

# Seed Testing

## INTERNATIONAL

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Dear Reader,

This Spring issue of Seed Testing International gives the final information and programme of the upcoming 29th ISTA Congress in Cologne, Germany. Once again, this will be a major event, with a three-day Seed Symposium on a number of important topics, with a total of 35 papers as well as poster sessions. To encourage the increase in quality of these contributions, the ISTA Seed Symposium Awards will be given to the three best papers and posters presented. You will find details of the awards procedure on page 19.

You will also find articles on certificates for sublots and performance-based germination testing, two proposals which will be discussed at the ISTA Ordinary Meeting following the Seed Symposium.

This being a Congress year for ISTA, the Ordinary Meeting will of course include the elections for the Executive Committee and Vice-President.

Being a member of the Executive Committee requires a certain amount of commitment in order to perform the required tasks. The code of conduct to be found on page 20 aims to show prospective candidates for a seat in the Executive Committee what they should expect in the case of their election. It also gives all ISTA Members an insight into the level of commitment that the Executive Committee members bring for the benefit of the Association.

I would like to take this opportunity to thank our outgoing President, Dr. Katalin Ertsey for her years of work for our Association as ECOM member, Vice-President and President, and especially for providing a voice in support of the countries of Eastern Europe and the former Soviet Union. In the past three years, several seed testing laboratories in this region have become accredited or are approaching accreditation.

Apart from the events at the Congress itself in Cologne, there will of course also be pre-Congress workshops (of which one is already full), and post-Congress tours, to give a glimpse of the seed industry in the host country, Germany, and of some of its cultural and natural attractions. Cologne itself is one of Germany's major cities, rich in history and culture, and a fitting venue for the ISTA Congress.

Next to all these articles related to the ISTA Congress and Ordinary Meeting, there is an especially interesting one on a new ergonomic system for purity testing, developed by Oregon State University. We would be happy to show you more such progress in practical seed testing, so if you have anything of this kind that you would like to share with others, please let us know.

So please enjoy this latest issue of Seed Testing International, and we hope to see as many of you as possible at the 29th ISTA Congress in Cologne.

Yours sincerely,

Michael Muschick



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# President's Report

Dr. Katalin Ertsey



Since the 27th Congress in Budapest, ISTA has held Annual Meetings to cope with the busy 21st century and the need for rapid communication. However, the three-yearly Congress still plays a significant role in the life of our Association, also this year, when the 29th Congress is being held in Europe again, in Cologne.

The organizers of the event, ISTA and the host country Germany, have put a great deal of work into the preparations.

In accordance with ISTA's aims, the Congress will take stock of the past three years, and prepare for the next, with the new and re-elected Executive Committee Members and the new Vice President, who will become President after three years. The founders of ISTA knew well that technical and scientific knowledge and practical experience are joint values, and our voting system combines these needs efficiently. The orderly election of the Executive Committee and Vice President is a guarantee for ISTA's stability and development.

The responsibility of the Designated Members is enormous; please use your votes with due consideration.

First, let us see what we achieved of the aims that we agreed upon three years ago.

At the end of February, we held an ECOM Meeting and a joint ECOM/TCOM Chair Meeting in Bonn. This in

itself was an achievement, since after the 28th Congress in Brazil we decided to have regular exchanges of experience between the TCOM chairs and the ECOM. There were several such meetings over the past three years, and the positive, open atmosphere of the February meeting with professional and strategic discussions verified that this kind of dialogue is necessary for future co-operation.

The ISTA Strategy was approved by the 2007 Ordinary Meeting. To emphasize its most important goals, method development and the validation programme continued on a high level, and culminated in Rules updates accepted by ISTA Members and all our stakeholders. The usage of ISTA Certificates was simplified, thanks to the Working Group led by Joël Léchappé, and today the Orange Certificate is the only certificate for seed lots. In the past three years, there has been quite some increase in the issuing of these certificates.

The laboratory accreditation programme is making good progress. Three years ago, we celebrated the 100th accredited laboratory; now, we can be proud of the 114th accreditation, and others are taking the first steps in the accreditation procedure.

It's an especially great pleasure for me to introduce the accreditation programme into the region which I represent within ISTA: Eastern Europe and the countries of the former Soviet Union. In the past three years there have been new accreditations of two company laboratories and the Ukrainian and Moldavian government laboratories, and Kyrgyzstan has renewed its accreditation. In the past years I had the opportunity to take part in many high-ranking consultations on this subject, and to emphasize the importance of ISTA. As far as I know, currently four laboratories from three countries in this region are nearing the end of the accreditation procedure. I hope they are successful.

There are also improvements in other areas. The importance of training is a key element in the strategy. The ISTA Seed Analyst Training Workshop, held in Zurich last

year with a large number of participants, was very successful. It was moderated by Pieter Oosterveld, our former President, who prepared a summary. This will be an ongoing project also for the next ECOM.

International co-operation works best when organizations are familiar with each other's activities, do not waste time on overlapping tasks, and use their efforts to strengthen each other for the success of their stakeholders, in our case for the seed sector. The 2nd World Seed Conference, held in Rome in September 2009, enhanced the position of the participating organizations (FAO, OECD, UPOV, ISF, ISTA). The Policy Forum and the press releases attracted the attention of breeders, seed multipliers and growers. For me, one of the most important points was in a presentation by an FAO representative: in regions of the world with a well-organized seed sector, there is no lack of food and the food system operates well. ISTA has a big role to play in supporting agriculture in the 21st century.

Of course, results from a former period never close discussions; on the contrary, they open new doors. It was also under discussion on the ECOM/TCOM Meeting in February but the agreement and acceptance of this new subject is part of the 2010 Meeting. I hope that as many current ECOM/TCOM members as possible will continue their activities in the next three-year period.

Speaking for myself, I am glad that I was working for ISTA when the economic and social challenges of the new millennium were identified, and that the Association was able to meet them with great structural and other changes. ISTA's strength comes from the collaboration of its members and I hope that this remains so in the future.

The next few weeks will be filled with the spirit of preparing for the Congress. It gives me pleasure to invite you to join us at the 29th ISTA Congress in Cologne, Germany. See you in June! ■

# The evolution of seed testing

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With the change from hunting and gathering to agricultural and animal production in the Neolithic revolution, seeds, or to be more precise, healthy seeds, have become one of the most important products for the survival of human beings. The knowledge that seeds are the parts of the plant that have the potential to produce new, healthy plants is the key to food production, food security and ultimately the survival of the population. It was obvious very early on that environmental conditions have a major influence on the successful realization of the potential of a seed to produce a healthy plant, and in all religions you will find examples of praying to God, or the gods, for favourable environmental conditions. However, it was not until the beginning of the 19th century that researchers and botanists started to study intensively the morphological characteristics of seeds, and to investigate their physiology and that of the germination process.

## The origins of seed testing

By the 19th century, the sale and trade of seed had become established in Europe. Merchants were travelling long distances from market to market to sell seed, and local farmers offered seed for sale or barter to their neighbours and at local markets. Nothing was known about the purity of the seed that was traded, nor even its potential to produce a crop (Nobbe, 1876).

In April 1869, a Saxon agronomist, the Count of Lippe-Weissenfeld, submitted several samples of grass seed, bought at the local market, for botanical identification to Prof. Dr. Johann Friedrich Nobbe, a botanist working at the Royal Academy for Foresters and Agronomists at Tharandt, Saxony, Germany (Nobbe, 1876). Surprisingly, one sample tagged “Tall Fescue” turned out to contain only 30% true seeds,

and other seed samples that were sent to the Academy for growing trials had similar shortcomings and other deficiencies.

Prof. Nobbe initiated further investigations into the quality of traded seed, and found that the situation was far from acceptable. He quickly realized that, in addition to the limited knowledge of traders and farmers regarding the identification of seed species, there was a great deal of cheating, swindling and fraud going on in the seed market. This resulted in his publication in May 1869 of an article entitled “On the Necessity for Control of the Agricultural Seed Market”.

In terms of seed quality, Prof. Nobbe considered what to measure, how to measure and when to measure. Addressing these questions, he proposed that measurements should be made of the trueness to species, the purity of seeds and the potential the seed has to produce healthy seedlings. He also came up with the revolutionary idea that these measures of quality should be assessed before the seed was sold to farmers, so that they could be sure that the seed they bought had the potential to give them a good harvest. This inspiration would not only tackle the cheating, swindling and fraud that existed in the seed market, but also give farmers an assurance that they had the necessary starting material for a successful harvest, provided that the environmental factors were reasonably favourable and the farmer applied the necessary cultivation and husbandry skills. Implementing Prof. Nobbe’s ideas was the key to an increase in overall plant production.

From a technical point of view, there were several questions:

- How can a representative sample be obtained from a lot?
- How can it be ensured that seed quality results represent the quality of the lot that has been tested?
- How can the seeds of different species be distinguished?
- How can the germination potential of different species be measured?

Finding the answers to these questions required an understanding of populations, and a detailed knowledge of the morphology of seeds and plant and seed physiology. Prof. Nobbe immediately rose to the challenge and worked out methodologies for sampling and testing (Nobbe, 1876). This was the starting point for seed testing, which consists, in effect, of measurements made to determine the potential and value of seed before it is planted in the field.

## 1869–1924: International spread of the idea and international collaboration

Nobbe’s revolutionary ideas spread rapidly around the world. By 1875, 12 seed-testing stations had already been established in Germany, Austria-Hungary, Belgium, Denmark and the United States, and 20 more were founded in the period 1876/77. By 1896, a good quarter of a century after Nobbe’s initiation, there were a total of 119 seed-testing stations in 19 different countries (Steiner and Kruse, 2006).

All of these stations were actively gathering information on the seed market, and working on species identification and the development of sampling, purity, germination and moisture methodologies for an increasing number of species. Seed health observations were also being made. It is obvious that this work involved the application of scientific principles, and a deep knowledge of plant morphology and physiology was required. This accounts for the fact that nearly all the heads of these seed-testing laboratories came from academia and had been botanists.

In 1875, a first meeting of directors of seed-testing stations took place in Graz, where experiences were shared on the development of the methodologies. It was recommended that the methods in the *Handbook of Seed Testing* by Nobbe, which would be published in 1876, should be standard use in seed-testing laboratories. A follow-up meeting took place in Hamburg in 1876. The motto “uniformity

in seed testing” was coined, and discussions were initiated on how to achieve it (Steiner and Kruse, 2006). Even today this topic remains on the agenda.

In 1906, a first conference on seed testing was held in Hamburg, Germany, and this can be viewed as the starting point for seed-testing conferences. The second conference was held in Münster/Wageningen, Germany/Netherlands, in 1910, the third in Copenhagen, Denmark, in 1921 and the fourth in Cambridge, UK, in 1924.

Since the first conference in 1906 there was a desire to work towards standards for seed testing, internationally approved methods and the uniform application of these methods. To help achieve this, the European Seed Testing Association was founded at the 1921 meeting in Copenhagen (MAF, 1925).

### 1924: the founding of the International Seed Testing Association

At the conference in 1924 in Cambridge, it was decided to enlarge the scope of the European Seed Testing Association and extend its activities to all the countries of the world in which the testing of seeds was practiced. It was also decided to re-constitute it under the name International Seed Testing Association (MAF, 1925).

Paragraph 1 of the 1924 ISTA Constitution stated:

“Under the name of the International Seed Testing Association, a union of Official Seed Testing Stations with legal domicile at the residence of its President exists for the purpose of advancing all questions connected with the testing and judgment of seeds. The Association seeks to attain this object through:

- Comparative tests and other research directed to achieving more accurate and uniform results than hitherto obtained.
- The formulation of uniform methods and uniform terms in the analysis of seeds in international trade.

The organization of international congresses attended by representatives of Official Seed Testing Stations for the purpose of mutual deliberation and information, the publication of treaties and reports on seed testing and mutual assistance in the training of technical officers.”

The first President was Mr. K Dorph Petersen from Denmark, and the Vice President Dr. Franck from the Netherlands. In addition to the office holders, there were three Executive Committee members: Prof. M.T. Munn, USA (who was also President of AOSA), Mr. W.V. Petery, Argentina, and Mr. A. Eastham, UK.

Nine Committees were established:

- Research Committee for Countries with a Temperate Climate
- Research Committee for Countries with a Warm Climate
- Provenance Determinations
- Hard Seeds and Broken Seedlings
- Moisture Content and Drying
- Investigations of Genuineness of Variety and of Plant Diseases
- Dodder Committee
- Publications and Registration
- Beet Sub-Committee

### 1931: the establishment of the International Rules for Seed Testing

The Chairman of the Research Committee for Countries with a Temperate Climate, Dr. W.J. Franck, Wageningen, Netherlands, presented the first draft of international rules for seed testing at the 5th Seed Testing Conference in Rome (ISTA, 1931). The draft was not, however, approved, owing to certain disagreements on purity tolerance and the evaluation of germination capacity.

At the 6th International Congress of Seed Testing, held in Wageningen, Netherlands, on 17 July 1931, a revised version of the International Rules for Seed Testing (ISTA Rules) was put to the vote and approved (ISTA, 1931).

These rules describe:

- Sampling
- Purity testing
- Germination
- Additional determinations:
  - Sanitary condition
  - Genuineness of variety
  - Provenance
  - Weight determinations
  - Determination of the moisture content
- Evaluation and reports
- Tolerances
- Hard seeds
- International certificates.

Since the establishment of the ISTA International Rules for Seed Testing, discussions have continued in all these different areas of seed testing, and new test concepts have been added. Existing chapters have continually been revised, modified and enhanced to increase uniformity, efficiency and effectiveness.

ISTA’s historical papers and journal publications (Proceedings of the International Seed Testing Association; renamed Seed Science and Technology in 1973) give a detailed insight into the discussions, developments and important milestones in the area of germination, seed health and purity testing (see Jensen, 2008; Klitgard, 2002; Mathur and Jorgensen, 2002). Today, the ISTA Rules are set out in 16 chapters, and sum up the findings of 140 years of worldwide research and the discussions at 28 seed-testing congresses.

### 1931: the establishment of ISTA International Certificates

With the establishment of the ISTA Rules and a uniform reporting system, a certificate that facilitated the international trade of seed was established. The 1931 Ordinary Meeting of the Association adopted two different certificates, the Orange International Seed Lot Certificate and the Blue International Seed Sample Certificate. The Orange Certificate gives results representing the average quality of a seed lot which has been sampled according to ISTA Rules. The Blue Certificate gives results that relate to the quality of the sample submitted for testing (ISTA, 1931).

### 1950: the 9th International Seed Testing Congress in Washington, DC, USA

The 8th International Seed Testing Congress took place in 1937 in Zurich, Switzerland. At this Congress, an invitation from the Association of Official Seed Analysts of North America to hold the next Congress in North America was submitted and accepted. Unfortunately, however, the war intervened and the Congress had to be postponed. After the end of the war, international connections were gradually re-established with the resumption of correspondence between the Executive Committee and other members of ISTA. The need for working towards “uniformity in

seed testing” was still obvious, and the 9th International Seed Testing Congress was held from 8–13 May 1950 in Washington, DC, USA. During this Congress, alterations to the ISTA Rules were tabled, and a new Constitution of the International Seed Testing Association was proposed, discussed and voted on (ISTA, 1951).

### 1966: introduction of seed health methods in the ISTA Rules

As early as 1907, Appel had drawn attention to the fact that information on the occurrence of seed-borne pathogens could be obtained during seed testing in the laboratory. In 1919, the seed testing station at Wageningen established a special division for studying the sanitary conditions of seeds. With the foundation of ISTA in 1924, the Committee for Investigation of Genuineness of Variety and of Plant Diseases was founded, and in 1928 a separate committee, the Plant Disease Committee (PDC). In 1928, the Chairman of the PDC suggested to the 5th ISTA Congress that information on the occurrence of certain fungi on seed samples should be reported on ISTA certificates (Mathur and Jorgensen, 2002). The Congress agreed that such information could be of advantage, but also realized that not many seed-testing stations had sufficient experience, and that before such information could be put on the Certificate, a number of comparative examinations should be undertaken to ensure that the results reported by the various stations agreed within reasonable margins.

The aim of the comparative testing programme was the establishment of internationally standardized seed health testing procedures. When selecting methods to be included in the ISTA Rules, the results of the comparative seed health tests had to be evaluated carefully in order to select methods producing uniform results among the laboratories applying them. In 1966, the first specific seed health testing methods were included in the ISTA Rules (Mathur and Jorgensen, 2002). Today, the ISTA Rules contain 21 standardized seed health testing methods, which can also be downloaded free of charge from the ISTA web site.

### 1966: introduction of the topographical tetrazolium test in the ISTA Rules

The topographical tetrazolium test is a biochemical test that may be used to make a rapid assessment of seed viability when seeds must be sown shortly after harvest, in seeds with deep dormancy, in seeds showing slow germination or in cases where a very rapid estimate of germination potential is required. Biochemical viability tests were introduced to seed testing by Hasegawa, and a report introducing the Eidmann-Hasagawa method was presented at the 1937 ISTA Congress in Zurich. In 1939, Lakon, at Hohenheim, Germany, started working in this field, and made a presentation at the 1950 ISTA Congress with the title: “Further research regarding the topographical tetrazolium test and the determination of viability”. In 1956, the ISTA Tetrazolium Committee was set up, and in 1966 the tetrazolium test was introduced as a standardized test into the ISTA Rules (Steiner, 1997).

### 1995: ISTA establishes an international accreditation standard for seed-testing laboratories

The achievement of accurate and uniform results, or in modern words, the reproducibility of results, has been an important point of discussion and consideration since seed testing was started by Prof. Nobbe in 1869.

Prof. Nobbe began comparative testing in 1877, and method validation has been a part of ISTA’s activities from its beginning. With the introduction of quality management systems, particularly those for analytical laboratories in the 1970s, quality management became a topic for discussion in seed-testing stations and at seed-testing congresses. The establishment by the OECD of the Guidelines for Good Laboratory Practice (GLP) was a starting point for this development. The aims of the GLP can be described as the traceability of analysis through documentation, the definition of responsibilities and clear, precise descriptions of the organization, and the production of accurate and reproducible results of products.

The overall development and discussion resulted in the generic standard ISO 17025 for the accreditation of all types of analytical laboratories. Nevertheless, at an early stage seed scientists realized that for seed-testing laboratories special conditions were required, and many of the requirements of ISO 17025 had already been implemented at seed-testing stations.

From 1992 to 1995, a Working Group developed the ISTA Accreditation Standard for seed-testing laboratories. This standard was approved at the Ordinary Meeting in 1995. The already existing “referee tests”, as they were called at that time, were modified, extended and adopted to become international proficiency tests, and an internationally operating accreditation department was formed at the ISTA Secretariat. This department was tasked with carrying out the required three-yearly quality assurance assessments of laboratories that had applied for ISTA accreditation. In addition, the 1995 Ordinary Meeting decided that from 2001 onwards, only ISTA-accredited laboratories could issue ISTA certificates (ISTA, 1993; ISTA, 1998).

### 2001: vigour methods

Seed vigour is the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments, and the objective of a seed vigour test is to provide information about the planting value of seed lots in a wide range of environments and/or their storage potential. Discussion on adding vigour methods to the ISTA Rules began at the 26th Congress in 1998 in Johannesburg, South Africa. However, critical voices were raised, and the proposal was withdrawn, revised and forwarded to the 27th Congress in 2001 at Angers, France. There, two vigour methods were added to the ISTA Rules – the conductivity test for *Pisum sativum* and the accelerated aging test for *Glycine max*. At the 2009 Ordinary Meeting in Zurich, the conductivity method was extended to include *Phaseolus* beans, and the controlled deterioration vigour test method was added for *Brassica* species (ISTA, 2001).



## 2004: performance-based methods for testing GM seed

With the introduction of genetically modified varieties and their commercial release in some countries, seed-testing laboratories were being faced with new challenges. Questions on the purity of GM seed lots and the adventitious presence of GM seed in non-GM seed lots were at the centre of the discussions. Since 2000, the ISTA GMO Task Force has discussed these questions intensively. For ISTA, the fundamental question was whether it would be possible to achieve international harmonization of the testing methods. It was concluded that method development in this particular area is so rapid that by the time agreement for a certain method had been achieved, it would most likely already be outdated. Furthermore, it was realized that the implementation of a standardized methodology in a laboratory could in this instance create major obstacles and produce a negative effect resulting in less accurate results. For these reasons the concept of performance-based methods was discussed, proposed and accepted for this particular area of testing. Under this approach, a laboratory may use any method it considers adequate, on condition that the laboratory provides sufficient performance data for the methodology according to clearly defined requirements. This approach received the backing of ISTA Member Governments, and today, Chapter 8 of the ISTA Rules specifies this test principle for biomolecular tests and bioassays used in testing for the presence of specified traits (ISTA, 2004).

## 2004: quality assurance programme extended to private-sector laboratories including the issuance of ISTA Certificates

At the 28th ISTA Congress in 2004 in Budapest, Hungary, a proposal was accepted that permitted private-sector laboratories to issue ISTA Certificates under the same conditions as public-sector laboratories, i.e. they must participate in the ISTA Quality Assurance Programme, successfully participate in ISTA proficiency tests and achieve ISTA accreditation. Therefore, the basis for issuing Certificates now depends on the individual performance of a laboratory rather than on its status. Strict

monitoring guarantees the performance of the laboratories (ISTA, 2004).

## Recent developments

Since the beginning of the 1990s, investments have been reduced in nearly all areas of seed technology at university level and within public seed-testing stations (Jensen, 2008). Important international training programmes at university level have ceased (e.g. the training programs at Edinburgh and Mississippi State Universities). This development must be seen as a threat to seed work in both the public and private sectors. Today, there are almost no universities offering specialized training in seed science and technology. The consequences of this development are unavoidable.

The reduction of capacities in the public sector and at large public seed-testing stations reduces activities in applied seed science. The reduction of resources means that the stations' activities are limited to the performance of simple routine control and monitoring tasks, and this reduces their ability to provide on-the-job training for seed analysts from developing countries. Furthermore, with the increasing activity of applied seed science in the private sector being used to competitive advantage in business, research results are not published, and uniformity in seed testing is threatened. This, without any doubt, may have negative implications for the international seed trade. It is recommended that governments and the public and private sectors carefully consider these developments, draw the right conclusions from them and take appropriate action to address them.

## Conclusions

Seed testing, as a concept to determine the value of seed before it is planted in the farmer's field, has spread rapidly throughout the world since its inception in 1869, and is used universally to provide farmers and legislators with information on the planting value of seed. An in-depth knowledge of plant and seed morphology, taxonomy and physiology were prerequisites for the development of seed-testing methods, and leading players in this field were scientists dealing with the wishes and needs of the seed trade and seed markets. Research and development activity in various areas

of seed science and technology has also increased rapidly throughout the world, and today's International Rules for Seed Testing are the result of the combined knowledge of 140 years of applied seed science and the essence of the discussions at 28 international seed congresses.

Quality management systems have been successfully introduced and put into practice at the global level. An evaluation of the results of this (e.g. proficiency tests and performance of accredited compared to non-accredited laboratories) demonstrates that this has been a success in optimizing the performance of laboratories and minimizing the risk of inaccurate testing.

From the founding of ISTA until around 1990, most ISTA seed laboratories received substantial financial support for both the running of their laboratories and support for international activities in ISTA and similar organizations. Due to decreasing government support and privatization of seed-testing services, the voluntary work within ISTA's Technical Committees, as well as the transparent sharing of recent research results, has become more and more limited. This lack of clarity of responsibility between the public and private sectors and the reduction of resources, as well as the use by some companies of recent research work as a competitive advantage, is seen as a threat and the biggest challenge to successful continuation of evolution in seed testing. Policy makers, the seed industry and farmers should keep this in mind.

It is obvious that the evolution of seed-testing methods is far from finished:

Continual improvements and research are necessary to increase the efficiency and effectiveness of seed testing and provide the tests needed to meet the changing needs of the market.

Quality assurance management needs to be further developed to minimize the risks and generate customer confidence.

DNA technology will lead to in progress and new needs and challenges for seed testing.

The evolution of seed testing must continue.

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The ISTA Secretariat at Bassersdorf, Switzerland, has a vacancy for a

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**Zurichstrasse 50**  
**8303 Bassersdorf**  
**Switzerland.**

Applications must be received by midnight, Friday 30 July 2010.

# Redesigning a purity testing system: development of an ergonomic, high-vision, continuous-flow seed inspection system

Adriel Garay, Sherry Hanning and Sabry Elias

Oregon State University  
Seed Laboratory  
Corvallis, OR 97331-3002, USA  
adriel.garay@oregonstate.edu

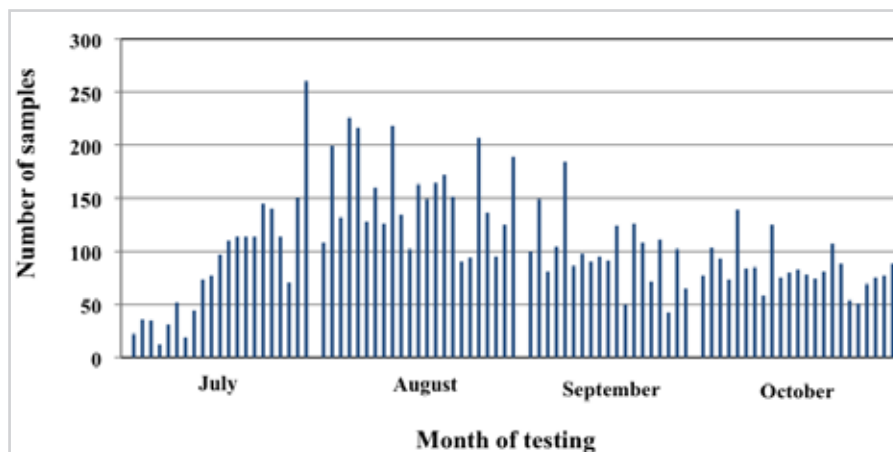
## The need and the opportunity

The changes in the seed industry require innovations in seed testing systems. The grass seed industry in Oregon, USA, has changed by quantum leaps to respond to the faster pace of the modern global grass seed industry, yet the purity testing system in general had not changed for many decades. Obviously, dramatic innovation to improve purity testing was needed.

Oregon is the home of a very modern and dynamic grass seed industry. In order to respond to market demands, grass seed has to be harvested, cleaned, tested, labeled and shipped to other states and countries around the world within a very short time. Thus, there is a strong seasonal surge in purity test requests (Fig. 1), and any delay in delivering purity testing results is a problem for seed growers, cleaners, dealers and users. The OSU Seed Laboratory found itself needing to respond to the stepped-up time demands of its customers.

If a grower could not deliver his product to the market in a timely manner, he could lose the sale of the seed to another. This meant that a faster, more effective and efficient system was urgently needed. Hiring a large number of temporary assistants and implementing extended hours of expensive overtime with the regular staff was not solving the problem and was seriously straining the budget.

Purity inspection depends on analysts painstakingly inspecting samples to distinguish and identify seeds correctly. In this regard, grass seeds present more challenges than larger seeds. For example, most other seeds (crop or weed) found in a grass seed sample typically belong to the same grass family, *Poaceae*. This is, in part, because selective herbicides cannot effectively control many grass species in the field, and



**Figure 1.** Number of samples that required a purity test in the busy season of 2007 at the OSU seed laboratory. The daily test requests can be higher in strong market years.

seed cleaners cannot separate other seeds of similar size and shape. Yet, any seed contaminant in a sample still needs to be distinguished and identified correctly based on fine morphological features, all of which require high-quality vision to make a correct determination of seed type. These factors indicated clearly that the purity testing system needed urgent innovations.

The following describes how the new system was conceived, the innovation process and the results achieved.

## Limitations of the conventional purity board system

The conventional purity board (Fig. 2) has not changed since purity testing began over 100 years ago. Anyone who has tested the purity of small seeds is familiar with this system, where the analyst is expected to carry out the work in a hunched-over working position, holding a hand lens in one hand and forceps or a slide in the other to move the seeds. If analysts are examining very small seeds, they are even afraid of breathing normally for fear of blowing the small seeds away.

Obviously, the lack of comfort and the equipment and tools used by the analysts had to be a limiting factor for productivity.

Another limitation in the system was the lack of preliminary preparations of the working samples. For example, samples that had abundant inert material had to be separated manually. The task of separating such particles one by one was not just difficult, but was limiting productivity, especially if the sample had a high proportion of fine, lightweight and small particles.

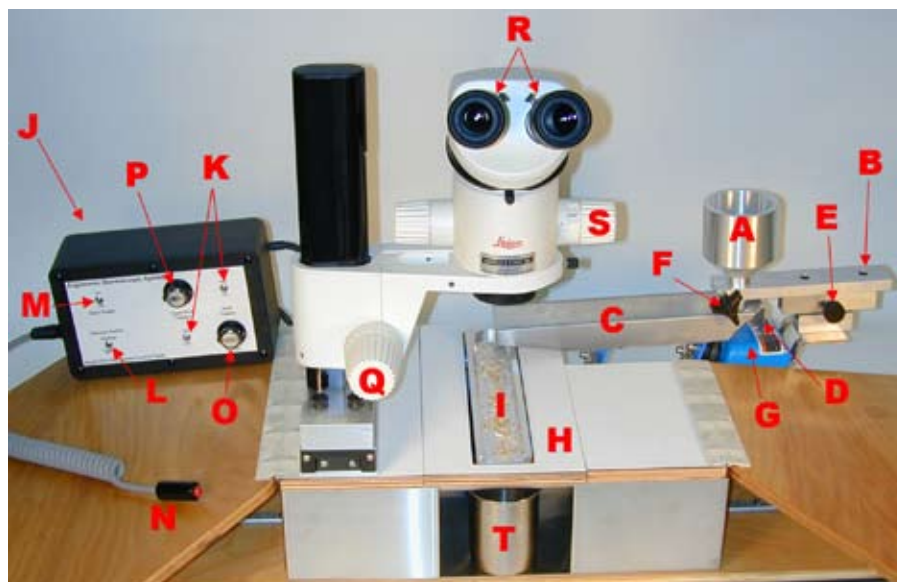
The testimonies from analysts that have experienced these situations may explain some of the difficulties and hardships



**Figure 2.** A photo from the middle of the last century from the OSU Seed Laboratory archive, showing the hunched working position of a seed analyst using a hand lens and forceps, which often caused discomfort and affected productivity.

presented by the conventional system: Gary Girdler, a purity analyst at OSU Seed Lab, recalled: "It used to be a rare day when I would go home after a shift bent over the purity board, without an ache in my back and one eye watering profusely from being stuck in a 7x hand lens for 8–10+ hours and the other eye almost functionless from focusing at nothing." Sherry Hanning, the supervisor of the purity-testing unit at OSU Seed Laboratory, adds: "Prior to the new system, many analysts in our laboratory had health problems caused by many years in the 'hunched' position required to conduct purity examinations. Several had back problems, and some required surgery to allow them to continue their seed testing careers. We had a larger staff, worked more hours and still got backlogged."

After examining the needs of the industry, the weaknesses of the conventional system and the slow flow of grass seed testing, the OSU Seed Laboratory concluded that a better system had to be developed in order to respond to industry needs. This new system also had to be faster and more cost effective.



**Figure 3.** Ergo Vision System (EVS) with microscope, developed by the OSU Seed Laboratory and Mater International.

A Funnel (feeds the sample). B Funnel holder plate. C Feeder tray. D Back plate of bulk feeder tray. E Funnel adjustment knob. F Funnel clamp knob. G Feeder vibrator. H Removable cover. I Inspection tray. J Feeder control panel. K Feeder switches. L Remote switch. M Main power switch. N Hand switch. O Bulk speed dial. P Inspection speed dial. Q Main focus knob. R Eyepiece focus. S Magnification setting. T Collection cup.

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

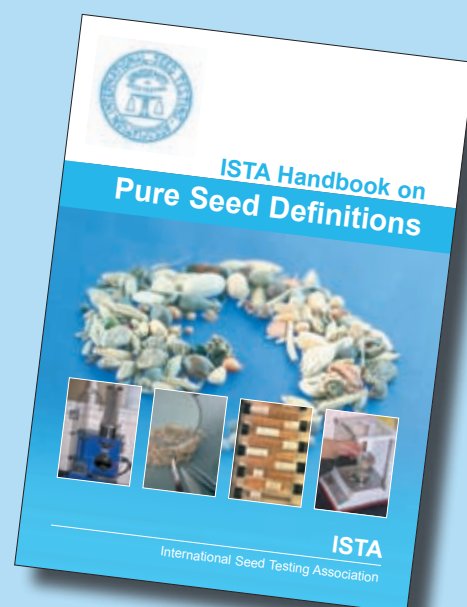
By the ISTA Purity Committee;  
editors M.R. Mannino, J. Taylor & S. Jones

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

A comprehensive glossary of scientific terms applying to seed purity is also included.

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ISTA Secretariat (for contact details, see back cover)

**Coming soon**





## The new Ergo Vision System

The new system was conceived as an integrated approach with two major components: first, the equipment with all the essential components needed to enhance analyst performance, and second, a procedure for preliminary preparation of samples by others to further enhance analyst performance. It was hoped that the equipment would allow the analysts to work more comfortably, differentiate particles clearly and correctly and achieve higher productivity. The preliminary preparations (blowing, screening, filtrations, etc.) would reduce the need to remove most particles by hand.

The OSU Seed Laboratory management contacted Dr. Jean Mater from Mater International of Corvallis, Oregon, a longtime supplier of tabletop seed-testing equipment. In turn, they partnered with Bradford Whiting of OEM, Inc. of Corvallis, Oregon, a manufacturing engineer of laboratory equipment. The team, which

included key OSU Seed Laboratory members and outside private experts, focused on assessing the flow of the seed purity inspection process. By applying a systems approach, the team identified multiple opportunities for process improvement.

After three years of incremental improvements, the team achieved the new Ergo Vision System or EVS. Figure 3 shows the system that is popular at the OSU Seed Laboratory. By design, the system is a flexible, modular system that integrates ergonomics, continuous seed flow, a choice of optical systems, precise feeder controls, interchangeable inspection trays and seed hoppers, and a hand or foot switch to stop and start the vibratory feeders. In addition, the whole microscope mount can be adjusted back and forth, and the eye piece can be adjusted to the needs of the operator. In one case, the optical system was mounted on the opposite side to accommodate a left-handed analyst. Other users may tailor the system to fit their needs and accommodate their physical characteristics

or applications. Video systems can be incorporated and are used by the lab for group teaching and demonstrations. In essence, the concept with the new system is to have the equipment, with all its essential components, fit the needs and potentials of each individual analyst.

## Operation of the Ergo Vision System

The procedure includes the following steps:

1. The working sample is placed in the sample holding funnel. Funnels and inspection trays of different sizes can be used to accommodate seeds of different sizes. The seed flows from the funnel to trays that are calibrated to the desired level.
2. The feeder tray moves the seeds to the inspection tray where the seeds are inspected. The speed of seed flow can be controlled by adjusting the vibration of the seed tray as desired by the analyst. The inspection trays are designed to spread the seeds uniformly. They are interchangeable, so that very small seeds such as bentgrass (*Agrostis* spp.) to large seeded species such as wheat stay within the field of view.
3. The seeds are examined using a high-quality microscope, Mantis Inspection Viewer or video camera. The magnification can be adjusted at will, depending on the kind of seed and the kind of contaminants being inspected.
4. The image clarity can be enhanced by fiber optic or LED lighting (not shown in Fig. 3) directed to the viewing area.
5. The flow of seed can be stopped at any time to make a closer examination of any object and to separate the contaminants from the sample.
6. The inspected seeds are automatically deposited from the inspection tray into the sample holding cup in the front of the inspection station.



**Figure 4.** The Ergo Vision System being used. The analyst sits in an ergonomically correct position and uses both eyes at optimum magnification, the seeds flow continuously and the process can be started and stopped any time to remove contaminants.



Once analysts use an Ergo Vision System inspection station, they can see the twin benefits of increased comfort and productivity. With the EVS, a long day of purity analysis is no longer as physically demanding. A survey conducted in the purity section of the laboratory provided the following testimonies:

Hortencia Borrero, purity analyst: “The EVS has been a good change in comparison with the work board because it has helped me focus better on the seeds, it is easy to clean and I can regulate the speed to fit my needs.”

Denise Goughner, purity analyst: “The EVS allows a person to sit properly. It allows me to use better magnification at my desk/work area. I can go faster because I can see better.”

Laura Youravish, purity analyst: “I like my EVS because it allows me to sit up straight and see the seeds at a high magnification. The lights we use with the EVS are better and help me see more clearly.”

Kimberly Diamond, purity analyst: “The EVS has greatly increased my production, which is valuable to the seed laboratory. I love my EVS because I can see every detail of every seed and work longer hours without neck and back discomfort.”

Scott Westen, purity analyst: “The main reason I like the EVS is because it provides relative comfort for long periods of time. I get to be in a normal seated position and the seeds come into my view on the vibrating tray, at the speed I choose. Since I am left-handed, my microscope was mounted on my right side so I can operate freely with my left hand.”

Chenhui Ho, purity analyst: “The EVS alleviates much shoulder and neck pain and this comfort allows me to increase my productivity.”

Mary Grey, purity analyst: “Why do I like the EVS better than the workboard? I can see the seeds more clearly with more comfort and have increased my speed and accuracy.”



**Figure 5.** A new application of the Ergo Vision System is the determination of seeds in the soil-seed bank tests at OSU Seed Laboratory. Due to the preliminary preparation of the sample, notice that seeds are clearly distinguishable and the rest of the sample does not look like soil any more.

Gary Girdler, purity analyst: “The EVS allows me a comfortable upright seating position, binocular vision, adjustable magnification and a better three-dimensional view of the seeds. The increase in physical comfort and visual acuity has made possible a significant increase in my daily productivity.”

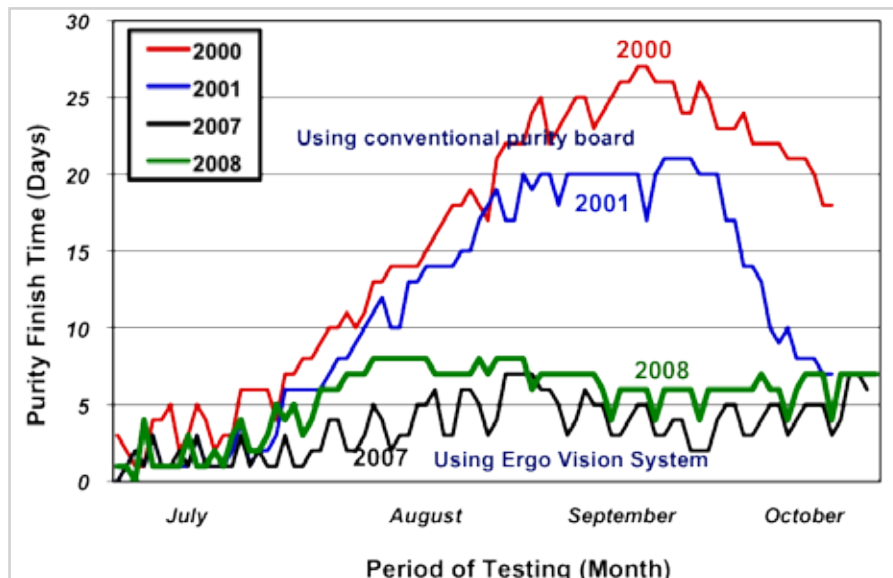
Sherry Hanning, the unit supervisor, who oversees the proficiency, accuracy and productivity in the unit, summarizes as follows: “Before we had this system, during the peak testing time, we used to work extended hours most weekdays and even weekends to process samples. Since we switched to the new system, I have seen the increase in accuracy and productivity due to the EVS. Our analysts are much happier and are able to enjoy the summer evenings and weekends with their families.”

## Current applications

The EVS was developed with features that can allow a broad range of applications. These features, combined with better preparation of the working sample before it is submitted to the analyst, have allowed the EVS to be used for many exams. Some successful examples at the OSU Seed Laboratory are:

- a) The working samples for purity separations are presented already blown, which removes lightweight inert material. This allows the analyst to examine the lightweight material separately from the heavier particles. Presenting particles in a discriminated manner by particle size (rather than completely mixed) allows more detailed inspection of each fraction and reduces the need for removing some particles manually by the analyst.

- b) The working sample for the bulk exam for noxious weed seeds (AOSA), or for other seed determination (ISTA) or sod quality exams (Oregon), is presented after precise screening. This makes it possible to examine each particle size fraction separately at optimum magnification at all times (Fig. 4).
- c) Soil seed-bank tests had always been difficult because seeds that are covered by soil are difficult to differentiate from other particles. To simplify the process, the soil sample goes through a filtration process to eliminate clay particles, followed by drying and screening. With this process, all sand particles, plant material and seeds become obvious and can be found and identified (Fig. 5).



**Figure 6.** Comparison of purity finish time using the conventional purity board (2000–01) and the new system (2008–09) during the busy grass testing season in Oregon.

In addition to seed identification, the EVS may be used for identifying other particles. For example, with the appropriate magnifying lens, it has been used for identifying different pollen grains. In theory, larva of different insects, minerals with different features, computer chips of different shapes, etc. may be identified and separated as long as there are proper descriptors for each type of particle. Our experience has demonstrated that with proper adaptations and validations it may be used for examinations that had not been possible before at such high production speed.

All of the above would be of academic value if the innovation did not have any impact on the service quality in terms of speed. The estimation at OSU Seed Laboratory has shown that, depending on the analyst, the use of the EVS has resulted in an improvement in efficiency of about 20–30%. Data collected through the years, for the peak testing season (August–October), demonstrate that the turn-around time has been improved (Fig. 6). For almost

a decade, previous to 2001, the delay in purity testing used to be more than 15–20 days. This situation made many customers very unhappy. Since the transition to the new system, around 2002–04, the purity testing results are reported consistently in less than a week, well within customer expectations.

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 info@gta-sensorik.com www.gta-sensorik.com

## Conclusions

The experiences of the analysts and the results achieved and presented in this article lead to the following conclusions:

The productivity of the analysts can be limited by the lack of an adequate system (including equipment, tools, sample preparation, proficiency, comfort, etc.) to carry out purity testing. This was clearly demonstrated in the conventional system, where the analyst was simply equipped with a purity board and a hand lens. Using such a system and despite the use of overtime and a large staff, large backlog situations were experienced.

An improved system, consisting of proper sample preparation and the use of an Ergo Vision System, can enhance the performance of each analyst and as a result the service capacity of the whole laboratory. This is demonstrated by the fact that after switching to the new system, the lab

has significantly reduced the waiting time for purity test results.

Improved systems for purity work, such as the one described, produce other important benefits: reduced health concerns, improved morale, reduction of absences, increased learning speed and greater proficiency.

In addition to accuracy and speed of services, one of the constant worries in a seed-testing laboratory, like in any other service business, is cost control. In the experience being described, cost control was derived from reduced temporary staff, reduced regular staff, reduction of paid overtime work, and higher proficiency and productivity of regular staff.

This overall development has been possible thanks to the contributions of a team (listed below) that worked on the design, development and evaluation of the Ergo Vision System. In addition, all the purity staff in the laboratory worked in the daily applications of the new system. It is worth

noting that we do not promote a certain company, but we promote the technologies aiming to advance purity testing in general.

## The team

Adriel Garay, Ph.D.

OSU Seed Lab Manager

Sabry Elias, Ph.D.

OSU Seed Lab Seed Scientist

Sherry Hanning, CSA-P

OSU Seed Lab Purity Supervisor

Richard Triplett, CSA-P

OSU Seed Lab Purity Analyst

Jean Mater, Ph.D. (deceased)

Mater International

Bradford Whiting, P.E.

OEM, Inc. ■



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# 29<sup>th</sup> ISTA Congress Cologne

# 2010



## Overview

Tues–Sun Pre-Congress workshops  
8–13 June (see page 42)

Tuesday Registration and welcome cocktail  
15 June

Wed–Thurs ISTA Seed Symposium  
16–17 June (see pages 15 and 22)

Friday ISTA Seed Symposium  
18 June Official Dinner

Sat–Sun Presentation of ISTA's technical work  
19–20 June (see page 17)

Monday Policy Forum: Harmonized seed testing  
21 June and global seed trade  
Reception hosted by the BMELV

Tuesday ISTA Ordinary Meeting with election of  
22 June Executive Committee (see page 18)

Wed–Fri Post-Congress tours to Bavaria, Baden-  
23–25 June Württemberg and Thuringia (see page 24)



# Final programme

## Tuesday 15 June 2010

14:00–20:00 Registration desk open

18:30–21:00 **Welcome Reception in the foyer of the Gürzenich**

## Wednesday 16 June 2010

### Seed Symposium day 1

07:00–18:00 Registration desk open

#### 08:30–09:40 **Opening Ceremony**

- Official Address by Dr. Robert Kloos, State Secretary of the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV)
- Official Address by Eckhard Uhlenberg, Minister for the Environment and Conservation, Agriculture and Consumer Protection of the State of North Rhine-Westphalia
- Opening of the ISTA Congress 2010 by the ISTA President, Dr. Katalin Ertsey
- Welcoming Address by the ISTA Secretary General, Dr. Michael Muschick

09:40–10:00 **The Seed Industry in Germany**  
Presentation by Dr. Hermann Freudenstein, Federal Plant Variety Office

10:00–10:30 Coffee break

10:30–10:40 **Opening of the Seed Symposium by the Symposium Convenor, Dr. Alison Powell**

10:40–12:30 **Session 1: Technologies for improved seed supply**

Keynote paper **Seed-led technology for better crop yield**  
Pramod K. Agrawal, Prasha Agri Consultants, India (Session Chair and Lead Speaker)

Paper 1 **Seed health in spinach seed by multispectral imaging**  
Merete Halkjær Olesen, Jens Michael Carstensen and Birte Boelt, University of Aarhus, Denmark

Paper 2 **Detection of three fungal pathogens infecting *Linum* seeds by one single method**  
Isabelle Serandat, Clement Poisplaud, Ronit Cohen, Quentin Brunelle and Valerie Grimault, GEVES-SNES, France

Paper 3 **Survey of the infestation of dwarf bunt (*Tilletia controversa*) and common bunt (*Tilletia caries*) of wheat on seeds and in the soil**

Markus Dressler, Benno Voit and Berta Killermann, Institut für Pflanzenbau und Pflanzenzüchtung, Germany

Paper 4 **The use of film coating on treated corn seeds**  
Suemar Alexandre Gonçalves Avelar, Fabianne Valéria de Sousa, Guilherme Fiss, Leopoldo Baudet and Silmar Teichert Peske, Federal University of Pelotas, Brazil

12:30–14:00 Lunch break

14:00–14:40 **Session 1 (continued)**

Paper 5 **Inert dusts better alternatives to manage Angoumois grain moth *Sitotroga cerealella* in stored rice**  
Mandali Rajasri, P. Sambasiva Rao and K.V.S Meena Kumari, Acharya N.G. Ranga Agricultural University, India

Paper 6 **Sustainable seed supply for orchid horticulture and conservation**

Hugh W. Pritchard, Philip T. Seaton, Y. Morales, N. Neto, P. León, P. Novoa, Hu Hong, H. Perner, J. Orejuela, J. Warner, Y. Yunelis Perez, J. Romero, C. Jijon, E. Sánchez, M. Plamieri, D. Puspitaningtyas, L. Pateña, Yam Tim Wing, K. Thammasiri, and Duong Tan Nhut, Royal Botanic Gardens, Kew, UK

14:40–15:30 **Poster session 1**

15:30–16:00 Coffee break

16:00–17:30 **Poster session 1 (continued)**

## Thursday 17 June 2010

### Seed Symposium day 2

08:30–10:00 **Session 2: Aspects of purity: genetic, technical and physical**

Keynote paper **Aspects of purity: genetic, technical and physical**  
Enrico Noli, University of Bologna, Italy (Session Chair and Lead Speaker)

Paper 1 **Assessment of mechanical damage in sweet corn seed by image analysis**  
Francisco Guillhien Gomes Jr. and Silvio Moure Cicero, University of São Paulo, Luiz de Queiroz College of Agriculture, Brazil

- Paper 2 **Image analysis of moving seeds in an indented cylinder**  
Ole Buus and Johannes Ravn Jørgensen, Aarhus University, Denmark
- Paper 3 **Free falling image acquisition system for Other Seed Determination of sunflower seed samples**  
Vincent Muracciole, Dominique Bertrand, Patrick Plainchault and Maria Rosaria Mannino, GEVES-SNES, France
- 10:00–10:30 Coffee break
- 10:30–11:30 **Session 2 (continued)**
- Paper 4 **Development of an ISTA DNA-based approach for testing variety identity**  
Ana Laura Vicario, E. Casarini, D. Perry, D. Zhang, C. Dollard and K. Hwu, Laboratorio Central de Análisis de Semillas, Argentina
- Paper 5 **Bonafide BDI™ – Pure PRG, a novel DNA-based diagnostic test that detects annual ryegrass contamination in perennial ryegrass seeds**  
Pegadaraju Venkatramana, Quentin Schultz and Benjamin Kaufman, BioDiagnostics Inc, USA
- Paper 6 **Estimate of the gene flow among transgenic and non-transgenic cultivars of soybeans**  
Antonio Carlos Albuquerque Barros, Silmar Teichert Peske, Gaspar Malone, Otavio Luis Mendes Leven and Lilian Madruga de Tunes, Federal University of Pelotas, Brazil
- 11:30–12:30 **Poster session 2**
- 12:30–14:00 Lunch break
- 14:00–15:00 **Poster session 2 (continued)**
- 15:00–15:30 **Session 3: Basic approaches to physiological processes in seeds (ISSS collaborative session)**
- Keynote paper **Comparative seed biology will lead the way: evolutionary conservation and biodiversity of physiological mechanisms that control germination**  
Gerhard Leubner, University of Freiburg, Germany (Session Chair and Lead Speaker)
- 15:30–16:00 Coffee break
- 16:00–17:30 **Session 3 (continued)**
- Paper 1 **Seed conservation in ex situ gene banks; genetical and physiological backgrounds for viability loss in wheat and barley after storage**  
Manuela Nagel, Ilse Kranner and Andreas Börner, IPK Gatersleben, Germany
- Paper 2 **Natural modifiers of seed longevity in the *Arabidopsis* mutants *abi3-5* and *lec1-3***  
Matteo Sugliani, Loïc Rajjou, Emile Clerckx, Maarten Koornneef and Wim Soppe, Max Planck Institute for Plant Breeding Research, Germany

- Paper 3 **The combined use of *Arabidopsis thaliana* and *Lepidium sativum* to find conserved mechanisms of seed germination within the *Brassicaceae* family**  
Ada Linkies, Kerstin Müller, Karl Morris, Kai Gräber, Stefanie Tintelnot, William Finch-Savage and Gerhard Leubner-Metzger, University of Freiburg, Germany

## Friday 18 June 2010

### Seed Symposium day 3

- 09:00–17:00 Registration desk open
- 08:30–09:30 **Session 3 (continued)**
- Paper 4 **Water activity measurement: demonstration of a single and non-specific optimal storage value for orthodox forest seeds**  
Fabienne Colas, Patrick Baldet and Michèle Bettez, Cemagref, France
- Paper 5 **Storability of ultra-dry wheat, rape and onion seeds**  
Michael Kruse and Yang Chi, University of Hohenheim, Germany
- Paper 6 **Seed storage and dormancy in *Myosotidium hortensia***  
Craig McGill, Myoung Joo Park, Jayanthi Nadarajan, Warren Williams and Bruce MacKay, Massey University, New Zealand
- 09:30–10:00 **Session 4: Approaches to the evaluation and improvement of germination**
- Keynote paper **The seed germination tests: ubiquitous and up-to-date tests over the years? Influence of external factors such as quality assurance and progress in research on the stability and the evolution of the tests**  
Joël Léchappé, GEVES-SNES, France (Session Chair and Lead Speaker)
- 10:00–10:30 Coffee break
- 10:30–12:30 **Session 4 (continued)**
- Paper 1 **Using climate data to predict optimum conditions for seed germination and dormancy breaking pre-treatments**  
Lindsay Robb, Robin Probert, John Dickie, Kenwin Liu and Fiona Hay, Royal Botanic Gardens, Kew, UK
- Paper 2 **Effectiveness of seed awn removal through chemical scarification in hybrids of *Pennisetum purpureum* × *P. glaucum* (elephant grass × pearl millet) and *Andropogon gayanus* (gamba grass)**  
R. Usberti and J. A. Usberti, Plant Protection Agency, Brazil

- Paper 3 **Optimal conditions for lettuce seed germination test**  
Sylvie Ducournau, Pierre Soufflet, Joël Léchappé and Angelo Vianello, GEVES-SNES, France
- Paper 4 **Rate of physiological germination relates to the percentage of normal seedlings in standard germination tests of naturally aged seed lots of oilseed rape**  
Mohammad Khajeh-Hosseini, Maryam Nasehzadeh and Stan Matthews, Ferdowsi University of Mashhad, Iran
- Paper 5 **Electrical conductivity could have a role in predicting germination and vigour in *Brassica* spp.**  
Zohair Mirdad and Alison A. Powell, University of Aberdeen, UK
- Paper 6 **Germination in recalcitrant seeds of Holm oak (*Quercus ilex* L.): effect of storage conditions**  
Sergio Pasquini, Elisa Petrusa, Enrico Braidot and Angelo Vianello, University of Udine, Italy
- 12:30–14:00 Lunch break
- 14:00–15:30 **Session 5: Assessment and improvement of seed performance in practice**
- Keynote paper **Seed vigour: from one hypothesis to many predictions and uses**  
Stan Matthews, United Kingdom (Session Chair and Lead Speaker)
- Paper 1 **Computer vision for monitoring seed germination from dry state till young seedlings**  
Marie-Hélène Wagner, Didier Demilly, Sylvie Ducournau, Carolyne Dürr and Joël Léchappé, GEVES-SNES, France
- Paper 2 **Development of a non-destructive germination test by measuring seed oxygen consumption**  
Sebastian Bopper and Michael Kruse, University of Hohenheim, Germany
- Paper 3 **A fast ethanol assay for seed vigour**  
Steven P.C. Groot, Jan Kodde, Corine de Groot and Wayne D. Buckley, Plant Research International BV, Netherlands
- 15:30–16:00 Coffee break
- 16:00–17:00 **Session 5 (continued)**
- Paper 4 **Hydration treatment improves the performance of low vigour seed lots of pepper through metabolic repair**  
Ibrahim Demir, Kazım Mavi, Burcu Begüm Kenanoğlu and Tuba Çelikkol, Ankara University, Turkey
- Paper 5 **Effect of pre-sowing and invigoration treatment for better crop establishment of mungbean**  
B. Gopal Reddy, P. Sambasiva Rao, M. Sreedhar, K.V. Radha Krishna and S. Kavitha  
Acharya N.G. Ranga Agricultural University, India
- Paper 6 **Changes in water sorption properties after priming increases the longevity of *Rhododendron griersonianum* seed**  
Ian P. Wood, Fiona R. Hay and Rosemary J. Newton, Royal Botanic Gardens, Kew, UK
- 17:00–17:30 **Conclusion of the Symposium**
- 19:00 **Official Dinner at the Tanzbrunnen Köln**
- Saturday, 19 June**
- Presentation of ISTA's technical work**
- 07:00–18:00 Registration desk open
- 08:00–09:00 Bulking & Sampling Committee  
Chair: Leena Pietilä, Finland
- 09:00–10:00 Flower Seed Committee  
Chair: Zita Ripka, Hungary
- 10:00–10:30 Coffee break
- 10:30–11:30 Forest Tree & Shrub Seed Committee  
Chair: Zdenka Procházková, Czech Republic
- 11:30–12:30 Germination Committee  
Chair: Ronald Don, United Kingdom
- 12:30–13:30 Lunch break
- 13:30–14:30 Moisture Committee  
Chair: Craig R. McGill, New Zealand
- 14:30–15:30 Statistics Committee  
Chair: Jean-Louis Laffont, France
- 15:30–16:00 Coffee break
- 16:00–17:00 Variety Committee  
Chair: Berta Killermann, Germany
- 17:00–18:30 GMO Task Force  
Chair: Christoph Haldemann, Switzerland
- Sunday, 20 June**
- Presentation of ISTA's technical work**
- 08:00–09:00 Purity Committee  
Chair: Maria Rosaria Mannino, France
- 09:00–10:00 Seed Health Committee  
Chair: Theresia A.S. Aveling, South Africa
- 10:00–10:30 Coffee break
- 10:30–11:30 Seed Storage Committee  
Chair: Hugh W. Pritchard, United Kingdom
- 11:30–12:30 Tetrazolium Committee  
Chair: Stefanie Krämer, Germany
- 12:30–13:30 Lunch break
- 13:30–14:30 Seed Vigour Committee  
Chair: Alison A. Powell, United Kingdom

- 14:30–15:00 Nomenclature Committee  
Chair: John H. Wiersema, USA
- 15:00–15:30 Editorial Board (Seed Science and Technology)  
Chair: Anne Bülow-Olsen, Denmark
- 15:30–16:00 Coffee break
- 16:00–18:30 Rules Committee  
Chair: Steve Jones, Canada

## Monday, 21 June

- 08:00–08:30 Advanced Technologies Committee  
Chair: Johan van Asbrouck, Thailand
- 08:30–09:00 Seed Analyst Training Committee  
Chair: John Hampton, New Zealand
- 09:00–10:00 Laboratory Accreditation & Quality Assurance Programme  
Günter Müller, Germany, ISTA Proficiency Test Committee Chair and Rasha El Khadem, ISTA Secretariat
- 10:00–10:30 Coffee break
- 10:30–13:00 Discussion on the Draft ISTA Strategy 2010–2013
- 13:00–14:00 Lunch break
- 14:00–15:30 **Policy Forum: Harmonized seed testing and global seed trade**
- 15:30–16:00 Coffee break
- 16:00–17:30 **Policy Forum: Harmonized seed testing and global seed trade (continued)**
- 18:00–21:00 **Reception hosted by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV)**

## Tuesday, 22 June

- 08:30–17:30 **ISTA Ordinary Meeting**
- 08:30–08:45 **Welcome by the ISTA President Dr. Katalin Ertsey**
- 08:45–10:00
1. Call to order
  2. President's address
  3. Roll call of Designated Members entitled to vote
  4. Reading and acceptance of Minutes
  5. Report of the Executive Committee
  6. Report of the Secretary General
- 10:00–10:30 Coffee break
- 10:30–12:30
7. ISTA Strategy 2010–2013
  8. Election of Officers & Members-at-large of the Executive Committee
  9. Constitution changes
  10. Fixation of the Annual Subscriptions
- 12:30–13:30 Lunch break
- 13:30–15:30
11. Consideration and adoption of the proposed Rules Changes
  12. Consideration and adoption of reports
  13. Announcement of the place and date of the next Ordinary Meeting of the Association
  14. Any other business raised by a Member, of which notice in writing has been received by the Secretary General two months prior to the date of the meeting
  15. Any other business raised by consent of the Executive Committee
- 15:30–16:00 Coffee break
- 16:00–17:30
16. Discharge of the Executive Committee
  17. Installation of new Officers and Members-at-Large
  18. President's closing address
  19. Adjournment

## Preparatory documents for the Ordinary Meeting

The following documents have been endorsed by the ISTA Executive Committee to be submitted to the ISTA Ordinary Meeting 2010 for acceptance by the nominated ISTA Designated Members voting on behalf of their respective Governments:

- 01-2010-OM Agenda of the Ordinary Meeting 2010 (voting document)
- 02-2010-OM Draft Minutes of the Ordinary Meeting 2009 (voting document)
- 03-2010-OM Activity Report 2009 of the ISTA Committees (voting document)
- 04-2010-OM Proposal for the Membership Fees 2011 (voting document)
- 05-2010-OM Proposed Changes to the ISTA International Rules for Seed Testing 2011 Edition (voting document)
- 06-2010-OM Method Validation Reports on Proposed Changes to the ISTA International Rules for Seed Testing 2011 Edition (supporting document)
- 07-2010-OM Constitution Change Proposals 2010 (voting document)
- 08-2010-OM Proposal from the Netherlands to allow issuing of multiple certificates of the same status and value for one seed lot (discussion document)
- 09-2010-OM Review of the ISTA Strategy – Compilation of Completed Questionnaires (supporting document)
- 10-2010-OM Draft ISTA Strategy 2013 (voting document)

The documents have been posted on the ISTA web site at [www.seedtest.org/OM2010](http://www.seedtest.org/OM2010).



# The Seed Symposium Awards

Alison A. Powell

ISTA Executive Committee Member, ISTA Seed Vigour Committee Chair and Seed Symposium Convenor

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There have been papers of high quality in both the oral and poster sessions of the ISTA Symposium over many years. This is recognised by ISTA in the presentation of awards for the three best oral papers and three best poster papers at each Symposium. The papers are assessed by two different Awards Committees (Table 1), drawn from people having experience in the presentation of both forms of paper. In

**Table 1.** Members of the Awards Committees for oral and poster papers

## Oral Paper Awards Committee

Anne Bulow-Olsen (Chair) (Denmark)  
Guro Brodal (Norway)  
Valerie Cockerell (United Kingdom)  
Fiona Hay (Philippines)  
Kae-Kang Hwo (Taiwan)  
Berta Killerman (Germany)  
Norbert Leist (Germany)  
Charlotte Leonhardt (Austria)  
Augusto Martinelli (Argentina)  
Craig McGill (New Zealand)  
Jose França Neto (Brazil)  
Silmar Peske (Brazil)  
Kirk Remund (United States)  
Marie-Hélène Wagner (France)

## Poster Paper Awards Committee

Sylvie Ducournau (Chair) (France)  
Terry Aveling (South Africa)  
Malavika Dadlani (India)  
Cheryl Dollard (Canada)  
Julio Marcos Filho (Brazil)  
Stefanie Krämer (Germany)  
Francisco Kryzanowski (Brazil)  
Andrea Jonitz (Germany)  
Zita Ripka (Hungary)  
Masatoshi Sato (Japan)  
Anny van Pijlen (Netherlands)  
Ana Laura Vicario (Argentina)

Cologne, the Oral Paper Awards Committee will be chaired by Anne Bulow-Olsen (Denmark) and the Poster Awards Committee by Sylvie Ducournau (France).

The committees base their assessment of the papers on previously agreed criteria. The authors of both forms of paper are informed of the criteria before the symposium and preparation of their paper. The criteria are as follows:

**Oral papers:** The assessment of oral papers is considered under four headings (Table 2), namely, material in the paper, presentation of the paper, the visual aids used and the overall impact made by the paper. Each aspect of the paper is given

a score by the members of the Awards Committee, who discuss the scores awarded to the different papers before making their final decision. Presenters of oral papers who have received awards at previous symposia are not eligible to win again.

**Poster papers:** Poster papers are also assessed under various headings (Table 3) and marks assigned to the various aspects by the Awards Committee.

The awards will be presented by the ISTA President at the Congress Dinner, to be held at the Tanzbrunnen Köln on Friday 18 June 2010. ■

**Table 2.** Criteria under which oral papers are assessed by the Oral Paper Awards Committee

### Material

**Quantity:** how many points are raised?

**Clarity:** is the paper concise, or with too much detail?

**Sequence:** is it logical, easy to follow?

**Suitability:** is the terminology/analysis appropriate?

**Content:** how original is the paper? Does it make a contribution to knowledge of seed technology or science?

### Visual aids

**Quantity:** are these relevant? Captivating?

**Quality:** are they well prepared? Hard to read?

**Use:** does the speaker address the audience? Is a pointer used? Is there contact with, or back turned to audience?

### Presentation

**Voice:** tone, diction, variation, loudness

**Speed of delivery:** is this too fast or slow?

**Overall style:** manner of delivery: does the speaker hold audience attention?

**Timing:** is the talk satisfactorily composed and rounded off?

### Overall impact

Level of informality, humour, audience reaction, handling of discussion and questions?

**Table 3.** Criteria under which posters are assessed by the Poster Awards Committee

**Scientific merit, contribution to seed science and technology:** is the work presented original?

**Quantity:** is there an unnecessarily large amount of text (or not enough) to explain the subject? Are there too many or too few graphs, tables or pictures?

**Message:** does the poster have a message that is stated clearly?

**Readability:** are the words, text, captions etc of adequate size; are the lines of print separated by sufficient space to be read easily from 2 m distance?

**Design quality:** is there a good balance to the poster? Is sufficient colour used to make the poster bright and interesting? Is the presentation of the lettering, graphs and diagrams crisp and neat?

# Code of conduct for members of the Executive Committee

This code of conduct has the aim of providing clarity, transparency and orientation to all who are willing to serve as members of the ISTA Executive Committee.

It is to be distributed at ISTA Congresses, prior to the elections for the ISTA Executive Committee, to inform candidates about the obligations that they must be willing to accept.

Furthermore, it is to inform ISTA Members about the commitment that Executive Committee members are willing to make for the benefit of the Association.

## Code of conduct for members of the Executive Committee (Members-at-large)

Prior to their election, candidates for membership of the Executive Committee must provide the membership with a declaration of potential conflict of interest. This declaration must be updated as required during the term of office.

Executive Committee members shall make their best efforts to attend punctually all meetings and functions of the Executive Committee, and shall plan to be in attendance at all times during the proceedings. Whenever Executive Committee members know in advance that they cannot attend a meeting, will be late for a meeting, or will have to leave a meeting early, they shall make best efforts to inform the President or Secretary General in advance of the meeting.

Executive Committee members are expected to attend at least five of the regular six Executive Committee meetings during their period of office.

Executive Committee members are expected to participate in all of the e-mail votes which are sent to them by the Secretariat within the timeframe set by the Secretariat.

Executive Committee members are expected to respond to all e-mails in which they are asked for views or opinions within the timeframe set for responses.

All members of the Executive Committee shall recognize that their individual behaviour is a reflection upon the Executive Committee as an entity; therefore, they shall at all times refrain from any public conduct within the Association or in public which would bring the Executive Committee into disrepute.

All members of the Executive Committee owe a duty of collective responsibility to the Executive Committee as an entity, particularly with respect to its formal votes and formally approved policies. If conducted civilly, robust disagreement between members of the Executive Committee is perfectly acceptable behaviour and even strongly encouraged, as it is often necessary and appropriate for the development of the best decision-making process; however, once the Executive Committee has formally voted on a matter, no member of the Executive Committee shall engage in any unauthorized activity which undermines the ability of the Executive Committee to successfully implement the results of the vote.

Collective responsibility requires dissenting members to work within the formal procedures of the Executive Committee to modify or revise the previously adopted votes or approved policies with which they disagree. Dissenting members of the Executive Committee may not voice their disagreements with any such votes or policies outside the Executive Committee, as this would show a lack of respect for the Executive Committee, the President and the democratic decisions of the Executive Committee. If a member of the Executive Committee is obliged to express an opinion contrary to that of the Executive Committee by reason of being a voting delegate, this shall be clearly stated.

Should a member of the Executive Committee wish to be absolved from the requirement of collective responsibility, they

are at liberty to resign from their position as a member of the Executive Committee.

All members of the Executive Committee shall recognize that all matters pertaining to the Association's business designated as confidential and conducted in executive session should be kept confidential and not disclosed to the Membership or to members of the public at large. The same applies to any written confidential communications. Executive Committee members shall not disclose confidential information under any circumstances to any person not on the Executive Committee without the express consent of a majority of the Executive Committee members voting on the matter.

In any instance when Executive Committee members might be confused about the confidentiality requirements, and in order to minimize the possibility of inadvertent disclosure, they shall consult the President or Secretary General before making any disclosure to any third party which might release any confidential information.

All confidential information is the property of the Association. Executive Committee members shall keep in strict confidence any information, documentation, records or devices containing confidential information. This applies both during and after the member's term of office.

Executive Committee members will be appointed as liaison officers to one or more ISTA Technical Committees. As such, they shall provide the committee with information from the Executive Committee as necessary, and shall ensure that the Technical Committee acts in accordance with its terms of reference. Prior to each Annual Meeting and Congress, the Executive Committee members shall provide the Executive Committee with an appraisal of the Technical Committee for which they are responsible. This appraisal shall give details of the performance of the Technical Committee and its progress in terms of meeting its objectives. ■

# Certificates for sublots: a proposal for your consideration

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The current ISTA Rules prescribe that seed lots are subject to a maximum size and that only one Orange International Seed Lot Certificate (OIC) marked 'ORIGINAL' may be issued with reference to the whole lot. This means that if parts of lots are to be sold, the ISTA system does not address this, with the result that trade is hampered. Often this problem is circumvented by issuing national certificates for such parts of lots, or duplicate OICs. This is an undesirable situation, for the seed trade as well as for ISTA, and it is suggested that something is done to address the issue.

## Why only one original certificate?

The philosophy of seed lots with a maximum size is based on the idea that the heterogeneity of larger lots cannot be guaranteed. Upon request of the seed trade, and based on practical experience rather than scientific evidence, ISTA has regularly increased seed lot sizes. It has proven very difficult to gather data on the heterogeneity of lots, mainly due to the prohibitive costs of heterogeneity testing. In order to meet the uniformity requirements, many companies have invested in modern large-scale blending and mixing equipment.

One may therefore assume that the present lot sizes guarantee sufficient homogeneity. Besides, if there is any sign of heterogeneity, the sampler must refuse to sample the seed lot for the issue of an OIC, and the company has to try and homogenise the lot.

At some point in time, in the past far beyond our recollection, ISTA decided on the principle of one lot – one submitted sample – one OIC. We understand the one lot – one submitted sample principle, but it is not clear why only one OIC should be issued.

## Why no longer one original certificate?

In practice, seed lots are usually prepared in larger amounts than strictly needed for trade. For instance, grass seed is almost always prepared in quantities of 10 t, but many lots are sold in parts, and for vegetable and flower seeds, the quantities prepared and traded usually differ greatly.

It is also apparent that quantities larger than the maximum seed lot size are prepared and have to be split into lots of the maximum size prescribed by ISTA. However, we will not discuss this here.

In grass seed production, like in many other crops, it is efficient to process one farmer's production in its entirety, resulting in a lot which may not amount to 10 t, but nevertheless will usually still be too large for one individual customer.

So, for operational reasons, lots are usually larger than needed for trade to individual customers. ISTA requires that the parts traded are treated as separate lots, and sampled and tested separately. As a consequence, costs become a problem, and the extra time needed for the sampling and testing can frustrate trade deals. ISTA requirements are therefore seldom followed.

## Possible objections

The argument has been raised that at present, seed lots show a certain range of heterogeneity, and that the present seed lot certificate represents the average value of all parts in the lot. This is very true. When one takes out a certain section of such a lot, it may well be that the average value of this part of the lot deviates from the average of the whole lot. That may be true, but if this deviation is larger than the tolerated range for that quality, that would be only accidental. Similarly, retests are sometimes but rarely out of tolerance, except when something has happened to the lot, for instance during transport. This is not the case here, because the lot (and its parts) are all handled on the same premises.

Buyers of seed lots should realise that they are dealing with natural material. This means that test results are based on samples and represent the nearest best estimate. It also means that individual bags may slightly differ in quality. One simply cannot test entire lots, and some variation and deviation from the mean must be accepted.

## The solution

In conclusion, we can say that if a lot has been tested once according to the ISTA Rules, there is no scientific objection against providing more than one OIC per lot. It will perhaps be desirable to specify which part of the lot is covered by an OIC. This could be done by mentioning label numbers on the certificate, but has the disadvantage of additional administration.

The value of ISTA Certificates as reliable documents in the seed trade and as bank collateral should of course remain intact. ■

Registration at [www.ista-cologne2010.de](http://www.ista-cologne2010.de)



# What has happened to the 'ISTA germination method'?

## A proposal for a performance-based approach to germination testing

Joost van der Burg  
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The last few years have seen a number of new substrates being validated and introduced into the Rules. In the past, the ISTA Rules had only a limited number of accepted media. These were paper (on top of paper, TP, or between paper, BP) and sand (S). These, in combination with one or more temperature regimes, were the 'ISTA methods'.

The new substrates or alternative uses of existing substrates that have recently been introduced are top of sand (TS), organic growing media (O), top of organic growing media (TO), and top of (crepe) paper sand (TPS). And this will probably not be the end of it, because many other media can be imagined and may be proposed in future, such as perlite, Rockwool®, clay beads, cocoa peat, agar, or combinations of these with each other or with the existing ones.

### The meticulous Method Validation process

The ISTA method validation procedures require that solid evidence must be presented in a validation report that has to be approved by the ISTA Ordinary Meeting. Reports usually include the results of comparative tests involving a number of labs. This means that much time and effort must be put in the introduction of a new medium into the Rules. And it is not sufficient to do this only once: each and every combination of media and species needs to be validated ISTA-wide! A recent example is the introduction of TPS for *Pisum* at the 2008 Annual Meeting in Bologna. This type of work requires an enormous amount of effort for those few labs that do most of the (voluntary) work in the Germination Committee. It does seem like an endless task.

TPS is a good example of a medium that may not be needed ISTA-wide: the pre-existing ISTA methods were quite satisfactory, and use of the TPS method is restricted to a few labs that have specially designed equipment. Why then adopt yet another method ISTA-wide when its uptake will be limited to specific labs or companies with a specific interest in it?

### Many methods mean less uniformity

Moreover, the introduction of many 'official methods' inevitably introduces more variation, or in ISTA terminology: less uniformity in seed testing! We now also face the situation that we lack a fixed reference for validation of new or in-house methods: the reference, the 'ISTA method', no longer exists, since there could be a multiplicity of ISTA methods.

Method development in the field of germination testing has become more dynamic lately, and like in GM testing, many in-house methods are being developed and are constantly evolving. With these methods one can achieve a similar degree of uniformity as with the current ISTA methods. This can be observed when labs are asked to perform a prescribed method alongside their preferred method. Then often the preferred methods, although different from lab to lab, may produce similar or even more uniform and equally reproducible results. This is because they are achieved using the preferred method, which is based on experience with the combination of equipment and personnel.

### Back to basics

Now that we have adopted the performance-based approach within ISTA for GMO testing, it is time for us to consider this approach for germination methods too. The Rules should be limited to the well-known and widely adopted basic methods and media, and create the possibility for individual labs to develop their

own methods. Of course, these need to be solidly validated and tested against the ISTA reference methods, and the results presented to the Accreditation Department and/or the next audit team. Currently, many in-house methods and procedures have already been accepted by auditors, if properly validated, such as methods of testing the performance of equipment, ways to prepare substrates, validation of home-made equipment or equipment of local suppliers.

### The result?

If ISTA restricted its methods to the basic ones and allowed labs to validate in-house methods against them, this would have a number of advantages.

First, what is meant with the 'ISTA method' would be clearer, now and in the future, resulting in more uniformity. Second, a limited number of ISTA methods, e.g. paper and sand, would provide a better reference for the validation of alternative methods than a whole list of methods. This would create flexibility and freedom for the individual labs and their methods, while maintaining a clear standard. Third, in cases where a result is disputed, we have again a clear reference, the ISTA method. Fourth, the Germination Committee can save a lot of time and energy that is presently spent validating substrates, and focus on other important issues, such as the introduction of new species into the Rules, the development of methods for testing seed mixtures, and guidelines for managing equipment and substrates.

### Next Congress

At the Ordinary Meeting of our next Congress in Cologne, this proposal will be discussed. We hope that you will join in the discussion, and we look forward to suggestions how to implement the principle of the performance-based approach to germination testing. ■

## Proposed changes to the *International Rules for Seed Testing* 2010 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.

This year, the bulk of the changes are to be found in Chapter 5: The Germination Test, which has been extensively revised by the Germination Committee.

Among the further changes are the following:

### Chapter 2: Sampling

- Cargo sampler for seeds of the size of *Triticum aestivum* and larger
- Hand halving method for *Gossypium* spp.
- Harmonization of sample sizes in Table 2A Part 1

### Chapter 5: The Germination Test

- Complete revision of Chapter 5
- Revision of Table 5A
- Revision of tolerance tables

### Chapter 6: The Tetrazolium Test

- Tetrazolium test for *Chloris gayana*

### Chapter 9: Moisture Content

- Resolution of inconsistency between 9.1.5.5 and 9.1.5.2
- Increase in moisture test duration for *Lolium* spp.

### Chapter 15: Seed Vigour Testing

- Conductivity test for *Glycine max*

### Chapter 17: Bulk Containers

- Amendment of Table in 17.5: Calculation and expression of results



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COMPETENCE IN COLOR

# Information about Cologne

## Registration

Different registration fees will be offered for three different periods:

- 16–22 June,
- 16–20 June or
- 19–22 June 2010.

Provision is made for ISTA Members and non-members. The table below gives an overview of the fees.

Deadline for final registration:  
15 May 2010

Any charges arising from bank transfers or credit card transactions shall be borne by the participant. The registration fee includes all sessions, coffee breaks and lunches, the welcome cocktail and the Official Dinner for the relevant period.

The fee for accompanying persons includes coffee breaks and lunches, welcome cocktail and the Official Dinner. the fee does not include sessions.

## Accommodation

Official Congress hotel:  
Hotel Mondial am Dom  
Kurt-Hackenberg-Platz 1  
50667 Cologne  
Phone: +49 (0)221 20630  
5 stars  
Room rates:  
Single: EUR 125 (including breakfast)  
Double: EUR 149 (including breakfast)

Distance to the Congress venue  
(Gürzenich): 400 meters

For other hotels, please see the Congress web site at:  
<http://www.ista-cologne2010.de/accommodation/Cologne>

## Cologne

Cologne (German: Köln) is Germany's fourth-largest city (after Berlin, Hamburg and Munich), and is the largest city both in the German Federal State of North Rhine-Westphalia and within the Rhine-Ruhr Metropolitan Area, one of the major European metropolitan areas with more than ten million inhabitants. It is one of the oldest cities in Germany, having been founded by the Romans in the year 38 BC.

Cologne lies on the River Rhine. The Cathedral (Kölner Dom) is the landmark of Cologne and the centre of the city.

## Registration fees

Periods	Events	Registration 1 March 2010 and after
<b>ISTA Members</b>		
16–22 June 2010	FULL Congress	750 €
16–20 June 2010	Seed Symposium & TCOM	700 €
19–22 June 2010	TCOM, Education, OM	650 €
<b>Non-members</b>		
16–22 June 2010	FULL Congress	1125 €
16–20 June 2010	Seed Symposium & TCOM	1050 €
19–22 June 2010	TCOM, Education, OM	975 €
<b>Students</b>		
16–20 June 2010	Seed Symposium & TCOM	150 €
<b>Accompanying persons</b>		
16–22 June 2010	FULL Congress	350 €
16–20 June 2010	Seed Symposium & TCOM	250 €
19–22 June 2010	TCOM, Education, OM	200 €



**Final registration deadline: 15 May 2010**





Cologne is located in the transition zone between the maritime and continental temperate climate, with mild winters (January average: 2.4 °C) and moderately warm summers (July average: 18.3 °C).

For further information about the city and its surroundings, please visit the web site [www.cologne.de](http://www.cologne.de).

For general information about Germany, please visit the web site [www.deutschland.de](http://www.deutschland.de).

### The venue: the Gürzenich

The Congress will be held in the traditional ballroom and reception hall of Cologne City Council, The Gürzenich (built 1441–1447). The building was restored after the Second World War and redeveloped in 1996–98 to become a stylish congress and special-events centre with the latest technical equipment. The name “Gürzenich” refers to the previous owner.

Today, it represents a harmonious blend of historic architectural styles with ultra-modern event technology and the ultimate in event facilities.

For more information, see the web site [www.koelnkongress.de](http://www.koelnkongress.de).

## Travel

### Flights to Cologne

Cologne Bonn Airport is located approximately 17 km (10.5 miles) east of Central Cologne, and within easy reach of the city centre.

S-Bahn S13 interurban trains leave from the airport every 30 minutes between 05:34 and 01:34. The average journey to Cologne Central Station (Köln Hauptbahnhof) takes 15 minutes. Price for a one-way ticket, tariff 1b, is EUR 2.30.

Taxis to or from the city cost approximately EUR 25 for a one-way trip.

Car rentals are available at the airport.

For further information, see the Cologne Bonn Airport web site at [www.koeln-bonn-airport.com](http://www.koeln-bonn-airport.com).

### Motorways to Cologne

A network of ten motorways from all directions lead into a motorway ring that encircles the city: A1, A3, A4, A57, A59, A555 and A559. In addition, Cologne is connected to several other main roads. We recommend using one of the over 150 Park and Ride facilities with more than 19 000 parking spaces all over the town, to avoid getting into traffic jams.

## Rail

The main railway station is situated next to Cologne’s Cathedral (Kölner Dom), in the city centre.

For further information, see the German rail web site at [www.bahn.de](http://www.bahn.de).

### Low-emission zone

Since 1 January 2008, Cologne city has been a low-emission zone. Such zones, recognizable by signs similar to signs designating 30 km/h speed limit zones, are regulated by traffic restrictions: low-emission vehicles are allowed, while high-emission vehicles are prohibited.

For further details please see the links on the Travel page of the Congress web site at: <http://www.ista-cologne2010.de>.

### Passports, visas

Please check first with the German Embassy in your country to see whether you will need a visa to enter Germany, or visit the web site of the German Federal Foreign Office at [www.auswaertiges-amt.de/](http://www.auswaertiges-amt.de/).

**Registration at [www.ista-cologne2010.de](http://www.ista-cologne2010.de)**

# Post-Congress tours

Three post-Congress tours are scheduled for 23–25 June 2010, directly after the ISTA Congress. All tours will depart from Cologne. The itineraries include interesting visits to seed laboratories, meetings with breeders, growers and multipliers, and visits to famous sights and cities.

The tours also end in Cologne, with stops at possible airports along the route (Cologne Bonn, Frankfurt, Munich), where participants can get off.

Deadline for booking is 15 May 2010.

## Tour I: Bavaria

**Organizer: Dr. Berta Killermann**

**Cost: EUR 220**

### First day: Wednesday, 23 June

After the trip from Cologne to Freising (Upper Bavaria), the participants will be welcomed at the ISTA Seed Testing Station of the Bavarian State Research Center of Agriculture. After a guided tour through the laboratories, we will have a typical Bavarian lunch (Bavarian veal sausages, pretzels and wheat beer). In the afternoon there will be a visit to the Bavarian Plant Breeding Station at Steinach (grasses, large- and small-grained legumes) in Lower Bavaria, followed by dinner. The first overnight stay will be in nearby Regensburg, a city on the river Danube in the Upper Palatinate founded by the Romans 2000 years ago. After dinner, the participants may join a guided tour through the city at night.

### Second day: Thursday, 24 June

In Niedertraubling we will visit the oldest Bavarian Plant Breeding Station at Bauer (wheat, barley, oats), founded in 1863, and also a modern seed-processing facility in nearby Obertraubling. After lunch we will have a trip to Teisendorf, a little village in the northern pre-alpine region where the Bavarian forest tree seed-testing and breeding station of the Bavarian State Ministry of Nutrition, Agriculture and Forestry is located. After a tour through the laboratories, participants can enjoy

Bavarian hospitality with a traditional Bavarian meal in an impressive and marvelous mountain scenery, accompanied by typical Bavarian folk music. The second overnight stay will be at nearby Chiemsee.

### Third day: Friday, 25 June

On the third day, participants can freely dispose of their time. They can make a trip to Chiemsee, where the famous Bavarian fairytale King Ludwig II built a beautiful castle on an island in the middle of the lake, or take a walking tour through the wonderful mountain scenery.

Return journey: by bus to Munich airport, Frankfurt airport and Cologne-Bonn airport. Final destination is Cologne.

## Tour II: Thuringia

**Organizer: Dr. Günter Müller**

**Cost: EUR 300**

### First day: Wednesday, 23 June

On the first day the participants will visit the Raiffeisen Waren-Zentrale Rhein-Main AG, a young company for seed cleaning located close to Cologne. This company operates with the latest standards for seed production to provide high-quality products.

Afterwards we will drive about 300 km eastwards to Eisenach in Thuringia. The history of Eisenach is connected with Wartburg Castle. The participants will have the opportunity to visit this famous castle built in 1067.

Martin Luther lived in Eisenach as a child and also later under the protection of Frederic the Wise, after having been persecuted for his religious views. During his stay at Wartburg Castle, he translated the New Testament into German.

Eisenach is also known as the birthplace of Johann Sebastian Bach, the German composer and organist whose sacred and secular works for choir, orchestra and solo instruments drew together the strands of the Baroque period and brought it to its ultimate maturity.

After visiting Eisenach's famous sights we will drive to Petkus Seed Technology, a company for producing seed cleaning and processing equipment. The participants will stay overnight in Erfurt, the capital city of Thuringia that is the closest city to the geographical centre of Germany.

### Second day: Thursday, 24 June

We will drive to Dachwig, where we will visit a trial station of the Bundessortenamt (Federal Plant Variety Office) to see variety testing of cereals, maize, legumes, oil plants, vegetables, medicinal and aromatic plants.

Afterwards we will drive to the company Dr. Marold, a 340-hectare farm for organic farming of seed, seed potatoes and aromatic plants.

In the afternoon we will visit the city of Erfurt with its two churches Erfurt Cathedral and Severikirche, standing side by side forming the emblem of the City.

Martin Luther studied at the university in Erfurt and during that time lived in the Augustinerkloster, an old Augustinian monastery, for a few years after 1505. In the evening we will have a traditional Thuringian meal in one of the famous restaurants in the city. The second overnight stay will also be in Erfurt.

### Third day: Friday, 25 June

On the last day, the tour will take us to Weimar, one of the great cultural sites of Europe, home to such luminaries as Goethe, Schiller and Herder, and the piano virtuosi Hummel, List and Bach. It has been a site of pilgrimage for the German intelligentsia since Goethe first moved there in the late 18th century.

At noon we will visit the company N.L. Chrestensen in Erfurt. This company has been engaged in plant breeding, variety maintenance and production of horticultural seeds since 1867.

Return journey: by bus to Cologne Bonn airport and Frankfurt airport.

### Tour III: Baden-Württemberg

**Organizer: Dr. Andrea Jonitz**

**Cost: EUR 325**

#### First day: Wednesday, 23 June

We start in Cologne with a drive through the romantic valley of the river Rhine, passing the famous Loreley rock and the city of Koblenz to the ancient cathedral city of Speyer. Here a guided tour and visit of the famous romanesque cathedral and the medieval city with royal palace gives a glimpse of a former centre of secular and clerical power. We continue the journey through the Rhine valley along the foot of the Pfälzer Berge with views of vegetable and tobacco fields on the one hand, and medieval castles on hilltops with sunny vineyards on the other.

We stop for a visit of grapevine research at the Geilweilerhof, an old farm located near the village of Siebeldingen. The working activities are focused on the resistance and climatic stress tolerance of grapevines, including various technologies of wine production, at the Julius Kühn Institute, the federal institute for cultivated plants financed by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV). The visit will be concluded with a wine tasting.

#### Second day: Thursday, 24 June

In the morning a short trip will bring us to the Center for Agricultural Technology Augustenberg, the second oldest seed testing station (150 years). It is situated on a hill near the historical city of Durlach, the former domicile of the Margrave of Baden, founder of the modern city of Karlsruhe.

The ISTA-accredited department of seed testing and applied botany will show their routine work and seed testing procedures. Afterwards, in a walk through the orchards (11 hectares), species and varieties of cultural relevance in Germany and parts of the orchard gene bank will be shown. The special aspects of the long-term storage system will be highlighted and a presentation of the in-house distillery will be given.

After lunch we will drive through a region rich in specialized crops to the heights of the Black Forest. Here we will be guests of the official "Klenganstalt" for harvesting and processing of seeds from trees and shrubs, especially spruce, fir, beech and oak.

Later, there will be an excursion to a tree population of foreign woody plants. There will be the opportunity for shopping of regional products such as the typical bacon, cuckoo clocks and the typical hats of the region. Dinner and overnight stay will be in the Black Forest near Nagold.

#### Third day: Friday, 25 June

The journey continues through the Black Forest, a mountainous region rising to about 1500 m, marked by dense forests of fir and spruce, and famous for its clean air. After passing the Daimler Benz manufacturing plant and the fertile hilly area of the Kraichgau we reach the Hohenlohe plateau, with its intensive animal husbandry. Here we visit the Plant Breeding Oberlimpurg, a medium-sized seed breeding business with nearly 100 years of family tradition in the breeding of wheat, spelt, field beans and intertillage.

After lunch, participants may join a guided tour through the city of Schwäbisch Hall in the Kocher valley, which was settled in the Stone Age. Schwäbisch Hall was an imperial city with saltworks, an important source of revenue. A Romanesque basilica and hall church in the late Gothic style were built. A market place with an impressive platform, surrounded by the renaissance houses of the nobility, demonstrate the early wealth of this town. There will be some time for shopping or free disposal. Then we drive back over the Kraichgau, with its huge production areas of wheat and sugar beet on best loamy soil, follow the valley of the river Rhine and pass Heidelberg to reach Cologne in the evening. ■

Seed Technology Institute  
Australia Pty Ltd  
seeks experienced

### Seed Analysts

for the Queensland Seed  
Technology Laboratory (AUDL02),  
Brisbane.

Candidates must have at least two years' demonstrated bench analyst experience in a recognised, preferably ISTA-accredited, seed-testing laboratory. A thorough knowledge and practical experience of purity and germination testing of grasses, cereals, pulses, sunflower, sorghum, vegetables and brassicas is an advantage. Experience with purity and germination testing of tropical species is also very desirable.

Employment may be on a short-term contract or as a full-time position.

Some assistance with relocation costs may be possible.

To apply, or for more information, contact the Laboratory Manager, Mrs Karen Hill, at [hillk@uq.edu.au](mailto:hillk@uq.edu.au).

This is an excellent opportunity for an enterprising person to enjoy the tropical climate of Queensland, Australia, and to work with a team of experienced and dedicated staff.

[www.seedinstitute.com.au](http://www.seedinstitute.com.au)

29<sup>th</sup> ISTA  
Congress Cologne

2010



# ISTA membership changes

Status 1 March 2010

## New members

### Argentina ARDL0101/ARDL0102

Laboratorio Central de Análisis de Semillas del Instituto Nacional de Semillas  
Laboratory representative: **Ignacio Aranciaga**  
Paseo Colón 922, 4° Piso, Buenos Aires, 1063  
Phone: +54 11 4349 2394  
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### Austria ATML0401/ATML0403

Pioneer Hi-Bred Services GmbH, Seed Quality Laboratory  
Laboratory representative: **Martina Pommer**  
Pioneer Str-Industriegelände, 7111 Parndorf  
Phone: +43 2166 2525 1440  
Fax: +43 2166 2525 62  
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### Canada CADL0801/CADL0802

Canadian Food Inspection Agency  
Saskatoon Laboratory  
Laboratory representative: **Steve Jones**  
301-421 Downey Road, Saskatoon, SASK.  
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Phone: +1 306 975 4240  
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### Chile CLPM0002

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### Denmark DKML0800/DKML0801

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### Germany DEDL0502/DEDL0503

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## Ghana GHML0100/GHML0101

Plant Protection and Regulatory Services  
Directorate (PPRSD), National Seed Testing  
Laboratory  
Laboratory representative: **Cletus Achaab**  
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## Greece GRML0200/GRML0201

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## India INML2100/INML2101

Karnataka State Seed Certification Agency  
Director of Seed Certification  
Laboratory representative:  
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## JPDL0401/JPDL0402

Forestry and Forest Product Research Institute  
Ministry of Agriculture, Forestry and Fisheries  
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Fax: +64 3 325 3844  
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## Russia RUML0301/RUML0302

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# New faces at the ISTA Secretariat

**Rasha El Khadem**
**Head of ISTA  
Accreditation  
Department**


Rasha El Khadem was born in Cairo, Egypt. She finished school in Germany and then studied biology at the Ruhr University Bochum in Germany with the main focus on botany. She completed an MSc in Austria at the University of Vienna, where her research topic was related to in-vitro propagation of endangered species needed for pharmaceutical purposes.

After completing her MSc she worked in the R & D department of a small company producing dietary supplement from seedlings, and was responsible for germination optimization and monitoring microbe propagation during germination. In cooperation with the Institute of Cancer Research, substances produced during early germination were tested on their ability to induce cancer. During this time she received a PhD in Natural Science from the University of Vienna. In 2002 she took over a temporary position in an ISTA-accredited laboratory as Electrophoresis Lab Leader. Upon completion of the contract she started working for a pharmaceutical company and built up and ran an in-process-control laboratory operating under GMP conditions. In 2005 she returned to the ISTA laboratory as the Seed Quality Manager. Rasha was responsible for quality control of seed lots, the quality management system and the continuous improvement process within the laboratory. This included the completion of Six-Sigma/Greenbelt and LEAN projects.

Rasha joined the ISTA Secretariat in October 2009. In her position she is responsible for all accreditation activities within the ISTA including auditing, organizing Quality Assurance Workshops and the Proficiency Testing Programme.

**Cannice Gubser**
**Membership  
and Financial  
Administration**


Cannice Gubser (Kwai-Ching Leung) is from Hong Kong, and studied financial accounting and business management. She worked for a business development director for several years in Hong Kong, extensively travelling in Asia, mainly focused on organizing events, setting up new companies, and taking responsibility for new projects in China and Taiwan. She gained a lot of knowledge of time management and public relations with clients worldwide. Before moving to Europe, she worked for the Chief Financial Officer at the Hong Kong Convention and Exhibition Centre, managing the CFO's back office and being responsible for financial closing and analysis.

In 1999, she completed her studies in hotel management at the DCT University Center in Lucerne, Switzerland, and joined the ISTA Secretariat. In summer 2004, she decided to have a change, and joined a wealth management group in Zurich as Senior Accounting Assistant, before taking over the position of Financial Controller for a life insurance company. Those few years were very demanding in the banking environment, and she expanded her knowledge of private banking within the Swiss investment sector.

Cannice Gubser was invited to work for the ISTA Secretariat again, and has been working part-time since September 2009, being responsible for Membership and Financial Administration. ■

# Seed viability testing of *Chloris gayana*

Stefanie Krämer<sup>1</sup> and Ronald Don<sup>2</sup>

<sup>1</sup>Chair, ISTA Tetrazolium Committee and <sup>2</sup>Chair, ISTA Germination Committee

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## Summary

A validation study on seed viability testing of *Chloris gayana* using tetrazolium was carried out. Six laboratories were involved, and each tested 400 seeds of four seed lots. The results demonstrated that the method is of sufficient repeatability and reproducibility to be included in the ISTA Rules.

## 1. Plant material

Four seed samples of *Chloris gayana* of commercial quality were obtained by the Queensland Seed Technology Laboratory, Australia, for this study. The seeds were stored at 10 °C prior to distribution to participants.

The samples were divided by the hand sampling method (ISTA Rules 2.5.2.2.4), and a purity test of 1 g was conducted on all samples prior to them being sent in December 2007. Lot 4 had a high content of empty seeds, but no attempt was taken to purify it and remove these. An in-house study by the Queensland Seed Technology Laboratory using 1000 seeds confirmed the homogeneity of the seed samples. Samples were sent to each of the participating laboratories in February 2009. The seeds were packed as blind samples (Lots 1–4).

## 2. Participating laboratories

Six laboratories from six countries participated in this validation study:

- Mrs. Valerie Blouin, GEVES-SNES, Beaucauzé, France
- Mrs. Karen A. Hill, Queensland Seed Technology Lab, Queensland, Australia
- Mrs. Stefanie Krämer, Landwirtschaftliches Technologiezentrum Augustenberg, Karlsruhe, Germany
- Miss Linda Maile, NIAB, Official Seed Testing Station for England and Wales, Cambridge, United Kingdom
- Mrs. Anny van Pijlen, General Netherlands Inspection Service (NAK), Emmeloord, Netherlands
- Mr. Garry Duffy, Seed Testing Laboratory, Celbridge, Co. Kildare, Ireland

In this report the laboratories are anonymously numbered as Laboratories 1–6; the sequence of these numbers is not identical to the list given above.

## 3. Procedure for the TTC test

The testing method is described in Table 1, which is the proposal for inclusion in the ISTA Rules. Each laboratory tested 4 × 100 seeds from each of the 4 lots.

## 4. Results

The results of the TTC viability tests were reported in April and July 2009. The results are given in Table 2 and shown in Figure 1.

The highest mean viability was 76 ± 3% for Lot 1, the lowest 32 ± 6% for Lot 4.

## 5. Statistical analysis

For statistical analysis the experimental error is quantified by the ratio *f* between the observed standard deviation (SD observed) and the expected standard deviation (SD expected) based on the binomial distribution:

$$f = \frac{SD_{(obs.)}}{SD_{(exp.)}}$$

$$SD_{(exp.)} = \sqrt{(p \times q) / n}$$

*p*: % TTC viability as mean;

*q*: 100 – *p*;

*n* = number of seeds.

### Experimental error among the replicates

Table 3 shows the factors *f* for experimental error among the 4 replicates within a viability test in each of the 6 laboratories. The average factor *f* for 6 labs and 4 lots is 0.98, which is below 1.00.

### Experimental error among tests in different laboratories

Table 4 shows the factors *f* for experimental errors among the 6 laboratories. The average factor *f* for 4 lots is 3.74. The individual *f* values for the lots are between 2.04 and 6.55. From Figure 1 it is clear that Laboratory 5 obtained a much higher viability for Lot 4 than other laboratories, and a plot of the mean viabilities obtained by the participating laboratories (Figure 2) demonstrates that Laboratory 5 obtained a higher mean viability. Analysis of the factor *f* experimental errors among participants when the results of Laboratory 5 are excluded show that the average factor *f*

**Table 1.** Testing method for *Chloris gayana* as proposed for the ISTA Rules Change Proposals 2010

Species	Pretreatment: type/min. time (h)	Preparation before staining	Staining solution (%)	Optimum staining time (h)	Preparation for evaluation	Permitted non-viable tissue	Remarks
1	2	3	4	5	6	7	8
<i>Chloris gayana</i>	Remove glumes before premoistening. BP/16 at 10 °C; W/3	Cut transversely near embryo	1	6	Observe surface of embryo and scutellum	1/3 radicle, measured from radicle tip; in total 1/3 of extremities of scutellum	Empty seeds are reported as non-viable

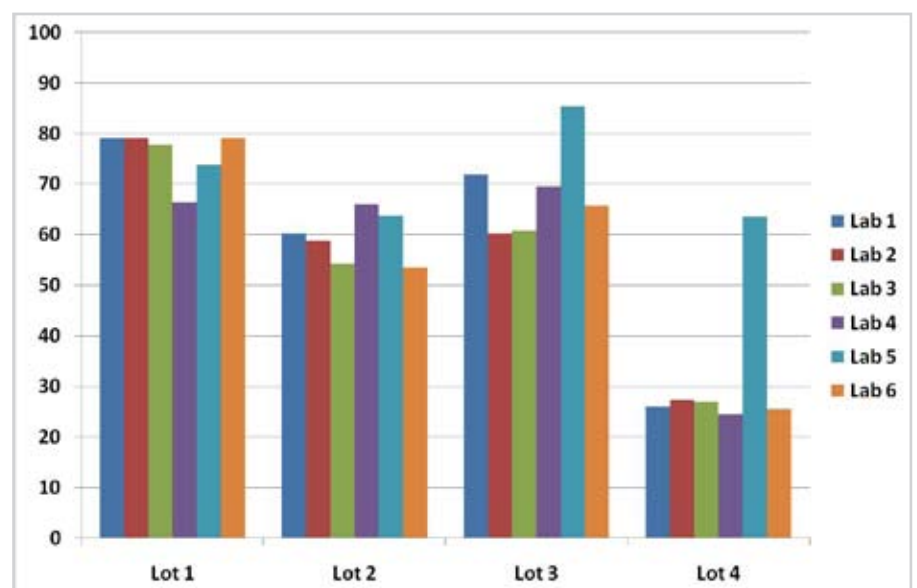


**Table 2.** Seed viability (%) as reported for the four *Chloris gayana* seed samples by the 6 participating laboratories (results of the four replicates each containing 100 seeds)

	Seed viability (%)			
	Lot 1	Lot 2	Lot 3	Lot 4
Lab 1	77	67	64	26
	79	58	74	27
	76	63	72	29
	84	53	77	22
Lab 2	82	58	66	31
	67	65	60	34
	77	54	50	25
	90	58	65	19
Lab 3	83	60	69	27
	79	48	62	26
	74	55	56	30
	75	54	56	25
Lab 4	68	70	73	23
	67	65	70	24
	64	68	67	28
	66	61	68	23
Lab 5	73	64	87	61
	80	60	87	65
	75	70	86	65
	67	61	81	63
Lab 6	80	54	62	26
	76	61	63	31
	77	49	67	25
	83	50	71	20
Mean	76	59	69	32
95% confidence interval	±3	±3	±4	±6

**Table 3: Experimental errors within the tests.** The table shows for each combination of lot and laboratory the mean, the observed standard deviation between the 4 replicates, the expected standard deviation (based on the binomial distribution) and the f values

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Mean
<b>Lot 1</b>							
Mean	79	79	77.75	66.25	73.75	79	
SD observed	3.56	9.63	4.11	1.71	5.38	3.16	
SD expected	4.07	4.07	4.16	4.73	4.40	4.07	
f value	0.87	2.36	0.99	0.36	1.22	0.78	1.10
<b>Lot 2</b>							
Mean	60.25	58.75	54.25	66	63.75	53.5	
SD observed	6.08	4.75	4.92	3.92	4.50	5.45	
SD expected	4.89	4.92	4.98	4.74	4.81	4.99	
f value	1.24	0.93	0.99	0.83	0.94	1.09	1.00
<b>Lot 3</b>							
Mean	71.75	60.25	60.75	69.50	85.25	65.75	
SD observed	5.56	7.32	6.18	2.65	2.87	4.11	
SD expected	4.50	4.89	4.88	4.60	3.55	4.75	
f value	1.24	1.50	1.27	0.57	0.81	0.87	1.04
<b>Lot 4</b>							
Mean	26.00	27.25	27.00	24.50	63.50	25.50	
SD observed	2.94	6.65	2.16	2.38	1.91	4.51	
SD expected	4.39	4.45	4.44	4.30	4.81	4.36	
f value	0.67	1.49	0.49	0.55	0.40	1.03	0.77



**Figure 1.** Viability test results for four *Chloris gayana* seed lots as reported by the six participating laboratories.

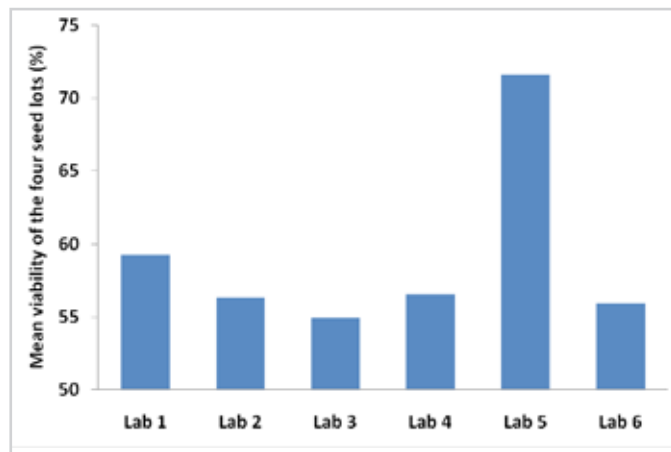


Figure 2. Mean viability test results for *Chloris gayana* seed lots as reported by the six participating laboratories.

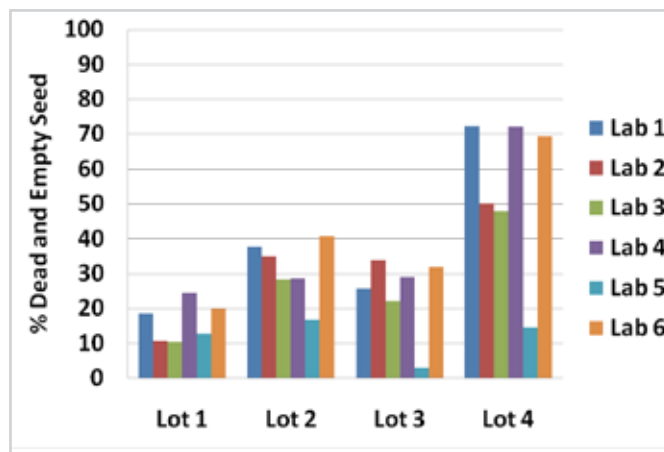


Figure 3. The levels of dead and empty seeds found in the 4 seed lots of *Chloris gayana* by the six participating laboratories.

Table 4. Experimental errors between the laboratories. The table shows for each lot the mean, the observed standard deviation (SD), the expected standard deviation (based on the binomial distribution) and the f values

Lot	Mean viability (%)	Observed SD (%)	Expected SD (%)	f value
Lot 1	80	5.10	2.14	2.38
Lot 1 without Lab 5	76	5.59	2.13	2.62
Lot 2	59	5.00	2.46	2.04
Lot 2 without Lab 5	59	5.06	2.46	2.62
Lot 3	69	9.24	2.32	3.99
Lot 3 without Lab 5	66	5.13	2.38	2.16
Lot 4	32	15.32	2.34	6.55
Lot 4 without Lab 5	26	1.12	2.19	0.51
Mean all labs				3.74
Mean without Lab 5				1.98

SD = standard deviation

for the 4 lots is now 1.98, with individual f values ranging from 0.51 to 2.62.

The f value used for establishing the tolerance tables for seed viability test results in the ISTA Rules is 2.82. Thus, even when including the results of Laboratory 5, the average f factor of 3.74 indicates a high but still acceptable experimental error among tests in different laboratories. When the results of Laboratory 5 are excluded, the average f factor of 1.98 indicates a totally acceptable experimental error among tests in different laboratories.

The reason for Laboratory 5 reporting higher results is that this laboratory was the only one of the participants to purify the seeds prior to carrying out the tetrazolium test. All other participants carried

out the test on the seeds as received, without making any attempt to remove empty seeds. Because of this, Laboratory 5 reported fewer dead or empty seeds than the others (Figure 3).

As a further test, the maximum tolerated ranges for the mean viabilities were calculated using the formula  $S = f \times SD \times F$ , as according to Miles (1963). This test was performed with and without the results of Laboratory 5. In only one case (Lot 4 including Laboratory 5) did the actual range exceed the tolerated range, and it did so by less than 2%. Thus, the range as a further measure indicates that the experimental error is acceptable.

Table 5. Maximum tolerated ranges S according to Miles (1963)

Lot	S (%)	Mean	f value	SD expected	F	Actual range
All Lot 1	33.9	72	2.82	2.13	5.62	12.75
All Lot 2	38.9	59	2.82	2.46	5.62	12.50
All Lot 3	36.7	69	2.82	2.32	5.62	25.00
All Lot 4	37.1	32	2.82	2.34	5.62	39.00
Lot 1 without lab 5	32.8	76	2.82	2.13	5.46	12.75
Lot 2 without lab 5	37.9	59	2.82	2.46	5.46	12.50
Lot 3 without lab 5	36.6	66	2.82	2.38	5.46	11.50
Lot 4 without lab 5	33.8	26	2.82	2.19	5.46	2.75

## 6. Conclusion

The f factors in Table 3 indicate an acceptable experimental error among the 4 replicates within the tests. Moreover, the maximum tolerated ranges in Table 5 indicate acceptable variation between participating laboratories. Laboratory 5 was the only participant to attempt purification of the samples prior to tetrazolium testing, and the results are even more impressive if the results from Laboratory 5 are excluded from the analysis. Thus, there is no reason to assume that the procedure given in Table 1 should not be introduced into the ISTA Rules. ■

# Extending the drying period for *Lolium* spp. for the high-temperature oven method from 1 to 2 hours

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## Summary

The PT round 08-1 included moisture determination of *Lolium multiflorum*. The results of this round indicated there was a difference in the moisture determined for *Lolium multiflorum* depending on which of the two methods (103 °C for 17 h or 130 °C for 1 h) permitted in the ISTA Rules (2009) was used. A comparative testing study was undertaken by four ISTA laboratories to determine whether moisture determination in *Lolium multiflorum* at 130 °C for 1, 2 or 3 h gave the same result as the reference method (17 h at 103 °C). The data from this comparative testing supports the proposal that the duration of the moisture test for *Lolium* spp. be increased from 1 to 2 h in the ISTA Rules (Table 9A Part 1).

## Introduction

In 2008 a new reference method was adopted for moisture testing (ISTA Rules, 2009). The new reference method is the low-temperature constant oven method, i.e. 17 h at 103 °C. The low-temperature constant oven method can be used for all species in Table 9 of the ISTA Rules. The high-temperature constant oven method can be used as an alternative method where indicated in Table 9. The PT round 08-1 included moisture testing of *Lolium multiflorum*. This was the first time that alternate methods could be used. The results of the PT round 08-1 indicated a difference in the moisture result for *Lolium multiflorum*, depending on the method used (Table 1). These results alone are not sufficient to support a change in the high-temperature constant oven method for *Lolium* spp. However, a comparative testing round was undertaken by four ISTA laboratories, under the leadership of LaRAS, to determine

whether the moisture contents determined by the low-temperature constant oven method and the high-temperature constant oven method for *Lolium* spp. are the same. The results of this comparative testing are the basis of this validation report.

## Materials and methods

Two seed lots of *Lolium multiflorum* were evaluated. These were the same seed lots used in PT round 08-1 and were obtained from the test organiser for moisture determination in PT round 08-1.

Four ISTA-accredited laboratories from three countries participated in the comparative testing:

- Ente Nazionale Sementi Elette, Laboratorio Analisi Sementi [ITDL0300] (Rita Zecchinelli);
- Forschungsanstalt Agroscope Reckenholz-Tänikon ART [CHDL0100] (Silvia Zanetti);
- GEVES (Station Nationale d'Essais de Semences [FRDL0200]) (Maria Rosaria Mannino);
- LaRAS (Laboratorio di Ricerca e Analisi Sementi [ITDL0100]) (Enrico Noli).

The low-temperature and high-temperature methods were followed as indicated in the ISTA Rules, with duplicate determinations carried out on each sample.

Samples were distributed to the laboratories in sealed (moisture-proof) aluminium packets. The laboratories were instructed to begin the moisture determination immediately after the packets were opened, and that all samples should be tested at the same time, i.e. only one experiment at 101–105 °C and one at 130–133 °C.

The moisture of the samples was determined in the following ways:

### High-temperature oven method

The moisture of the samples was first determined using the high-temperature oven method as described in Chapter 9.1 of the ISTA Rules (2009). At the end of the

prescribed drying period (1 h at 130 °C), samples were allowed to cool and then weighed. Samples were then returned to the oven for a further 1 h drying. At the end of the second hour of drying, samples were again allowed to cool before reweighing and were then returned to the oven for a further 1 h drying. Samples were again allowed to cool before reweighing.

### Low-temperature oven method

The moisture of the samples was first determined using the low-temperature oven method as described in Chapter 9.1 of the ISTA Rules (2009). At the end of the prescribed drying period (17 h at 103 °C), samples were allowed to cool and then weighed. Samples were then returned to the oven for a further 2 h drying. At the end of the second two-hour drying period, samples were again allowed to cool before reweighing. The second 2 h drying period was based on ISTA Rule 9.1.4.2 (ISTA Rules, 2009) for checking the ventilation of the oven.

All drying periods were begun when the oven had returned to the set temperature.

## Data analysis

The reference method for moisture determination is 17 h at 103 °C. However, a shorter determination at 130 °C may be used if properly validated. A tolerance of 0.3% is permitted for the comparison between the reference method and a shorter duration test at 130 °C. The shorter-duration 130 °C method is accepted if 75% or more of the differences between the mean of the two replicates for each method are within the tolerated range of  $\pm 0.3\%$  (ISTA, 2007). This tolerance was used in this validation study to compare the moisture determinations for each sample by each laboratory at 103 °C for 17 h with 103 °C for 19 h and 130 °C for 1, 2 and 3 h.

To investigate the interactions between different laboratories, samples, temperatures and duration, the data was subjected to an analysis of variance (ANOVA). A

**Table 1.** Moisture content determined in *Lolium perenne* seed lots in ISTA PT round 08 using the low-temperature and high-temperature oven methods

Seed lot	Oven method	
	Moisture after 17 h at 103 °C (%) (average of 6 labs)	Moisture after 1 h at 130 °C (%) (average of 100 labs)
1	10.7	9.6
2	11.6	10.7
3	15.1	14.2

**Table 3.** Comparison between the moisture determined for two seed lots of *Lolium multiflorum* at 103 °C for 17 h (low-temperature (reference) oven method) with that determined using the high-temperature oven method of 130 °C for 1 h. Samples with a difference in moisture content of ±0.3% or greater are out of tolerance. Only two (25%) of the moisture determinations were in tolerance.

Lab	Sample	Reference method moisture (%)	Moisture (%) determined after 1 h at 130 °C	Difference (%)	In tolerance (±0.3%)
1	1	11.37	10.77	0.40	No
2	1	11.37	11.23	0.14	Yes
3	1	11.47	11.10	0.37	No
4	1	11.49	11.04	0.45	No
1	2	11.59	10.67	0.08	Yes
2	2	11.63	11.04	0.59	No
3	2	11.76	11.02	0.74	No
4	2	11.78	10.92	0.86	No

**Table 2.** Time taken for the moisture ovens used in the comparative testing to return to 103 °C or 130 °C

Laboratory	Time for the oven to return to the set temperature (min)	
	High-temperature method (130 °C)	Low-temperature method (103 °C)
Laboratory 1	5–15	4–5
Laboratory 2	30	25
Laboratory 3	5–15	4–5
Laboratory 4	4	2–3

**Table 4.** Comparison between the moisture determined for two seed lots of *Lolium multiflorum* at 103 °C for 17 h (low-temperature (reference) oven method) with that determined using the high-temperature oven method of 130 °C for 1 h, plus an extra 1 h drying. Samples with a difference in moisture content of ±0.3% or greater are out of tolerance. All moisture determinations were in tolerance.

Lab	Sample	Reference method moisture (%)	Moisture (%) determined after 1 h at 130 °C + 1 h at 130 °C	Difference (%)	In tolerance (±0.3%)
1	1	11.37	11.38	0.01	Yes
2	1	11.37	11.59	0.22	Yes
3	1	11.47	11.54	0.07	Yes
4	1	11.49	11.55	0.06	Yes
1	2	11.59	11.43	0.16	Yes
2	2	11.63	11.60	0.03	Yes
3	2	11.76	11.67	0.09	Yes
4	2	11.78	11.61	0.17	Yes

general liner model (GLM) was used to determine significant interactions between treatments. Where significant effects were detected in the ANOVA ( $P = 0.05$ ), means were compared using the Tukey test. Prior to analysis, data were checked for normality using the univariate procedure in SAS (Release 8.2 (TS2M0), SAS Institute Inc., Cary, NC, USA). No transformation of the data was necessary.

**Results and discussion**

The recovery times for the moisture ovens used by the four laboratories are given in Table 2.

Recovery times for the ovens are all with the limit prescribed in the ISTA Rules (9.1.4.2).

The results of the moisture determinations of the four laboratories and the difference in the moisture determinations from each laboratory are given in Tables 3–6.

The ANOVA table (Table 7) indicates that there were significant differences in moisture determination between the laboratories (Table 8), duration (Table 9) and seed lots. The moisture content of the seed lots was 11.54% and 11.42% (minimum significant difference ( $P < 0.05$ ) = 0.044). While this difference may be statistically significant, the actual difference is very small; less than the difference that would be

acceptable for duplicate determinations on the same sample (ISTA Rule 9.1.6.2; ISTA Rules, 2009). In practical terms, therefore, this difference is not important.

The ANOVA indicates that the moisture content determined by laboratory 1 differs from that determined by laboratories 2, 3 and 4. The difference in the moisture content determined by the different laboratories is 0.2% or less. There are no tolerance tables for comparing moisture determinations between laboratories however a tolerance of 0.2% is used for comparisons between duplicate moisture determinations performed in the same laboratory at the same time on the same sample (ISTA Rule 9.1.6.2; ISTA, 2009). It is not unreasonable to expect that, because of the increased



**Table 5.** Comparison between the moisture determined for two seed lots of *Lolium multiflorum* at 103 °C for 17 h (low-temperature (reference) oven method) with that determined using the high-temperature oven method of 130 °C for 1 h, plus an extra 2 h drying. Samples with a difference in moisture content of  $\pm 0.3\%$  or greater are out of tolerance. Seven out of eight (87.5%) moisture determinations were in tolerance.

Lab	Sample	Reference method moisture (%)	Moisture (%) determined after 1 h at 130 °C + 1 h + 1 h at 130 °C	Difference (%)	In tolerance ( $\pm 0.3\%$ )
1	1	11.37	11.61	0.24	Yes
2	1	11.37	11.70	0.33	No
3	1	11.47	11.73	0.29	Yes
4	1	11.49	11.76	0.27	Yes
1	2	11.59	11.73	0.14	Yes
2	2	11.63	11.78	0.15	Yes
3	2	11.76	11.85	0.09	Yes
4	2	11.78	11.90	0.12	Yes

**Table 6.** Comparison between the moisture determined for two seed lots of *Lolium multiflorum* at 103 °C for 17 h (low-temperature (reference) oven method) with that determined using the low-temperature (reference) oven method of 103 °C for 17 h, plus an extra 2 h drying. Samples with a difference in moisture content of  $\pm 0.3\%$  or greater are out of tolerance. All moisture determinations are in tolerance.

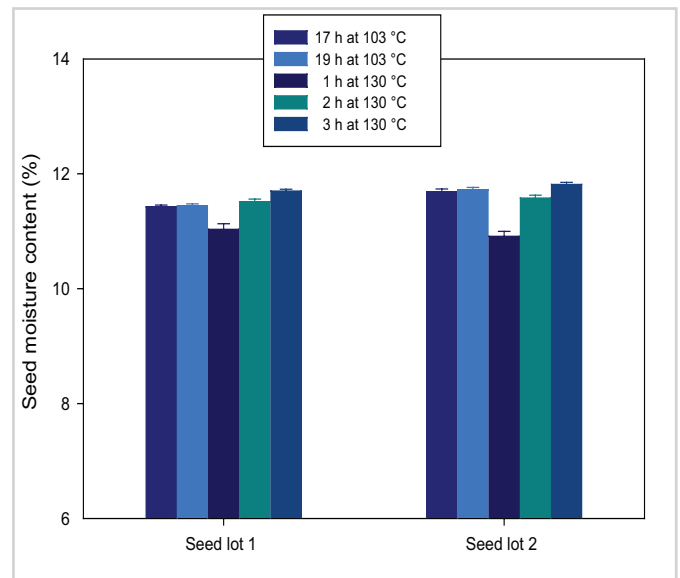
Lab	Sample	Reference method moisture (%)	Moisture (%) determined after 17 h at 103 °C + 2 h at 103 °C	Difference (%)	In tolerance ( $\pm 0.3\%$ )
1	1	11.37	11.37	0	Yes
2	1	11.37	11.42	0.05	Yes
3	1	11.47	11.52	0.05	Yes
4	1	11.49	11.47	0.02	Yes
1	2	11.59	11.62	0.03	Yes
2	2	11.63	11.69	0.06	Yes
3	2	11.76	11.80	0.04	Yes
4	2	11.78	11.78	0	Yes

sources of error, a tolerance calculated for the difference in moisture determinations on the same sample in different laboratories would be greater than 0.2%. Therefore, while there may be a statistically significant difference in the moisture determination between laboratory 1 and laboratories 2, 3 and 4, the actual difference is very small, and there is no reason to remove the results from laboratory 1 from the analysis.

There was no significant difference in the moisture determined at 103 °C for 17 h or 19 h or 130 °C for 2 h. The moisture content determined after 1 h drying at 130 °C was significantly lower than that determined for any other method. Similarly moisture content determined after 3 h drying at 130 °C was significantly higher than that determined for any other method.

There was also a significant interaction effect between the sample, the temperature at which the moisture determination was performed and the duration of the moisture determination (Table 7, Figure 1).

For both seed lots there was a significant difference in the moisture determined using 103 °C for 17 h compared to 130 °C for 1 h with more water being lost after 17 h at 103 °C. This suggests that 1 h at 130 °C



**Figure 1.** Moisture content (%) determined in two seed lots of *Lolium multiflorum* using the low-temperature oven method or the high-temperature oven method for different durations. Error bars are the standard error of the mean for each moisture determination method.

**Table 7.** ANOVA table for moisture determination in two lots of *Lolium multiflorum*

Source	DF	Type I SS	Mean square	F value	Pr > F
Laboratory	3	0.233	0.078	16.71	<0.0001
Seed lot	1	0.143	0.143	30.72	<0.0001
Temperature	1	0.201	0.201	43.29	<0.0001
Duration	3	2.634	0.878	188.85	<0.0001
Seed lot · temperature	1	0.154	0.154	33.02	<0.0001
Seed lot · temperature · duration	3	0.062	0.021	4.48	0.0112

**Table 8.** Moisture content determined by all laboratories for both seed lots at all temperatures and durations.

Laboratory	Moisture content (%)
1	11.35
2	11.51
3	11.55
4	11.53
Minimum significant difference (P < 0.05)	0.0834

**Table 9.** Moisture content determined by all laboratories for all seed lots for different drying durations.

Test method	Moisture content (%)
103 °C for 17 h	11.56
103 °C for 19 h	11.58
130 °C for 1 h	10.97
130 °C for 2 h	11.55
130 °C for 3 h	11.76
Minimum significant difference (P < 0.05)	0.100

**Table 10.** Percentage of moisture of total lost (at 130 °C) over time as influenced by absolute moisture level for *Lolium perenne* (Nijenstein, n.d.)

Moisture content level after 6 h	Percentage of total lost over time (minutes)				
	15	30	60	120	360
12.37	60	81	89	95	100
16.92	76	85	92	97	100
20.70	73	86	95	97	100
23.91	82	89	94	97	100
27.64	84	91	95	98	100
30.50	78	91	96	97	100
32.56	71	91	96	98	100
35.04	69	93	97	99	100
39.34	65	90	97	99	100

is insufficient to remove all the moisture from *Lolium multiflorum*.

There was no significant difference in the moistures determined using 103 °C for 17 h and 130 °C for 2 h, suggesting that a drying duration of 2 h is more appropriate for *Lolium multiflorum*. The results for 3 hours drying at 130 °C are less clear. For sample one significantly more weight was lost after 3 h drying at 130 °C than after 17 h drying at 103 °C, but not for the second sample. The data is therefore inconclusive as to whether more water is being lost after 3 h at 130 °C.

There was no significant difference in the moisture content determined when the duration of the low-temperature method was extended from 17 to 19 h confirming that after 17 h at 103 °C no further moisture is lost from *Lolium multiflorum*.

The comparative testing has been performed using *Lolium multiflorum*. Table 9 Part 1 (ISTA Rules, 2009) does not distinguish between species of *Lolium*. An assumption made in this validation is that moisture determination on other species of *Lolium* would give similar results. There is experimental evidence available to support this assumption. Grabe (1984) presents data that indicates that moisture determination in *Lolium perenne* using 17 h at 103 °C and 2 h at 130 °C gives similar results.

**Limitations of the validation study**

A limitation of this validation study is that one moisture content only was used to compare the high-temperature and low-temperature oven methods. There is data

published (Benjamin & Grabe 1988) that indicates there is no single drying period at 130 °C that gives an accurate moisture determination over a range of moisture contents in *Lolium perenne*, i.e. 6 h for whole seed at around 6% moisture, 3 h at around 9% and 2 h at around 15%. Different drying durations for seed at different moisture levels are not practical, as they require prior knowledge of the seed moisture content. Table 10 gives the percentage of moisture lost (at 130 °C) from *Lolium perenne* as a percentage of the total moisture in the seed. In contrast to Benjamin & Grabe (1988), these data suggest that 6 h drying is required to remove 100% of the moisture, including samples with high moisture content. Nonetheless, the data do show that 1 h is too short a drying duration, and that 2 h may be a good compromise.

**Conclusions and recommendations**

Only one moisture content level was used to compare the high-temperature and low-temperature oven methods; therefore, the study may not have given a clear or correct drying period. Nonetheless, the data from PT round 08-1 (Table 1) and this validation study have demonstrated that the one-hour duration for the high-temperature oven method is too short to accurately determine the moisture content in *Lolium multiflorum*, and that a change from this duration is required immediately. Previously published data suggest that no single drying period at 130 °C gives an accurate moisture determination over a range of

moisture contents in *Lolium* spp., but that 2 h may be a good compromise. The PT round 08-1, this validation study and the literature combined provide evidence to support the recommendation that the duration of the high-temperature oven method for *Lolium* spp. be increased to 2 h.

**Acknowledgements**

The Moisture Committee would like to thank Enrico Noli from LaRAS for initiating the comparative testing that forms the basis of this validation study, and the four laboratories, LaRAS, ENSE Agroscope and GEVES for participating in the comparative testing.

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# Seed vigour conductivity test for *Glycine max*

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## Summary

Five seed lots of *Glycine max*, all having a laboratory germination of >80%, were tested by seven laboratories using the electrical conductivity test, as described in the ISTA Rules for *Pisum sativum*. All laboratories consistently identified the same significant differences in the seed lot conductivity and the data was repeatable within laboratories and reproducible between laboratories. The results of all tests gave a z-score between +2.00 and -2.00 and all data fell within the tolerance levels established for peas in the ISTA Rules. This provides evidence in support of the inclusion of *Glycine max* within the ISTA Rules as a species to which the conductivity test can be applied.

## Introduction

The conductivity test is currently validated in the ISTA Rules as a test that can be applied to *Pisum sativum*. In 2008, the test was also validated for application to *Phaseolus vulgaris* (see Method Validation Report) and the addition of *P. vulgaris* to the ISTA Rules as a species to which the conductivity test can be applied is a Rules Proposal for 2010. The basis of the conductivity test is the solute leakage from seeds into water. The extent of solute leakage can be attributed to impaired membrane integrity and the development of dead tissue on the living cotyledons as the result of seed ageing or imbibition damage (Matthews and Powell, 2006), both of which are common to most grain legumes (Powell, Matthews and Oliveira, 1984). It is therefore not surprising that measurements of solute leakage, using the conductivity test, identified differences in the vigour of soya

bean (*Glycine max*) seed lots, as reflected in their field emergence (Oliveira *et al.*, 1984; Yaklich *et al.*, 1979). The aim of this study was to demonstrate that the conductivity test applied to *Glycine max* is both repeatable within laboratories and reproducible between laboratories.

## Materials and methods

Samples of five seed lots of *Glycine max* were supplied by Rasha El-Khadem, from Pioneer HiBred, Austria. The seeds originated from Italy and had standard germinations above 80%. Coded samples of the seed lots were sent from Aberdeen UK to the participating laboratories, namely SNES, GEVES, Angers, France; LaRAS, Bologna, Italy; OSTs, SASA, Edinburgh, UK; OSTs, NIAB, Cambridge, UK; Department of Horticulture, Ege University, Izmir, Turkey; Department of Crop Science, University of Ferdowsi, Mashhad, Iran; Seminis, Enkhuizen, The Netherlands. The participants in the test were limited to those in countries to which the soyabean seeds could be readily exported.

Each laboratory completed the conductivity test using the same method as that described for peas in the ISTA Rules (ISTA, 2009) i.e. 4 replicates of 50 seeds, each soaked in 250 mL deionised/distilled water for 24 h at 20 °C.

The data was analysed using (a) Analysis of Variance, (b) calculation of z-scores and (c) the statistical tool developed by S. Grégoire according to ISO 5725-2 and available for download at the ISTA website: <http://www.seedtest.org/upload/cms/user/ISO572511.zip>

## Results

The seed lot means (Table 1) revealed clear and significant differences in seed leachate conductivity and hence vigour. Seed lot E had the highest conductivity (23.5  $\mu\text{S cm}^{-1} \text{g}^{-1}$ ), that is the lowest vigour, followed by lot D, lots A and B (not

significantly different from each other) and lot C (16.21  $\mu\text{S cm}^{-1} \text{g}^{-1}$ , highest vigour). Lots E and D were consistently identified as having the highest conductivity (lowest vigour) in every laboratory (Table 1), while lot C always had the lowest conductivity (highest vigour). Application of the tolerance tables from Chapter 15 of the ISTA Rules (ISTA, 2009) revealed that, the replicate data (Appendix 1) for each lot in each laboratory were in tolerance with one another, as were the test results for each lot from the seven different laboratories. There were small, but significant, differences in the overall means from the seven laboratories (Table 1). The coefficient of variation for the comparative test was 6.4%, a value comparable with that reported (4.3%) for the method validation of conductivity for *Phaseolus vulgaris* (Powell, 2009).

Calculation of the z-scores (Table 2) revealed that all data fell within the values +2.0 to -2.0 that are acceptable within ISTA proficiency tests

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

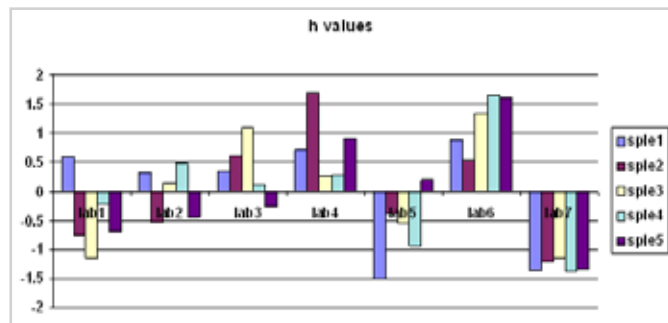
There was only one significant h-value, namely for lot 2, in lab 4 (Figure 1) which indicated that the result was significantly overestimated. Significant k values were found for two lots in each of two laboratories (lab 3, lots 1 and 2; lab 6, lots 3 and 4), indicating that there was greater variability between replicates. Even so, the replicates were in tolerance (Appendix 1; Chapter 15, ISTA Rules, [ISTA 2009]).

Repeatability and reproducibility values are affected by the seed quality of the lots tested, with low vigour seeds often having

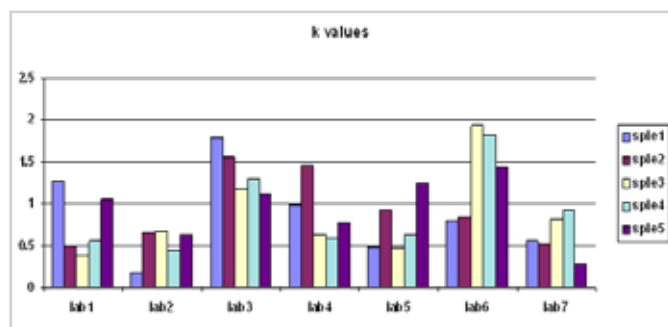
**Table 1.** Comparison of laboratory and seed lot means of five seed lots of soya beans tested by seven laboratories using the conductivity test

Lab	Lot					Lab means
	A	B	C	D	E	
1	18.9 <sup>C</sup>	17.4 <sup>D</sup>	15.3 <sup>F</sup>	21.2 <sup>B</sup>	22.2 <sup>A</sup>	19.0 <sup>d</sup>
2	18.6 <sup>B</sup>	17.8 <sup>B</sup>	16.4 <sup>C</sup>	21.8 <sup>A</sup>	22.7 <sup>A</sup>	19.4 <sup>cd</sup>
3	18.5 <sup>D</sup>	19.4 <sup>C</sup>	17.1 <sup>E</sup>	21.5 <sup>B</sup>	23.0 <sup>A</sup>	19.9 <sup>bc</sup>
4	18.9 <sup>C</sup>	20.8 <sup>B</sup>	16.3 <sup>D</sup>	21.4 <sup>B</sup>	25.1 <sup>A</sup>	20.5 <sup>ab</sup>
5	15.8 <sup>D</sup>	18.0 <sup>C</sup>	15.8 <sup>D</sup>	20.6 <sup>B</sup>	23.9 <sup>A</sup>	18.8 <sup>d</sup>
6	19.3 <sup>C</sup>	19.3 <sup>C</sup>	17.4 <sup>D</sup>	22.8 <sup>B</sup>	26.3 <sup>A</sup>	21.0 <sup>a</sup>
7	16.0 <sup>CB</sup>	16.8 <sup>B</sup>	15.3 <sup>C</sup>	20.2 <sup>A</sup>	20.9 <sup>A</sup>	17.8 <sup>e</sup>
Lot means	18.0 <sup>c</sup>	18.5 <sup>c</sup>	16.21 <sup>d</sup>	21.4 <sup>b</sup>	23.5 <sup>a</sup>	

For lot and lab means, different lower-case letters indicate that values are significantly different using LSD at the 5% level. Within a row (laboratory), different upper-case letters indicate that values (lots) are significantly different using LSD at the 5% level.



**Figure 1.** h-values for five seed lots of *Glycine max* tested using the conductivity test in seven laboratories.



**Figure 2.** k-values for five seed lots of *Glycine max* tested using the conductivity test in seven laboratories.

higher values. It is therefore not possible to compare directly the data from comparative tests using different seed lots. However, the values obtained for soya bean (Table 3) were similar to and lower than values previously obtained for *Phaseolus vulgaris* (repeatability: 0.9511–2.2287; reproducibility: 1.6850–4.2581).

### Discussion

The conductivity test consistently identified differences between seed lots in each of seven laboratories. The test was both repeatable within laboratories and reproducible in different laboratories. In addition, the replicates within the laboratories

**Table 2.** Comparison of means, standard deviations (SD) and z-scores for five seed lots of soya beans tested by seven laboratories using the conductivity test

Lab	Lot				
	A	B	C	D	E
a) means					
1	18.9	17.4	15.3	21.2	22.2
2	18.6	17.8	16.4	21.8	22.7
3	18.5	19.4	17.1	21.5	23.0
4	18.9	20.8	16.4	21.4	25.1
5	15.8	18.0	15.8	20.6	23.9
6	19.3	19.3	17.4	22.8	26.3
7	16.0	16.8	15.3	20.2	20.9
Mean	17.99	18.47	16.21	21.37	23.46
SD	1.463	1.373	0.827	0.836	1.822
b) z-scores					
1	0.62	-0.78	-1.10	-0.60	-0.69
2	0.39	-0.52	0.17	0.53	-0.43
3	0.38	0.65	1.12	0.19	-0.23
4	0.59	1.66	0.06	0.08	0.92
5	-1.50	-0.34	-0.50	-0.92	0.24
6	0.91	0.57	1.39	1.71	1.57
7	-1.37	-1.22	-1.15	-1.36	-1.38

**Table 3.** Values for repeatability and reproducibility of results from the conductivity test on *Glycine max*

Lot	Repeatability	Reproducibility
A	1.0097	1.7318
B	1.2622	1.8014
C	0.7503	1.0589
D	1.1318	1.2908
E	1.8131	2.4901

and the mean values obtained for each lot in different laboratories all fell within tolerance, using the tolerance tables in the ISTA Rules (ISTA, 2009). This provides evidence in support of the addition of *Glycine max* to the ISTA Rules as a species for which the conductivity test can be applied.



## Acknowledgements

I am very grateful to Rasha El-Khadem, from Pioneer Hi Bred, Parndorf, Austria, for supplying the seeds, and to Emanuela Casarini, Hulya Ilbi, Mohammad Khajeh Hosseini, Tim Loeffler, Gillian McLaren, Jane Taylor and Marie-Hélène Wagner for participating in this comparative test.

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**Appendix 1.** Data for each replicate conductivity reading for each of five lots taken in each of seven laboratories

Lot	Replicate	Lab						
		1	2	3	4	5	6	7
A	1	17.44	18.31	20.44	18.01	16.22	18.33	15.84
	2	19.11	18.87	16.31	18.17	15.06	19.16	15.28
	3	20.49	18.4	17.91	19.11	16.04	20.17	16.56
	4	18.67	18.64	19.51	20.16	15.78	19.67	16.24
	Mean	18.93	18.56	18.54	18.86	15.8	19.33	15.98
B	1	16.58	18.73	18.38	22.58	19.75	17.82	17.32
	2	17.38	16.72	22.17	21.5	17.28	20	16.22
	3	17.60	17.68	17.77	20.62	17.61	19.09	17.39
	4	18.11	17.93	19.11	18.3	17.31	20.14	16.22
	Mean	17.41	17.76	19.36	20.75	17.99	19.26	16.79
C	1	15.29	16.5	17.57	16.43	15.60	17.14	15.29
	2	15.58	16.02	16.13	16.11	15.56	18.27	14.46
	3	14.95	15.86	18.11	15.67	15.59	15.45	15.95
	4	15.27	16.99	16.75	16.82	16.26	18.6	15.34
	Mean	15.27	16.35	17.14	16.26	15.75	17.36	15.26
D	1	21.27	21.52	19.71	22.25	20.72	21.74	20.09
	2	21.91	21.34	23.24	20.77	19.82	20.53	18.91
	3	21.31	22.38	21.93	21.07	20.41	24.88	20.51
	4	20.35	21.99	21.22	21.67	21.54	24.05	21.4
	Mean	21.21	21.81	21.53	21.44	20.62	22.8	20.23
E	1	20.85	23.67	25.15	26.22	22.86	27.37	20.91
	2	25.05	21.08	21.45	25.79	21.83	22.8	21.59
	3	21.81	22.64	24.47	23.1	27.02	28.5	20.35
	4	21.20	23.34	21.1	25.45	23.93	27.85	20.94
	Mean	22.23	22.68	23.04	25.14	23.91	26.32	20.94

# Seed Symposium: have you registered?

## Final deadline: 15 May 2010

[www.ista-cologne2010.de](http://www.ista-cologne2010.de)

## Laboratory accreditation changes

Status 1 March 2010

### Re-accreditations

#### Argentina ARDL0100

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#### ATML0400

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Seed Quality Laboratory  
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# A survey of seed science research amongst ISTA Members

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on behalf of the ISTA Seed Science Working Group (Françoise Corbineau, Joël Léchappé, Robin Probert, Alan Taylor)

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Seed science is an important aspect of the ISTA Strategy, but the extent to which ISTA Members are participating in research projects has not been clear. Therefore, an e-mail survey was sent out by the Seed Science Working Group to all Members in March 2009 to determine whether they were involved in research, whether any research was being done alone or in collaboration with other organisations, the nature of any collaborators, sources of funding and the topics of the research.

There were 47 responses: 35 from Member Laboratories, 3 from Associate Members, 8 from Personal Members and 2 from non-members who are active in the Association. The majority of responses originated

**Table 1.** Countries of origin of the responses to the seed research survey of ISTA members (1 response from each country, unless indicated otherwise)

Active	Not active
Armenia	Australia
Austria	Belgium (2)
Brazil	Bolivia
Chile	Denmark
Czech Republic	Germany (5)
France	Hungary
Germany (2)	India
Israel	Japan (2)
Italy	Latvia
Japan	Lithuania
Netherlands (2)	Netherlands
New Zealand	Norway
Norway	Philippines
South Africa	Romania
Taiwan	Slovak Republic
UK (3)	Sweden
USA	Switzerland
Zambia	Turkey
	UK

from Europe (32), with further responses from South America (3), Australasia (2), Asia (6), Africa (2) and North America (1) (Table 1). The absence of responses from many members may indicate their lack of involvement in research work.

A total of 21 responses (45% of the total) indicated that these laboratories or Members are active in seed research. These included 11 Member Laboratories (31% of the responding Member Laboratories), 2 (out of 3) Associate Members and 7 (out of 8) Personal Members.

In 5 of the laboratories that are active in research, some of the research work is within TCOM projects. Two are involved in projects associated with more than 1 TCOM (3 and 6 TCOMs), and the remainder in projects associated with 1 TCOM. Four of the 5 laboratories are also involved in projects that are not TCOM based.

The majority of the non-TCOM research projects are national projects, with 2 being international and 4 both national and international. The sources of funding reflect the national nature of the projects, with most funds coming from the ministry of agriculture, provincial government or national research council of the country. In a few cases seed companies are involved with funding, usually when they are involved in the research project directly, and the EU contributed to a few projects.

The work is carried out in collaboration with seed companies (6 responses), universities (11), research institutions (4), other ISTA laboratories (3), international agencies (1) and seed trade or professional organisations (3). In some cases (9), there is collaboration with a number of organisations in one or more projects.

The nature of the research projects is very diverse. The responses from university-based members and laboratories tended to be very general, stating that their research was on 'aspects of seed physiology and production' or 'improving seed quality and evaluation methods', probably reflecting the student-based nature of the

research. Eight laboratories are involved in projects related to seed health, ranging from determining inoculation thresholds to biocontrol of specific diseases and distribution of seed-borne diseases, weeds or plant propagules, and two projects focus on mycotoxins. Other projects investigate aspects of seed production (faba beans, clover and forage brassicas), priming (wild-flower seed, tree species), dormancy (tree species), effect of chemicals on germination, GM issues, DNA-based methods of variety testing, and new testing and sorting techniques. There is also one project with a greater social input, which supports seed producers as part of the development of rural enterprises and small-scale agriculture. In some cases the seed work forms part of a project with broader aims, such as maize breeding for tolerance to low nitrogen and drought, and the breeding, preservation and reproduction of forest tree species. The emphasis is clearly on applied seed science, although two laboratories are also involved in more basic work.

The skills that the ISTA Members contribute to research projects are largely those of practical seed testing, such as evaluation of physical purity, seed health, germination, variety, vigour and GMO testing. However, in many cases the experience of Members in the management and co-ordination of projects is also employed.

To summarise:

1. There is a strong core of research by ISTA Members, with 45% of respondents (21 out of 46) doing research;
2. Only 5 laboratories were involved in TCOM-based projects;
3. Research projects are largely nationally based and funded;
4. Most of the research is applied seed science;
5. ISTA Members contribute to research through their skills in seed quality evaluation, and project management and co-ordination.

# ISTA Workshop on GMO Testing

## Oberschleissheim, Germany, 8–12 June 2010

This workshop is a pre-Congress workshop of the 29th ISTA Congress, Cologne, Germany.

### Location

Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (Bavarian State Office for Health and Food Safety)  
Oberschleissheim, Germany

### Organizers

Dr. Ulrich Busch, Head of the biomolecular unit  
Benjamin Kaufman, Member of the ISTA GMO Task Force

### Registration fees

450 Euro for ISTA Members  
675 Euro for non-members

### Preliminary programme

Tuesday, 8th June, 2010  
08:00–08:15 Opening and introductions  
08:15–08:45 **Introduction to GMO testing & workshop overview**  
(Beni Kaufman)  
Testing Plans  
(Jean-Louis Laffont)  
08:45 Theory 1: Theory and basic concepts  
09:30 Coffee break  
10:00 Qualitative testing plans – Introduction to Seedcalc  
12:30 Lunch  
13:30 Theory 2: Testing plans 2–Quantitative testing plans  
14:30 Computer exercises  
16:00 End of the day one

Wednesday, 9th June, 2010

### From Seed To DNA

(Beni Kaufman)  
08:00 Theory: Sample Preparation: 1. DNA Extractions  
09:00 Theory: Sample Preparation: 2. DNA quantification, normalization, and sample tracking  
10:30 Experimental: DNA extraction using NucleoSpin method  
12:30 Lunch  
13:30 Experimental: DNA visualization, quantification and normalization  
16:00 End of day 2 (lab work here may require for additional time)

Thursday, 10th June, 2010

### The Polymerase Chain Reaction

(Cheryl Dollard, Bruno Zaccomer, Jean-Louis Laffont)  
08:00 Theory: Introduction to PCR (Cheryl Dollard)  
09:00 Theory: PCR for GMO testing: Definitions and practices  
(Bruno Zaccomer)  
10:00 Coffee break  
10:30 Experimental: Qualitative PCR set-up  
12:30 Lunch  
13:30 Outing & Official Dinner

Friday, 11th June, 2010

### Real-Time PCR

(Bruno Zaccomer, Beni Kaufman, Cheryl Dollard, Jean-Louis Laffont)  
08:00 Theory: Real-time PCR for GMO quantification (Bruno Zaccomer)  
09:00 Experimental: Real-time PCR set –up  
10:00 Coffee break  
10:30 Experimental: PCR result visualization & and documentation  
11:30 Theory: ISTA rules for GMO detection (Cheryl Dollard)  
12:30 Lunch  
13:30 Experimental/Theory: Real-Time PCR, data and results analysis and interpretation (Jean-Louis Laffont)  
15:00 Theory: Protein based methods (Cheryl Dollard)  
16:00 End of day 4

Saturday, 12th June, 2010

### Laboratory Best Practices

(Beni Kaufman, Bruno Zaccomer, Cheryl Dollard)  
09:00 Theory: Assay and process validation (Beni Kaufman)  
10:00 Coffee break  
11:00 Theory & discussion: Management and practices unique to the GMO testing lab (Beni Kaufman, Bruno Zaccomer, Cheryl Dollard)  
12:00 Conclusion

### Registration

[www.ista-cologne2010.de](http://www.ista-cologne2010.de)

# ISTA Workshop on Viability and Germination Testing

## Augustenberg, Germany, 10–13 June 2010

This Workshop is fully booked.



# ISTA Workshop on Species and Variety Testing and Protein Electrophoresis

Hanover, Germany, 11–13 June 2010

This workshop is a pre-Congress workshop of the 29th ISTA Congress, Cologne, Germany.

## Location

Bundessortenamt (Federal Plant Variety Office), Hanover, Germany

## Organizers

Gabriele Kerschbaumer, Provisional Head of the Laboratory Section  
 Cornelia Tepper, Senior Analyst  
 Nora-Sophie Schmidt, Federal Plant Variety Office and Member of the National Organizing Committee for the ISTA Congress 2010

## Registration fee

EUR 250 for ISTA Members  
 EUR 375 for non-members

## Preliminary programme

Friday, 11 June 2010  
**Polyacrylamide gel electrophoresis (PAGE) methods for *Triticum*, *Hordeum*, *Avena*, *Pisum*, *Lolium* and others (×*Triticosecale*, *Poa*, ...)**  
**A(cid)-PAGE, SDS-PAGE, different methods of native PAGE**  
 08:30 Registration of participants  
 09:00 Opening of the Workshop  
 Addresses of welcome  
 Introduction to the Federal Plant Variety Office  
 Introduction of participants and staff members  
 10:15 Coffee break  
 10:45 Theory of PAGE: A(cid)-PAGE, SDS-PAGE, Native PAGE (pH 7.9, pH 8.9)  
 11:45 Practice: casting of polyacrylamide gels; sample preparation: *Triticum*, *Hordeum*, *Triticosecale*, *Avena*  
 Demonstration of sample preparation: *Pisum*, *Zea mays*  
 12:45 Lunch  
 14:00 Practice: sample preparation: *Lolium*, *Poa*; casting of polyacrylamide gels; A-PAGE E-run and staining; SDS-PAGE E-run and staining  
 16:00 Coffee break  
 16:30 Practice/demonstration: A-PAGE E-run and staining; SDS-PAGE E-run and staining; native PAGE (pH 7.9 and pH 8.9)  
 18:00 to 18:30 Questions and discussion

Saturday, 12 June 2010

## Isoelectric focusing (IEF) for *Zea mays*, *Helianthus annuus* Preliminaries for starch gel electrophoresis (SGE)

08:30 Theory of IEF  
 09:00 Practice: IEF (Start of E-run)  
 09:15 Demonstration: casting of IEF gels; casting of starch gels  
 10:15 Practice: IEF (stop of E-run)  
 10:45 Coffee break  
 11:15 Practice: destaining and fixation of PAGE gels  
 11.45 Questions and discussion  
 12:30 Lunch  
 13:30 Excursion  
 19:00 Official Dinner

Sunday, 13 June 2010

## SGE for *Zea mays*, *Brassica*, *Beta*, *Helianthus annuus*

09:00 Theory of starch gel electrophoresis (SGE)  
 09:30 Practice: SGE: Start of E-run  
 10:00 Practice: SGE: preparation of staining solutions  
 10:30 Coffee break  
 11:00 Time for questions or in-depth discussion of special issues (individual requests can be accommodated)  
 12:00 Lunch  
 13:30 Practice: SGE: end of E-run, slicing, staining the slices, fixation of gels  
 14:30 Coffee break  
 15:00 Introduction to ISTA and the Variety Committee (activities, tasks and goals, search for members, what should be done in the future? Where should the main focus be? Which crops should be analysed?)  
 16:30 Final discussion on several aspects on species and variety testing; Conclusion

If there is enough time in between, we can give further theoretical lectures on species and variety testing (i.e. objectives, overview of conventional methods, comparison of UPOV and ISTA).

## Registration

[www.ista-cologne2010.de](http://www.ista-cologne2010.de)

# Germination Committee Workshop

## Emmeloord, the Netherlands, 21–25 September 2009

Anny van Pijlen<sup>1</sup> and Ronald Don<sup>2</sup>

<sup>1</sup>Member, ISTA Tetrazolium and Germination Committees, <sup>2</sup>Chair, ISTA Germination Committee

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Over the past year, the activity of the Germination Committee concentrated on a revision of the Germination Chapter in the Rules (Chapter 5). The Committee held a Workshop at the NAK in the Netherlands, where the current Rules were carefully scrutinised with the aim of making them more precise and easier to interpret. Evidence considered by the committee during the Workshop included:

- the results of a questionnaire sent out to all ISTA labs requesting suggestions for change, and the identification of areas where the current rules were unclear and where clarification was required;
- feedback from ISTA auditors regarding areas of the Rules that for QA purposes needed greater precision, and areas that had led to differences in interpretation by accredited laboratories;
- questions on interpretation posed to the committee either directly or via the ISTA web site.

The Committee would like to thank all laboratories who completed questionnaires, ISTA auditors for their feedback

and all those who contributed to the revision through the questions they asked of the committee. The result of these contributions and the subsequent work and deliberations of the Committee is a major revision of the Germination Chapter of the Rules, which will be presented to the Cologne Congress for approval by the Ordinary Meeting.

Revisions include:

- correcting errors;
- providing greater clarity and precision as to what is required when testing germination;
- providing definitions currently missing;
- ensuring that the numbering of the types of abnormal seedlings is the same in both the Rules and the Handbook on Seedling Evaluation;
- providing methodology for testing fewer than 400 seed
- providing enhanced guidance on re-testing and new tolerance tables that are required when more than one retest has to be carried out;
- providing tolerance tables that should be used when fewer than 400 seeds are tested;
- making Table 5A more user friendly.

Revision of the tolerance tables would have been impossible without the invaluable assistance of Jean-Louis Laffont, who developed a comprehensive tool that enables the calculation of germination tolerances

between replicates and tests conducted in one laboratory and between tests conducted in different laboratories. This tool will be available for use in the Germination Committee Toolbox on the ISTA web site.

In addition to the revisions that the Committee are proposing, other matters were discussed at the Workshop. These included areas where changes to the Rules was not considered appropriate, but where further guidance will need to be provided in the Handbook on Seedling Evaluation. Some major philosophical issues were also considered following workshop presentations given by Harry Nijenstein, Joost van der Burg and Max Soepboer:

- finishing germination tests once a predetermined germination level is achieved;
- not differentiating ungerminated seed;
- introducing performance-based germination procedures;
- testing seed mixtures.

A flavour of these issues will be presented at Cologne, and it is clear that these issues will be a major consideration for the next Germination Committee.

In addition to thanking everyone who contributed to the technical aspects of the workshop, the Germination Committee would like to extend special thanks to the NAK for the use of their facilities and their generous hospitality. ■



Some of the participants at the Germination Workshop.

Germination Tolerances for tests in 1 laboratory based on methodology of Miles (1963) Tables G1 and G2, columns D, H and L	
# of tests	2
# of seeds/test	100
Average germination	93
Maximum range	8
Change any value in a yellow cell	

Screenshot of the tolerance calculator available in the Germination toolbox on the ISTA web site.

# CALENDAR

## 2010

- 8–13 June **ISTA Workshop on GMO Testing, Oberschleissheim, Germany**  
[www.ista-cologne2010.de](http://www.ista-cologne2010.de)
- 10–13 June **ISTA Workshop on Viability and Germination Testing, Augustenberg, Germany**  
[www.ista-cologne2010.de](http://www.ista-cologne2010.de)
- 11–13 June **ISTA Workshop on Species and Variety Testing and Proteinelectrophoresis, Hanover, Germany**  
[www.ista-cologne2010.de](http://www.ista-cologne2010.de)
- 16–22 June **29th ISTA Congress, Cologne, Germany**  
[www.ista-cologne2010.de](http://www.ista-cologne2010.de)

## 2011

- 13–16 June **ISTA Annual Meeting, Tsukuba, Japan**  
[www.seedtest.org](http://www.seedtest.org)

### Advertising rates 2010

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
2/3 page (vertical)	600	1000	133 × 297 mm	139 × 303 mm
1/3 page (vertical)	250	500	71 × 297 mm	77 × 303 mm
1/2 page (landscape)	400	800	210 × 148.5 mm	216 × 154.5 mm
1/3 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<sup>2</sup>

Cover pages: glossy coated 170 g/m<sup>2</sup>

#### 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

See inside front cover



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