as the governing body of the Association) (c) Financial Auditors
	Article 9 Capacity to Act
	(a) Legal entities have capacity to act once the (governing) bodies required by law and their articles of Association have been appointed.
	(b) The (governing) bodies express the will of the legal entity.
	(c) They bind the legal entity by concluding transactions and by their other actions.
	(d) The governing officers are also personally liable for their wrongful acts.
	A. The General Meeting of Members Article 10 Powers
	(a) The General Meeting of Members appoints the Officers and the Executive Committee and decides all matters which are not reserved to other governing bodies of the Association.
	(b) It supervises the activities of the Executive Committee and Financial Auditors and may at any time dismiss – whenever justified by good cause – the latter without prejudice to any contractual rights of those dismissed.
~~ARTICLE X~~ ~~Meetings of the Association~~	Article 11 Meetings
(a) An ~~ordinary meeting~~ of ~~the~~ Members ~~of the Association~~ shall normally be held every year, but ~~extraordinary meetings~~ may be held when considered necessary by the Executive Committee or ~~when requested by two-thirds of the Designated Members~~.	(a) An Ordinary General Meeting of Members shall normally be held every year, but Extraordinary General Meetings may be held when considered necessary by the Executive Committee or by law when requested by one-fifth of the Members.
(b) Matters in dispute at ~~meetings~~ of the Association shall be referred to a vote.	(b) Matters in dispute at General Meetings of the Association shall be referred to a vote.
(c) In the event of a tie in a vote, the President, or in his/her absence the Vice-President shall have a deciding vote at ~~meetings~~ of the Association and of the Executive Committee. In all other committees of the Association, in the event of a tie, the acting Chairman shall have a deciding vote.	(c) In the event of a tie in a vote, the President, or in his/her absence the Vice-President shall have a deciding vote at General Meetings of the Association and of the Executive Committee. In all other committees of the Association, in the event of a tie, the acting Chairman shall have a deciding vote.

Current	Proposed
(d) Designated Members designated by forty percent of the Designated Authorities shall constitute a quorum at ~~meetings~~ of the Association. In determining the percentage, fractions less than 0.50 shall be dropped and those 0.50 or greater shall be regarded as a whole number. If the ~~ordinary meeting~~ is not quorate a 'by correspondence' vote will be held to allow the adoption of ~~ordinary meeting~~ agenda items.	(d) Designated Members designated by forty percent of the Designated Authorities shall constitute a quorum at General Meetings of the Association. In determining the percentage, fractions less than 0.50 shall be dropped and those 0.50 or greater shall be regarded as a whole number. If the Ordinary General Meeting is not quorate a 'by correspondence' vote will be held to allow the adoption of Ordinary General Meeting agenda items.
(e) The agenda for an ~~ordinary meeting of the Association~~ shall include:	(e) The agenda for an Ordinary General Meeting shall include:
(1) Call to order.	(1) Call to order.
(2) President's address.	(2) President's address.
(3) Roll call of Designated Members entitled to vote.	(3) Roll call of Designated Members entitled to vote.
(4) Comments about the minutes of the previous ~~meeting~~.	(4) Comments about the minutes of the previous General Meeting.
(5) Report of the Executive Committee.	(5) Report of the Executive Committee.
(6) Report of the Secretary General.	(6) Report of the Secretary General.
(7) Fixation of annual subscriptions.	(7) Fixation of annual subscriptions.
(8) Consideration and adoption of reports.	(8) Consideration and adoption of reports.
(9) Announcement of the place and date for the next ~~ordinary meeting of the Association~~.	(9) Announcement of the place and date for the next Ordinary General Meeting.
(10) Any other business raised by a Member, of which notice in writing has been received by the Secretary General at least two months prior to the date of the ~~meeting~~.	(10) Any other business raised by a Member, of which notice in writing has been received by the Secretary General at least two months prior to the date of the General Meeting.
(11) Any other business raised by consent of the Executive Committee.	(11) Any other business raised by consent of the Executive Committee.
(12) President's closing address.	(12) President's closing address.
(13) Adjournment.	(13) Adjournment.
~~And a~~dditionally at the ~~ordinary meeting~~ held in the third year after the ~~ordinary meeting~~ at which officers and members-at-large of the Executive Committee were appointed:	(f) Additionally at the Ordinary General Meeting held in the third year after the Ordinary General Meeting at which officers and members-at-large of the Executive Committee were appointed:
(14) Discharge of the Executive Committee.	(14) Discharge of the Executive Committee.
(15) Election of Officers and members-at-large of the Executive Committee.	(15) Election of Officers and members-at-large of the Executive Committee.
(16) Installation of new Officers.	(16) Installation of new Officers.

Current	Proposed
~~(f)~~ The Executive Committee approved minutes of the ~~ordinary meeting~~ will be published on the ISTA website within two months of the ~~ordinary meeting~~. If there are no comments requiring amendment to the minutes within the subsequent two month period, the minutes will be considered approved. If there are comments and the comments are accepted by the Executive Committee, then the minutes including the comments will be considered approved and published on the ISTA website.	(g) The Executive Committee approved minutes of the Ordinary General Meeting will be published on the ISTA website within two months of the Ordinary General Meeting. If there are no comments requiring amendment to the minutes within the subsequent two month period, the minutes will be considered approved. If there are comments and the comments are accepted by the Executive Committee, then the minutes including the comments will be considered approved and published on the ISTA web site.
Any comments about the minutes of the previous ~~meeting~~ will be considered at the next ~~ordinary meeting~~ under agenda Article ~~X~~ (e) 4.	(h) Any comments about the minutes of the previous General Meeting will be considered at the next Ordinary General Meeting under agenda Article 11 (e) (4).
~~ARTICLE IX Voting~~	Article 12 Voting
(a) Irrespective of the number of Designated Members designated by a single Government, only one vote may be cast on behalf of that Government.	(a) Irrespective of the number of Designated Members designated by a single Government, only one vote may be cast on behalf of that Government.
(b) The following categories of motions require for adoption a two-thirds majority of those voting: ~~m~~otions to alter this ~~Constitution~~, ~~m~~otions to dissolve the Association, ~~and m~~otions arising during ~~meetings~~ and relating to temporary adjournment, closing of debate, or postponement of action. All other motions require a simple majority of those voting for adoption.	(b) The following categories of motions require for adoption a two-thirds majority of those voting: (1) Motions to alter these Articles. (2) Motions to dissolve the Association. (3) Motions arising during General Meetings and relating to temporary adjournment, closing of debate, or postponement of action. All other motions require a simple majority of those voting for adoption.
(c) On urgent matters as determined by the Executive Committee, and in which the Executive Committee is not authorised to act, voting members may be requested by the President to vote by correspondence during the period between ~~ordinary meetings~~ of the Association in accordance with ~~paragraph~~ (a) and (b) of this ~~article~~.	(c) On urgent matters as determined by the Executive Committee, and in which the Executive Committee is not authorised to act, voting members may be requested by the President to vote by correspondence during the period between Ordinary General Meetings of the Association in accordance with letter (a) and (b) of this Article. (d) Each Member is by law excluded from voting on any resolution concerning a transaction or dispute between him or her, his or her spouse or a lineal relative on the one hand and the Association on the other.

Current	Proposed
~~ARTICLE V~~ ~~Officers~~	Article 13 Officers
(a) The Officers of the Association shall be: President Vice-President	(a) The Officers of the Association shall be: (1) President. (2) Vice-President.
(b) The tenure of office of the President and Vice-President shall be from the adjournment of the ~~ordinary meeting~~ at which they were appointed to the adjournment of the ~~ordinary meeting~~ held in the third year after the ~~ordinary meeting~~ at which they were appointed. If the ~~ordinary meeting~~ at which elections are held is not quorate the tenure of the existing Executive Committee will continue until a 'by correspondence' vote can be held to discharge the Executive Committee and to appoint a new Vice-President and new Executive Committee.	(b) The tenure of office of the President and Vice-President shall be from the adjournment of the Ordinary General Meeting at which they were appointed to the adjournment of the Ordinary General Meeting of Members held in the third year after the Ordinary General Meeting at which they were appointed. If the Ordinary General Meeting at which elections are held is not quorate the tenure of the existing Executive Committee will continue until a 'by correspondence' vote can be held to discharge the Executive Committee and to appoint a new Vice-President and new Executive Committee.
(c) On completion of the tenure of office the outgoing President shall not at any time in the future be eligible for reappointment as President or for appointment as Vice-President.	(c) On completion of the tenure of office the outgoing President shall not at any time in the future be eligible for reappointment as President or for appointment as Vice-President.
~~ARTICLE VI~~ ~~Functions of Officers~~	Article 14 Functions of Officers
(a) The President shall call and preside at ~~meetings~~ of the Association and of the Executive Committee. The President shall be an ex-officio member of all committees of the Association.	(a) The President shall call and preside at General Meetings of the Association and meetings of the Executive Committee. The President shall be an ex-officio member of all committees of the Association.
(b) The Vice-President shall assist the President and, in the event of the inability of the President to serve, shall carry out such duties as pertain to the office of the President. In the event that a President cannot continue in office for the remainder of his/her term, the Vice-President will be referred to as the President for the remaining period of that Presidency and will also serve for the expected period of his/her own Presidency.	(b) The Vice-President shall assist the President and, in the event of the inability of the President to serve, shall carry out such duties as pertain to the office of the President. In the event that a President cannot continue in office for the remainder of his/her term, the Vice-President will be referred to as the President for the remaining period of that Presidency and will also serve for the expected period of his/her own Presidency.
~~ARTICLE VII~~ ~~Executive Committee~~	Article 15 Executive Committee
(a) The Executive Committee shall consist of the President and Vice-President, together with nine members-at-large who shall be Designated Members.	(a) The Executive Committee shall consist of the President and Vice-President, together with nine members-at-large who shall be Designated Members.
(b) The tenure of office of the members-at-large shall be the same as that for Officers as provided in Article ~~V~~ (b).	(b) The tenure of office of the members-at-large shall be the same as that for Officers as provided in Article 13 (b).
(c) The functions of the Executive Committee shall be as follows:	(c) The functions of the Executive Committee shall be as follows:
(1) The Executive Committee shall manage and direct the affairs of the Association according to the provisions of this Constitution and to decisions arrived at by the Association at ~~ordinary~~ or ~~extraordinary meetings~~.	(1) The Executive Committee shall manage and direct the affairs of the Association according to the provisions of this Constitution and to decisions arrived at by the Association at Ordinary or Extraordinary General Meetings.
(2) The Executive Committee shall …	(2) The Executive Committee shall …
(3) In the event of vacancies in the panel of Officers or members-at-large of the Executive Committee, the remaining members of the Committee are empowered to appoint substitutes to serve until the next ~~ordinary meeting~~ of the Association at which the election of officers and members-at-large will be held.	(3) In the event of vacancies in the panel of Officers or members-at-large of the Executive Committee, the remaining members of the Committee are empowered to appoint substitutes to serve until the next Ordinary General Meeting of the Association at which the election of officers and members-at-large will be held.
(4) The meetings of the Executive Committee shall be called in accordance with the provisions of Article ~~VI~~ or on the written request of six or more of its members.	(4) The Meetings of the Executive Committee shall be called in accordance with the provisions of Article 14 or on the written request of six or more of its members.
(5) The Executive Committee is …	(5) The Executive Committee is …
(6) Responsibility for the finances of …	(6) Responsibility for the finances of …
(7) The Executive Committee shall appoint …	(7) The Executive Committee shall appoint …
(8) The Executive Committee shall approve …	(8) The Executive Committee shall approve …
(9) The Executive Committee is empowered to appoint, at each ~~ordinary meeting~~, an Auditor who shall not be an Officer or member-at-large of the Executive Committee and who need not be a Designated Member.	(9) The Executive Committee is empowered to appoint, at each Ordinary General Meeting of Members, an Auditor who shall not be an Officer or member-at-large of the Executive Committee and who need not be a Designated Member.
(10) The Executive Committee shall render to each ~~ordinary meeting~~ of the Association a full account of its proceedings and of the activities of the Association and shall present to said ~~meeting~~ an audited statement of accounts up to the end of the preceding calendar year.	(10) The Executive Committee shall render to each Ordinary General Meeting of Members of the Association a full account of its proceedings and of the activities of the Association and shall present to said General Meeting an audited statement of accounts up to the end of the preceding calendar year.

Current	Proposed
(11) The Executive Committee is empowered to call and summon an International Seed Testing Congress in conjunction with an ~~ordinary meeting~~ of the Association. All such Congresses shall be devoted to the reading of scientific papers, discussions and demonstrations on seed investigations, and such related subjects as appertain to the objects of the Association.	(11) The Executive Committee is empowered to call and summon an International Seed Testing Congress in conjunction with an Ordinary General Meeting of Members of the Association. All such Congresses shall be devoted to the reading of scientific papers, discussions and demonstrations on seed investigations, and such related subjects as appertain to the objects of the Association.
(12) The Executive Committee is empowered to employ …	(12) The Executive Committee is empowered to employ …
(13) The Executive Committee is empowered to approve …	(13) The Executive Committee is empowered to approve …
(14) The Executive Committee is empowered to delegate ...	(14) The Executive Committee is empowered to delegate …
(15) The Executive Committee is empowered to approve and publish …	(15) The Executive Committee is empowered to approve and publish …
(16) The Executive Committee shall prior to an ~~ordinary meeting~~ decide the place of the next ~~ordinary meeting~~ of the Association.	(16) The Executive Committee shall prior to an Ordinary General Meeting of Members decide the place of the next Ordinary General Meeting of Members of the Association.
(17) Six members of the Executive Committee shall constitute a quorum. Between meetings, business shall be transacted by correspondence in which at least 6 members must participate to effect a decision.	(17) Six members of the Executive Committee shall constitute a quorum. Between meetings, business shall be transacted by correspondence in which at least 6 members must participate to effect a decision.
~~ARTICLE VIII~~ ~~Nomination and Election~~	Article 16 Nomination and Election
(a) At the ~~ordinary meeting of the Association~~ which completes the tenure of office of the President and Vice-President, the outgoing Vice-President, provided that person was duly elected to that office at the ~~ordinary meeting~~ three years previous, without further election shall be appointed President for the ensuing period. If at this ~~ordinary meeting~~, for whatever reason, the outgoing Vice-President is not available for appointment as President, the office of the President shall be filled by election by the procedure prescribed for officers in paragraphs (b) and (c) of this Article.	(a) At the Ordinary General Meeting of Members which completes the tenure of office of the President and Vice-President, the outgoing Vice-President, provided that person was duly elected to that office at the Ordinary General Meeting of Members three years previous, without further election shall be appointed President for the ensuing period. If at this Ordinary General Meeting, for whatever reason, the outgoing Vice-President is not available for appointment as President, the office of the President shall be filled by election by the procedure prescribed for officers in the letters (b) and (c) of this Article.
(b) Subject to the provisions of paragraph (a) of this Article, the election of Officers and members-at-large of the Executive Committee shall be by ballot at an ~~ordinary meeting~~ of the Association.	(b) Subject to the provisions of the letter (a) of this Article, the election of Officers and members-at-large of the Executive Committee shall be by ballot at an Ordinary General Meeting of the Association.
(c) Subject to the provisions of paragraph (a) of this Article, nominations for the election of Officers and of members-at-large of the Executive Committee may be submitted only by Designated Members. Such nominations shall be in writing supported by a mover and a seconder (both being Designated Members) and must be received by the Secretary General at the latest on the day prior to the ~~ordinary meeting~~ at which the elections are to take place.	(c) Subject to the provisions of the letter (a) of this Article, nominations for the election of Officers and of members-at-large of the Executive Committee may be submitted only by Designated Members. Such nominations shall be in writing supported by a mover and a seconder (both being Designated Members) and must be received by the Secretary General at the latest on the day prior to the Ordinary General Meeting at which the elections are to take place.
	C. Auditors Article 17 Figures
	(a) The Association must submit its accounts to a full audit by external auditors if two of the following figures are exceeded in two successive business years: (1) Total assets of CHF 10 million. (2) Turnover of CHF 20 million. (3) Average annual total of 50 full-time staff.
	(b) The Association must submit its accounts to a limited audit by external auditors if a Member with personal liability or an obligation to provide further capital so requests.
	(c) The provisions of the Code of Obligations on external auditors for companies apply mutatis mutandis.
	(d) In all other cases the Articles of the Association and the General Meeting are free to make such auditing arrangements as they deem fit.
	(e) The Association must be registered if it is subject to an audit requirement or if it conducts a commercial operation in pursuit of its objects.
	V. Assets of the Association and Liability
~~ARTICLE XI~~ ~~Finances~~	Article 18 Assets of the Association
(a) Payment of monies belonging to the Association …	(a) Payment of monies belonging to the Association …
(b) The income of the Association shall be derived …	(b) The income of the Association shall be derived ...
(c) The financial year of the Association shall be from …	(c) The financial year of the Association shall be from …

Current	Proposed
(d) The amount of the annual subscription for Members and the additional fee for Accredited Laboratories shall be determined annually at an ~~ordinary meeting~~ of the Association, due consideration being given to statements submitted in accordance with Article ~~VII~~(c)(9) and ~~paragraph~~ (g) of this Article. Notification of proposals to change the rate of annual subscriptions shall be sent to the Designated Authorities and Members of the Association at least two months prior to an ~~ordinary meeting~~.	(d) The amount of the annual subscription for Members and the additional fee for Accredited Laboratories shall be determined annually at an Ordinary General Meeting of the Association, due consideration being given to statements submitted in accordance with Article 15 (c)(9) and letter (g) of this Article. Notification of proposals to change the rate of annual subscriptions shall be sent to the Designated Authorities and Members of the Association at least two months prior to an Ordinary General Meeting.
(e) (1) The representation by any Designated Member … (2) The membership of any Member, the subscription …	(e) (1) The representation by any Designated Member … (2) The membership of any Member, the subscription …
(f) Accounts of all monies received …	(f) Accounts of all monies received …
(g) A statement showing the financial position of …	(g) A statement showing the financial position of …
	Article 19 Liability
	The Association is liable for its obligations with its assets. This liability is limited to the assets. The Members are not personally liable.
~~ARTICLE XII~~ ~~Amendments~~	VI. Amendments and Dissolution Article 20 Amendments
The provisions of this Constitution may be amended as follows:	The provisions of this Constitution may be amended as follows:
(a) Any proposal to alter the provisions of this Constitution must be received in writing by the Secretary General at least three months prior to the date of the ~~meeting~~ of the Association at which it is to be considered.	(a) Any proposal to alter the provisions of this Constitution must be received in writing by the Secretary General at least three months prior to the date of the General Meeting of the Association at which it is to be considered.
(b) The Secretary General shall communicate any such proposal to each Member of the Association at least two months prior to the date of such ~~meeting~~ of the Association and shall maintain records showing evidence of such communications.	(b) The Secretary General shall communicate any such proposal to each Member of the Association at least two months prior to the date of such General Meeting of the Association and shall maintain records showing evidence of such communications.
(c) Amendments of this Constitution shall come into force only if they receive the support of at least two-thirds of the Designated Members voting at a ~~meeting~~ of the Association, provided a quorum is present.	(c) Amendments of this Constitution shall come into force only if they receive the support of at least two-thirds of the Designated Members voting at a General Meeting of the Association, provided a quorum is present.

Current	Proposed
~~ARTICLE XIV~~ ~~Dissolution of the Association~~	Article 21 Dissolution of the Association
Dissolution of the Association can take place when a ~~meeting~~ called for this purpose shall have voted ~~therefore~~ by a two-thirds majority of the Designated Members voting, provided a quorum is present. The funds remaining after dissolution of the Association shall be given to (an) institution(s) granted exemption from taxes with the same or similar objects. Remaining funds cannot be allocated to the Membership.	(a) Dissolution of the Association can take place when a General Meeting called for this purpose shall have voted for the dissolution of the Association by a two-thirds majority of the Designated Members voting, provided a quorum is present. The funds remaining after dissolution of the Association shall be given to (an) institution(s) granted exemption from taxes with the same or similar objects. Remaining funds cannot be allocated to the Membership.
	(b) The Association is dissolved by operation of law if it is insolvent or if the Executive Committee may no longer be appointed in accordance with the Articles of the Association.
	(c) Where the objects of the Association are unlawful or immoral, the competent authority or an interested party may apply for a court order of dissolution.
	VII. Final Provisions: Coming into Effect and Interpretation Article 22 Coming into Effect
	Once the Articles, or changes to the Articles, have been adopted by the voting members at a General Meeting and they are signed on behalf of the Association they come into effect. The Articles adopted as Constitution in previous meetings of the Association are therefore annulled once the Articles are signed.
~~ARTICLE XIII~~ ~~Interpretation~~	Article 23 Interpretation
In any case where the interpretation of this Constitution is in doubt, the English text thereof shall govern.	In any case where the interpretation of this Constitution is in doubt, the English text thereof shall govern.
	The Articles adopted as Constitution in the Zurich 2011 meeting are now annulled. These revised Articles come into immediate effect on: Date: Signed on behalf of the Association
	ISTA President
	ISTA Vice-President/Officer

30th ISTA Congress Seed Symposium
Antalya, Turkey, 12–14 June 2013

Alison A. Powell
ISTA Executive Committee member; Chair, ISTA Seed Vigour Committee; Seed Symposium Convenor

1st call for papers

The 30th ISTA Seed Symposium, to be held in Antalya, Turkey from 12 to 14 June 2013, will cover a wide range of seed related topics including:

- Genetic conservation
- Seed pathology
- Habitat regeneration
- Seed germination and dormancy
- Seed quality and plant breeding
- Application of molecular markers
- Seed quality evaluation
- Seed physiology and stress responses

This is the first invitation to people interested in presenting a paper during the Seed Symposium of the 30th ISTA Congress under the theme:

Evaluation of seed quality: a key step in exploiting the benefits of plant breeding and genetic conservation

The symposium will be made up of five oral sessions (see below), and two poster sessions, each of 2 hours, 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.

Intending participants are encouraged to present oral and poster papers dealing with a range of 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 tropical and temperate crop species, wild species, flowers, trees and shrubs, including species with potential for use in plant breeding and in habitat regeneration.

Submission of papers:

Offers of papers should be submitted **online only** (www.seedtest.org/ss2013) in the form of an abstract in English of 1600 characters (maximum). Papers will be presented orally and in poster form, both forms having equal status. 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.

Deadlines

19 October 2012: Deadline for submission of all papers; papers will be selected for presentation by the symposium convenor and a small scientific committee. Authors of papers considered for oral presentation will be contacted for further information on experimental results, additional to the abstract.

5 November 2012: Authors informed whether papers submitted for poster presentation have been accepted.

10 December 2012: Authors informed whether papers have been accepted for oral presentation. Authors of papers not accepted for oral presentation will be invited to present their work as a poster.

8 February 2013: 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.
Deadline for acceptance of an invitation to present an offered oral paper as a poster.

8 March 2013: 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

30th ISTA Congress, Antalya, Turkey, 12–18 June 2013

as early as possible. **ISTA cannot offer any financial support to authors of papers.** However, a letter of acceptance of a paper for oral presentation (subject to funding) can be provided to assist in funding applications from early December 2012; letters of acceptance of poster papers can be provided on request from early November 2012.

Session topics

Session 1: Role of quality evaluation in seed production

Chair and Lead Speaker: Francisco Kryzanowski, Brazil

Multiplication of new cultivars; maintenance of genetic purity; environmental and maternal effects on quality; production and processing; conventional and organic seed production; epidemiology and modelling; seed treatments.

Session 2: Seed storage for commercial use and genetic conservation

Chair and Lead Speaker: Robin Probert, UK

Seed collection and handling effects on germination and longevity; seed moisture content and water activity; storage conditions in relation to quality; orthodox and recalcitrant seeds, identification of quality traits in non-crop species

Session 3 (ISSS collaborative session): Physiological, biochemical and molecular markers of seed quality

Chair and Lead Speaker: Françoise Corbineau, France

Stress and desiccation tolerance, genomics, proteomics, development and maturation, regulation and induction of dormancy, germination; seed longevity; disease resistance

Session 4: Advanced methods in seed quality evaluation

Chair and Lead Speaker: Beni Kaufman, USA

Automatic and computer based methods; image analysis; DNA-based methods; variety identification; purity analysis; seed pathology; germination

Session 5: Evaluation and improvement of physiological quality

Chair and Lead Speaker: Alison Powell, UK

Evaluation of germination and vigour; seed quality in relation to field establishment, transplant production, land reclamation / regeneration; response to stress (e.g. pathogens, drought, salinity, soil contaminants).

Poster Sessions

Chair: Hulya Ilbi, Turkey

Posters will be presented on topics from all the above oral sessions. ■

Errata

We would like to correct the following errors in the article "Perspectives on horticultural, forestry and agricultural seed storage: analysis of the ISTA laboratories" by H. W. Pritchard *et al.* (Seed Testing International 142, October 2011).

Page 23, column 2, paragraph 1:

"Some members of the Committee also provided information, but this was not included in the analysis."

This should read:

"Some non-ISTA Laboratory members of the Committee also provided information, but this was not included in the analysis."

Page 25, column 1, paragraph 2:

"… about 2 billion seeds across all ISTA laboratories."

This should read:

"… about 20 billion seeds across all ISTA laboratories." ■

New Chief Editor for Seed Science and Technology

In October 2011, Fiona Hay took over from Alison Powell as Chief Editor of Seed Science and Technology.

Fiona's seed career began as a sandwich year Plant Science student working for the Seed Bank of the Royal Botanic Gardens Kew in the UK. After completing her PhD on the development of longevity in seeds, still based at the Royal Botanic Gardens Kew, she started working for the Millennium Seed Bank Project. For three years, she studied the germination and storage behaviour of seeds from aquatic species, touring wetland sites across the UK to make seed collections. Her focus then moved to more general problem solving, involving studies of seed germination and dormancy, and seed longevity.

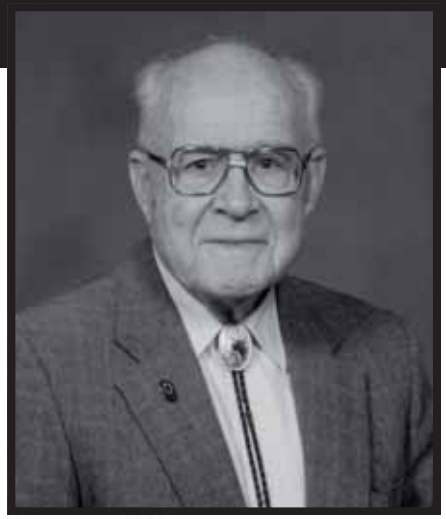
In late 2009, Fiona moved to the International Rice Research Institute in the Philippines, where she is working to ensure best-practice gene bank procedures in the rice gene bank at the T. T. Chang Genetic Resources Center.

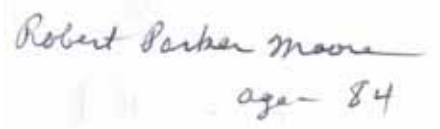
She has numerous peer-reviewed publications, including six in *Seed Science and Technology*, and has contributed to a variety of book chapters.

Fiona joined the Editorial Board of *Seed Science and Technology* in 2010 and is now looking forward to working with the other members of the Board and with ISTA, to make sure that *Seed Science and Technology* continues to publish a wide range of papers that are of interest to its subscribers and readers. ■

Centenary of Robert Parker "Bob" Moore (30 January 1912–11 October 2000)

A. M. Steiner[1], M. Kruse[2] and N. Leist[3]
[1]ISTA Alumnus; [2]ISTA Personal Member; [3]ISTA Past President and Honorary Life Member





University of Hohenheim and Karlsruhe Institute of Technology, Germany
am.steiner@.uni-hohenheim.de
mkruse@uni-hohenheim.de
norbert.leist@bio.uni-karlsruhe.de

On the occasion of his 100th birthday, we remember our dear ISTA friend of outstanding merit, Prof. Dr. Robert Parker Moore, known as Bob, born on 30 January 1912 near Blackburn, Oklahoma, and deceased on 11 October 2000 at the old age of 89 years in Raleigh, North Carolina.

He grew up on a 160-acre farm near Blackburn, run by his parents. Moore earned a B.S. at Oklahoma State University in Stillwater in 1934, a M.S. at Iowa State University in Ames in 1935 and a Ph.D. at Ohio State University in Columbus in 1940, all in agronomy. He gained promotion through several academic and administrative positions, eventually becoming Professor of Research in Crop Stands at North Carolina State University in Raleigh, a chair which he held from 1953 to 1977.

Prof. Moore's professional career and outstanding merits are described and highlighted in his laudation on the occasion of his receiving the high-ranking AOSA Award of Merit in 1985 (*Newsletter of the AOSA*, 59 (No. 3) 63–64, 1985).

Here, we would like to commemorate Prof. Moore's important and substantial ISTA activities, which are mentioned, understandably enough, only marginally in the AOSA laudation.

After attaining his professorship in 1953, Moore became increasingly interested in tetrazolium testing for determining seed viability. In 1962, he visited the Seed Testing Station Hohenheim, where Prof. Dr. Georg Lakon had established tetrazolium testing in the 1940s. There, he met Lakon's successor, Prof. Dr. Werner Lindenbein, and Lakon's close co-worker since 1942, Dr. Helene Bulat, who were doing pioneering work in tetrazolium testing. Lindenbein (1959–1965) and later Bulat (1965–1971) were both Chairs of the ISTA Tetrazolium Committee.

From then on, Moore's contacts with Hohenheim were never broken. His last handwritten letter to the first author of this commemorative paper dates from 21 December 1999, ten months before he passed away. In this letter, he wistfully brought to mind his first encouraging visit to Hohenheim, almost 40 years previously, and he added sadly that before long he would have to cease his seed-testing activities, because of increasing sight disorders. From retirement up to the age of 88 years, Moore had run a private advisory seed testing laboratory in the basement of his home, serving predominantly local farmers and gardeners.

In the middle of the 20th century, there was a successive triad of pioneers in tetrazolium testing. First, there was Lakon, who had invented tetrazolium viability testing. Then came Bulat, who devised and verified testing for numerous species by extensive comparative testing methods, and who established Chapter 6: Biochemical Test for Viability: The Topographical Tetrazolium Test of the International Rules for Seed Testing, which was approved at the ISTA Congress in Munich in 1966. Finally, there was Moore, who became the worldwide proponent of tetrazolium testing, by further improving testing techniques and extending tetrazolium testing to species not yet covered by the ISTA Rules. It was hence only natural that, after Bulat's retirement in 1971, Moore took over the Chair of the ISTA Tetrazolium Committee until 1977. He had been a committee member since 1962, and remained a member after retiring as Chair until 1986, altogether an impressing commitment over eight working periods. In addition, he was a member of the ISTA Vigour Committee from 1959 to 1974 in order to advance tetrazolium vigour testing.

During Moore's service as Chair, new species were introduced to Chapter 6, e. g. important species of cereals and forest tree seeds. After retiring as Chair in 1977, he began to prepare what was to become in 1985 the ISTA Handbook on Tetrazolium Testing, with 99 pages and 19 illustrations, and a then very valuable up-to-date reference list. An example of his diligence and commitment towards the preparation of the handbook is the fact that, in order to include forest tree seeds properly, he visited at his own expense his ISTA colleague Perry Overaa in Ås, Norway (who succeeded him as Chair of the Tetrazolium Committee from 1977 to 1986). Overaa had wide experienced in forest tree seed testing. For several weeks, under Overaa's guidance, Moore verified by hands-on experience the suggested prescriptions for his forthcoming Handbook.

As far as tetrazolium testing was concerned, there were never insuperable obstacles for Moore. His commitment to ISTA is shown not only by the example of his responsible and long-term committee work and by creating the ISTA Handbook on Tetrazolium Testing, but also by his participation at the ISTA Congresses in Lisbon 1962, Munich 1965, Warsaw 1974, Madrid 1977, Vienna 1980, Ottawa 1983, Brisbane 1986, Edinburgh 1989 and Buenos Aires 1992. In addition, as ISTA representative he attended many other national and international congresses and meetings promoting tetrazolium testing. Simultaneously, he published research-level papers, and, in particular, numerous articles at the technical level. His list of around 300 publications overall included many papers on aspects of seed quality and the diagnosis of seed disorders in general. In parallel, he contributed to many workshops and training courses. Moore thus became the principal champion of tetrazolium testing of his time.

However, it was not easy for him to pursue these activities. A great deal of his travelling to congresses and workshops took place after his retirement, on his own account. Moreover, since the 1960s, his wife Ruth had unfortunately been suffering from multiple sclerosis. Therefore, whenever he was away from home, he needed to find help for his wife and take over the concomitant expenses.

To everyone's surprise, Moore did not attend the ISTA Congress 1995 in Copenhagen. Previously, however, he had informed the first author of this paper by letter that during the period of the Congress he would be honeymooning. What had happened?

In 1990, Moore's wife Ruth Elizabeth nee Findley passed away. Shortly thereafter, the husband of a couple living next door in their street also died. Both couples had long been close friends. So it happened that, after a period of mourning and a good deal of thought, widower Robert Moore and widow Mable Nordstrom, both well advanced in years, married, and Mable moved to the Moore home. Subsequently, in a letter dated 20 February 1996, Moore wrote the wonderful poetical words: "Mable and I thoroughly enjoy sharing our remaining lives together." Very likely, Mable well knew that she had to share her new husband Bob with tetrazolium testing in his basement laboratory.

However, next to tetrazolium testing, there were other remarkable kinds of merit. A Raleigh, North Carolina, newspaper reported in Moore's obituary of October 2000: "Besides being a well published and respected scholar, teacher, and researcher in the field of crop science, Dr. Moore was well known for his loving service to all that he knew. In addition to fixing things for people, he provided everyone an abundance of vegetables, roses, and enthusiastic support".

Vegetables, roses and enthusiastic support – Prof. Dr. Robert Parker Moore belonged to a generation of ISTA exponents who not only used ISTA services or participated somehow or other in ISTA activities, but who considered ISTA to be an intrinsic part of their lives. Bob Moore lived for ISTA, and shared this attitude and the splendid experience of ISTA friendship with many like-minded colleagues of his generation. At that time, ISTA was a vividly flourishing interconnected community of go-getting scientists autonomously working on a voluntary and self-organizing basis. Bob was one of the well-known highly esteemed exponents of what was then called the ISTA Family.

Note: documents, including a detailed curriculum vitae, personal correspondence and photographs of Prof. Robert Parker Moore, have been presented to the ISTA archives for use to everybody of valid interest. ■

ISTA membership changes

Status 1 March 2012

New members

Brazil BRAM0005

Denise Cunha Dias
Universidade Federal de Viçosa
UFV-Depto. de Fitotecnia
Av. P.H. Rolfs, s/no
Viçosa - Minas Gerais, 36570-000
Phone: +55 31 8991118
Fax: +55 31 8992614
Mail: dcdias@mail.ufv.br

Brunei BNPM0001

Faizah Metali
University Brunei Darussalam
Biology, Faculty Of Science
Jalan Tungku Link
Bandar Seri Begawan, BE1410
Phone: +673 246 3001 ext. 1381
Fax: +673 2461502
Mail: faizah.metali@ubd.edu.bn

China CNPM0004

Rui He
Guangzhou University of Chinese Medicine,
Research Centre of Chinese Herbal Resource Science and Engineering
Higher Education Megacentre, Panyu
Guangzhou, 510006
Phone: +86 20 393 58331
Fax: +86 20 393 58066
Mail: rayhe618@hotmail.com

Denmark DKML0900/DKML0901

Laboratory representative: Steen Jensen
Eurofins Steins Laboratory A/S
Seed Testing
Pedersmindevej 1
8363 Hoerning
Phone: +45 2020 3092
Fax: +45 7660 4066
Mail: dstj@eurofins.dk

Egypt EGDM0005

Laboratory representative: Gamal El Azzab
Central Administration for Seed Certification (CASC), Giza Seed Testing Station
8 Gamaa Street Giza, P.O. Box 237
Rabee El Gezee-Giza, 12211
Phone: +202 35 72 4721
Fax: +202 35 72 4721
Mail: casc@casc-org.eg; gamal.alazab@gmail.com

Hungary HUPM0001

Katalin Ertsey
Corvinus University of Budapest
Faculty of Horticultural Science
Dept of Vegetable & Mushroom Growing
Villanyi ut 29-43, 1118 Budapest
P.O. Box 10518 Budapest pf 53
Phone: +36 1 482 6225
Fax: +36 1 482 6397
Mail: katalin.ertsey2@uni-corvinus.hu

Indonesia IDML0200/IDML0201

Laboratory representative: Junaidi
PT East West Seed Indonesia
Quality Assurance
Desa Benteng, P.O. Box 1
Campaka, Kabupaten Purwakarta
41181 West Java
Phone : +62 264 201 871
Fax : +62 264 201 875
Mail : junaidi@ewsi.co.id

India INML2700/INML2701

Laboratory representative: Jaysing C. Rajput
Nirmal Seeds Pvt Ltd.
Seed Testing Laboratory, Quality Control
Bhadgaon Road, Tal. Pachora, Dist- Jalgaon
Pachora, 424201
Phone : +91 259 6244396
Fax : +91 259 6244045
Mail : info@nirmalseedsindia.com; drjcrajput@nirmalseedsindia.com

INML28000/INML2801

Laboratory representative: Rita Pawade-Tonge
Yashoda Hybrid Seeds Pvt. Ltd
Yashoda Hybrid Seed Testing Laboratory
Hinganghat, Quality Control
248 Near Laxmi Talkies Dist. Wardha
Hinganghat, 442301
Phone : +91 94 2290 3931
Fax : +91 7153 200868
Mail : ritatonge@gmail.com

INML2900/INML2901

Laboratory representative : Haresh D. Ganar
Vibha Agrotech Ltd.
Seed Testing Laboratory "Inspire", Plot #21,
Sector 1, HUDA'S Techno Enclave, Madhapur
Andhra Pradesh
Hyderabad, 500 081 (AP)
Phone : + 91 40 3041 5852
Fax : + 91 40 3041 5761
Mail : hareshganar@vibhaseeds.com

Israel ILAM0004

Barry Nadel
Agecotec Ltd, Plant Breeding
Meshek 74
Kfar Pines, 37920
Phone : +972 4 637 6694
Fax : +972 4 627 3439
Mail : ns_agacotek@bezeqint.net

Netherlands NLML0800/NLML0801

Laboratory representative:
Frank Vanwijnsberghe
Monsanto Holland B.V.
Monsanto Vegetable Germination Laboratory
Leeuwehoekweg 52
2661 CZ Bergschenhoek
Phone: +31 105295311
Mail: frank.vanwijnsberghe@monsanto.com

United States USAM0019

Donald Ogawa
Crookham Company
P.O. Box 520
Caldwell, ID 83606
Phone: 208-459-7451
Fax: 208-454-2108
Mail: dogawa@crookham.com

USML0900/USML0901

Laboratory representative: Susan Alvarez
US Agriseeds, Seed Laboratory
3424 Roberto Court
San Luis Obispo, 93401
Phone: +1 805-547-9391
Fax: +1 805-547-9319
Mail: salvarez@usagriseeds.com

Membership cancellations

Denmark DKPM0002

Laboratory representative: Jan Torp
Danish Seed Health Centre
Thorvaldsensvej 40, 1
1871 Fredriksberg, C
Phone: +45 35 28 37 37
Fax: +45 35 28 37 01
Mail: seedpath@post7.tele.dk

DKAM0003

Marianne Pedersen
Levinsen & Abies Ltd.
Bollemosevej 9-11
3550 Slangerup
Phone: +45 48187527
Fax: +45 48187481
Mail: mail@treeseed.com

DKPM0005

Dot Vittrup Pedersen
DLF Trifolium A/S
Ny Østergade 9
P.O. Box 59
4000 Roskilde
Phone: +45 46 330 330
Fax: +45 46 320830
Mail: dot@dlf.dk

France FRPM0004

Christelle Forzy
Syngenta Seeds SAS
Rte de Francescas BP37
47600 Nerac
Phone: +33 553 976 200
Fax: +33 553 976 296
Mail: Christelle.forzy@syngenta.com

Moldova MDDM0001

Ion Paraschiv
State Center for Certification of
Seed Material
str. Mesterul Manole 4
1508 office
Chisinau, 2004
Phone: +373 22 47 60 35
Fax: +373 22 47 60 35
Mail: sccsm.rm@gmail.com

Netherlands NLML0700/NLML0701

Laboratory representative: Henk Burg
Monsanto Holland B.V.
Germination Laboratory Enkhuizen
Quality Assurance
P.O. Box 22
1600 AA Enkhuizen
Phone: +31 228 357794
Fax: +31 228 357713
Mail: Henk.Burg@seminis.com

Peru PEML0100/PEML0101

Laboratory representative: Ysabel Montoya
SENASA-Agricultural Health National Service
Ministry of Agriculture of Peru
Diagnostic Plant Health Unit Center
Avenue La Molina 1915
Lima, 12
Phone: +511 313 3300
Fax: +511 313 3303
Mail: ymontoya@senasa.gob.pe

Slovenia SIML0400/0401

Laboratory representative: Darja Vouk
Semenarna Ljubljana d.d.
Seed Testing Laboratory
Dolenjska C. 242
1000 Ljubljana
Phone: +386 1 4759 240
Fax: +386 1427 3538
Mail: darja.vouk@semenarna.si

S.C.T. of Taiwan, Penghu, Kinmen and Matsu TWDM0001

Han-Ching Hsu
Agriculture and Food Agency
Council of Agriculture, Executive Yuan
8 Kuan-Hwa Road, Chung-Hsing Village
Nantou, 540-44
Phone: +886 49 234 1000
Fax: +886 886 49 235 1706
Mail: cwd@mail.afa.gov.tw

Thailand THPM0001

Johan Van Asbrouck
Astec Global Rung Rueng Consulting (Rhino Research)
Moo baan sai samphan, 66/17 Moo 4
66000 Phichit
Phone: +1 307 674 8970
Fax: +1 307 673 1265
Mail: jvanasbrouck@astecnet.com

United States USPM0015

Laboratory representative: Brad Storrs
Monsanto
Seed Biology, Mail Zone U2B
800N. Lindbergh Blvd
St. Louis, 63167
Phone: +1 314 694 6470
Mail: s.bradley.storrs@monsanto.com

USAM0006

John Heaton
Seed Services Program
California Department of Food and Agriculture
1220 N Street
Sacramento, 95814
Phone: +1 916 654 0435
Fax: +1 916 654 0986
Mail: jheaton@cdfa.ca.gov

USAM0009

Cynthia Finneseth
Kentucky State Seed Laboratory
University of Kentucky
Division of Regulatory Services
103 Regulatory Services Bldg.
Lexington, KY, 40546-0275
Phone: +1 859 2572785
Fax: +1 859 2577351
Mail: chfinn0@email.uky.edu

USAM00100

Dale Krolikowski
Germain's Technology Group
Technical Services
8333 Swanston Lane
Gilroy, California, 95020
Phone: +1 408 848 8120
Fax: +1 408 848 2124
Mail: dkrolikowski@germains.com

New face at the ISTA Secretariat

Manuel Raithelhuber

Membership Administrator

Manuel Raithelhuber grew up in Germany in a bicultural environment, with a French mother and a German father. Owing to his interest in the world's different cultures, he enrolled in the International Business Management programme of the University of Applied Science of Northwestern Switzerland at Basle, the Université de Haute-Alsace, and the University of Cooperative Education, and had the opportunity to spend a term at the Hong Kong Baptist University. During his studies he specialized in the field of Customer Service, in which he conducted his Bachelor's Thesis about how to improve service quality in a call centre. His international working experience in Asia, Germany and the Netherlands in the field of Communication, Marketing and Customer Service prepared him for work at an international association such as ISTA.

Manuel Raithelhuber joined the ISTA Secretariat in November 2011. His main areas of responsibility are Membership Administration and supporting the ISTA Secretariat during the Annual Meetings.

PCR as a new identification method of *Xanthomonas campestris* pv. *campestris* on *Brassica* spp. seed

V. Grimault[1], C. Andro[2] & A. Politikou[3]

[1]Vice-Chair, ISTA Seed Health Committee

Erratum: the correct address for A. Politikou is:
[3]International Seed Federation
1260 Nyon, Switzerland

[1, 3]GEVES-SNES and [2]BioGEVES
49071 Beaucouzé CEDEX, France
valerie.grimault@geves.fr
celine.andro@geves.fr
liana.politikou@ufs-asso.com

Summary

The efficiency of the PCR technique for the identification of *Xanthomonas campestris* pv. *campestris* (Xcc) on *Brassica* spp. seed was compared to the pathogenicity test described in ISTA Seed Health Method 7-019 in a peer validation study organized by the International Seed Health Initiative for Vegetables. Four laboratories from the Netherlands and France together tested 1472 suspect bacteria isolates of *X. campestris* pathovars by conducting in parallel a PCR and a pathogenicity test. Xcc was identified using the DLH primer sets by Berg *et al.* (2005) and the Zup primer sets by Rijlaarsdam *et al.* (2004), and the results of the PCR and pathogenicity test were summarized and compared. There is a negligible risk of a false-positive PCR result for Xcc, caused by primer sets targeting *X. campestris* pv. *incanae*. It is highly unlikely that *X. campestris* pv. *incanae* isolates are present on cultivated *Brassica* spp. seeds. The study showed comparable results for the PCR and pathogenicity tests for 97.21% of the total of suspect isolates. Compared to the pathogenicity test, the PCR produced a false-negative result in only 0.41% of the suspect isolates tested. The PCR technique was shown to provide complementary information in cases where the pathogenicity test did not show clear symptoms, and to give additional information on the suspected occurrence of *X. campestris* pv. *armoraciae* or *X. campestris* pv. *raphani* (Xca/Xcr). Similarly, the pathogenicity test would be valuable when an indeterminate PCR result appears. The risk of a final false-negative result on a seed lot is minimized by testing at least six suspect isolates per seed subsample, as instructed by ISTA Method 7-019. The use of a PCR technique is highly recommended as an alternative or complementary method for Xcc identification in seed health testing laboratories.

Introduction

Xanthomonas campestris pv. *campestris* (Xcc) is a seed-borne, pathogenic bacterium on cruciferous plants and the causal agent of black rot disease that can cause severe economic losses worldwide (Qian *et al.*, 2005). Both ISTA Seed Health Method 7-019 for untreated seed, and the ISHI-Veg method (www.worldseed.org >> Trade Related Topics >> Phytosanitary Matters >> Seed Health >> ISHI-Veg) for disinfested seed comprise a pathogenicity test following dilution plating as a confirmation step of suspect Xcc colonies. Symptoms on inoculated bioassay plants are recorded 10 to 14 days after inoculation. Although this confirmation method is reliable, the duration of the test is of concern to laboratories which routinely test *Brassica* spp. seeds for the presence of Xcc. The PCR technique is faster than the pathogenicity test, and could be of use for these laboratories.

For several years, a PCR technique using pathogen-specific primers has been used to identify several pathogenic *Xanthomonas* species on various hosts (Pan *et al.*, 1999; Fargier and Manceau (2007); Palacio-Bielsa *et al.*, 2009). Comparative studies between the pathogenicity test and PCR for *X. hortorum* pv. *carotae* (Xhc) identification on carrot seed showed that PCR was a reliable and rapid confirmation tool (Asma *et al.*, 2002). PCR as an alternative to the pathogenicity test for confirming suspect Xhc colonies is part of ISTA Seed Health Method 7-020 (ISTA, 2006).

Berg *et al.* (2005) developed the primer set DLH120-125, which is specific to all *X. campestris* pathovars (*X. c.* pv. *campestris*, *X. c.* pv. *armoraciae*, *X. c.* pv. *raphani* and *X. c.* pv. *incanae*), while Rijlaarsdam *et al.* (2004) and Zaccardelli *et al.* (2007) developed primer sets for identification of Xcc. Fargier and Manceau (2007) validated the specificity of the Zup2309-2310 and Zup2311-2312 primer sets developed by Rijlaarsdam *et al.* (2004) and the primer sets of Berg *et al.* (2005) on a collection of 47 *X. campestris* isolates by comparing PCR results to pathogenicity tests. In that study, the Rijlaarsdam *et al.* (2004) primer sets were found to amplify the DNA of Xcc and *X. c.* pv. *incanae* isolates. However, *X. c.* pv. *incanae* isolates were pathogenic only on *Matthiola* spp. and *Erysimum cheiri* (previous name *Cheiranthus cheiri*) plants. Given Fargier and Marceau's results, as well as the fact that it is highly unlikely to have *X. c.* pv. *incanae* on cultivated *Brassica* spp. seeds, the combination of primer sets by Berg *et al.* (2005) and Rijlaarsdam *et al.* (2004) was considered appropriate in the present study for validation of the identification of Xcc on *Brassica* spp. seeds without posing any risk of a Xcc false-positive PCR result.

Vicente *et al.* (2006) did not confirm the existence of *X. c.* pv. *armoraciae* after testing one isolate that was received as such. Moreover, Fargier and Manceau (2007), after testing three isolates received as *X. c*. pv. *armoraciae*, didn't support the existence of another leaf spot disease caused by this pathogen. In further validation studies, Porcher *et al.* (2008) and Mathis *et al.* (2009) found a PCR result that was negative with Rijlaarsdam *et al.* (2004) primers and positive with Berg *et al.* (2005) primers. This PCR result, after taking into consideration that Xcc and *X. c.* pv. *raphani* (Xcr) are carried by *Brassica* spp. seed and are pathogenic on *Brassica* spp. plants, was interpreted as the suspected presence of Xcr . However, final conclusions on the presence of Xcr should be legitimate only after validation by epidemiological studies and identification of *Xanthomonas* sp. strains.

Despite the conclusions of Fargier and Manceau (2007) and Vicente *et al.* (2006) on *X. c.* pv. *armoraciae* (Xca) nomenclature, the names of both "*armoraciae*" and "*raphani*" pathovars are included in the list of Bull *et al.* (2010). Moreover, no change has been made to section 7.7 of ISTA Method 7-019 regarding the denomination of the causal agent (leaf spot *Xanthomonas*) of the leaf spot disease symptoms on the pathogenicity test. Thus, for consistency with ISTA Method 7-019, this study uses the names of both pathovars as Xca/Xcr wherever appropriate.

Scope and objective of the peer validation study

The scope of this peer validation study is to compare the efficiency of a PCR test with a pathogenicity test to identify suspect Xcc isolates among a large number of suspects. For this purpose, participating laboratories were called to provide data generated over the past years on the comparison of the two tests. This study was performed in addition to the work done on primer validation by Fargier and Manceau (2007). The objective of the study is to use PCR, if found to be efficient and comparable, as an alternative to the pathogenicity test described in ISTA Method 7-019 for the identification and confirmation of suspect Xcc colonies isolated from *Brassica* spp. seed.

Materials and methods

Bacterial isolates

A total of 1472 bacterial isolates of *Xanthomonas campestris* pathovars were identified by four laboratories: BioGEVES and SNES (Beaucouzé, France), Clause Tézier (Valence, France), Naktuinbouw (Roelofarendsveen, the Netherlands) and Rijk Zwaan (De Lier, the Netherlands). The isolates were from the collections or the participant laboratories or other company collections, or extracted from *Brassica* spp. seeds or plants. The numbers of isolates tested by each laboratory are provided in Grimault *et al.* (2012), Table 3. The laboratories used Xcc and Xca/Xcr reference isolates as positive controls. Mock inoculation with sterile water and/or inoculation with isolates of other *Xanthomonas* species were used as negative controls.

Pathogenicity test and PCR protocols

A detailed description of the pathogenicity test and PCR protocol used by each laboratory is provided in Table 1 of Grimault *et al.*, 2012. All laboratories used the pathogenicity test described in ISTA Method 7-019 or a slightly modified version thereof. Lab 1 and lab 2 used the same PCR protocol. Lab 3 used a PCR mix to analyse 1145 isolates, with primers by Rijlaarsdam *et al.* (2004) and UpBacF/UpBacR universal primers adapted from Eden *et al.* (1991). Ten isolates showed Xcc false-positive identification with PCR compared to the pathogenicity test result, and 5 isolates showed Xca/Xcr symptoms in the pathogenicity test and didn't react with Rijlaarsdam *et al.* (2004) primers. These were retested by lab 3 using Berg *et al.* (2005) primers in the aforementioned PCR mix with the same amplification program. Lab 4 used an adapted version of the PCR protocol utilized by lab 3. The sequences of all primer sets used are presented in Table 2 of Grimault *et al.*, 2011.

The 22 isolates that were initially tested by lab 4 were retested by lab 2 using a pathogenicity test and PCR to confirm the original results. Five isolates (Table 3 of Grimault *et al.*, 2012) that had been originally tested by lab 1 were retested by lab 4 by PCR in parallel to a pathogenicity test. Three of these isolates had originally shown ambiguous results, while the other 2 had corresponding results and served as a control to lab 4.

Results and discussion

The pathogenicity tests and PCR results of the isolates tested were validated by the results of reference isolates and PCR controls used at each laboratory. Reference isolates used as positive or negative controls showed corresponding results from pathogenicity tests and PCR in all four laboratories (data not shown). The results of the comparisons of the pathogenicity test and PCR for identifying Xcc and Xca/Xcr isolates are shown in Grimault *et al.* (2012), Table 3. Examples of PCR amplification products are shown in Figures 1 and 2.

The summarized results in Table 1 showed that corresponding results were obtained from the pathogenicity test and the PCR for 1431 isolates (97.21 %), of which 729 were identified as not Xcc, 694 as Xcc and 8 as Xca/Xcr. For the remaining 41 isolates (2.79 %), there was no correspondence between the pathogenicity test results and the PCR results. These conflicting results for the 41 isolates were broken down into categories A–E for better analysis and discussion. The percentages given refer to the percentages of the total number of isolates tested.

In the categories A1 and A2 (0.47%), the pathogenicity test failed to identify isolates as Xcc or Xc. However, the PCR showed a positive Xcc identification of the A2 isolates, but not of the A1 isolates. In both categories A1 and A2, no comparison between the pathogenicity test and PCR results can be made, and no final result can be given on the identification of these isolates thereafter.

An unexpected PCR result was shown for 13 bacterial isolates (0.88%) in category B1. These isolates showed a negative Xcc identification with the pathogenicity test, but with PCR reacted positively with the Zup primers (Zup+) and negatively with the DLH primers (DLH–). This PCR result is considered to be indeterminate.

For the four bacterial isolates (0.27%) in category B2, the same indeterminate PCR result was found, while the pathogenicity test showed Xca/Xcr symptoms.

It should be noted that in none of the isolates tested was there a positive Zup+ result in combination with a positive pathogenicity result for Xcc. Therefore, a positive PCR result using the Zup primers is not an indication of the presence of Xcc.

In categories C1 and C2, seven isolates (0.48%) showed a negative Xcc or Xca/Xcr identification in the pathogenicity test. However, the PCR indicated a suspected presence of Xca/Xcr in one of these isolates, and positive Xcc identification in the other six. It could not be determined whether the results of the pathogenicity test were negative because of loss of bacterial virulence, or whether the PCR result was a false positive. In either category, a seed lot would be considered to be contaminated based only on the PCR test results.

In categories D1 and D2, six isolates (0.41%) showed a negative Xcc identification with the PCR test. However, in the pathogenicity test, five of these were identified as being Xca/Xcr and one as Xcc. When compared to the pathogenicity test

results, the PCR results in both categories would be considered to be false negatives. In both categories D1 and D2, a seed lot would be considered to be contaminated based only on the pathogenicity test results.

In two isolates from category E1 (0.13%), the pathogenicity test showed Xca/Xcr symptoms, but the PCR showed a positive identification for Xcc. The contrary was shown for two other isolates from category E2 (0.13%), in which the pathogenicity test showed Xcc symptoms, but the PCR indicated suspected Xca/Xcr presence. In both categories E1 and E2 the seed lot would be considered to be contaminated.

The pathogenicity tests and PCR results for the five isolates (*a, b, c, d, e;* Grimault *et al.,* 2012, Table 4) that were retested by lab 4 agreed with the original results of lab 1. Lab 2 confirmed both the pathogenicity test and PCR results originally found by lab 4 on all 22 isolates provided by this laboratory.

Conclusions and recommendations

In this study, the efficiency of the PCR test was compared to the pathogenicity test of ISTA Method 7-019 for the identification of Xcc isolates. For a high percentage of isolates tested (97.21%), the results of the PCR test corresponded to those of the pathogenicity test. This shows that the PCR technique can be used as an alternative to the pathogenicity test for the identification and confirmation of Xcc colonies isolated from *Brassica* spp. seed.

In cases where the pathogenicity test gave ambiguous results, the PCR test provided complementary information on the identification of isolates. Therefore, the PCR test could be considered as a complementary method to the pathogenicity test. Moreover, the Zup–/DLH+ result would provide additional information on the suspected presence of Xca/Xcr. However, in the case of the indeterminate Zup+/DLH– result, the pathogenicity test would be recommended, since bacterial isolates would remain suspect.

The PCR test gave false negative results compared to the pathogenicity test in 0.41% of all cases. Thus, using the PCR technique, in 99.6% of cases a laboratory will obtain an accurate result, or will get an indication to perform an additional pathogenicity test to confirm the PCR result.

When following the instructions of ISTA Method 7-019 to test at least six suspect isolates per subsample, the risk of a final false negative result in a seed lot is minimized.

Given that all 27 isolates showed the same PCR results when tested with the protocols of labs 2 and 4 (the latter being a slight variant of the lab 3 protocol), these protocols can be considered to be equivalent. Consequently, this report supports two PCR options, each comprising different primers and amplification regimes for identifying or confirming suspect Xcc isolates.

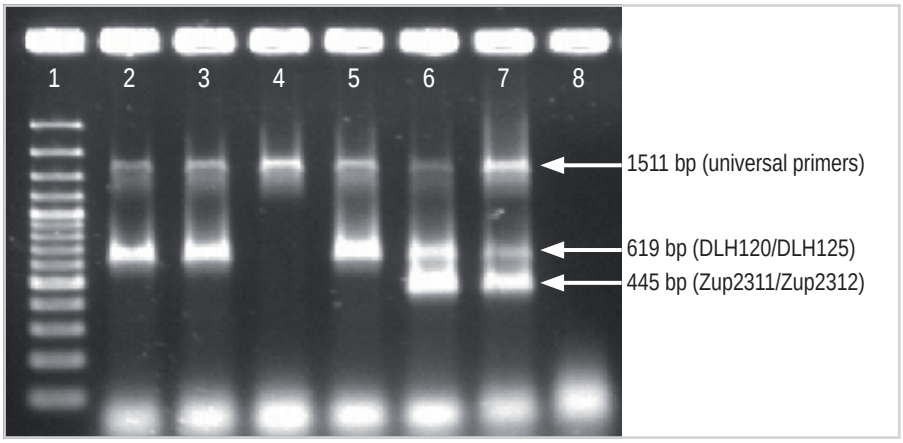


Figure 1. Examples of PCR amplification using primer sets by Berg *et al.* (2005) and Rijlaarsdam *et al.* (2004), and adapted from Eden *et al.* (1991). 1 100 bp ladder; 2, 3, 5 Two bands (619 bp, 1511 bp): positive sample with *Xanthomonas campestris* (Xca/Xcr suspected presence); 4 One band (1511 bp): negative sample, no *Xanthomonas campestris* (Xc); 6, 7 Three bands (445 bp, 619 bp, 1511 bp): positive sample with *Xanthomonas campestris* pv. *campestris* (Xcc)(with or without Xca/Xcr); 8 Water (negative PCR control): no reaction.

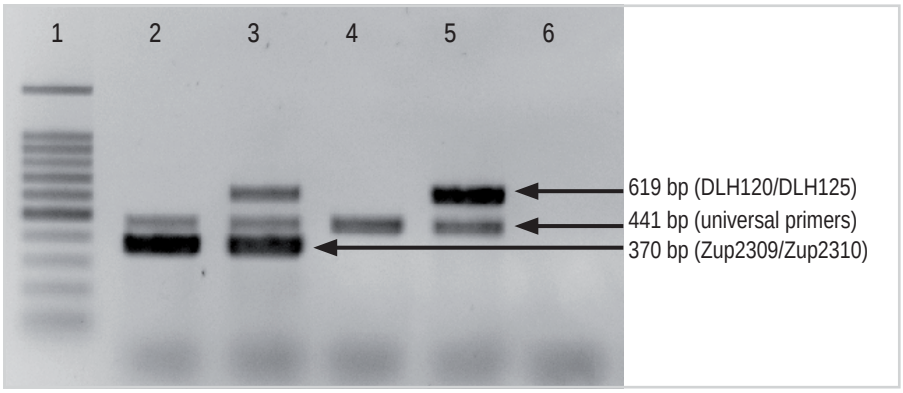


Figure 2. Examples of PCR amplification using primer sets by Berg *et al.* (2005) and Rijlaarsdam *et al.* (2004), and adapted from Eden *et al.* (1991). 1 100 bp ladder; 2 Two bands (370 bp, 441 bp): indeterminate PCR result; 3 Three bands (441 bp, 619 bp, 370 bp): positive sample with *Xanthomonas campestris* pv. *campestris* (Xcc)(with or without Xca/Xcr); 4 One band (441 bp): negative sample, no *Xanthomonas campestris* (Xc). 5 Two bands (441 bp, 619 bp): positive sample with *Xanthomonas campestris* (Xca/Xcr suspected presence); 6 Water (negative PCR control): no reaction.

Acknowledgements

The participating laboratories Bio-GEVES and SNES (Beaucouzé, France), Clause Tézier (Valence, France), Naktuinbouw (Roelofarendsveen, the Netherlands) and Rijk Zwaan (De Lier, the Netherlands) are gratefully acknowledged for providing bacterial isolates and carrying out this peer-validation study.

Table 1. Summary of the pathogenicity test and PCR results

	Number of tested isolates	Pathogenicity test result[1]	PCR result[2]	Discussion	No. of tested isolates per lab	Isolates retested by lab 4
Isolates with correspondence in results	729	–	–			(a, b)
	694	+	+			
	8	Xca/Xcr	Xca/Xcr			
Total	1431					
Isolates with differing results	3	?	–	A1	3 (lab 1)	
	4	?	+	A2	4 (lab 2)	
	13	–	Zup+	B1	3 (lab 1), 10 (lab 3)	(c, d)
	4	Xca/Xcr	Zup+	B2	4 (lab 2)	
	1	–	Xca/Xcr	C1	1 (lab 2)	
	6	–	+	C2	3 (lab 1), 3 (lab 2)	(e)
	5	Xca/Xcr	–	D1	5 (lab 2)	
	1	+	–	D2	1 (lab2)	
	2	Xca/Xcr	+	E1	1 (lab 2), 1 (lab 4)	
	2	+	Xca/Xcr	E2	2 (lab1)	
Total	41					

[1] Symbols used for pathogenicity test results:

+	Xcc symptoms
Xca/Xcr	Xca/Xcr symptoms
–	No Xcc or Xca/Xcr symptoms
?	Ambiguous result

[2] Symbols used for PCR results:

+	Zup and DLH primers positive (Xcc positive identification)
–	Zup and DLH primers negative (Xcc negative identification)
Xca/Xcr	Zup primers negative and DLH primers positive (*X. campestris* positive identification, Xca/Xcr suspected presence)
Zup+	Zup primers positive and DLH primers negative (Indeterminate result)

References

Asma, M., de Vogel, R., Woudt, B. & Krause, D. (2002). *Evaluation of pathogenicity testing, rep-fingerprinting and PCR for the identification of Xanthomonas campestris pv.* carotae. ISHI Report Bejo Zaden BV, Research Report P9317-16.

Berg T., Tesoriero L. & Hailstones D. L. (2005). PCR-based detection of *Xanthomonas campestris* pathovars in *Brassica* seed. *Plant Pathology,* **54,** 416–427.

Bull, C. T., De Boer, S. H., Denny, T. P., Firrao, G., Fischer-Le Saux, M., Saddler, G. S., Scortichini, M., Stead, D. E. & Takikawa, Y. (2010). Comprehensive List Of Names Of Plant Pathogenic Bacteria, 1980-2007. *Journal of Plant Pathology,* **92,** 551–592.

Eden, P. A., Schmidt, T. M., Blackemore, R. P. & Pace, N. R. (1991), Phylogenetic Analysis of *Aquaspirillum magnetotacticum* using Polymerase Chain Reaction Amplified 16S rRNA specific DNA. *International Journal of Systematic Bacteriology,* **41,** 324–325.

Fargier, E. & Manceau, C. (2007), Pathogenicity assays restrict the species *Xanthomonas campestris* into three pathovars and reveal nine races within *X. campestris* pv. *campestris*. *Plant Pathology,* **56,** 805–818.

Grimault, V., Andro, C. and Politikou, A. (2012). *Report on validation of PCR as a new identification method of* Xanthomonas campestris *pv*. campestris *on* Brassica *spp. seed.* ISTA Method Validation Reports 2012, 1–17. International Seed Testing Association, Bassersdorf, Switzerland.

ISTA (2007). Detection of *Xanthomonas campestris* pv. *campestris* on *Brassica* spp. *International Rules for Seed Testing*. Annexe to Chapter 7: Seed Health Testing Methods 7-019, 16 pp.

ISTA (2006) Detection of *Xanthomonas hortorum* pv. *carotae* on *Daucus carota*. *International Rules for Seed Testing. Annexe to Chapter 7: Seed health Testing Methods 7-020*, 19 pp.

Mathis, R., Porcher, L., Fargier, E., Briand, B., Guillaumès, J., Andro, C., Grimault, V., Valette, N., Darrieutort, G. & Manceau, C. (2009). *A new method to detect Xanthomonas campestris in cruciferous seeds by enrichment – PCR*. The EPPO Conference on Diagnostics, York (UK).

Palacio-Bielsa, A., Cambra, M. A. & Lopez, M. M. (2009). PCR detection and identification of plant-pathogenic bacteria: Updated review of protocols (1989-2007). *Journal of Plant Pathology,* **91** (2), 249–297.

Pan, Y.-B., Grisham, M. P., Burner, D. M., Legendre, B. L. & Wei, Q. (1999). Development of polymerase chain reaction primers highly specific for *Xanthomonas albilineans*, the causal bacterium of sugarcane leaf scald disease. *Plant Disease,* **83,** 218–222.

Porcher, L., Mathis, R., Fargier, E., Briand, B., Guillaumès, J., Grimault, V., Valette, N., Guyot, L., Darrieutort, G. & Manceau, C. (2008). Development of a new detection method of living *Xanthomans campestris* in cruciferous seed lots by Bio-PCR. *6th ISTA Seed Health Symposium,* 14–18 April 2008, Kruger National Park (South Africa).

Qian, W., Jia, Y., Ren, S.-X., He, Y.-Q., Feng, J.-X., Lu, L.-F., Sun, Q., Ying, G., Tang, D.-J., Tang, H., Wu, W., Hao, P., Wang, L., Jiang, B.-L., Zeng, S., Gu, W.-Y., Lu, G., Rong, L., Tian, Y., Yao, Z., Fu, G., Chen, B., Fang, R., Qiang, B., Chen, Z., Zhao, G.-P., Tang, J.-L. & He, C. (2005). Comparative and functional genomic analyses of the pathogenicity of phytopathogen *Xanthomonas campestris* pv. *campestris*. *Genome Research,* **15,** 757–767. ISSN 1088-9051/05; www.genome.org.

Rijlaarsdam, A., Woudt, B., Simons, G., Koenraadt, H., Oosterhof, J., Asma, M., Buddiger, P., Roorda, P., Grimault, V. & De Koning, J. (2004). Development of specific primer for the molecular detection of *Xanthomonas campestris* pv. *campestris*. *EPPO Conference on Quality of Diagnosis and New Diagnostic Methods for Plant Pests.* Noordwijkerhout, NL, 2004-04-09/22.

Vicente, J. C., Everett, B. & Roberts S. J. (2006). Identification of Isolates that Cause a Leaf Spot Disease of *Brassica*s as *Xanthomonas campestris* pv. *raphani* and Pathogenic and Genetic comparison with Related Pathovars. *Phytopathology*, **96** (7), 735–745.

Zacardelli, M., Campanile, F., Spasiano, A. & Merighi, M. (2007). Detection and identification of the crucifer pathogen, *Xanthomonas campestris* pv. *campestris*, by PCR amplification of the conserved Hrp/type III secretion system gene *hrc*C. *European Journal of Plant Pathology*, **118,** 299–306.

Detection of tobamoviruses on *Lycopersicon esculentum* seed by local lesion assay on *Nicotiana tabacum* plants

V. Grimault[1], H. M. S. Koenraadt[2] and A. Politikou[3]

[1]Vice-Chair, ISTA Seed Health Committee; Member[2], ISTA Seed Health Committee

Erratum: the correct address for A. Politikou is:
[3]International Seed Federation
1260 Nyon, Switzerland

[1, 3]GEVES-SNES
49071 Beaucouzé CEDEX, France
valerie.grimault@geves.fr
liana.politikou@ufs-asso.com
[2]Naktuinbouw
2370 AA Roelofarendsveen, the Netherlands
h.koenraadt@naktuinbouw.nl

Summary

Local lesion assay (indexing) with *Nicotiana tabacum* 'Xanthi NN' resistant plants was evaluated as a detection method for infectious tobamoviruses on tomato (*Lycopersicon esculentum*) seed in a comparative test between nine laboratories, organized by the International Seed Health Initiative Vegetable group (ISHI-Veg). Two naturally infected seed lots with medium and high infection levels, one virus-free seed lot and two infection levels of reference material (RM) were used. Subsamples of the seed lots and RM samples were distributed to laboratories who inoculated their extracts onto the leaf surface of two assay plants. For each combination of (sub)sample × plant × leaf, the number of necrotic local lesions was recorded 5–7 days post-inoculation under specific incubation conditions. No false positives were recorded, and most laboratories were able to detect the expected number of positive seed subsamples and RM samples, and to distinguish between the various infection levels. The growth stage of the tobacco assay plants and the incubation temperature were found to be critical factors for the detection of positive seed and RM samples. The local lesion assay was found to be repeatable and reproducible for both seed subsamples and RM samples. The use of a known infected seed sample or a reference material sample is indispensable for the validation of the results. The local lesion assay with *Nicotiana* plants resistant to tobamoviruses is considered a reliable detection method of infectious tobamoviruses on tomato seed and is highly recommended in routine tomato seed testing.

Introduction

Tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) belong to the genus *Tobamovirus* (Lewandowski and Dawson 1998). TMV and ToMV are seed-borne and seed-transmitted viruses (Hadas et al., 2004), commonly found on tomato (*Lycopersicon esculentum*) seed, localized on the seed coat and sometimes in the endosperm (Huttinga and Rast, 1995). They are able to survive for long periods outside the plant tissue and can be easily transmitted mechanically on tomato plants, causing significant economic yield losses (Averre and Gooding, 2000; Demski, 1981; Huttinga and Rast, 1995). A seed health program certifying virus-free seed is considered an important tool for the control of infection of tomato plants (Hadas et al., 2004).

Several serological methods based on ELISA tests have been broadly used for the detection and identification of various plant viruses (Clark and Adams, 1977; Maury *et al.*, 1987). It is known, however, that ELISA tests detect both infectious and non-infectious virus particles; therefore, they do not allow for the evaluation of virus seed transmission and yield false positive results (Maury *et al.*, 1987; Nolan and Campbell, 1984).

TMV and other tobamoviruses have played a significant role in virology research in studies on plant-virus interactions (Dawson, 1999). The infection mode, pathway, replication and expression of tobamoviruses have been extensively studied on the TMV-tobacco (*Nicotiana* spp.) plant model (Rhee *et al.*, 2000; Diaz-Griffero *et al.*, 2006; Bawden, 1964; Padmanabhan *et al.*, 2008; Demski, 1981). Many references are available in the literature about the induction of hypersensitive-response (HR) reaction in tobacco plants from tobamoviruses, a plant defence mechanism against the attack of viruses (Ehrenfeld *et al.*, 2008; Takahashi, 1956; Erickson *et al.*, 1999b; Whitman *et al.*, 1994; Taliansky *et al.*, 1994). The HR reaction is the outcome of the gene-for-gene resistance (Flor, 1971; Kiraly *et al.*, 2007), mediated in tobacco plants carrying the dominant *N* resistant gene (Holmes 1938; Hammond-Kosack and Jones 1996; Erickson *et al.*, 1999a; Whitman et al., 1994; Boovaraghan *et al.*, 2007). The HR is characterized by confinement of the virus at the initial infection site through cell death and the development of local necrotic lesions (Holmes 1938; Takahashi, 1956; Dawson, 1999).

Holmes (1929), by developing the local lesion assay, was able to quantify the virus infectivity. Based on this local lesion assay, Hadas (1999) and Hadas *et al.* (2004) proposed an ELISA prescreening and an indexing of tomato seed on resistant tobacco plants (*Nicotiana tabacum* 'Xanthi NN') as a detection method, and an infectivity evaluation of ToMV on commercial tomato seed lots. The latter study highlighted the importance of standardizing factors that influence performance and results of the indexing assay, such as temperature, light and the physiological condition of the plants.

The HR mediated by the N gene in resistant *Nicotiana* plants has been shown to be temperature sensitive (Whitman *et al.*, 1994; Ordog *et al.*, 2002; Dijkstra *et al.*, 1977). TMV infection induces HR local lesions at temperatures below 28 °C, while no necrotization occurs above this temperature; instead, the virus multiplies systemically in the plant (Samuel, 1931; Kiraly *et al.*, 2008; Takahashi, 1975; Weststeijn, 1981; Padgett *et al.*, 1997; Dawson, 1999). Day length and light intensity have been shown to affect virus replication as well as expression of the disease (Matthews, 1991). The physiological age and developmental stage of a host-assay plant have been shown to influence the virus infections and subsequently the number of local lesions

on inoculated leaves (Takahashi, 1972). Old plants have been generally reported to be less susceptible to viral infections than younger ones (Padmanabhan *et al*., 2008).

Based on the above described literature, the seed industry and seed health testing laboratories have developed a local lesion assay for the detection of tobamoviruses on tomato seed. The method requires mechanical inoculation of plants resistant to tobamoviruses, such as *N. tabacum* 'Xanthi NN' (Stange *et al*., 2004; Diaz-Griffero *et al*., 2006), with tomato seed extract, resulting in local HR lesions on the inoculated leaf surface. Nine laboratories from the Netherlands, Israel, the USA and France participated in this comparative test, organised by the ISHI-Veg Tomato and Pepper International Technical Group. It has previously been reported that one infected seed in a subsample of 500 healthy seeds can be detected with this method (Hadas *et al*., 2004). However, in this ISHI-Veg comparative test, a subsample size of 250 seeds was used for validation purposes, which increased test sensitivity.

Aim and objective

The aim of this ISHI-Veg comparative test was to evaluate the local lesion assay as a detection method for infectious tobamoviruses on naturally infected tomato seed. The ultimate objective is to obtain an internationally accepted seed health testing method for the detection of tobamoviruses on tomato seed.

Materials and methods

Seed lots and seed subsamples

Three tomato seed lots (E27315, E08543 and E12505) with a range of levels of natural infection with tobamovirus were selected by GEVES-SNES, following the described detection method. Lot E27315 was disinfected with calcium hypochlorite, lot E08543 was untreated, and lot E12505 was treated with TSP. The infection levels of the lots were evaluated by testing 12 subsamples of 250 seeds, a total of 3000 seeds per lot. The number of positive seed subsamples out of the 12 tested from the selected lots E27315, E08543 and E12505 was zero, 12 and 12 respectively. However, the average number of local lesions (2 repetitions per seed subsample) of each of the two infected lots was variable. As this outcome demonstrated the possibility of a seed lot showing all subsamples positive, though having a low, medium or high virus incidence, it served as a criterion for the final characterization of the lots. Lots E08543 and E12505 showed an average number of 10 and 123 lesions respectively, and were characterized as Medium and Highly infected. Lot E27315 showed zero lesions and was characterized as Healthy. For each laboratory a total of 20 subsamples were prepared: ten subsamples of 250 seeds from the Medium infected lot, and five subsamples of 250 seeds from each of the Highly infected and Healthy lots. Subsamples were prepared based on the thousand-seed weight of each lot. The subsamples were coded randomly in order to ensure a blind comparative test and were distributed to participating laboratories along with *Nicotiana tabacum* 'Xanthi NN' seeds. The *N. tabacum* 'Xanthi NN' seeds came from the same production line, aiming to keep potential variations in the comparative test as low as possible.

Positive and negative controls

Although a known tobamovirus-infected seed subsample can be used as a positive control in a local lesion assay, the preparation of identical seed subsamples for all laboratories of the comparative test was assessed as unrealistic, due to the potential for uneven virus distribution in the seed subsamples. Therefore, a reference material (RM), prepared and provided by NAKT, which would provide higher consistency in preparation of identical samples was used as a positive control. This RM consisted of *Nicotiana occidentalis* leaves infected with freeze-dried pepper mild mottle virus (PMMoV) and diluted at two different levels with dry pea flour. The RM that was 50× diluted and produced an average of 40 local lesions was characterized as Highly infected, while the RM that was 500× diluted and produced an average of 10 local lesions was characterized as Medium infected. The participating laboratories received 5 randomly coded samples of 0.5 g of each of the two RM levels. For the negative control, phosphate buffer saline (PBS) was prepared by dissolving 8.0 g NaCl, 1.15 g Na_2HPO_4 and 0.2 g KH_2PO_4 in 1 L of distilled water. The pH was adjusted to 7.2–7.4 and the solution was autoclaved at 121 °C at 15 p.s.i. for 15 min.

Local lesion assay

Production of *N. tabacum* 'Xanthi NN' plants

Plants of *N. tabacum* 'Xanthi NN' were grown from the supplied seeds by individual laboratories at 20–28 °C under sufficient light intensity for 6–8 weeks until they reached the 4–5 true leaves growth stage. Two tobacco plants per tomato seed subsample, two per RM sample and two plants for the negative control were selected, resulting in 62 plants in total. Plants were watered the day before the mechanical inoculation to ensure leaves with high turgor. Two consecutive leaves of each pair of plants were labelled with the corresponding code of the sample for inoculation.

Virus extraction from seed and reference material

In each laboratory the 20 tomato seed subsamples of 250 seeds were ground in 10 mL PBS seed extraction buffer with a grinder. The 10 samples of 0.5 g of each RM were similarly ground in 5 mL PBS seed extraction buffer.

Mechanical inoculation of plants

The two labelled leaves of each pair of plants were dusted with a fine layer of carborundum powder, and 100 µL of the corresponding ground extract was placed on each leaf's surface. The 100 µL of extract was smeared over the entire leaf surface by applying light pressure with fingers. Plastic finger tips or gloves were used and were changed between (sub) samples. Special care was taken to avoid leaf damage that might be caused by too much pressure. Two consecutive leaves of one pair of plants were inoculated in a similar manner with 100 µL of PBS and served as negative control plants. All inoculated leaves were rinsed with tap water a few minutes after inoculation and plants were incubated at 20–25 °C in an alternating 12 h light–12 h dark regime for 5–7 days. Plants were examined for the development of typical local necrotic lesions by comparison to

positive and negative control plants, and the number of local lesions which developed on each leaf per plant and per (sub) sample was counted.

Data analysis

Following characterization of the seed lots based on the average of developed lesions, another local lesion assay was conducted for each infected lot with a smaller number of subsamples per lot and seeds per subsample. The aim of this assay was to calculate the infection rate of each lot with the spreadsheet application Seedcalc version 8 (http://www.seedtest.org/en/statistical-tools-for-seed-testing-_content---1--1143--279.html), and to calculate the probability of expected positive subsamples in the comparative test at a confidence level above 99% with the spreadsheet application developed by J. L. Laffont. Two positive subsamples of 32 seeds (1/8 of 250) out of 6 subsamples tested revealed an infection rate of 1.26 % and 8–10 expected positive subsamples out of the 10 total distributed to laboratories for a confidence level above 99% for the Medium level lot. Likewise, 3 positive subsamples of 62 seeds (¼ of 250) out of 6 subsamples revealed an infection rate of 1.11 % and 4–5 expected positive subsamples out of the 5 total distributed to laboratories for a confidence level above 99% for the High level lot.

For each combination of laboratory × infection level × (sub) sample × plant × leaf, the number of developed local lesions was recorded. A seed subsample or a RM sample was considered positive when at least one local lesion developed in at least one leaf. Values of the Medium level seed subsamples of laboratory 1 were excluded from the analysis, as they were all recorded as zero. Values of the High level seed subsamples of laboratory 9 were also excluded, as the values of 4 of the 5 subsamples were zero.

The average number of local lesions was calculated for each combination of laboratory × infection level × (sub) sample. The number of local lesions of positive seed subsamples from both Medium and Highly infected level seed lots was analysed by Analysis of Variance (ANOVA) implemented in Statgraphics Plus 5.0 statistical program. Likewise, an ANOVA on the number of local lesions from positive RM samples – and excluding the missing values – from both Medium and High infected levels was performed. Prior to the analysis, the original values of seed subsamples and RM samples were transformed with the natural logarithm plus one and with the natural logarithm respectively, which allowed for their normal distribution based on Levene's test. The number of lesions from positive seed subsamples and positive RM samples were also analysed by ANOVA for each individual laboratory. Prior to the analysis, the original values of seed subsamples and RM samples were transformed with the natural logarithm which allowed for their normal distribution based on Levene's test.

The repeatability (within-laboratory variability) and reproducibility (between-laboratory variability) was evaluated using the original values of the above mentioned set of data for seed subsamples and RM samples of all laboratories with the use of ISO 5725 (http://www.seedtest.org/en/statistical-tools-for-seed-testing-_content---1--1143--279.html).

Results

Seed subsamples

No local lesions were recorded on plants inoculated with seed extract from the Healthy lot subsamples or on plants inoculated with the negative control (PBS seed extraction buffer).

The numbers of the detected and expected positive seed subsamples of the total tested per infection level lot and per laboratory is presented in Table 1. All laboratories except 1 and 4 detected the expected number of positive seed subsamples from the Medium infected seed lot. With regard to the Highly infected seed lot, all laboratories, except laboratory 9, detected the expected number of positive seed subsamples (Table 1).

The ANOVA for the average number of local lesions from both the Medium and High level lots showed a significant difference between laboratories (P = 0.0000) and infection levels (seed lots)(P = 0.0000). No test on laboratory × infection level interaction was reported by the Statgraphics program, as not all combinations between the values of these two factors were possible (e.g. Lab 1 Medium level seed subsamples were excluded). The average number of the natural logarithm plus one of local lesions from positive seed subsamples of both Medium and High level lots for all nine laboratories is presented in Grimault *et al.* (2012), Figure 1. The highest number of local lesions was recorded by laboratories 5 and 9 and the lowest by laboratory 4.

The ANOVA for the average number of local lesions per infection level for each laboratory showed an infection level effect in each analysed laboratory: Lab 2 (P = 0.000), Lab 3 (P = 0.015), Lab 4 (P = 0.006), Lab 5 (P = 0.008), Lab 6 (P = 0.002), Lab 7 (P = 0.026) and Lab 8 (P = 0.000). The average number of the natural logarithm plus one of local lesions from positive seed subsamples for each of the Medium and High level lots per laboratory is depicted in Grimault *et al.* (2012), Figure 2. All laboratories detected a higher number of local lesions in the High than in the Medium level lot.

The standard deviation of repeatability and reproducibility based on the mean of local lesions from positive seed subsamples of Medium and High infected lots are shown in Grimault *et al.* (2012), Table 2. No (h) critical values were reported while there were (k) critical values at the 5% confidence level for Lab 5 for both Medium and High levels, for Lab 7 for High level and for Lab 9 for Medium level (Grimault *et al.*, 2012, Figs. 5, 6).

Reference material samples

The numbers of detected positive subsamples and the expected positive reference material (RM) samples out of the total tested per infection level and per laboratory is indicated in Grimault *et al.* (2012), Table 1. In both Medium and High infection levels, all laboratories detected five positive samples out of five tested except laboratory 1 where one negative RM sample was recorded. Laboratory 6 inoculated only two RM samples due to the lack of sufficient tobacco assay plants. However, both RM samples were recorded positive.

An ANOVA on the average number of lesions for Medium and High infected RM showed a significant difference between

Table 1. Detected and expected (99% confidence) positive seed subsamples and reference material (RM) samples per laboratory, with incubation temperatures and growth stages of assay plants

Labs	Detected subsamples/total	Expected	Detected subsamples/total	Expected	Detected RM samples/total	Detected RM samples/total	Expected	Temperature (°C)	Growth stage (No. true leaves)
	Medium infection	Medium infection	High infection	High infection	Medium RM	High RM	Medium/High RM		
1	0/10	8–10/10	4/5	4–5/5	4/5	5/5	5/5	24**	3–4
2	10/10	8–10/10	4/5	4–5/5	5/5	5/5	5/5	20	4–5
3	9/10	8–10/10	5/5	4–5/5	5/5	5/5	5/5	25 ± 2	4–5
4	4/10	8–10/10	4/5	4–5/5	5/5	5/5	5/5	26 ± 3	5–6
5	10/10	8–10/10	4/5	4–5/5	5/5	5/5	5/5	25**	4–5
6	10/10	8–10/10	5/5	4–5/5	2/2*	2/2*	5/5	24 ± 2	7–8
7	10/10	8–10/10	5/5	4–5/5	5/5	5/5	5/5	28	3–4
8	10/10	8–10/10	4/5	4–5/5	5/5	5/5	5/5	27**	4–5
9	10/10	8–10/10	1/5	4–5/5	5/5	5/5	5/5	29**	4–5

*Inoculation of 2 samples of each RM1 and RM2 due to not enough assay plants.
**Incubation of inoculated assay plants in a greenhouse.

laboratories (P = 0.000) and between infection levels (P = 0.000). No significant laboratory × infection level interaction was shown (P = 0.901). The average number of the natural logarithm plus one of local lesions from positive RM samples of both Medium and High infected RM levels per laboratory is presented in Grimault *et al.* (2012), Figure 3. The highest number of local lesions was detected by laboratories 5, 8 and 9 and the lowest by laboratory 1.

An ANOVA for this number of lesions showed an infection level effect for the following laboratories: Lab 1 ($P = 0.001$), Lab 4 ($P = 0.004$), Lab 5 ($P = 0.000$), Lab 7 ($P = 0.001$), Lab 8 ($P = 0.000$) and Lab 9 ($P = 0.000$). There was no infection level effect shown for Laboratories 2, 3 and 6 . The average number of the natural logarithm plus one of local lesions from positive RM samples for each of the Medium and High infected levels per laboratory is depicted in Grimault *et al.* (2012), Figure 4. All laboratories detected a higher number of lesions in the High infection level than in the Medium.

The standard deviation of repeatability and reproducibility based on the mean of local lesions from positive RM samples of Medium and High infection are shown in Grimault *et al.* (2012), Table 3. No (h) critical values were reported while there were (k) critical values at 5% confidence level for Lab 5 for Medium and High level and for Lab 8 for High level (Grimault *et al.*, 2012, Figs. 7, 8).

Discussion

No false positives were reported in the local lesion assay, and no cross contamination occurred, as all laboratories found all seed subsamples from the Healthy lot negative and did not record any lesions on the negative control plants. This finding is in agreement with the Hadas *et al.* (2004) findings.

Most laboratories detected the expected number of positive seed subsamples from the Medium and High infection level lots. Laboratories 1, 4 and 9, which detected less than the expected number of positive seed subsamples, reported assay performance under conditions considerably variable to the optimal ones (*e.g.* plants under or over the optimal growth stage, suboptimal or fluctuating incubation temperature). The growth stage of plants is well known to affect the infection of tobamoviruses and the number of lesions on the inoculated leaves. In older plants, less severe virus infections are generally reported than in younger plants (Takahashi 1972; Padmanabhan *et al.*, 2007). At temperatures higher than 28 °C, tobacco plants inoculated with tobamoviruses do not show a hypersensitive reaction (Whitman *et al.*, 1994; Ordog *et al.*, 2002). Generally, the desired optimal temperature cannot be easily maintained in greenhouse conditions, resulting in deviations. Thereafter, it is postulated that the detection of positive seed subsamples by these laboratories was affected by uneven virus distribution in the seed subsamples in the preliminary tests for the characterization of the seed lots, by suboptimal incubation conditions, and by the potential mishandling of the subsamples, either individually or in combination.

The laboratory effect that was shown in the number of local lesions from seed subsamples of the lots with Medium and High infection levels is also attributed to the variations in the growth stage of the plants, and variations in the incubation temperatures applied by each laboratory. This statistical outcome was expected by the organizers of this comparative test, who were aware that the number of local lesions developed as an HR product of tobamovirus-inoculated tobacco plants from seed lots with variable infection levels cannot be considered as an absolute number that could be expected to be reproduced by the participants.

However, since no (h) critical values for the reproducibility were reported by ISO 5725-2, no lab × level interaction was shown in the ANOVA, and since most of the laboratories detected the expected number of positive seed subsamples, the local lesion assay was evaluated as being reproducible for the seed subsamples.

The Medium and High infection levels of the seed lots were distinguishable by the laboratories as indicated by the level effect that was shown on the number of local lesions from the seed subsamples. All laboratories recorded a higher average number of lesions in seed subsamples from the High level lot than from the Medium.

Moreover, this difference in infection level was demonstrated within each laboratory by the level effect that was shown in each of them. The (k) critical values shown for Labs 5, 7, and 9 indicate that the uneven virus distribution in the subsamples and/or the variations from the optimal incubation conditions may result in variations between subsamples in the number of developed local lesions . However, since the majority of the laboratories detected the expected number of positive seed subsamples, the local lesion assay was considered to be repeatable for seed subsamples.

All laboratories were able to detect all RM samples from the Medium and High infection levels, with the only exception at the Medium level being Laboratory 1, as it recorded one negative sample. The previously mentioned factors that can influence the assay and/or suboptimal manipulation of the sample can explain the results of Laboratory 1.

The significant differences shown between laboratories in the number of lesions from RM samples of the Medium and High infection levels are also attributed to the same influencing factors mentioned for the seed subsamples. However, as no (h) critical values for the reproducibility were reported by the ISO 5725-2, and no lab × level interaction was shown in the ANOVA, the local lesion assay was evaluated as being reproducible for the RM samples.

The level effect on the tobamovirus lesions from RM samples shown for most laboratories showed that they were able to distinguish the Medium and High infection level of the RM. All laboratories recorded a higher average number of lesions in RM samples from the High infection level than from the Medium. The level effect given for individual laboratories demonstrated that they were able to distinguish the Medium and High infection levels of RM samples. The absence of the level effect in Laboratory 6 is due to the small number of inoculated plants. The (k) critical values shown for Labs 5 and 8 indicate that the variations from the optimal incubation conditions may result in variations in the number of developed local lesions between subsamples. However, as all laboratories except Lab 1 detected all RM samples, the local lesion assay was considered repeatable for RM samples.

Conclusions and recommendations

The local lesion assay (indexing) as a detection method of infectious tobamoviruses does not lead to false positive results, as it is based on the mediation of the HR reaction of tobacco plants that carry the *N*-resistant gene (Holmes 1938; Hammond-Kosack and Jones 1996; Erickson *et al*., 1999a; Whitman et al., 1994; Boovaraghan *et al*., 2007).

The local lesion assay allows for the detection and distinction between three different tobamovirus infection levels on seed lots and two different tobamovirus infection levels on RM samples.

The growth stage of the tobacco assay plants and the incubation temperature of the tobamovirus assay plants were found to be critical factors.

The use of a positive control is considered to be indispensable for validation of the results. In this comparative test, ground *N. occidentalis* leaves infected with tobamovirus mixed with pea flour were used as a positive control. However, other types of reference material (*e.g*. liquid plant extract of leaves of solanaceous hosts infected with TMV/ToMV/PMMoV) or a known positive seed sample can be also used. The local lesion assay can moreover be performed after ELISA pre-screening on the same seed sample ground on ELISA buffer (Hadas *et al*., 2004), on condition that the final results are validated through comparison with results of positive controls of both the assay and the ELISA test, which will have been prepared and stored under the same laboratory conditions.

The local lesion assay as a detection method of infectious tobamoviruses was found to be repeatable and reproducible for both seed subsamples and reference material samples.

When 3000 seeds (12 subsamples of 250 seeds) of a lot are tested, based on the "Qual Impurity Estimation" spreadsheet of SeedCalc the minimum infection level that can be detected is 0.03%, which corresponds to one infected seed in 3000. The desirable tolerance level for infectious tobamoviruses on tomato seed has been set by the international seed industry at zero. According to the "Quality Plan design" spreadsheet of SeedCalc, when 3000 seeds of a lot are tested, a 0.1% infection is avoided with a 95% confidence level. Therefore, the minimum recommended sample size for the detection of infectious tobamoviruses is 3000 seeds.

The local lesion assay has been found to be a reliable detection method of infectious tobamoviruses on tomato seed. It is therefore highly recommended to seed health laboratories for routine analysis.

Acknowledgements

The input of participating laboratories from France, Israel, the Netherlands and the USA in this comparative test, and of M. El Yaklifi in the analysis of the results is greatly acknowledged. Special thanks to Bejo NL, Enza Zaden, Clause Tezier, NAKT and GEVES-SNES for providing testing material.

References

Averre, C. W. and Gooding, G. V. (2000). *Virus Diseases of Greenhouse Tomato and Their Management.* North Carolina State University, http://www.ces.ncsu.edu/depts/pp/notes/oldnotes/vg15.htm.

Bawden, F. C. (1964). *Plant viruses and virus diseases.* 4th edition. The Ronald Press, New York.

Boovaraghan, B., Cawly, J., Angel, C., Zhang, Z., Palanichelvam, K., Cole, A. and Schoelz, J. (2007). Silencing of the *N* family of Resistance Genes in *Nicotiana edwardsonii* Compromises the Hypersensitive Response to Tobamoviruses. *Molecular Plant-Microbe Interactions,* **20**, 1262–1270.

Clark, M. F. and Adams, A. N. (1977). Characteristics of the microplate method of enzymelinked immunosorbent assay for the detection of plant viruses. *Journal of General Virology,* **34,** 475–483.

Dawson, W. O. (1999). Tobacco mosaic virus virulence and avirulence. *Philosophical Transactions of the Royal Society of London Biological Sciences,* **354**, 645–651.

Demski, J. W. (1981). Tobacco mosaic virus is seed borne in pimiento peppers. *Plant Disease,* **65**, 723–724.

Diaz-Griffero, F., Espinoza Cancino, C., Medina Arevalo, C. and Arce-Jonhson, P. (2006). Expression of the crucifer-infecting TMV-Cg movement protein in tobacco plants complements *in trans* a TMV-U1 trafficking-deficient mutant. *Biology Resistance,* **39**, 269–279.

Dijkstra, J., Bruin, G. C. A., Burgers, A. C., Van Loon, L. C., Ritter, P., Van de Sanden, P. A. C. M. and Wieringa-Brants, D. H. (1977). Systemic infection of some N-gene-carrying *Nicotiana* species after inoculation with tobacco mosaic virus. *Netherlands Journal of Plant Pathology,* **83**, 41–59.

Ehrenfeld, N., Gonzalez, A., Canon, P., Medina, C., Perez-Acle, T. and Arce-Johnson, P. (2008). Structure-function relationship between the tobamovirus TMV-Cg coat protein and the HR-like response. *Journal of General Virology,* **89**, 809–817.

Erickson, F. L., Dinesh-Kumar, S. P., Holzberg, S., Ustach, C. V., Dutton, M., Handley, V., Corr, C. and Bakker, B. J. (1999a). Interactions between tobacco mosaic virus and the tobacco *N* gene. *Philosophical Transactions of the Royal Society of London Biological Sciences,* **354**, 653–658.

Erickson, F. L., Holzberg, S., Calderon-Urea, A., Handley, V., Axtell, M., Corr, C., and Baker, B. (1999b). The helicase domain of the TMV replicase proteins induces the *N*-mediated defence response in tobacco. *The Plant Journal,* **18**, 67–75.

Flor, H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology,* **9**, 275–296.

Grimault, V., Koenraadt, H. M. S. and Politikou, A. (2012). *Detection of tobamoviruses on* Lycopersicon esculentum *seed by local lesion assay on* Nicotiana tabacum *plants.* ISTA Method Validation Reports 2012, 13–25. International Seed Testing Association, Bassersdorf, Switzerland.

Hadas, R. (1999). A report of comparative test for Tobamoviruses in tomato seeds. ISHI-Veg Research Report 1-1999-Tomato-Tobamo. International Seed Federation, Nyon, Switzerland.

Hadas, R., Pearlsman, M., Gefen, T., Lachman, O., Hadar, E., Sharabany, G. and Antignus, Y. (2004). Indexing system for Tomato mosaic virus (ToMV) in commercial tomato seed lots. *Phytoparasitica,* **32** (4), 421–424.

Hammond-Kosack, K. E. and Jones, J. D. G. (1996). Resistance Gene-Dependent Plant Defense Responses. *The Plant Cell,* **8**, 1773–1791.

Holmes, F. O. (1929). Local lesions in tobacco mosaic. *Botanical Gazette* (Chicago), **87**, 39–55.

Huttinga, H. and Rast, A. T. B. (1995). Tomato mosaic tobamovirus. In *Viruses of Plants* (eds. A. A. Brunt, K. Crabtree, M. J. Dallwitz, A. J. Gibbs, and L. Watson), pp. 1302-130, CAB International, Wallingford, UK.

ISO 5725 (http://www.seedtest.org/en/statistical-tools-for-seed-testing-_content---1--1143--279.html)

Kiraly, L., Barna, B. and Kiraly, Z. (2007). Plant Resistance to Pathogen Infection: Forms and Mechanisms of Innate and Acquired Resistance. *Journal of Phytopathology,* **155**, 385–396.

Kiraly, L., Hafez, Y. M., Fodor, J. and Kiraly, Z. (2008). Suppression of tobacco mosaic virus-induced hypersensitive-type necrotization in tobacco at high temperature is associated with downregulation of NADPH oxidase and superoxide and stimulation of dehydroascorbate reductase. *Journal of General Virology,* **89**, 799–808.

Lewandowski, D. J. and Dawson, W. O. (1998). Tobamoviruses. In *Encyclopedia of Virology*, 2nd edition (eds. A. Granoff and Webster, R. G.), *Academic Press,* **3**, 1780–1783.

Matthews, R. E. F. (1991). *Plant Virology.* 3rd edition, Academic Press, Inc., New York, N.Y.

Maury, Y., Bossennec, J. M., Boudazin, G., Hampton, R. O., Pietersen, G. and Maguire, J. (1987). Factors influencing ELISA evaluation of transmission of pea seed-borne mosaic virus in infected pea seed: seed-group size and seed decortication. *Agronomie,* **7**, 225–230.

Nolan, P. A. and Campbell, R. N. (1984). Squash mosaic virus detection in individual seeds and seed lots of cucurbits by enzyme-linked immunosorbent assay. *Plant Disease,* **68**, 971–975.

Ordog, S. H., Higgins, V. J. and Vanlerberghe, G. C. (2002). Mitochondrial alternative oxidase is not a critical component of plant viral resistance but may play a role in the hypersensitive response. *Plant Physiology,* **129**, 1858–1865.

Padgett, H. S. Watanabe, Y. and Beachy, R. N. (1997). Identification of the TMV Replicase Sequence That Activates the N Gene-Mediated Hypersensitive Response. *Molecular Plant-Microbe Interactions* **6**, 709–715.

Padmanabhan, M. S., Kramer, S. R., Wang, X. and Culver, J. N. (2008). Tobacco mosaic virus replicase-auxin/indole acetic acid protein interactions: reprogramming the auxin response pathway to enhance virus infection. *Journal of Virology,* **82**, 2477–2485.

Rhee, Y., Tzfira, T., Chen, M.-H., Waigmann, E., and Citovsky, V. (2000). Cell-to-cell movement of tobacco mosaic virus: enigmas and explanations. *Molecular Plant Pathology,* **1**, 33–39.

Samuel, G. (1931). Some experiments on inoculating methods with plant viruses and on local lesions. *Annals of Applied Biology,* **18**, 494–507.

Seedcalc version 8 (http://www.seedtest.org/en/statistical-tools-for-seed-testing-_content---1--1143--279.html).

Stange, C., Matus, J. T., Elorza A. and Arce-Johnson, P. (2004). Identification and characterisation of a novel tobacco mosaic virus resistance N gene homologue in *Nicotiana tabacum* plants. *Functional Plant Biology,* **31** (2), 149–158.

Takahashi, W. N. (1956). Increasing the sensitivity of the local-lesion method of virus assay. *Phytopathology* **46**, 654-656.

Takahashi, T. (1972). Studies on viral pathogenesis in plant hosts. III. Leaf age-dependent susceptibility to tobacco mosaic virus infection in "Samsun NN" tobacco plants. *Phytopathology*, Z. **75**,140–155.

Takahashi, T. (1975). Studies on viral pathogenesis in plant hosts VIII. Systemic virus invasion and localization of infection on "*Samsun NN*" tobacco plants resulting from tobacco virus infection. *Phytopathology*, Z. **84**, 75–87.

Taliansky, M., Aranda, M. A. and Garcia-Arenal, F. (1994). Differential invasion by tobamoviruses of *Nicotiana megalosiphon* following the hypersensitive response. *Phytopathology,* **84**, 812–815.

Weststeijn, E. A. (1981). lesion growth and virus localization in leaves of *Nicotiana tabacum* cv. Xanthi nc. after inoculation with tobacco mosaic virus and incubation alternately at 22°C and 32°C. *Physiological Plant Pathology,* **18**, 357–368.

Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C. and Baker, B. (1994). The product of the tobacco mosaic virus resistance gene N: similarity to Toll and the interleukin-1 receptor, Cell, 78, 1101–1115.

Laboratory accreditation changes

Status 1 March 2012

Re-accreditations

Australia AUDL0100

SA Seed Laboratory
Primary Industry & Resources SA
Plant Research Centre, Waite Campus, Urrbrae
GPO Box 1671
Adelaide SA 5001
Phone: +61 8 83039549
Fax: +61 8 83039508
Mail: Heather.Bennet@sa.gov.au

AUDL0200

Queensland Seed Technology Laboratory
The University of Queensland
Gatton Campus QLD 4345
Phone: +61 75460 1487
Fax: +61 75460 1486
Mail: seedlab@uq.edu.au

AUDL0700

AGWEST Plant Laboratories
Department of Agriculture and Food
Seed Testing Station
3 Baron-Hay Court
South Perth WA 6151
Phone: +61 8 9368 3844
Fax: +61 8 94742658
Mail: agwestplantlabs@agric.wa.gov.au

Greece GRDL0100

Seed Testing Station of Athens
Ministry of Rural Development and Food
Antheon 2, Marousi
151 23 Athens
Phone: +30 210 683 0471
Fax: +30 210 6830917
Mail: an2u001@minagric.gr

India INML0600

Maharashtra Hybrid Seeds Company Ltd.
Quality Assurance Laboratory
Jalna-Aurangabad Road, Dawalwadi,
Badnapur Tq
P.O. Box 76
Jalna 431 203 (M.S.)
Phone: +91 2482 262001 / 02
Fax: +91 2482 262002
Mail: chandrakumar.saragur@mahyco.com

INML0700

Indo-American Hybrid Seeds (India) Pvt. Ltd.
Seed Testing Laboratory
7th. Km, Banashankari-Kengeri Link Road
Uttarahalli Hobli, Channasandra Village
Subramanya Pura Post
Bangalore 560 061
Phone: +91 80 286 4499
Fax: +91 80 286 029 12
Mail: indamseeds@gmail.com

Italy ITML0700

C/O Cooperativa Agricola Cesenate
Laboratorio Analisi Sementi
Via Calcinaro 1450
47521 Martorano di Cesena (FC)
Phone: +39 0547 643511
Fax: +39 054 7383417
Mail: laboratoriocac@libero.it

Japan JPML0600

Takii & Co. Ltd.
Quality Assurance Department
180 Umekoji, Inokuma
Shimogyo-KU
C.P.O. Box 7
Kyoto 600-8686
Phone: +81 75 365 0123
Fax: +81 75 3650110
Mail: mkomaba@takii.co.jp

New accreditations

France FRML0300

Monsanto SAS
Quality Assurance Laboratory
P.O. Box 21
40305 Peyrehorade CEDEX
Phone: +33 558 736099
Fax: +33 558 730929
Mail: bruno.zaccomer@monsanto.com

Tajikistan TJML0100

Republican Seed Inspectorate
Central Seed Laboratory
Mirzobek set. 1
734063 Dushanbe
Phone: +99 237 225 8249
Fax: +99 237 221 1628
Mail: sabohat82@gmail.com

New arrival

Cannice Gubser, ISTA Membership Administration, is currently on maternity leave.
Our world is now complete!
We are so happy to announce the arrival of our baby girl,
Fiona Keren
born: 24 January 2012
weight: 2920 g, length: 47 cm
Filling our arms with love and our hearts with happiness!
Cannice, Peter & Philip Gubser

ANNOUNCEMENT

ISTA Workshop on Flower Seed Testing
Roelofarendsveen, the Netherlands 6–9 June 2012

The ISTA-accredited seed testing laboratory Naktuinbouw, the Netherlands, will host an ISTA workshop on Flower Seed Testing prior to the ISTA Annual Meeting 2012 (see page 16).

Location

Naktuinbouw, Roelofarendsveen, the Netherlands

Organizers

Anton Grim (a.grim@naktuinbouw.nl)

Lecturers

– Stefanie Krämer
– Rita Zecchinelli
– Zita Ripka
– Sylvie Ducournau
– Anton Grim

Aim of the workshop

To gain knowledge and some experience in flower seed testing.

Target group

Staff of laboratories willing to gain experience on flower seed testing.

Workshop content

– Purity analysis
– Germination testing
– Tetrazolium testing
– Statistics in seed testing
– Excursion to flower auction and flower seed company

Registration deadline

Monday, 30 April 2012

Registration fee

ISTA Members: EUR 400
Non-members: EUR 600

Fee includes instruction materials, manuals and proceedings; coffee breaks, lunches and official dinner; excursion to flower auction and flower seed company.

Registration will be limited to 40 participants.

Accommodation

Tulip Inn Leiderdorp: EUR 80.00
(breakfast included, tourist tax not included)
www.tulipinnleiderdorp.nl
leiderdorp@autogrill.net
+31 71 5899 302

For accommodation registration code, please contact Anton Grim (a.grim@naktuinbouw.nl)

Registration form

www.seedtest.org/workshops

ANNOUNCEMENT

ISTA Workshop on Variety Testing
Roelofarendsveen, the Netherlands 6–9 June 2012

The ISTA-accredited seed testing laboratory Naktuinbouw, the Netherlands, will host an ISTA workshop on Variety Testing prior to the ISTA Annual Meeting 2012 (see page 16).

Location

Naktuinbouw, Roelofarendsveen, the Netherlands

Organizers

Dr. Hedwich Teunissen (h.teunissen@naktuinbouw.nl)

Lecturers

– Dr. Hedwich Teunissen (Naktuinbouw)
– Daniel Perry (Grain Research Laboratory, Canada)
– Berta Killermann (Bayerische Landesanstalt für Landwirtschaft, Germany)
– Bruno Pot (Applied Maths N.V., Belgium)

Aim of the workshop

Overview of the various methods that can be applied to variety testing. The main focus is on DNA-based methods for variety ID and statistics. Hands-on experiments of PCR-based marker systems, fingerprint analysis and clustering.

Workshop content

– Overview of conventional and protein ID methods
– Overview of sampling for Variety Testing and preparation of working samples
– Different methods for DNA extraction and quantification
– Polymerase Chain Reaction, optimalisation, primer design etc.
– Molecular markers for Variety ID – Different types of markers
– Data analysis in BioNumerics (different outputs of fingerprint data)
– Data mining/Database development
– Genetic similarity analyses and statistics (clustering, PCA, MDS)
– Statistical aspects of purity testing
– Molecular markers applications at Naktuinbouw
– Future (technological) developments for variety testing

Registration deadline

Monday, 30 April 2012

Registration fee

ISTA Members: EUR 450
Non-members: EUR 650

Fee includes instruction materials, manuals and proceedings; coffee breaks, lunches and official dinner.

Registration will be limited to 20 participants.

Accommodation

Tulip Inn Leiderdorp: EUR 80.00
(breakfast included, tourist tax not included)
www.tulipinnleiderdorp.nl
leiderdorp@autogrill.net
+31 71 5899 302

For accommodation registration code, please contact
Dr. Hedwich Teunissen (h.teunissen@naktuinbouw.nl)

Registration form

www.seedtest.org/workshops

ANNOUNCEMENT

ISTA Workshop on Vigour Testing
Nisku, Edmonton, Canada 11–14 September 2012

The ISTA Vigour Committee and 20/20 Seed labs Inc invite you to a Workshop on Seed Vigour Testing. The workshop will be made up of lectures, interactive seminars and practical experience in vigour testing. It will also offer the opportunity for general discussion of seed vigour and time for participants to ask specific questions regarding vigour testing procedures

Workshop content

- Introduction to seed vigour and its importance in crop production
- Conductivity test for *Pisum sativum, Phaseolus vulgaris, Glycine max*
- Controlled deterioration test for *Brassica* spp.; application to other small seeded vegetables
- Radicle emergence test: development and validation. Examples: maize, cotton, *Brassica*, pepper, cucurbits, general applicability
- Accelerated ageing test for soya beans
- Seed vigour and seed treatments, with particular reference to canola (*Brassica napus – B. rapa – B. juncea*)
- Physiological basis of seed vigour
- Factors affecting seed vigour
- Future developments in vigour testing

Practical work

All participants will complete the conductivity test, carry out stages in the radicle emergence and controlled deterioration tests and assess results from the controlled deterioration and radicle emergence tests.

Question and answer sessions

These will consider questions on all aspects of seed vigour and any vigour test.

Lecturers

- Dr Alison Powell (Chair of ISTA Vigour Committee, University of Aberdeen, UK),
- Dr Stan Matthews (ISTA Vigour Committee, University of Aberdeen)
- Dr Bob Elliott (ISTA Vigour Committee, AgriCanada).

Local organisers

Carey Matthiessen, 20/20 Seed labs Inc.
Carey@2020seedlabs.ca

Location

The workshop will be hosted by:
20/20 Seed Labs Inc.
Suite 201
09–11 Avenue
Nisku, Alberta.

20/20 Seed Labs Inc is a private, independently owned seed testing laboratory, accredited by the Canadian Food Inspection Agency for all grade tables of the Canada Seed Act. The company is ISO 9001: 2008 registered and is also the first private laboratory in Canada with ISTA certification. Our core business is testing seed for purity, germination, vigour and seed health. We have a seed health laboratory as well as a molecular diagnostics laboratory. We have been in business serving Canadian and international clients since 1989. Find out more about 20/20 Seeds Labs: http://www.2020seedlabs.ca/

20/20 Seed labs is located just outside Edmonton, Alberta. Edmonton is the capital city of Alberta and home to a diverse cultural and economic climate. Find out more information about Edmonton at: http://www.edmonton.ca/

Accommodation

The Varscona Hotel is located in the heart of Whyte Avenue, a trendy, central location for some of Edmonton's premier restaurants, entertainment, and local businesses.

The cost of a hotel room per night is CAD 130, which includes breakfast.

Participants should book reservations themselves, booking under the group "2020seedlabs" to receive the group rate.

Please follow this link for booking information:
http://www.varscona.com/reservations
or call the hotel at +1-866-465-8150.

Travel to and from Nisku

The nearest airport is Edmonton International Airport.
Shuttle bus to city hotels: CAD 18
Taxi to city hotels: approximately CAD 60.
This cost should be paid personally by the participants.

Registration deadline

Deadline for registration: 29 June 2012

Registration fee

ISTA Members: CAD 500
Non-members: CAD 750

Fee includes participation in the workshop, all supporting literature, coffee and tea breaks, lunches and workshop dinner.

There will be a minimum number of participants required for this workshop to take place, with a maximum number of 20.

Registration form

www.seedtest.org/workshops

ANNOUNCEMENT

ISTA Workshop on Germination Testing
Saskatoon, Canada 17–19 September 2012

Local organiser

Steve Jones (ISTA Executive Committee Member and ISTA Rules Chair) for the ISTA Accredited Canadian Food Inspection Agency (CFIA), Seed Science and Technology Section, Saskatoon

Main lecturers

Sylvie Ducournau (Chair of ISTA Germination Committee) plus one other Germination committee member

Aim of the workshop

This workshop aims at presenting and discussing the principles of the ISTA germination test, seedling evaluations for different species, results calculations and reporting plus aspects of quality management linked to germination testing.

Workshop content

- Introduction to ISTA
- The history of germination testing
- Seedling evaluations
- Germination calculations and tolerances
- Group work
- Reporting results
- QA aspects of germination testing: e.g. how to check the vacuum planting heads, proficiency testing, trending of analyst performance
- Discussion of common errors in proficiency tests, issues and possible Rules proposals
- Future work for the Germination Committee
- Tour of the seed laboratory and National Seed Herbarium
- Workshop dinner

The theoretical background will be given through lectures. The workshop language is English. Participants of this workshop will be actively involved through group work, discussions and practical work. The ISTA Rules and the ISTA Seedling Evaluation Handbook will be discussed and used during the workshop.

Now also in German – International Rules for Seed Testing Edition 2012

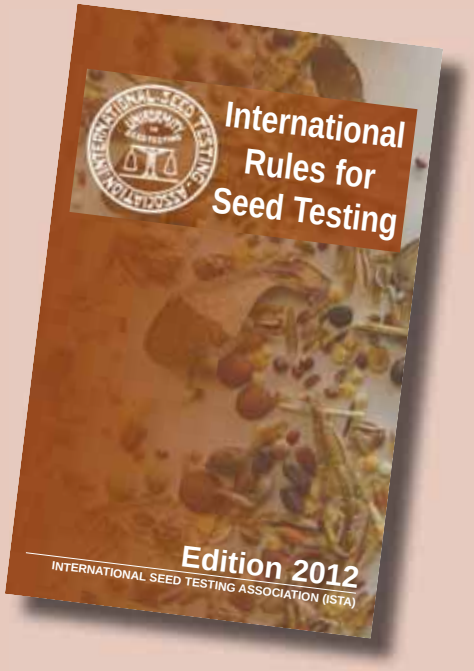
ISBN 978-3-906549-68-2
ISBN 978-3-906549-69-9 (Annexe to Chapter 7)
ISBN 978-3-906549-70-5 (French, incl. Annexe to Chapter 7)
ISBN 978-3-906549-71-2 (German, incl. Annexe to Chapter 7)

The Edition 2012 (effective 1 January 2012) includes the latest changes passed at the ISTA Ordinary Meeting 2011, held at Zurich, Switzerland. Updates, in the form of additions or replacements of existing pages, are published as Amendments and can be inserted separately into the binder.

Price (incl. Annexe to Chapter 7):
CHF 417.00 (approx. USD 437.00/EUR 345.00)

Amendments:
CHF 114.00 (approx. USD 90.00/EUR 94.00

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



ISTA Workshop on Statistical Aspects of Testing for GMOs
Mexico City, Mexico, 25–29 July 2011

Adriana Otero Arnaiz and Martha Graciela Rocha Munive

Centro Nacional de Investigación y Capacitación Ambiental (CENICA)
Instituto Nacional De Ecología
04530 México D.F., Mexico

A workshop was held in Mexico City, Mexico on the Statistical Aspects of Testing for the presence of Genetically Modified Organisms. The workshop was an initiative of the Biosafety Program at the National Ecology Institute and the Autonomous Metropolitan University (UAM), and was organized in collaboration with, and delivered by, the Statistics and GMO Technical Committees of ISTA.

The National Ecology Institute (INE) at the Ministry of Environment and Natural Resources is building capacity for monitoring and testing for the presence of GMOs in Mexico. It has integrated a curriculum and a diploma program in collaboration with the UAM, and it is implementing ongoing sampling and analysis of GMO presence in the environment. As part of this effort, and to strengthen the statistical base that goes into the design and validation of field sampling plans and laboratory testing, this workshop was initiated.

The workshop was organized locally by Dr. Adriana Otero Arnaiz, Biosafety Program Coordinator and Dr. Martha Graciela Rocha Munive, Deputy Director of Genetic Analysis, both at INE. Additional support was provided by Berenice Bustos Zúñiga, head of research department of GMOs at INE and Dr. Beatriz Rendón Aguilar, professor of Biology at UAM. The ISTA organizers and lecturers were Dr. Benjamin (Beni) Kaufman, of Pioneer Hi–bred, USA, Mr. Jean Louis Laffont, Pioneer Génétique SARL, France, Chair of the Statistics Committee, and Dr. Kirk Remund, Monsanto, USA, Vice-Chair of the Statistics Committee. All ISTA lecturers are members of the GMO Committee as well.

The workshop was held on the campus of the UAM Iztapalapa, located in eastern Mexico City. The extensive university facilities had provided a well equipped computer room with 30 computers running Windows 7, as well as a projector and computer equipment for the teacher.

Twenty-two people registered for the workshop, representing regulating bodies, academia, and industry. The attendees included officials from the Mexican Secretariat of Environment and Natural Resources (SEMARNAT), the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA), the National Metrology Center (CENAM) and the Inter ministerial Commission on Biosafety of Genetically Modified (CIBIOGEM), researchers from the National Institute for Forestry, Agriculture and Livestock (INIFAP), the National Center for Genetic Resources, the Center for Scientific Research of Yucatan (CICY) and the UAM campus Xochimilco. International participants came from the National Seed Institute of Uruguay, the ANSES GEVES Plant Health Laboratory in France, and from the Saskatchewan Research Council, Canada. The private sector was represented by attendees from Pioneer Hi-Bred International, Monsanto, BASF Plant Science, Bayer Crop Science Canada and the Kenya Seed Company Ltd.

During the first day Dr. Beni Kaufman gave an introduction to the workshop and an overview of the detection methods used for GMO testing as well as the challenges in conducting the testing in seed. The concept and practice of method and process validation were presented and discussed. The day was concluded with a presentation of ISTA's GMO technical committee activities.

On the following days Mr. Laffont and Dr. Remund reviewed some of the statistical theoretical concepts that form the basis of sampling, testing plan design and analysis, such as sample and population, tools to explore data, and probability distributions. This was followed by the statistical tools for the design of GMO testing: pool testing, analysis of variance (ANOVA), repeatability and reproducibility and uncertainty estimation. The criteria for assessing the performance of different testing plans were discussed, and the SeedCalc tool was presented. There was also a review of an approach for performing calculations on stacked traits. All classes were followed by practical exercises done by the participants during the practice sessions.

The instructors demonstrated broad knowledge of the subject matter and issues, and much interaction between participants and instructors ensued with many questions and interesting discussions. Importantly, participants showed much interest in the issues that had risen and the instructors encouraged the participation and discussion, which greatly enriched the workshop.

To balance the intense intellectual effort, and to enhance the social interaction amongst the participants, an outing and an official dinner were organized; the excursion took the participants to the "trajinera" boats of Xochimilco, where attendees enjoyed the Mexican folklore, snacks and some traditional music while boating along the beautiful channels on the trajineras. The official dinner took place in a typical Mexican restaurant where everybody enjoyed traditional food, tequila, music and folk dances.

At the conclusion of the workshop participants were asked to fill evaluation forms and provide feedback regarding various quality parameters of the workshop by rating between 1 ("poor") to 6 ("excellent"), and to identify opportunities for improvement. The feedback reflected satisfaction with lecturers (average: 5.5 for preparation, enthusiasm, and knowledge), organization and the deductive materials (average 5.1) and advancements of their knowledge. The overall satisfaction rank averaged at 5.6! Which is as close to excellent as one can expect. More importantly, the majority of the participants agreed that the workshop was relevant to their jobs and the skills they have acquired or improved upon in the work shop will most likely improve their daily work.

For the local organizers, it was a pleasure to have interacted so successfully with the representatives of ISTA and hope that these experiences will be repeated in the future. ■

ISTA Workshop on Purity Testing
Saskatoon, Canada, 27–29 September 2011

Steve Jones[1] and Ruojing Wang
[1]Chair, ISTA Rules Committee

Canadian Food Inspection Agency
Saskatoon Laboratory
S7N 4L8 Saskatoon, Sask.
Canada
steve.jones@inspection.gc.ca

In September 2011 the Canadian Food Inspection Agency's official seed testing laboratory was host to an ISTA Purity Workshop. The workshop brought together two very experienced lecturers: Jane Taylor from the National Institute of Agricultural Botany (NIAB), UK and Deborah Meyer from the California Department of Food and Agriculture, Plant Pest Diagnostics Center, USA. Jane is currently the Vice-Chair of the ISTA Purity Committee, and Debbie is a long-term member of the ISTA Purity Committee and is also very active within AOSA.

Janine Maruschak (Section Head, Seed Science & Technology Section), Steve Jones (Chief of Germination and Purity) and Marc Sabourin (Saskatoon Laboratory Director) opened the workshop for the 27 people on the workshop.

Participants had the unique opportunity to share experiences and check their own understanding of the methods and techniques of ISTA analytical purity testing, and the Other Seed Determination test, as well as pure seed definitions and seed id. The history of ISTA and its purity test objectives, the use of the blower, the half-seed rule, the new *Orobanche* spp. test, QA related to purity testing, tolerances, calculation and reporting of results were also covered. The workshop participants came from six different countries: Denmark, the Netherlands, Thailand, Taiwan, the UK, the USA and of course Canada. Most people came from North America, therefore species of particular interest to North America were featured, but a range of common species traded around the world were also studied.

The workshop was hands on with presentations, practical work, course reference

material, demonstrations and active participation. During the workshop the use of resources for seed identification like the ISTA Pure Seed Definition Handbook, GRIN, the ISTA Universal list and the ISTA PT program were included. Linked to this, Ruojing Wang, the Head of the CFIA's National Seed Herbarium, gave participants a tour of the unique seed reference collection based in Saskatoon, and talked about seed imaging and the use of LUCID keys in seed identification.

During the visit to the Seed Science & Technology Section (SSTS) at Saskatoon of course saskatoons had to feature, with saskatoon pies, chocolates and tea available. For those that do not know them, saskatoon berries (*Amelanchier alnifolia*) are very tasty, grow wild and are also grown commercially.

As well as enjoyable workshops and time to talk over the break periods the social visit was great opportunity to talk and visit the Western Development Museum. After supper at the museum, everyone could wander around the exhibits featuring the aboriginal cultures, the first European immigrants and their lives on the prairies.

The feedback from the course was very positive, and people made good use of the opportunity to ask and exchange views on seed testing and purity issues. Ideas for new and improved Rules proposals were another positive result from the workshop.

Both Jane and Debbie enjoyed the workshop. As the local organisers we would like to thank them for all their hard work and excellent presentations. We also thank all the participants for their involvement and also especially all the people at SSTS in Saskatoon who prepared the samples for use at the workshop.

Thanks also to NIAB and CDFA for allowing Jane and Debbie to provide this ISTA Purity Workshop.

ISTA Workshop on Biotechnology Trait Detection
Shanghai, China, 5–9 December 2011

Beni Kaufman[2], Kirk Remund, Bruno Zaccomer, Dabing Zhang and Cheryl Dollard[1]
[1]Vice-Chair and members, ISTA GMO Committee

[2]Pioneer Hi-Bred
Johnston, IA 50265, USA
beni.kaufman@pioneer.com

The ISTA GMO Technical Committee Information Exchange Working Group held a workshop on the theory and methods of biotechnology trait detection at the Shanghai Jiao Tong University (SJTU)School of Life Sciences and Biotechnology, Shanghai, China, from December 5–9, 2011. There were 21 participants from seven different countries – China, India, South Korea, the USA, Croatia, Separate Customs Territory of Taiwan, Peghu, Kinmen and Matsu, and Brazil – from a variety of backgrounds, including academia, industry and government. The lecturers were Benjamin (Beni) Kaufman (Pioneer, USA), Bruno Zaccomer (Monsanto, France), Kirk Remund (Monsanto, USA), and Cheryl Dollard (Canadian Food Inspection Agency, Canada). Prof. Dr. Dabing Zhang, Deputy Dean of the National Testing Center for Molecular Characterization of GMOs (NCMCG) at SJTU, coordinated local activities, with excellent support from Dr. Litao Yang, Dr. Sheng Quan and a team of dedicated students.

The first day of the workshop opened with a welcome by Beni Kaufman. The lecturers and participants introduced themselves. Kirk Remund then delivered a full day of statistics and test plans as an overview to statistical considerations for seed testing. Students learned how to develop qualitative and quantitative testing plans and how to use many of the functions of SeedCalc. This included an introduction to the newly developed box-plot macro that had just been added to the statistics toolbox of the ISTA Statistics Committee.

The participants worked through practical exercises to become familiar with the software and principles that they would need for adventitious-presence (AP) testing, and for the exercises which would follow during the workshop. Each of the following days would bring theoretical lectures complemented by hands–on laboratory work.

On the second day, there was an introduction to AP testing, commonly known as genetically modified organism (GMO) testing, and a lecture on sample preparation delivered by Beni Kaufman. The topics included DNA extraction, quantification, normalization and sample tracking. Later that day, the students started the laboratory portion of the workshop where they prepared their own DNA from ground seed samples.

On days three and four, Bruno Zaccomer and Cheryl Dollard introduced the polymerase chain reaction (PCR) and the application of this technique for GMO testing, including an overview of PCR and how it works, definitions and practices of using qualitative end-point techniques, and quantitative real-time approaches. Protein-based test methods were also introduced. The data collected was examined using the statistical analyses taught by Kirk

Remund. A presentation on ISTA's Rules for GMO detection was also given by Cheryl Dollard, including an overview of the performance-based approach for Biotech trait testing in seed and the process for ISTA accreditation within this system.

The last lecture of the fourth day was delivered by Prof. Dr. Dabing Zhang, who presented the current GMO situation in China and gave an overview of some of the current research activities in this area at the NCMCG.

On the final day of the workshop, Beni Kaufman discussed the concepts of assay and process validation, and Kirk Remund gave an introduction to handling stacked traits using SeedCalc.

The laboratory portion of the workshop was designed to illustrate and exercise the concepts and technologies presented in the lectures. Participants worked together in teams of three. Each team chose a name: "Potato", "Tomato", "IT", "Shanghai", "Rice"," Number 1", and "Late". all teams worked through the exercises with great enthusiasm. The laboratory facility was excellent, with each team being able to work at a workstation with the dedicated equipment and reagents required to carry out the bench work. The experimental design of the hands-on program involved comparing semi-quantitative pooled testing approaches to real-time quantitative procedures. The design was intended to shed light on some of the special challenges involved in seed GMO testing in general, and in comparison of such testing in feed and food.

The experiments were conducted using corn seed, and involved the analysis of four different spiked samples containing different numbers of different types of transgenic corn seeds. The groups worked collectively to perform replicated DNA extractions, and then proceeded to analyze them by endpoint semi-quantitative PCR and by real-time quantitative PCR. The data from all groups were combined and analyzed. An exercise in protein detection by lateral flow strip tests was also included.

These exercises allowed participants to experience at first hand the workflow of this type of testing – from initial subsampling of flour, through extraction and visualization, up to the final analysis of the data using SeedCalc. The collective results were a concrete illustration of the complexities of GM testing, and demonstrated the many variables that must be considered. The final discussion covered quality assurance and ideas on how to apply such principles to biotech trait testing for seeds.

There are many challenges that must be addressed for any lab wishing to perform this type of testing, and these concepts were discussed openly.

The hosting facility was excellent. The lecture rooms in the Academic Activities Center and the teaching lab in the Biological Pharmacy Building provided ample space and equipment for delivery of the course material. Aside from the science, the Jiao Tong University campus is very beautiful and the dining facilities were admirable – a wonderful selection of delicious food was available every day, and great snacks and coffee during breaks.

In addition, a delicious welcoming dinner was held for the workshop participants at the end of the first day at the Liu Yuan Restaurant at SJTU, hosted and sponsored by Prof. Dabing Zhang and the NCMCG.

Later in the week, an official outing was arranged, sponsored by Pioneer Hi-Bred, a DuPont business. The evening included a visit to the beautiful rock gardens and ponds in Yuyuan Garden, the Shanghai Old Street with buildings displaying classic Ming and Qing architecture, and shops filled with curios, antiques, tea, and traditional souvenirs.

A wonderful meal of traditional Chinese dishes at Xiqo-Nan-Guo restaurant, followed by a night cruise along the Huangpu River along the Pudong area, featuring the Oriental Pearl TV tower, the World Financial Center and the Jinmao building, among many others, all gloriously illuminated for spectacular night-time views.

Although the weather was a little damp, the mist of rain seemed only to enhance the beauty of the city and everyone thoroughly enjoyed themselves.

Overall, the participants were friendly, very eager and quick to learn. They were open, asked questions and shared their own experiences, which led to excellent exchanges.

The workshop was a great success in no small part due to the great support from Dr. Litao Yang, who helped us with many details, including local logistics and travel arrangements, and the small army of students who were available to us at all times for technical support (especially Fang, Hong, Jun, and Miao).

Generous support from Pioneer, Monsanto, KBioScience Life Technologies, Agilent Technologies, Envirologix, Roche, Quanta BioSciences, Qiagen, and Macherey-Nagel helped make this workshop the success it was. Finally, thanks go to Martina Haefeli and many others at the ISTA Secretariat for helping to facilitate the organization. The work program was busy and technical, but the overall atmosphere was informal, and the combination of lecture and lab work created many opportunities for discussion among the participants and lecturers.

The week ended with great success, with presentation of completion certificates to all the participants and many expressions of thanks. ■

ISTA Workshop on Quality Assurance in Seed Testing
Shanghai, China, 5–9 December 2011

Ronald Don[1] and Rasha El-Khadem[2]

[1]Honorary ISTA Member; [2]Head, ISTA Accreditation Department

[2]ISTA Secretariat, Accreditation Department
8303 Bassersdorf, Switzerland
auditor@ista.ch

The 10th Quality Assurance Workshop was held in conjunction with the ISTA Biotechnology Trait Detection Workshop. The National Center of Molecular Characterization of GMOs (NCMCG) in Shanghai Jiao Tong University, China hosted the five-day workshop in the University premises. Participants from China, Indonesia, Iran, Brazil and Spain participated in the event. The participants represented Ministries of Agriculture, Governmental Seed Testing Station, Universities, Research Centers, Certification Agencies and quality departments of the Seed Industry.

The support of our Chinese colleagues from the NCMCG and their contribution to a very successful workshop in China was overwhelming.

The aim of the event was to present and discuss the basic principles of quality management. It focused on the needs of seed testing laboratories that wish to comply with the ISTA Accreditation Standard and was designed to be suitable for those preparing to attain ISTA Accreditation and for those aiming to maintain it. The expectations of the participants covered the aims as outlined for the workshop announcement. One participant is working in a laboratory holding ISTA accreditation. The majority of participants were interested to gain information about the management of a QA system and some were from laboratories in the preparatory stages of having an ISTA accreditation audit.

Rasha El-Khadem, head of the ISTA Accreditation Department, and Ronald Don, an ISTA technical auditor, gave the lectures. Presentations on the first two days dealt with document control and management of equipment. This was followed by more detailed presentations about how to check whether riffle/soil seed dividers or hand dividing leads to the suitable division of a composite sample. Several possibilities were presented on how to check if containers are moisture proof and therefore suitable to hold samples intended for the moisture determination analysis. The determination of the water holding capacity of germination substrate was explained and information with regards to germination substrate checks in general was provided.

Some of the lectures and presentations were followed by group work. Detailed explanations were given with regards to the Purity and the Other Seed Determination Analysis. Participants practiced their knowledge by filling in work cards using data provided for both tests. ISTA's Accreditation Scheme and the ISTA Proficiency Test Programme were presented and participants gained experience of completing ISTA Orange Certificates. The interest was very high and many questions were addressed and answered.

The group visited the laboratory where the participants had to undertake in small groups the practical exercises (water holding capacity and pH measurement of germination paper; riffle/soil divider check versus Boerner divider check and verification; check on hand dividing; and check of suitability of moisture proof containers). The raw data generated during the practical exercises in the host laboratory was evaluated and discussed with the participants. Hand division was performed using a mixture of soybean seed and rice which made the exercise challenging. All group members had to assist in preventing the loss of too many seeds during the dividing process. The data reflected this challenge showing a broad range of variation. Some groups started to compete between each other and some participants competed with members of their own group. Using a riffle/soil divider and Boerner divider the segregation of different sized seeds was estimated and discussed.

The workshop dinner was held in a restaurant famous for its Chinese hot pot dishes. At this social event participants and lecturers with less experience using chopsticks had the opportunity to practice for a short period of time. The participants enjoyed the huge variety of meat, fish, mushrooms, vegetables and plant leafs which they cooked for themselves at the table in the mild or spicy hot pot. A large selection of sauces and dips was available to compliment the hot pot food and the participants shared a very pleasant and unique experience together.

Detailed information was also given on conducting internal Audits. The requirements of the ISTA Accreditation Standard were discussed and how a checklist can be created was demonstrated. The demonstration SOP "Sample Mixing, Calibration of Seed Dividers and Operation of the Seed Divider Register" was used to develop a checklist for an internal Audit on the calibration of a soil/riffle divider. Participants working in groups had to perform this internal Audit while the auditees (both lecturers) performed the practical divider check. All auditors had also to conduct a closing meeting presenting their audit findings to the lecturer they had audited and the other lecturer who acted as the Laboratory Manager. They were provided with feedback on the audits they performed. Due to the excellent performance of the groups, the auditees decided to put special efforts in disturbing the internal auditors during the audit process. This led to unforgettable funny situations and the participants were able to experience challenging audit situations.

After completing the lectures Professor Dabing Zhang guided us through the laboratories of the National Center of Molecular Characterization of GMOs. Their spacious facilities, which are excellently equipped, gave us a good impression of the huge range of pioneering research activities carried out at within the university department. A nearby field demonstration and trial site used by the University students for their studies was also visited.

Most of the participants had not participated in an ISTA workshop before. While the first day was a little bit formal, the participants were put at ease after the first group works. Over the next days the participants melted into a thoroughly collaborating group showing an extraordinary team spirit. The participants shared experience and questions that were addressed and discussed during the workshop. Valediction after the five days was difficult and we have gained new colleagues within ISTA and have pleasant memories of China.

The ISTA Secretariat invited the participants to share their feedback regarding the workshop using an internet-based anonymous questionnaire. Ten of the fourteen participants submitted their impressions and suggestions for improvement. The majority of responses contained very positive comments and for nine out of ten participants their expectations towards the workshop were satisfied completely. All participants gained new insights and could take home new knowledge and experiences from the workshop

The staff of the National Center of Molecular Characterization of GMOs (NCMCG) in Shanghai Jiao Tong University have to be thanked sincerely for the success of the workshop. The efforts put into the organisation and smooth workshop flow, as well as the continuous support they provided during the workshop, were tremendous. Their contribution was very much appreciated by the lecturers and participants. We would also like to thank the ISTA GMO Committee for the possibility of holding this event in conjunction with their workshop.

2012

20-24 May	AOSA/SCST Annual Meeting	Des Moines, Iowa, USA www.aosaseed.com
6–9 June	ISTA Workshop on Flower Seed Testing	Roelofarendsveen, Netherlands www.seedtest.org/am12
6–9 June	ISTA Variety Workshop	Roelofarendsveen, Netherlands www.seedtest.org/workshops
11 June	ISTA Seminar "New developments and technologies in seed testing"	Venlo, Netherlands www.seedtest.org/am12
11–14 June	ISTA Annual Meeting	Venlo, Netherlands www.seedtest.org/am12
20–23 June	ASTA 129th Annual Convention	Washington, DC, USA www.amseed.org
26-28 June	ISF World Seed Congress	Rio de Janeiro, Brazil www.worldseed2012.com/
09-13 July	OECD Seed Schemes Annual Meeting	Helsinki, Finland www.mmm.fi/en/index/frontpage/Agriculture/upcomingevents.html
29-31 August	FELAS Pan-American Seed Congress	Santa Cruz de la Sierra, Bolivia
11–13 September	ISTA Workshop on Vigour Testing	Nisku, Edmonton, Canada www.seedtest.org/workshops
17–19 September	ISTA Workshop on Germination Testing	Saskatoon, Canada www.seedtest.org/workshops
14-16 October	ESA Annual Meeting	Brussels, Belgium www.euroseeds.org
17-26 October	ILAC/IAF Conference	Rio de Janeiro, Brazil
1 November	UPOV Council	Geneva, Switzerland www.upov.int
4-7 December	ASTA Corn & Sorghum and Soybean Seed Research Conference	Chicago, Illinois, USA www.amseed.org

Advertising rates 2012

Position/size	Monochrome (euros)	Colour (euros)	Dimensions (trimmed)	Dimensions of artwork (+ 3 mm bleed overall)
Outside back cover	–	2100	210 × 297 mm	216 × 303 mm
Inside front/back cover	–	1800	210 × 297 mm	216 × 303 mm
Full page	800	1200	210 × 297 mm	216 × 303 mm
⅔ page (vertical)	600	1000	133 × 297 mm	139 × 303 mm
⅓ page (vertical)	250	500	71 × 297 mm	77 × 303 mm
½ page (landscape)	400	800	210 × 148.5 mm	216 × 154.5 mm
⅓ page (landscape)	250	500	210 × 99 mm	216 × 105 mm

All rates include bleed if required.

Front of page (right-side page): +10%

For other sizes or special requests, please contact us directly.

Discounts

ISTA Members: –10%

2 ads in same issue (can be different): –5%

Repeat ad in following issue: –5%

Artwork specifications

PDF; images 300 dpi; text & line art 600 dpi; all fonts and images embedded; colour space CMYK Euroscale coated

Technical information

Circulation: 1500 copies worldwide

Inside pages: semi-matt coated 135 g/m^2

Cover pages: glossy coated 170 g/m^2

Deadlines

Publication dates: April/October

Booking advertising space: 15 February/15 August (confirmation of placement by e-mail)

Artwork delivery: 1 March/1 September

Contact details

Phone: +41 44 838 60 00

Fax: +41 44 838 60 01

E-mail: ista.office@ista.ch

Seed Testing
INTERNATIONAL

ISTA News Bulletin No. 143 April 2012
ISSN 1999-5229

International Seed Testing Association (ISTA)

ISTA Secretariat
Zürichstrasse 50
CH-8303 Bassersdorf
Switzerland

Phone: +41 44 838 60 00
Fax: +41 44 838 60 01
E-mail: ista.office@ista.ch

www.seedtest.org