

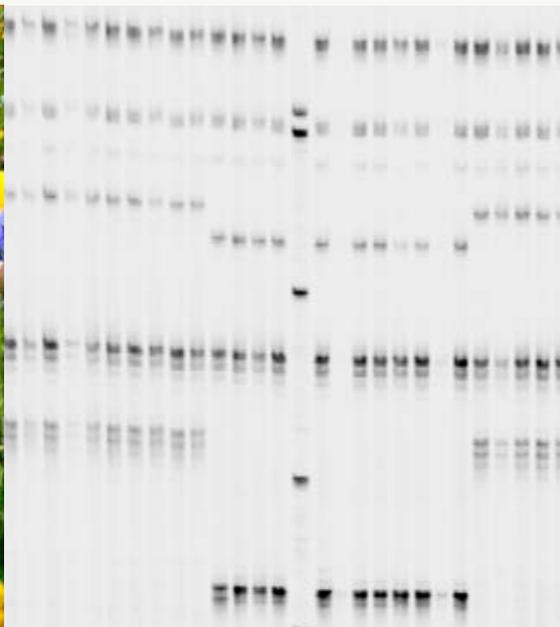
Seed Testing

INTERNATIONAL

ISTA News Bulletin No. 135 April 2008



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Dear Reader,

you have in your hands the Spring 2008 issue of Seed Testing International (STI), the ISTA membership magazine. The Spring issues are always published shortly before the ISTA Congress or Annual Meeting, and you will therefore find a lot of information regarding this upcoming important event.

However, on top of that you will find in this issue an overview regarding the Seed Industry in Italy, the host country of the ISTA Annual Meeting 2008.

One of the focal points in this year's Annual Meeting will be the use of DNA methodology in the area of seed testing. Aspects of specified-trait testing (including GMO testing for adventitious presence of GM seeds in non-GM seed lots, as well as GMO lot purity) and aspects regarding the identification and purity of single varieties will be discussed. Concrete training for seed analysts in a laboratory will be provided in a GMO workshop shortly before the Annual Meeting, and the Meeting itself will be opened by a Seminar on specified-trait testing. Please find in this STI issue the very interesting detailed programme of this Seminar.

There is also a report of the Variety Committee on a first comparative test on DNA-based methods.

Proficiency tests are extremely important and useful methods to evaluate the performance of single laboratories and to work towards uniformity in seed testing. ISTA continuously tries to enlarge its range of proficiency tests to areas where no such tests have hitherto been offered. It is therefore a great pleasure for me to announce that ISTA has enlarged its proficiency test programme to seed health testing. In this issue you will find a report about the first ISTA seed health proficiency test for *Botrytis cinerea* on *Helianthus annuus*. You should not miss this interesting article.

Seed quality control is a question not only for commercially used agricultural species for food production, but also for seed conservation, biological diversity and environmental restoration initiatives. Therefore please find an article in this STI issue about quality control in wildflower seed production in the UK.

You will find many more interesting articles about and around seed testing, and I'm extremely happy about the vigour and enthusiasm of all the authors who have provided so many highly interesting and technically and scientifically precise articles. I would like to thank them all for all their efforts and their support for our association.

Talking about the people who support and work for ISTA: please find in this issue also profiles of the newly elected members of the ISTA Executive Committee, and the Secretariat staff who recently joined the ISTA Secretariat.

In closing, I wish you a lot of fun reading this issue, and I hope to welcome personally as many of you as possible to the ISTA Annual Meeting 2008, in Bologna, Italy.

Yours sincerely,

Michael Muschick



Seed Testing International
No. 135 April 2008

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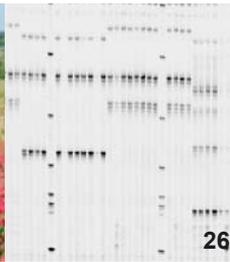
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President's Report

Dr. Katalin Ertsey



The last six months has again been a busy period in the life of our association.

First of all was the meeting of the newly elected Executive Committee (ECOM) and the Technical Committee (TCOM) chairs in October in Zurich. The fact that 13 of the 17 TCOM chairs participated shows the importance of this meeting. The ISTA strategic priorities were revised and the Working Programmes for 2007–2010 finalized and approved by the ECOM. All those present agreed that more and better communication is needed between the various ISTA bodies. To realize this object and support this procedure is one of my main goals.

The ECOM/TCOM Chairs Meeting was followed by the ECOM Meeting. According to our decision made in Brazil, Steve Jones as Rules Chair participated at the meeting as observer without voting rights. In concert with the strategic priorities, two new important Working Groups were established: Publications and Workshops.

The autumn was also rich in international events. Michael Muschick, our Secretary General, was at the Asian Seed Congress in Manila. I myself visited the 7th Eastern European Seed Network (EESNET) Meeting in Novi Sad, Serbia (17–21 December). The EESNET is a regional integration of national seed associations and other such groups in Central and Eastern Europe.

There were 122 participants, mostly from Eastern Europe, including Turkey. The main topics were GM plants and coexistence. On request, I summarized in a short presentation ISTA's activity in this field since our decisions in Bangkok in 2005. The next EESNET meeting will be in Siófok, Hungary, in October 2008. Following the suggestion of the ISF, the topic will be the different ways of seed certification in Eastern Europe.

I was invited to participate at the Annual Meeting in Moscow of the Co-ordinating Committee on Questions of Seed of the Independent Republics. This is the forum for the experts of the independent republics of the former Soviet Union to discuss the plant breeding situation and questions of the seed sector. The participants finalized a proposal for their agricultural ministries to support ISTA membership of their countries, and to establish ISTA-accredited seed testing laboratories. A further opportunity for ISTA to maintain contacts and strengthen our position in central Asia is the Joint FAO/ECO/ASA/ICARDA Meeting in Bishkek, Kirgizstan, in June 2008.

Africa is also in the focus of ISTA's interest. We provided information about our quality assurance system for seed testing laboratories at the African Seed Trade Association (AFSTA) Meeting on 26–29 February.

After these preliminaries, the new ECOM had become accustomed by the time of our usual February Meeting. All members were able to attend this meeting, prepared very professionally by the Secretariat; this allowed us to work very efficiently.

The ECOM accepted the modified 2008 Rules Proposals, which together with the Method Validation Reports will be distributed to Members by the end of March.

Moreover, we asked the Publications Working Group to elaborate a Position Paper on ISTA Rules Policy to be presented at the Ordinary Meeting (OM) 2008.

The ECOM discussed changing the number and tasks of ISTA officers. These constitutional changes will be discussed

with the membership at the OM 2008, and presented for vote at the OM 2009. Another important issue was the future of the ISTA copyright policy.

ISTA's role in the global seed industry is an important one. Seed testing laboratories worldwide can rely on ISTA methods, but this relationship is bilateral. ISTA is recognized by international organizations such as the ISF, OECD, UPOV, FAO and others. In this context, the new and outgoing Secretaries General of the ISF, Marcel Bruins and Bernard Le Buanec, joined the ECOM Meeting on 12 February. During the discussion, both partners agreed on the procedure of the final phase of the ISTA/ISF Herbage Seed Lot Size Experiment. We agreed to hold an annual meeting with the ISF at the executive level, the first to be next year in Geneva.

Discussion is in progress with the above-mentioned international organizations to hold the 2nd World Seed Conference (the 1st Conference, organized by the ISF, UPOV, OECD and ISTA, took place in Cambridge in 1999).

The 1st Global Conference on GMO Analysis on 24–27 June 2008 at Como, Italy, is also in preparation, Michael Muschick, our Secretary General, is a member of the Organizing Committee.

The ECOM discussed and accepted the modified draft programmes for the Annual Meeting of 2009 and the Congress of 2010, and approved the topics, sessions and contents of the ISTA Seed Symposium 2010 in Cologne, Germany.

Before then, we have other important events and a lot of work to do. ISTA is offering eight different workshops in 2008, four of which are still open for registration. Dates and further information can be found on pages 54 to 57, and on the ISTA website (<http://www.seedtest.org/en/workshop.html>).

And we must not forget our Annual Meeting 2008, on 16–19 June in Bologna, Italy, one of the birthplaces of seed testing in Europe. I look forward to seeing you in Bologna. ■

The seed business in Italy

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In Italy today, the total area of cultivated land is 50% of what it was 100 years ago. Nevertheless, Italy is still the European Union (EU) country with the largest proportion of Utilized Agricultural Area, with about 45% of its territory used for this purpose, or around 13 000 000 ha. 665 different species are cultivated all over the country, but only a small number is of larger interest. The most important group are the herbaceous species, and among them, with more than 3 500 000 ha, the cereals, in particular durum wheat (1 300 000 ha), common wheat (700 000 ha), barley (350 000 ha), rice (220 000 ha) and maize (1 100 000 ha). Other important crops are forage crops, vegetables and oil seeds. In recent years, the total number of agricultural farms decreased from 1 963 816 in 2003 to 1 728 528 in 2005 (–12%), while their average size increased from 6.7 ha in 2003 to 7.4 ha in 2005 (+10%).

The seed market

The annual turnover of the Italian seed industry in Italy is estimated to be around 520–530 million euros. The various sectors contribute as follows:

- cereals: 150 million;
- vegetables: 140 million;
- maize: 130 million;
- herbage: 50 million;
- seed potatoes: 35 million;
- sugar beet: 15 million;
- oil species: 15 million.

(Estimates by the Italian Seed Trade Association AIS, a member of ESA and ISF, for the working year 2006/07)

In 2007, 336 companies were involved in seed production of agricultural species, based all over the country, but in particular in Emilia Romagna (16%), Sicily (14%), Apulia (14%), Veneto (8%), Piedmont (8%)

and Lombardy (7%). Some of them deal with only a small number of species, or even only one (e.g. durum wheat seed production and marketing in southern areas), while others include a larger number of species and of groups of species in their catalogues. Figure 1 shows the distribution of some agricultural species in Italy.

As far as vegetable seed production is concerned, the total number of companies in 2007 was 97. A number of vegetable species are of high interest in Italy, and companies are based mainly in the central-northern regions (Emilia Romagna 29%,

Veneto 15%, Lombardy 11%, Piedmont 9%), although production areas are mostly based in the south, in Campania, Apulia and Sicily.

Regarding import–export statistics, the balance is constantly negative. The figures show different values depending on the years, due to many factors, but mainly climate conditions and EU policies influencing internal productions and seed needs differently. However, in 2006, seed imports reached 247 million euros and exports 124 million euros.

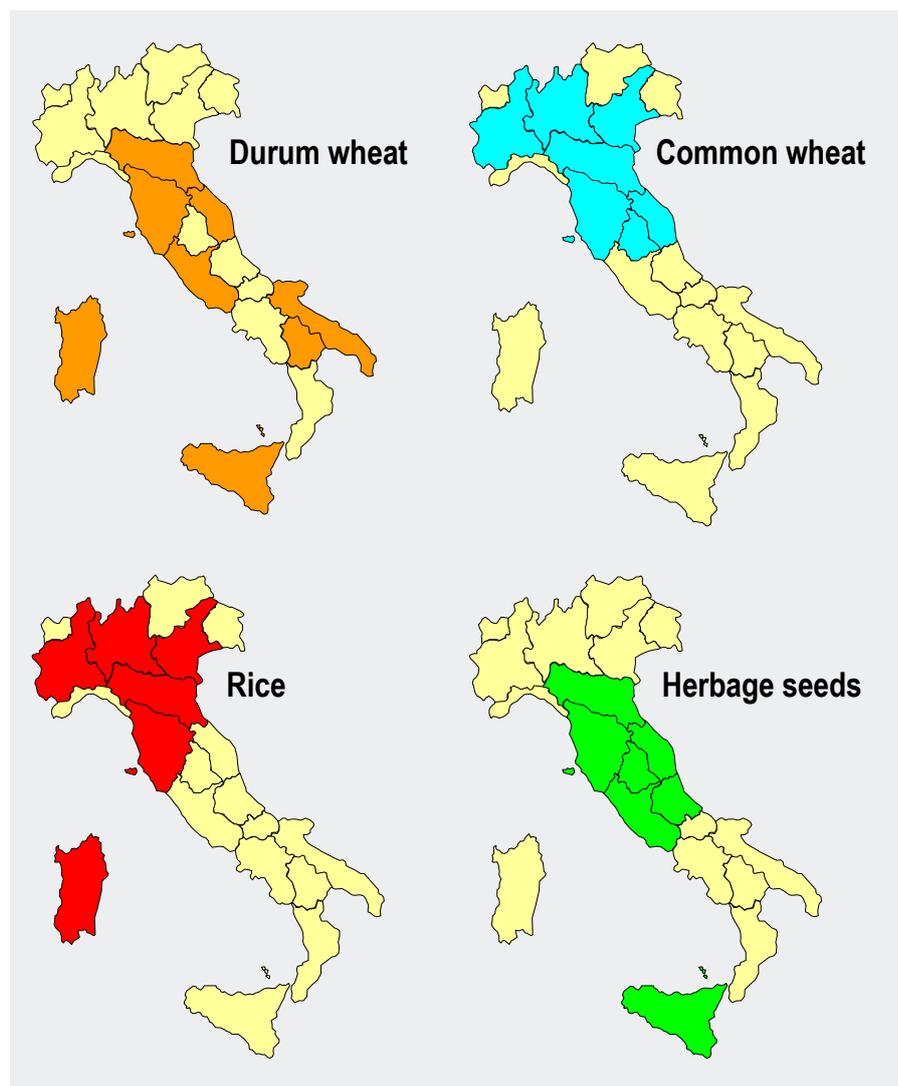


Figure 1. Distribution of seed crops of some species in Italy

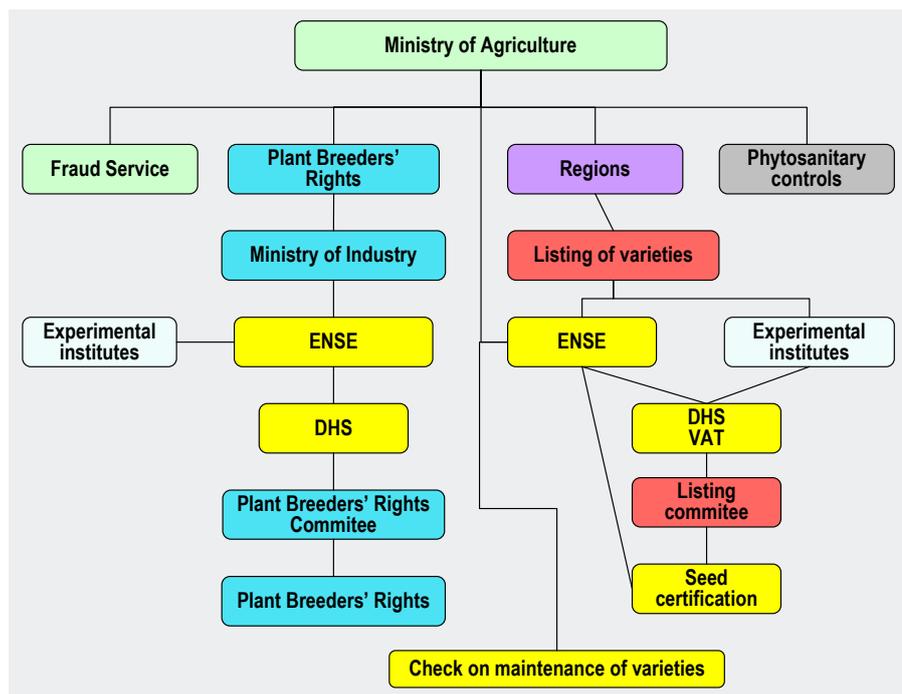


Figure 2. Flow chart of the Italian system for seed controls

Seed regulation

The normative regulation of the seed sector in Italy is obviously related to that of the EU. The early EU Seed Directives have been implemented by the law N. 1096, published in 1971. Subsequently, a mandatory certification system was established. The Ente Nazionale Sementi Elette (ENSE) is the Italian public body carrying out seed certification under the supervision of the Ministry of Agriculture and Forestry.

Even before 1971, ENSE made a certification scheme available on a voluntary basis, and many seed companies used to participate in this scheme, with the aim of certifying their products and to offer Italian farmers seed of good and certified quality.

In 2001, Italy implemented the EU Directive 98/96/CEE, concerning field inspections carried out under official control. Since then, ENSE organizes annual training courses and exams to authorize field inspectors, and carries out monitoring activities as the official control organ. More than 100 technicians belonging to 55 seed companies have been authorized to carry out field inspections under official supervision. In principle, all species are included, but applications received by ENSE concerned in particular durum and soft wheat, barley, soybean, maize and annual ryegrass.

Similarly, Italy is definitely implementing EU Directive 2004/117/CE, which covers the examinations carried out under official control in the framework of certification (seed sampling and seed testing). The Italian regulation was published in 2007, and its practical application is therefore still at an early stage.

In past years, Italy has authorized seed samplers and seed analysts in the framework of the common experiment organized within EU countries on non-official sampling and testing of seeds (Decision 98/320/CE). The experiment focused on a few species (common and durum wheat, barley, maize and sugar beet), while in the near future all species will be considered. The implementation of EU Directive 2004/117/CE will introduce other innovations, including the possibility to authorize independent laboratories. For these labs, ISTA-accreditation status is foreseen. Another new implementation will be the extension to all the seed categories, while the experiment was restricted to certified seed categories (basic seed excluded).

Seed certification

Figure 2 shows the organization of the seed sector, from the point of view of the responsibilities given to public bodies.

As far as seed certification is concerned, ENSE activity refers both to agricultural

(including seed potato) and vegetable species, according to domestic and EU regulations and international agreements related to seed production and marketing.

An average of 200 000 ha has been inspected annually in recent years (as much as 250 000 ha in the recent past), and about 600 000 tonnes of seed is certified by ENSE, corresponding to about 20 million official labels granted. Table 1 shows the evolution of seed certification in Italy in the past 5 years, related to both seed cultivation and seed production.

Tables 2 and 3 show some general figures regarding seed certification of various

Table 1. Development of seed certification of agricultural species 2003–2007 (source: ENSE)

Year*	Cultivation (ha)	Certification (t)
2003–04	252 827.90	698 163.36
2004–05	263 616.05	659 427.83
2005–06	193 632.99	533 648.84
2006–07	174 980.03	579 509.63
2007–08	200 856.77	–

* Working year 1 July–30 June

Table 2. Field inspections (ha) (source: ENSE)

	2006	2007
Sugar and fodder beet	3 104.41	3 867.52
Cereals	126 383.12	151 976.92
Feed legumes	33 388.02	34 030.24
Grasses	3 158.04	3 317.83
Oil and fibre plants	8 011.69	6 695.64
Potatoes	229.38	270.94
Other crops	705.37	697.68
Total	174 980.03	200 856.77

Table 3. Seed certification 2006–2007 (source: ENSE)

Crop	Certification (t)
Sugar and fodder beet	6 489.89
Cereals	518 600.58
Feed legumes	25 620.21
Grasses	5 050.11
Oil and fibre plants	11 610.50
Potato	2 797.22
Other species and mixtures	9 341.12
Total	579 509.63



Figure 3. Durum wheat plots at an ENSE corporate centre (M. Leandri)

groups of species in Italy. Table 2 focuses on groups of species, showing the areas inspected in 2006 and 2007, while table 3 shows the amount of seeds certified for the same groups of species in the working year 2006–07 (starting from field production 2006), as 2007–08 figures are not yet available (the working year will end at the end of June 2008).

Five ENSE units take care of certification, each with its own territory (Milan, Bologna, Verona, Battipaglia and Palermo).

The number of farms inspected yearly in the framework of certification is about 15000, with almost 50 species.

Five ENSE laboratories analysed more than 40000 samples per year for certification (Tavazzano, Vercelli, Verona, Battipaglia and Palermo). One is an ISTA-accredited laboratory: the ENSE station at Tavazzano, located between Milano and Lodi.

Around 7500 post-control plots are checked at three corporate ENSE centres (Tavazzano, Battipaglia, Palermo). Post-control activities are also based in other locations, set up year by year.

Today, ENSE's tasks also cover other fields: registration of new varieties, seed health testing, GMO testing, organization of an organic seed database, research and experimental activities on sampling and testing methodologies, genetic characteristics, seed production and seed science in general.

Wheat

Durum wheat (fig. 3) is the most important sowing crop in Italy, for both the production of typical Italian pasta and seed production.

Seed production has been always important, but its development varied widely, due to changes in European policies and regulations. The use of certified seeds was made compulsory in the early 1990s, as an additional aid to producers. This led to a significant increase in the cultivation of durum wheat seed. Demand for certified seed doubled compared to previous years, and the area under seed production increased from between 60000 and 70000 hectares to the figures shown in table 4 (more than 170000 hectares in 2004).

In 2004, changes in EU policies caused an opposite trend. In 2005 the area was reduced to about half, and in 2006 to even less. Production also decreased, but not by

Table 4. Durum wheat seed certification in Italy 2003–2007 (source: ENSE)

Year*	Cultivation (ha)	Production (t)
2003–04	165 172.49	437 830.80
2004–05	171 487.86	359 903.46
2005–06	91 472.16	233 525.90
2006–07	73 807.48	262 846.35
2007–08	95 884.40	–

* Working year 1 July–30 June

the same rate. In the south, fields of smaller productivity were abandoned, while more productive soils all over the country remained under seed production. Moreover, new areas of the centre and the north of the country have been sown with durum wheat, thanks to the availability of new Italian and foreign varieties suitable for these cultural zones.

In very recent years, a new positive trend has been seen, due to the increase in prices on the global cereal market.

Wheat breeding in Italy has a long tradition. Italian breeders have been working with this species since the beginning of the 20th century, introducing characteristics such as earliness and drought resistance, which are relevant for cultivation in southern regions, but also characteristics targeted at the good pasta-making qualities required by the industry. Italian varieties are also well known outside Italy, especially in other Mediterranean countries, where they are market leaders.

Nowadays an additional EU aid of 40 euros per hectare is granted for the use of varieties included in a special list and recognized for their good qualities.

At the beginning of the 1990s, the cultivation of common wheat showed a relevant decrease, due to changes in the EU agricultural policy, which led the farmer toward different crops. Cultivation therefore dropped from about 1250000 to 700000 hectares. At the same time, production only decreased by around 30%, thanks to a higher yield per hectare. Soft wheat still remains one of the top species, even for seed production. In the past 5 years the production of certified seed has shown a positive trend, and the perspective for the near future is that this trend will remain stable or even increase (table 5).

Table 5. Soft wheat seed certification in Italy 2003–2007 (source: ENSE)

Year*	Cultivation (ha)	Production (t)
2003–04	24 424.27	98 322.32
2004–05	22 459.10	111 369.26
2005–06	24 063.99	112 929.01
2006–07	21 682.02	126 740.15
2007–08	24 376.80	–

* Working year 1 July–30 June



Figure 4. Rice cultivation in Italy (L. Tamborini)



Figure 5. Rice seed harvesting (L. Tamborini)

Rice

Italy is the largest producer of rice in the EU, both for consumption and seed. The total area is around 220000 ha, with over 12000 ha for seed production, and has remained almost stable in recent years. The industry foresees a possible increase in the market, and consequently also in prices, mainly due to demand by new EU members. Rice seed cultivation is mainly located in traditional areas where paddy rice is also produced (Piedmont, Lombardy, Sardinia, Emilia Romagna) (figs. 4, 5). The cultivated varieties are all obtained from Italian breeders. They range from the short-grain varieties, favourites in Italy, to the long-grain varieties, similar to the indica type, well appreciated by Northern European consumers, cultivated since the 1990s. Italian research investments in rice are still important, and particularly the breeding of new varieties, offering a wide range of varieties suitable for different areas of cultivation and agricultural and industrial needs.

In Italy, the utilization rate of certified rice seed is near to 100%, and seed production is highly specialized: growers are very skilled and able to guarantee high quality standards. In Italy, a higher germina-

tion standard than the EU is required: a minimum of 85% instead of 80%.

Rice seed production in Italy covers domestic needs and is also intended for export. Over the past 10 years, the proportion of exported seed has stayed at around 15–20% of total production, and in 2006 amounted to more than 8600 t, or 17%. The importing countries are Portugal, Spain, France, Greece, Hungary, Romania, Bulgaria, Morocco and Turkey.

Rice seed certification over the last five years is summarized in table 6.

Maize

Maize has been a key crop in Italy for a long time, albeit not the most important. 75% of maize cultivation is for grain production, 25% for silage. About 85% is located in the north (Po Valley) and only 15% in central or southern areas. 60% of the national market is covered by FAO Classes 500 and 600, and about 20% each by earlier hybrids (Classes 200, 300, 400) and the later Class 700 (source: AIS, 2006).

Maize cultivation is still very important, despite the decrease and the range of uncertainty which characterize the sector, which is closely connected with the situation on the global market.

This situation is reflected in seed production, and is also influenced by the strategy of a few multinational companies whose policies can lead to important changes from one year to the next.

Table 7 gives an idea of maize seed certification in the past five years, showing the reduction in seed cultivation from 2005 to 2006 (–40%). Until 2005, Italy was the third largest EU producer, after France (around 40000 ha) and Hungary (around 22000 ha), but in 2006 it was also overtaken by Austria (around 4000 ha). Please also note the new positive trend in 2007.

The production data shown in table 7 include both national seed production and not finally certified seeds from other countries.

A significant increase in maize cultivation is foreseen for the coming season. The reasons are the increased prices of all cereal products, the increasing uses of maize for energy production and the abandoning of set-aside policy by the EU.

Nevertheless, the future is difficult to foresee. Many different factors will affect the development of maize cultivation, and in particular seed production.

At a global level, the demand for and price of bioethanol seems to be increasing, but it is not clear which consequences we will have at a national level, as Italy is still an importing country and national production does not cover internal demand.

The GMO issue and its development at the EU level will also have an influence on the maize sector.

Finally, and not surprisingly, climate conditions, water availability and nitrate limit rules in particular will also affect positive or negative trends too, due to the relevance of these factors for production costs.

Table 6. Rice seed certification in Italy 2003–2007 (source: ENSE)

Year*	Cultivation (ha)	Production (t)
2003–04	12 407.47	51 394.98
2004–05	12 261.48	48 348.69
2005–06	11 489.48	48 882.38
2006–07	11 976.72	51 162.64
2007–08	12 560.69	–

* Working year 1 July–30 June

Table 7. Maize seed certification in Italy 2003–2007 (source: ENSE)

Year*	Cultivation (ha)	Production (t)
2003–04	5 208.58	28 834.26
2004–05	4 935.53	30 981.28
2005–06	5 525.10	25 753.03
2006–07	2 944.89	24 757.14
2007–08	3 328.85	–

* Working year 1 July–30 June

Sugar beet

While there was a significant decrease in sugar beet cultivation in all EU countries, due to the restructured Common Market Organization in 2005, seed production in Italy did not follow this trend. On the contrary, after the initial reduction in 2005, the total area cultivated for beet seed production seems to be increasing. One of the reasons is the increasing demand for high-quality sugar beet seed by Eastern European countries, such as Russia and Ukraine. About half the sugar beet seed marketed in Europe is produced in Italy. Its production is concentrated in a few areas of Emilia Romagna, where there are both favourable climatic conditions (mild winters and sunny and dry summers) and a high degree of specialization of growers and companies. For this reasons, Italy plays a leading role in beet seed production, together with France.

Beet seed produced in Italy is mainly intended for export as not finally certified seed (either as rough or preprocessed lots). It means that field production is usually carried out on behalf of foreign companies and then exported to be processed in their own countries.

Italian production is characterized by high quality standards: high germination, high vigour and good performance (high productivity in certifiable seed) can be guaranteed, together with the necessary flexibility in the organization for the required turnover of varieties. Beet seed production requires strong co-operation between breeders, growers and seed companies.

Beet seed production is based on the indirect cultivation method: seeds are sown in nurseries, where the small plants spend the winter, to be transplanted in open fields the following spring, according to a defined scheme of male-sterile and female-sterile

Table 8. Sugar beet seed certification in Italy 2003–2007 (source: ENSE)

Year*	Cultivation (ha)	Production (t)
2003–04	3 678.45	8 046.82
2004–05	3 541.91	10 163.84
2005–06	2 625.82	6 839.63
2006–07	3 012.60	6 372.80
2007–08	3 766.37	–

* Working year 1 July–30 June



Figure 6. Flowering plants of lucerne (A. Sommovigo)

plants. The vernalization assures uniform flowering.

Sugar beet is an allogamous species, and its production necessarily requires cultural isolation distances to be respected. A regional law in force in Emilia Romagna prescribes technical standards and requires the definition of cultivation programmes in advance in the aim of a preliminary verification of isolation distances.

A similar situation exists with regard to forage beet seed production, while its significance is less relevant.

Table 8 shows some figures on sugar beet seed certification in Italy.

Table 9. Herbage seed certification in Italy 2003–2007 (ha) (Source: ENSE)

	Lucerne	Field bean	Berseem clover	Crimson clover	Common vetch	Other feed legumes	Grasses	Total
2003	10 990	1 461	5 073	1 569	3 141	1 246	2 924	26 406
2004	12 782	1 130	6 312	1 448	3 297	1 411	4 432	30 813
2005	14 306	2 833	6 527	1 679	3 737	2 258	4 604	35 944
2006	14 995	5 324	4 203	1 649	4 481	2 737	3 158	36 546
2007	15 090	5 285	3 617	2 367	4 658	3 013	3 318	37 348

Table 10. Herbage seed production in Italy 2003–2007 (t) (source: ENSE)

	Lucerne	Feed broad bean	Berseem clover	Crimson clover	Common vetch	Other feed legumes	Grasses	Total
2003–2004	4 059	1 048	2 097	494	4 042	1 117	6 362	19 219
2004–2005	5 384	2 122	3 345	781	3 856	1 734	6 981	24 203
2005–2006	5 561	4 776	3 335	992	4 882	3 162	6 736	29 444
2006–2007	5 548	6 790	2 438	1 116	5 642	4 086	5 050	30 670

Herbage seeds

Forage crops are still important in Italy, even if the interest is not as large as in the past. The sector has been through changing fortunes, as a consequence of many factors, but of course this group of species has been always one of the most important for Italian Agriculture.

Lucerne is the top species (fig. 6); in some areas its relevance is related to the production of Parmigiano Reggiano cheese, since the cows must be fed only on lucerne hay.

A different case is that of field bean, which recently raised increasing interest as a possible alternative to soybean, because



Figure 7. Agricultural landscape in Tuscany, with a French honeysuckle (*Hedysarum coronarium*) field in the foreground (A. Sommovigo)

of demand for “GMO-free” feed products for organic farming.

Some figures on seed production are shown in tables 9 and 10.

Despite the negative trend in forage crops in general, seed cultivation and production has increased in the past 5 years. This has various reasons. Export of lucerne seeds to other European countries, the Mediterranean, South America and – recently – even North America, has become more and more important. This is due of course to good prices, but also to the quality of Italian materials and in particular to the quality of Italian seed, well appreciated for its high germination value.

Mediterranean clovers are further important species (fig. 7). Seed is aimed at the national market and for export. Overproduction in some years led to large stocks, now probably exhausted, and a possible positive trend is foreseen for the coming season.

Seed production of feed legumes has a long tradition, in contrast to grasses, which recently generated some interest especially as far as Italian ryegrass is concerned.

Vegetables

The vegetable sector includes an important number of vegetable species, as well as aromatic and flower species.

As far as seed production of this large group is concerned, three sectors need to be considered: multiplication from buyer’s seed stocks, production for professional growers, and production for the hobby market.

Seed multiplication in Italy is carried out in a similar way to sugar beet seed multiplication. Again, Italy and France are the most important seed producers in Europe. Again, the environmental conditions together with the specialization of the growers allow Italy to play a very important role. The standards maintained by Italian seed producers and especially high and uniform germination are particularly appreciated, even for sprouting seed products.

Currently, increasing prices of cereal products are causing some alarm, as the cultivation areas are the same. Changing climate conditions and the euro exchange rate are also cause for worry.

Despite the concerns about the near future, in recent years the cultivated area has remained stable. No official statistics are available for vegetable seed field production, but a survey by the Italian Seed Association (AIS) shows that around 10000 ha

Table 11. Vegetable, herb and flower seed production in 2006 (source: Associazione Italiana Sementi)

Species	Cultivation (ha)
Radish	1510
Seed pea	1457
Chicory	1113
Onion	919
Coriander	689
Cabbage	557
Brassicaceae	366
Beet	335
Carrot	326
Lettuce	317
Broad bean	260
Spinach	244
Bean	237
Rocket	227
Leek	156
Chick pea	154
Turnip-top	149
Parsley	113
Cucumber	110
Turnip	103
Basil	102
Spring onion	82
Roscano (<i>Salsola soda</i>)	50
Endive	41
Chives	38
Squash	33
Fennel	31
Pumpkin	17
Celery	17
Water-cress	16
Dill	15
Cardoon	7
Pepper	6
Egg-plant	2
Tomato	2
Melon	1
Other vegetables	5
Other herbs	63
Flowers	24
Total	9894

are used for vegetable seed production (see table 11), confirming Italy's role in this particular sector.

Multiplication is mainly for export to Asia and Europe. For example, radish is multiplied in Italy mainly on behalf of Japanese companies. In the recent past, vegetable seed amounted to up to 40% of the estimated value of Italian seed export.

Many foreign seed companies are present in Italy, with their own facilities or with trading networks. These companies mainly offer hybrids and in general high-quality products for professional growers.

Italian companies are usually of small to medium size. They focus their activities on typically Mediterranean species such as onion, chicory, basil, fennel, turnip-top and rocket. Their products are for professional horticulture, but also for hobby farmers who mainly ask for traditional and typical Italian varieties. Of course, these products are also exported, together with other species, as required on the international market. A recent example is coriander, which has recently become one of the top vegetable species. Coriander seed is only produced for export.

Table 12. Organic seed supply

Working year	Quantity (t)
1999–2000	1 700
2000–2001	5 200
2001–2002	10 916
2002–2003	16 740
2003–2004	17 918
2004–2005	11 388
2005–2006	11 635
2006–2007	10 036
2007–2008*	14 442

*up to 2007-10-31

Table 13. Availability of organic seed 2006/07

Crop	Quantity (kg)
Herbs	0.65
Cereals	8 543 005
Herbages	1 381 565
Vegetables	53 552
Oil and fibre seeds	20 484
Seed potatoes	37 500
Total	10 036 107

Organic farming

Italy accounts for nearly 18% of total organic crops in the EU, and is the largest such producer in the Union. These crops cover more than 1 000 000 ha, about 8% of the Utilized Agricultural Area (source: Eurostat Organic Farming Statistics 2005). Organic farming is therefore important in Italy, and is still showing a slight upward trend (2005/2006: +7.6%).

EEC Regulation 2092/91 was the starting point for organic farming regulations in EU. As far as organic seed is available, its use is mandatory in the framework of organic farming. It means that a information platform is to be organized by each member state, in order to check demand and supply of organic seed and, in certain cases, to allow the use of conventional seed (derogation).

For many years, ENSE has been managing a database of organic seed, where farmers can check supply of species and varieties of interest. ENSE is the public body which grants derogation in cases of unavailability.

Table 12 shows very general figures on organic seed supply, while table 13 shows organic seed availability in 2006/07. Figures of both tables are taken from the ENSE database (www.ense.it/).

GMO issues

It is common knowledge that GMOs represent a delicate issue in both the EU and Italy. This is not the place to discuss this issue, but to give an overview of the situation. Currently, no GMO varieties are cultivated in Italy, where cultivation of GMOs is subject to a specific authorization by the Ministry of Agriculture. As far as is known, no application has yet been submitted.

Table 14. GMO monitoring in conventional maize and soybean seed lots

	Number of tests					
	Maize		Soybeans		Total	
	Negative	Positive	Negative	Positive	Negative	Positive
2004	776 (96.6)	27 (3.4)	167 (98.2)	3 (1.8)	943 (96.9)	30 (3.1)
2005	1 509 (96.5)	55 (3.5)	206 (95.4)	10 (4.6)	1 715 (96.3)	65 (3.7)
2006	2 530 (98.7)	34 (1.3)	539 (97.5)	14 (2.5)	3 069 (98.5)	48 (1.5)
2007	2 138 (98.5)	32 (1.5)	389 (94.2)	24 (5.8)	2 527 (97.8)	56 (2.2)

Figures in parentheses are percentages.

Since 2002 a monitoring programme has been conducted for maize and soybean seed lots produced and marketed in Italy. In recent years, the programme has been based on a national regulation issued in November 2003: some public bodies (ENSE, Fraud Repression Inspectorate, Customs Agency, Phytosanitary Regional Services) are appointed to sample and analyse seed lot samples to verify the absence of GMO seeds in conventional maize and soybean seed lots intended for the national market. Since the beginning, the number of lots included in the programme has increased; in 2006 more than 3000 samples were checked, and in 2007 more than 2500.

Table 14 gives an idea of the number of seed samples included in the monitoring programmes and test results. When these are positive (presence of GMOs), the seed lot is excluded from the market.

It must be stressed that all maize and soybean seed lots marketed in Italy are checked to verify the absence of GMOs, by both the above-mentioned public bodies and seed companies.

ISTA Laboratories

In Italy, three laboratories are ISTA accredited:

- ITDL01: Laboratorio di Ricerca e Analisi Sementi LaRAS, DISTA, University of Bologna;
- ITDL03: Ente Nazionale Sementi Elette, Laboratorio Analisi Sementi, Tavazzano (LO);
- ITML06: Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale, Peri (VR).

The scope of accreditation of each lab can be found on the ISTA website (www.seedtest.org). ■

1st Global Conference on GMO Analysis

24–27 June 2008, Cernobbio (CO), Italy

Over the last few years, detection of genetically modified organisms (GMOs) in the environment, and in food and feed, has become increasingly important. Issues such as monitoring, traceability from farm to fork and co-existence are being increasingly discussed. In addition, a number of emergencies concerning unauthorized GMOs have further alerted us all of the need for a rigorous but affordable control system.

Particularly in recent years there have been significant new developments in the regulatory framework, in the rapid development of qualitative and quantitative test methods and in sampling.

However, there has been little opportunity for experts to discuss these developments at an international level.

Therefore the Joint Research Centre of the European Commission has taken the initiative with the European Network of GMO Laboratories to organize, together with an organizing committee, the first Global Conference on GMO Analysis. ISTA Secretary General Michael Mutschick is also a member of the organizing committee.

The conference aims to address the science and technology underpinning GMO control and analysis by bringing together international experts willing to share knowledge and participate in promoting international scientific dialogue across diverse yet interdependent areas, such as:

- Sampling for GMO analysis, along the seed, food and feed production and supply chain (“from farm to fork” and vice versa)
- Analytical tools and applied procedures along the commodity production chains;
- Consistency of test results, result interpretation and reporting;
- Harmonizing standards for detection of genetically modified traits.

The event is aimed at all stakeholders involved in GMO control and analysis, including industry and regulators, and beyond. It will be held at the Villa Erba, Como, Italy (www.villaerba.it).

Further information can be found by visiting the official web site of the conference (<http://gmoglobalconference.jrc.it>), or by contacting:



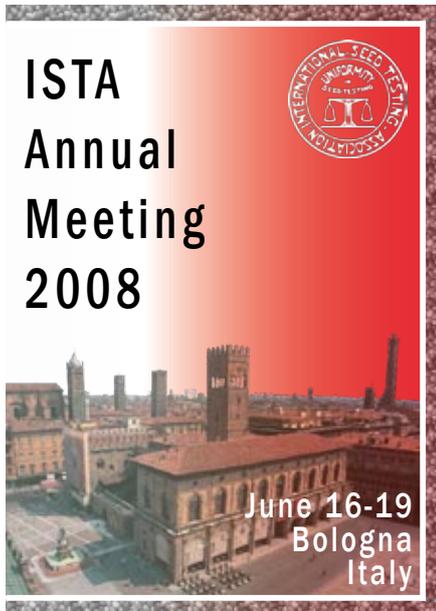
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The conference venue: the Villa Erba, Como, Italy

ISTA Annual Meeting 2008

16–19 June 2008, Bologna, Italy



Dear colleagues,
The International Seed Testing Association (ISTA) takes pleasure in inviting you to participate at the ISTA Annual Meeting 2008, to be held from 16–19 June in Bologna, Italy.

Bologna is one of the birthplaces of the seed testing in Europe. The seed testing laboratory as part of the University of Bologna was established in the academic year 1907/1908 by Professor Francesco Todaro, with the purpose of protecting and promoting quality seed production, national and international seed trade, and seed growers and users. The successor of the former laboratory today is an ISTA “Designated Laboratory” of the Italian Government. On the occasion of its 100th anniversary, our Honorary President Professor, Attilio Lovato, invited ISTA to organize the Annual Meeting 2008 in this historical place.

The meeting is aimed at discussing and deciding on proposals for changes to the

ISTA *International Rules for Seed Testing*, and business items of the Association, with the international participation of ISTA delegates and representatives from both the seed industry and governments, including experts in seed technology, scientific research and laboratory accreditation.

At this year’s Annual Meeting there is a special focus on the testing of specified traits, including the testing of the adventitious presence of GM seeds in non-GM seed lots. All aspects regarding this important area in seed testing will be covered in a one-day seminar on 16 June. A highlight therein will be to draw conclusions from data gathered in recent rounds of the ISTA Proficiency Test Programme on GM Testing.

I look forward to seeing you in Bologna!

Katalin Ertsey
President of ISTA

ISTA Seminar on Specified Trait Testing

Monday, 16 June 2008, Bologna, Italy

This year, on 16 June, the Monday of the ISTA Annual Meeting, the GMO Task Force will present the ISTA Seminar on Specified Trait Testing. The aim of the Seminar is to provide useful information regarding the overall situation of specified trait testing in seeds, as well as to provide education regarding the work in the laboratory.

The seminar is structured in three main parts (see following page). In part one,

important information regarding the political and technical surroundings of specified trait testing in seeds will be presented with a focus on the international trade aspects, the coexistence questions in Europe and the situation in a GM seed growing country.

In the second more technical part, information and education will be provided regarding the work in the laboratory itself

with a focus on the establishment of appropriate testing plans.

In the third part, detailed information will be provided regarding the work of ISTA and the outcome and conclusions of this work.

The meeting will be chaired by Christoph Haldemann, Chairman of the ISTA GMO Task Force. The invited speakers are experts in their fields, coming from the private and the public sectors, from universities and seed companies.

ISTA Seminar on Specified Trait Seed Testing: final agenda

08:20–08:30 Welcome by the Chairman of the Seminar, Christoph Haldemann, ALP, Switzerland, Chairman of the ISTA GMO Task Force)

Part 1: Information regarding the political and technical situation

08:30–09:00 The international seed trade and the difference in the use of GMO seeds – challenges and consequences (Marcel Bruins, Secretary General of the ISF)

09:00–09:30 The situation in Europe – report from the Co-Extra project (Yves Bertheau, INRA, France)

09:30–10:00 Genetically modified crops in Argentina – overview of the regulatory framework, current status and challenges (Moisés Burachik, Biotechnology Office, SAGPyA, Argentina)

10:00–10:30 Coffee break

Part 2: Useful information for the work in the laboratory

10:30–11:00 Latest developments in the area of detection methods – an overview (Enrico Noli, LaRAS, Italy)

11:00–11:30 How to use Seedcalc for the design of testing plans (Sylvain Grégoire, GEVES, France and Kirk Remund, Monsanto, US)

11:30–12:00 The design of appropriate testing plans for testing of adventitious presence of GM seed in non-GM seed lots (Sylvain Grégoire)

12:00–12:30 The design of appropriate testing plans for the testing of genetic purity of a seed lot in the case of a GM variety (Kirk Remund)

12:30–13:30 Lunch break

13:30–14:00 Units of measurement in specified trait testing in seed – the influence of different units on the test result. (Sylvain Grégoire and Kirk Remund)

14:00–14:45 Assessing stacked genes in conventional seed lots (Jean-Louis Laffont, Pioneer, France)

14:45–15:15 Coffee break

Part 3: Work and progress within ISTA

15:15–15:45 Testing for specified traits in seed – the ISTA strategy and the ISTA aim (Christoph Haldemann)

15:45–16:15 The ISTA Accreditation Programme for laboratories testing for specified traits in seed (Martina Roesch, ISTA Accreditation Dept., Switzerland)

16:15–16:45 ISTA GM proficiency tests: strategies and results after 9 rounds (Gerhard Schuon, ISTA Technical Committee Dept., Switzerland)

16:45–17:15 Retrospective analysis of the ISTA GM proficiency tests (Jean-Louis Laffont)

17:15–17:30 Adjournment by the Chairman (Christoph Haldemann)

ISTA Annual Meeting 2008, Bologna, Italy
TRADE FAIR SPACES AVAILABLE
Prime location immediately outside auditorium
200 seed experts expected

EUR 1000 per space and 1 person
including coffee, lunches and Official Dinner
EUR 180 for each further person

For more information, contact Maria Francesca Mannone at:
meetings@ista.ch

ISTA Annual Meeting 2008: final programme

SUNDAY 15 June 2008

16:00–20:00 Registration of participants and welcome cocktail at Savoia Regency Hotel

MONDAY 16 June 2008

08:30–18:00 ISTA SEMINAR ON SPECIFIED TRAIT SEED TESTING

19:00 Cocktail party in celebration of the 100th anniversary of LaRAS, the Bologna Seed Research and Testing Laboratory at the Faculty of Agriculture

TUESDAY 17 June 2008

PRESENTATION OF ISTA's TECHNICAL WORK

08:30 Opening by the ISTA President

08:30–10:00 Session I

- a) Report from the Purity Committee
- b) Report from the Germination Committee
- c) Report from the Moisture Committee

10:00–10:30 Coffee break

10:30–12:30 Session II

- a) Report from the Tetrazolium Committee
- b) Report from the Vigour Committee
- c) Report from the Seed Health Committee
- d) Report from the Variety Committee

12:30–13:30 Lunch break

13:30–15:30 Session III

- a) Report from the Flower Seed Committee
- b) Report from the Forest Tree and Shrub Seed Committee
- c) Report from the Storage Committee
- d) Report from the Committee on Advanced Technologies

15:30–16:00 Coffee break

16:00–16:30 Session IV

- a) Report from the Editorial Board of Seed Science and Technology

16:30–19:00 Time allocated for meetings of single ISTA Committees

WEDNESDAY 18 June 2008

PRESENTATION OF ISTA's TECHNICAL WORK

08:30 Opening by the ISTA President

08:30–10:30 Session V

- a) Report from the Bulking and Sampling Committee
- b) Report from the Statistics Committee
- c) Report from the Nomenclature Committee
- d) Report from the Method Validation Advisory Group

10:30–11:00 Coffee break

11:00–12:00 Session VI

- a) Report from the Proficiency Test Committee
- b) Report from the Audit Programme

12:00–13:00 Lunch break

13:00–13:30 Session VII

- a) Report from the Seed Analyst Training Committee

13:30–14:30 Session VIII

- a) Meeting of the Rules Committee

14:30–15:00 Coffee break

15:00–18:00 Session VIII (continued)

19:30–22:00 OFFICIAL DINNER

THURSDAY 19 June 2008

ISTA ORDINARY MEETING

09:00–10:00 Welcome by the ISTA President, Dr. Katalin Ertsey

Presentation on the development of the Italian seed industry, by Dr. Marco Nardi, Secretary General of the Associazione Italiana Sementi (Italian Seed Trade Association)

10:00–10:30 Coffee break

10:30–12:30 1. Call to order

2. President's address

3. Roll call of Designated Members entitled to vote

4. Reading of Minutes

5. Report of the Executive Committee

6. Report of the Secretary General

12:30–13:30 Lunch break

13:30–14:30 7. Constitution changes

8. Fixation of annual subscriptions

9. Consideration and adoption of the proposed Rules changes 2008

15:00–15:30 Coffee break

15:30–17:30 10. Consideration and adoption of reports

11. Announcement of the place and date for the next Ordinary Meeting of the Association

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

13. Any other business raised by consent of the Executive Committee

14. President's closing address

15. Adjournment

Proposed changes to the *International Rules for Seed Testing*

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 sees the completion of the project to re-arrange the chapters and merge the Rules with the Annexes. The final part of this project is to revise the 'old' Chapter 1, which was the Introduction, and make this into a separate introduction. This is to allow the renumbering of the most important chapter of the ISTA Rules, Chapter 17 Certificates, into the 'new' Chapter 1.

Among the further changes are the following:

New species

Addition of *Cucumis* spp., *Cucurbita* hybrids, *Cucurbita* spp., *Lycopersicon* hybrids and *Lycopersicon* spp.

Chapter 1: Introduction

- Revised Introduction

- Renumbering of Chapter 17, Appendix A and Appendix B

Chapter 2: Sampling

- Increase in seed lot weights for sorghum and pulses

Chapter 3: The Purity Analysis

- Modification to reporting of results for the purity analysis
- Reporting the weight of the purity working sample
- Modification to the PSD for *Spinacia*
- Modification to 3.2.1.2 to include other family names covered by the same rule

Chapter 4: Determination of Other Seeds by Number

- Modification to the precision to use when weighing samples for other seeds determination by number

Chapter 5: The Germination Test

- Changes to germination conditions for *Cynara cardunculus*

- Addition of Top of Crepe Paper with Sand (TCS) method to the ISTA Rules for *Pisum sativum*

Chapter 6 Tetrazolium Test

- Addition of new procedures for *Allium*, *Lycopersicon*, *Lactuca* and *Cucumis*

Chapter 7: Seed Health Testing

- Addition of new method: 7-025: Detection of *Aphelenchoides besseyi* on *Oryza sativa* L.

Chapter 8: Species and Variety Testing

- Improvements to the testing method to detect bitter seeds in lupin samples (*Lupinus* spp.) method 8.8.2

Chapter 17: Certificates

- Improvements to processes described; renumbering of Chapter 17 to Chapter 1

International Rules for Seed Testing Edition 2008

ISBN 978-3-906549-38-5

ISBN 978-3-906549-48-4 (Annexe to Chapter 7)

Complete set in two parts (Rules & Annexe to Chapter 7, including Amendments 2008)

The *International Rules for Seed Testing* are ISTA's primary instrument to promote uniformity in seed testing. The ISTA Rules have 17 sections that provide definitions and standardized methods to be used in, for example, sampling, testing seed lot quality and reporting results for international trade. The ISTA Rules are also a useful reference guide to germination conditions and methods for over 1000 species.

The *International Rules for Seed Testing* are approved by and amended at ISTA Ordinary Meetings on the basis of advice tendered by the ISTA Technical Committees. The Edition 2008 (effective 1 January 2008) includes the latest changes which were passed at the ISTA Ordinary Meeting 2007, held at Iguazu Falls, Brazil, on 11 May.

Updates, in the form of additions or replacements of existing pages, are published as Amendments to the *International Rules for Seed Testing* and can be inserted separately into the binder.

Price: CHF 389.00 (approx. USD 400.00/EUR 250.00) from the ISTA Secretariat (for contact details, see back cover)



Membership requests for the Working Programme (Terms of References) 2007–2010

On 16 October 2006, a letter was sent by the ISTA Secretariat to all ISTA Designated Authorities, Members and Stakeholders, asking them to state their wishes and needs. The letter was sent to a total of 322 addressees.

Replies received by 1 April 2007 are included here.

A total of 15 answers were received.

Summary of expressed needs and wishes regarding ISTA Working Programme 2007–2010

A. Method development

Species missing in the ISTA Rules

- *Limnanthes alba*
- *Diploaxis* sp.
- A few rules for vegetables
- Tropical seeds: *Carica papaya*

Methods missing in the ISTA Rules

- Methods for analyses (purity, germination, moisture content) of seed mixtures
- Image analysis for Purity and determination of other seed by number
- SHT methods
- Crepe cellulose paper (TC method in AOSA), (TCS method in AOSA, MWSS working on a referee through ISTA Germination committee), multiple floret factors, bowing points
- We need to think about methods of evaluating seedlings at a later stage than is now required: special tests could be developed for “useful plants”

In view of the progress of technology, new rapid tests could be included, for instance:

- to determine “germinability” of seeds, for instance based on the automatic measurement of the appearance of root tips in time, enabling to determine quality factors such as maximum germination, T50 or T75, expressing speed of germination, which can be related to vigour
- automated determination of normal seedlings or useful plants

- tests to determine weeds or ergot in large quantities of seeds (other seed determination by number) using serological or molecular techniques on ground seeds (meal) or seed extracts
- We need sampling methods in identification of species and varieties test

Tests for seed quality missing in the ISTA Rules

- Compost on corn (e.g. Pb with specific treatment)
- Purity, OSD, but not immediately. They need improvement and standardization
- Many more methods for SHT
- Vigour testing of vegetables seeds is currently receiving much attention in the private sector; is ISTA aware of that?
- Taking of seed mixture samples and samples for seed health need to be improved. Also, validation to use cargo samplers for some species could be included.

Proposals for new or improved test methods

Germination Vigour Test

Purity and other seed determination

- Molecular methods for strain identification or for detection
- Co-operation with stakeholders, e.g. for tropical species, not only ISF but also governments, institutes, etc.
- Fast methods to detect damage and/or different quality in seed lots (e.g. fast green, C1Na.)
- We know that in sampling, stick sleeve triers are not allowed for ISTA sampling. We have made an experiment: sampling seed lot with stick sleeve trier and stick trier with compartments. The samples were analysed for purity, other seed determination and germination. Results are in tolerance. We sampled cereals and rape seed, at different places and times. Also, we tried to take samples from *Phleum pratense*, but the sleeve trier jammed. For very small seeds, it is not suitable. But for everyday work the sleeve trier is easy to use. We

have application to speed up the validation procedure.

- About weight determination: we carried out testing with 8 replicates and compared results with counting 1000 seeds from the working sample. The results are the same. What do we have to do: how many tests are required to prove that it is not necessary to count 8 replicates and calculate coefficient and 1000-seed weight or to count the entire working sample?

Proposals for changes/improvements for the current ISTA Rules

- Chapter 9: Moisture: needs more clarifications and descriptions, especially point 9.1.5.3. Working sample

Germination:

- use of BP for *Lolium*, *Festuca*, *Brassica* (NZDL04 is prepared to organise Method Validation for the use of BP for *Lolium* and *Festuca*)
- final count for *Trifolium repens*, *Trifolium pratense* and *Medicago sativa* shortened from 10 to 7 days
- final count for *Lolium* spp. shortened from 14 to 12 days
- 20–25 °C as an optional temperature for *Lolium* spp.
- removal of seed coat before testing *Crambe abyssinica* (i.e. include in “Additional directions” column)
- GA₃ as a dormancy-breaking option for *Crambe abyssinica*
- the amount of water required for sand tests is unclear. What does “moist” actually mean? A better explanation is required
- Table 5A of the ISTA Rules states that *Oryza sativa* has to soak in HNO₃ (24 h) to break dormancy. Our observations resulted that no *Oryza sativa* can germinate after soaking in HNO₃ (they all exactly dead). We usually soak *Oryza sativa* in KNO₃ 3% for 24–48 h depending on how deep the dormancy occurs (paper moistened by water not KNO₃) and it is successful in breaking dormancy of *Oryza sativa*.

- to conduct paper quality control, we should compare unknown quality paper with known quality paper to germinate sensitive seed, i.e. *Phleum pratense* etc. Currently we have no species that we can use to conduct paper quality control.
- Viability Test:
- there are no procedures to conduct TZ test on *Vigna unguiculata*
- Vigour Test:
- we need vigour test for tropical seeds such as *Oryza sativa*, *Zea mays*, vegetable seed etc.
- Species and variety testing:
- we need methods to conduct species and variety testing for other seeds, especially tropical seed
 - we need methods to identify specific traits (morphological, chemical and biomolecular DNA) in the species and varieties test
- Seed health testing:
- we propose that ISTA should develop a method for the seed health sample for fungi in general. We often find that in one seed sample there are many species of fungus.
 - if our test shows that there is a high percentage of storage fungi such as *Aspergillus* sp, *Penicillium* sp, *Rhizopus* sp. etc., should we report it in the certificate, even though they are not seed-borne diseases?
 - there should be seed testing procedures for other food crops and horticulture seeds to detect and identify bacterial seed-borne diseases (in the ISTA Rules there are only methods for *Brassica* and carrot). Also, methods to detect and identify viruses and nematodes causing seed-borne diseases, especially in tubers, rhizomes and fruit seeds. We need this because we often find that bacteria, viruses and nematodes cause poor germination.
- Moisture content:
- there is no method for determination of moisture content in *Ipomea aquatica*, *Mamordica charantia* and *Luffa acutangula*.
 - we test mainly *Oryza sativa*. We have a seed grinder (Cemotec), but the smallest scale could not reduce to the appropriate seed size because the hulls could not be ground well, hence they could not pass the sieve. What shall we do?
- Weight determination:
- seed weight is affected by moisture content. We propose that moisture content should be in the weight determination report.
- International Orange Certificate
- we conduct method development and we use these methods for routine tests. Can we report it in the Orange Certificate and where?
- Other:
- A question about calculating and expressing germination results (5.8.A) when the mean of the four replicates is 99,5% normal seedlings: according to the Rules, the result to go on the Certificate is 100%, but this ignores the fact that in at least one of the replicates one or more abnormal/hard/fresh/dead seeds occurred. Is this ethically correct?
 - With annual changes to the ISTA Rules, a new set of pages is sent for the one ISTA Rules provided to each laboratory. However, when a laboratory has purchased extra copies of the Rules, a new set of pages is not sent, and the laboratory then has to order them. Surely the system could be improved so that the Secretariat has a register of every purchaser of extra sets of the Rules, and new pages (plus the account) get sent automatically?
 - A method for testing the presence of endophytic fungi in *Lolium* would be useful.
 - SHT: not always. Need general methods for several pathogens of a same crop and not 2 methods for 1 crop and similar pathogens.
 - Need methods for treated seeds
 - Update methods that have variability
 - The key to help us to find the proper tolerance table may be clearer. The choice between 3.2 and 3.3 (one-way or two-way test) is not clear, and a better description in the key would be helpful. In the headings of the table 3.3 it says: "...when the results of the second test is poorer than that of the first test". What is actually meant is that we use this table to see whether the second lower result is within the limits of natural variation and still acceptable, and that there is no scientific basis for a possible claim.
 - It is striking that different levels of probability are being used, not reflecting the natural variation of such tests. In other words, we feel that for all two-way tests it would be logic to use 2.5% and for one-way test 5%. Now all kinds of different combinations are being used depending on the kind of test. The 2.5-5% case we find in the tables of germination capacity, but in purity we observe the opposite and with a large jump from 5% (3.1) to 1% (3.2). For the other seed by number test it remains at 5% in both cases.
 - Now that we have accredited laboratories we can reduce the number of tables and simplify the instructions that Miles (1963, p. 641) suggested: "Therefore, to be realistic, the tolerances for tests made in different laboratories include true variation that existed in the 1950's among laboratories. Laboratories should strive to eliminate real variation among laboratories. If this is accomplished, tolerances for tests in different laboratories should be recomputed for random sampling variation only. This would reduce tolerances." Or in other words, do no longer distinguish between the situation when a re-test is done on the same sample or on two different samples that have been independently drawn (=at different moments). It may be time to decide that we have reached the situation as described by Miles after 40 more years of striving for uniformity in seed testing. Therefore the suggestion is made that the Statistics Committee has a close look at the various tables in use, they are based on variation of labs during the 1950's, the choices made from Miles' tables look sometimes arbitrary and a possible reduction of the number of tables may be possible. (The cases of Controlled Deterioration and Accelerated Aging testing may serve as examples of a simple solution.)
 - The Accelerated Aging tables are supposedly all based on Miles' Table G1, column L. This is a typographical error: 15.4 is indeed column L, and 15.5 could be traced to column M, but the third table is a mystery.
 - One company mentioned that ISTA could be more alert and respondent to actual seed trade problems. Mention was made of the current problems with *Xanthomonas* in (treated) cabbage seed. ISHI NL jumped onto the problem immediately, has ISTA taken any action?

- Avoid changing rules with “new” concepts and ideas that really nothing improve
- ISTA Rule description. 50% rule. In first count of germ test, when we find 50% healthy cotyledon (seed coat cover less than 50% cotyledon area), we begin to evaluate. In this situation we would be very happy that you give us some typical picture of 50% cotyledon.
- Anyway we need Seedling evaluation; more descriptions with pictures

Gaps in the text of ISTA Rules

- (no comments)

Distribution channels for the ISTA Rules

- Paper
- In addition of the existing ones: meeting, e-mail
- electronic
- electronic distribution is needed, may be not for the complete ISTA Rules, but for separate chapters
- Today’s distribution channels for the ISTA Rules are fine, but if possible send updates by e-mail

B. Seed Science

Proposals on enhancements for the ISTA Seed Symposium

- For New Zealand’s seed analysts the Seed Symposium is only something they can read about – their system does not have the funding to allow seed analysts to attend ISTA meetings/seminars/workshops
- New technologies in testing and information management
- The Seed Symposium should be restricted to two days: there is not enough substance to fill three days. It proves difficult to fill the three days, and some presentations are embarrassingly below the mark.
- The Seed Symposium should come after the General Meeting; so the sequence should be: Technical Meetings, General Meeting, Symposium. We feel it is not appropriate to force people to attend a symposium they are not interested in. No interruption between technical issues. In this way we serve our three main groups best:
- the technical people can follow the Technical Meeting and the General Meeting, and may decide to stay on for

the Symposium (for many the Congress is too long however)

- the policy people can come solely for the General Meeting and may decide to attend either the Technical Meetings or the Symposium, or both. (for all the entire “Congress” is too long)
- the scientists may decide to come to the Symposium only, as very often happens now
- We need symposium or seminars in tropical seeds only. We hope ISTA could arrange a suitable place for us (for example South-East Asia or Asia region) and free of charge also, including accommodation and travel costs, especially for ISTA members who come from government laboratories.

More seed science activities on special topics or areas demanded?

- Scientific presentations
- Yes
- More meetings of scientists, like seminars, do not seem necessary. Scientists can interact at many occasions, and through electronic ways. It is reported that the scientific level of the ISSS congress is higher. Company seed scientists come to listen at the ISTA Symposium, they rarely contribute. This one-way traffic is not good: we need to know what goes on in the whole seed science world, not only in the official testing stations. So we propose to expressly invite them to contribute in future to the Symposium (i.e. mailings outside the ISTA circle). In this way we may get a better picture of the world of seed science.

One suggestion could be that ISSS and ISTA jointly organise a symposium once every 3 years. An idea could be to choose a theme per meeting: ISSS is now preparing a meeting on Storage; other themes could for instance be organic seed treatments or priming.

- Seek co-operation with organisations for joint organisation of congresses, seminars, workshops etc.
- More seed science in seed health (*Fusarium* problems)
- Encourage ISTA members to involve the national science and research institutions to participate and initiate seed science.

Provision in regards to seed science

- For applied research which has no chance to be formed in scientific projects
- ISTA could enter a little bit more on science

Comments on scientific journal “Seed Science and Technology”

- Preference on receiving the hard copy
- The articles should be accessible per computer via direct access to (pre-publication) abstracts, and more importantly, access to full articles through paid electronic subscription. For instance, at Universities one may have access to over one thousand electronic journals (like Seed Science Research of CAB), including their back volumes. SS&T is not among them. The present CD can only be an intermediate solution.
- STI contains several semi-scientific papers, SST for real scientific papers only? Perhaps it should be made clearer what types of papers ISTA will publish in both series. Validation papers to be included in STI, and no longer a separate series?
- This journal could be used much more by looking up the papers that could go directly to the Technical Committee for discussion
- We are satisfied to see Seed Science and Technology (SST) and ISTA News bulletin. It is very helpful to improve our knowledge.
- We would like to see special issues of SST with certain topics (germination test edition, moisture content edition, seed storage edition, etc.).

C. Training

Proposal for new training tools

- Molecular biology
- Webinars
- We need training and technical assistance for all aspects of seed testing including method development and validation, especially for tropical seed. We hope free of charge, including accommodation and travel costs, especially for ISTA members from government laboratories.

Comments on the quality of ISTA Training and Education tools

- Enlarge exchange of information about possibilities to participate in ring tests between accredited seed laboratories, as ring tests are considered very positive tools to maintain proficiency of laboratory experts not taking part directly in ISTA Workshops
- The likelihood of any New Zealand analysts actually getting to a Workshop is very small
- The opportunity to participate in a career development training course which would lead to an ISTA-recognised Certificate of Proficiency in Seed Analysis is exciting for new analysts. This system would be particularly beneficial for small laboratories where staff training is difficult to organise. Having an international qualification would also be useful for seed analysts who go to work in other countries. There will be no doubts of quality of staff when they are the holders of an ISTA Certificate of Proficiency in Seed Analysis.
- Good!
- Good documentation on seed characteristics of other seeds is still lacking. An electronic database of seed images should become available; at least for those species on the “common list”. Alternatively, perhaps the GRIN database could include links to seed images, like it has now to foliage and flower images.
- Members would also be better served with an updated list of stabilised plant names at the ISTA website, not via GRIN. ISTA should take its responsibility here. GRIN gives too much information that is not relevant or understandable for non-taxonomists; analysts tend to get lost in the wealth of taxonomical information. Alternatively, a special ISTA window can perhaps be made in GRIN: valid name, current synonyms, link to seed image, link to the full content page.
- The Hohenheim Seed Collection is an excellent tool: however it is biased towards temperate species. Both temperate and tropical labs would be very well served with a good collection of the most common tropical seeds. A good collection of seeds occurring in tropical crops, such as in rice, should be organised relatively quickly: the need is high.
- Develop ISTA-wide training programmes or guidelines; at the moment every lab has to develop its own. This is a waste of time and money. Labs can use them, and pay ISTA a percentage per course or analyst?
- Especially workshops are very useful and should be carried out at different places (regions) and should be announced enough time in advance, so that laboratories can prepare staff (learn English) for workshop
- Any workshops on seed testing, especially on purity, other seed determination, germination, tetrazolium (on agricultural and tree species) and moisture content (on cereals) are the priorities for our laboratory

High priority on what kind of distance learning programme

- An online seed analysis distance (online) learning programme is a very good idea. This will help especially the candidate and new labs.
- Distance learning in the end has to be checked in a practical exam for analysts. Materials (photos, illustrations, flow charts, handbooks, texts) to be used could be available on the ISTA website (uniformity, one central place known to all involved).

Handbooks which are not in the ISTA Publications Catalogue

- A very detailed handbook on pure seeds for specific purity (photos)
- Seed identification, Quality management requirements for different kind of analysis
- SHT and under progress. One for molecular methods? Or included in Handbooks with molecular techniques
- Suggestion to make a handbook to prepare for ISTA accreditation with examples of quality handbooks, SOPs, how to develop them, etc.

Proposals on enhancements for the existing ISTA Handbooks

- A Handbook of weed seeds would be very useful
- Preference on the Handbooks being posted to each laboratory
- French version of Handbooks
- A Handbook in use of the different tolerance tables and other necessary use of statistics in seed testing

- There should be statistical analyses for calculation and expressions of results in method development or validation.
- Materials (photos, illustrations, flow charts, handbooks, texts) to be used could be included in handbooks?

Distribution channel for ISTA Publications

- Paper and mail
- Internet
- Rules and Handbooks are getting more and more beautiful and hence expensive. This is an obstacle for their use.
- Paper and electronic
- E-mail or as downloads from the ISTA website
- It is very helpful if we can access and download ISTA Handbooks by internet and free of charge

D. Certificates**Proposals for increasing the demand for ISTA Certificates**

- Not only documents for export purpose but also an insurance of quality of analysis for internal seed trade
- Electronic certificates
- PR in US-News releases on acceptance in other countries. Promoting importing countries to request ISTA Orange Certificates from US Seed Industry more
- In order to get a better picture of the use of certificates, and for what categories, it could be an idea to collect data on seed moving in international trade, with and without certificates. This can be done via member labs that also have a market inspection task. A market survey will enable the identification of effective actions.
- We had several companies explaining why they did not go for ISTA accreditation and what would be needed for them to do so. Vegetable seed companies test their seed batches intensively and according to protocols that meet market requirements. Tests are repeated periodically to assure reliable quality. Vegetable seed companies take their responsibility regarding seed quality in order to keep clients satisfied and to avoid (financial) claims. Considering this:
 - for Orange International Certificates (OIC) samples are being taken from a ready-to-ship-delivery,

- the often dynamic vegetable seed market can demand several varieties for different segments and growers; variety choices in many cases are being made relatively shortly before the sowing season,
 - new varieties often have to be delivered out of new harvest,
 - within the shipment often several varieties are present with limited quantities (e.g. new introductions),
- it is concluded that the OIC does not fit well in the routine of the vegetable seed industry. The OIC cause a considerable delay of shipment and the costs for the OIC are for several batches not in balance with the value of the goods.

Vegetable seed companies therefore try to limit the use of the OIC to those cases that this certificate is absolutely obligatory from the side of the government of the country of import.

Most seed companies are using methods and systems to verify that seed lots qualify for delivery to customers that deviate from the germination methods required for Orange Certificates.

For example, seed companies may base their decision on an average of results of multiple fractions from the same production lot, before having the final test result of the lot delivered to customers.

Or seed companies may base their decision on test protocols that predict the percentage of usable plants under growing conditions, protocols that differ significantly from ISTA methods.

For the vegetable seed industry to facilitate companies in participating in the ISTA laboratory accreditation system, it is proposed that ISTA undertakes a study to take into account the quality assurance systems and test methods currently in use by the seed industry. Many companies are NAL certified, and use NAL certificates based on in-house methods and procedures. This is a non-stop procedure that does not take extra weeks of testing after the lot has been prepared. Maybe ISTA could consider allowing such approaches as well.

- With the size of companies in the seed industry increasing, more and more countries will accept regional accreditation systems; difficulties in moving seed across borders between countries will decrease.

I only see a decrease in the demand for certificates. Don't spend time on this issue, as the chances for success are low.

- Accept that it is possible to use an ISTA Certificate for only one single analysis: germination/tetrazolium – purity/number count test – seed health
- Make agreements with the EC/OECD about use of ISTA Certificates in international trade of seeds
- ISTA Secretariat should have a programme to publish “the benefits of ISTA Certificates” hence it can increase demand for ISTA Certificates

E. Accreditation of seed-testing laboratories

Proposals on improvements to the ISTA Proficiency Test

- Could be useful to show not only test results but different methods used too, if it is a case of more than one ISTA-validated method
- would be useful to have the packaging of proficiency test samples strengthened
- More frequent
- the ISTA PT programme could benefit from the large experience of companies with seed health testing. In this way the methods of ISTA and ISHI could come closer together.
- PT is now concentrating on purity, germination, with moisture and TZ at a less frequent level. I think all tests that are described in the ISTA Rules should be subject to PT.
- ISTA could organise Proficiency Tests using e-mail. The laboratories can give their opinion about different abnormal seedling for example or the laboratories must identify several weed seeds by e-mail.
- We are missing the rating system in purity (Proficiency Test)
- We have planned to be accredited by ISTA in 2009 for tropical seed in moisture content, purity analysis and germination test. We took part in three Proficiency Tests (2006), i.e. *Sorghum bicolor*, *Beta vulgaris* and *Phaseolus vulgaris*, but these are not our main species and there was no moisture determination. We hope that ISTA will arrange special proficiency tests for tropical seeds (such as *Oryza sativa*, *Zea mays*, *Glycine max*, *Vigna unguiculata*, Brassicaceae, *Capsicum*, *Lycopersicum*

etc. including tropical other seed) in moisture content, purity analysis and germination test.

Proficiency Test missing

- SHT but under progress

Proposals on improvements for the ISTA Audit Programme

- Calibrating a soil divider: two different technical auditors have recommended the use of *Brassica* seeds to calibrate the divider. This should be changed, as *Brassica* seeds are the wrong type for this purpose (they roll and bounce and therefore are not practical).
- Audit schedule: if an accredited laboratory continues to perform to expectation in proficiency testing, why can't the audit schedule be moved to every 5 years?
- Technical Auditor: receive training on basics of auditing. Evaluation of audit is standard process
- The audit programme is expensive for small labs, and for companies with more than one lab (in one or more countries). Find ways to decrease the costs.

F. Products

Products missing

Other comments on the products

- The new format of International Rules for Seed Testing is appreciated but the book cover breaks quite quickly, especially Annex to Chapter 7 Seed Health Testing methods
- ISTA should consider expanding its range of products, include seed testing supplies such as germination paper. For long established laboratories in Europe, supply chains are organised, but for new laboratories in many other parts of the world, it is often difficult to know where to go for supplies of seed testing materials/equipment.
- Suggestion to ask new members about their motivation for becoming a member. It is important to know whether lower membership prices are a main incentive to become an ISTA member. Even the free items are probably not the greatest incentive for personal members.

(continued on page 22)

The new Executive Committee 2007–2010

Katalin Ertsey

President



Katalin Ertsey is from Hungary, and studied at the Budapest University of Horticulture, qualifying as a horticultural engineer in 1974. From 1979 to 1987 she was head of the Seed Testing Laboratory. Further studies led to a doctorate in horticultural science in 1985, and a PhD in Agriculture and Horticulture Sciences in 1995.

From 1987 to 2004 she was Head of the Seed Certification Department of the Central Agricultural Office, and since 2004 has been Director at the Directorate Plant Production and Horticulture, responsible for plant variety registration certification for seed seedlings and vegetative propagation material.

Katalin Ertsey is a member of the Technical Board of the Hungarian Food Safety Committee, and has been a member of the ISTA Executive Committee since 1992.

John Hampton

1st Vice-President



John Hampton is from New Zealand. He gained in 1973 a BAgSc in Plant Pathology from Lincoln College and in 1975 an MAgSc from Canterbury University, Christchurch, New Zealand, and in 1983 a PhD in Agronomy from Nottingham University, England.

He started his career in seed technology in 1974 at the Palmerston North Seed testing Station, and has since held several university posts in this field, finally becoming Professor of Seed Technology in 1999, and

Director of the Bio-Protection and Ecology Division in 2005, at Lincoln University, Christchurch.

His current research interests are seed quality evaluation, particularly seed vigour and seed-borne pathogens; seed sampling protocols for border biosecurity; development of seed quality assurance systems, and seed production in conventional and organic systems.

John Hampton is a member of the Editorial Board of Seed Science and Technology, and of other professional bodies and societies too numerous to mention here. He has been a member of the ISTA Executive Committee since 2001.

Udo von Kröcher

2nd Vice-President



Udo von Kröcher gained his diploma as Ingenieur-Agronom from the University of Göttingen, Germany in 1984. Since then, he has worked as manager of an arable farm (1985–1986), as project manager at the Hanover Chamber of Agriculture (1986–1989), and in supervisory positions at the Ministry of Agriculture of Lower Saxony, responsible for plant production incl. plant variety, seed certification and seed control issues (1989–2000).

For two years during this period (1993–1994) he was national expert at the EC Commission in Brussels, involved in examination of national agri-environmental programmes.

Since 2000, Udo von Kröcher has been President of the German Bundessortenamt (Federal Plant Variety Bureau).

As 2nd Vice-President of ISTA, he is responsible for organizing the 29th ISTA Congress in 2010 in Cologne, Germany.



Back row, left to right: Joël Léchappé, Grethe Tarp, Mary Chipili, Rita Zechinelli, Masatoshi Sato, Jorge Rosales King. Front row, left to right: Susan Maxon, Udo von Kröcher (2nd Vice-President), Katalin Ertsey (President), John Hampton (1st Vice-President), Alison Powell, Michael Muschick (Secretary General)

Grethe Tarp**Member-at-Large**

Grethe Tarp, from Denmark, received her MSc in Horticulture from the Royal Veterinary and Agricultural University, Copenhagen, in 1976. After graduate studies in plant pathology at the University of Illinois and training in seed testing at the Danish State Seed Testing Station, Lyngby, she went to Swaziland as an Agricultural Officer for the FAO in 1978. From 1980 to 1988, she was Seed Quality Control Expert, Officer-in-Charge and acting Director at the National Seed Service, Maputo, Mozambique.

Returning to Denmark in 1988, she first worked as Research Officer in Certification at the Danish State Seed Testing Station at Lyngby, before promotion to Head of the Department of Germination at the Danish Plant Directorate in 1990, and of the Seed Department in 1992. Apart from a period in Vietnam as an Senior Adviser on Seed Sector Development, she has remained in that post to the present day.

Grethe Tarp has been a member of the ISTA Germination Committee since 1992, and was elected to the Executive Committee in 1995.

Joël Léchappé**Member-at-Large**

Joël Léchappé graduated at the Universities of Nantes and Rennes (France) in Botany, Zoology, Ecology, Biochemistry and Plant Physiology.

After a PhD in Plant Pathology (root diseases on *Phaseolus*), he joined INRA (National Institute for Agronomical Research) in the Group for Study and Control of Varieties and Seeds (GEVES) as head of the Germination Laboratory of

the National Seed Testing Station. He has been Director of the Station since 1993.

He made contact with ISTA in 1987 with Professor Lennart Kåhre in Uppsala. Since then, he has contributed to ISTA work via the Germination, Proficiency Test, Vigour and Rules Committees. He has been a Member of the Executive Committee since 2001.

Being part of the ISTA team of technical auditors offers him a great opportunity to learn and exchange more about the situation in the seed world and the world in general.

Spare time is shared with family and hobbies, among which are botany, bird watching and fly fishing.

Rita Zecchinelli**Member-at-Large**

Rita Zecchinelli is from Italy, and is Head of the Seed Testing Laboratory in Tavazzano, not far from Milano. The laboratory is part of the Ente Nazionale Sementi Elette (ENSE), the Italian public body which carries out seed certification on behalf of the Ministry of Agriculture and Forestry.

Before joining the laboratory in 1998, she worked in the seed certification unit of Milan for eleven years, being involved in various tasks related to seed certification.

The laboratory is organized in various departments, carrying out a wide range of analyses (traditional seed testing and variety and GMO tests). The lab has been ISTA-accredited since 2000. In 2006, new tests were included in the scope of accreditation, including of specified traits.

Rita is a member of two ISTA Technical Committees: the Flower Seed Testing Committee and the Proficiency Test Committee. She is also an ISTA technical auditor.

This is Rita's second three-year term in the Executive Committee.

Alison A. Powell**Member-at-Large**

My interest in seed science began with my PhD studies at the University of Stirling, Scotland, and my first ISTA Congress was in Madrid in 1977. Subsequently, as a member of staff at the University of Aberdeen, I worked with postgraduate students and visiting researchers from more than 20 countries on aspects of seed vigour in both temperate and tropical crops. My contribution to seed science was recognised by the award of the degree of DSc in 2004.

I have always been interested to see research into practice, and I have been able to work towards this during my 13 years with the Vigour Committee as new vigour tests have been introduced. Communication of science has always been important to me, both written and in oral presentations. As an editor of three international botanical journals, including *Seed Science and Technology*, I am able to keep up with aspects of research and to assist authors. I also have the opportunity to help in the communication of science to a wider audience as the Convenor of the ISTA Seed Symposium since 2002.

Although no longer in University teaching, I am able to continue my interest in education and training as well as clear presentation by participation in ISTA Workshops.

Mary Chipili**Member-at-Large**

Mrs. Mary Mwanza Chipili has been the Controller of Seeds in Zambia, and Director of the Seed Control and Certification Institute, the country's seed certifying Authority, since 2003. She is the Seeds Focal Point for the Southern African Development Community (SADC) in Zambia.

She holds a Master's Degree in Seed Technology from Edinburgh University (Scotland), having previously gained her BSc degree in Agronomy at Tsaul Agricultural Institute, Moldavia, USSR. She has training in the Organization and Management of Seed Programs (Sweden), Forage Seed Quality Control (Ethiopia), International Seed Testing on Tropical Seed Species (Austria), Varietal Identification and GMO Detection (South Africa) and Management Development for Senior Managers (Zambia).

With about 23 years of experience in seed services, seed quality is her paramount interest. This is her first term of office as Member of the ISTA Executive Committee.

Masatoshi Sato

Member-at-Large



I work in the Seed Inspection Division of the National Center for Seeds and Seedlings in Tsukuba, Japan. I have been a seed inspector since 2003 and also the head of the Seed Health Testing Laboratory, the only seed health testing laboratory accredited by ISTA in my country. Before my current position, my specialty of the past

20 years was mainly focused on developing detection and diagnostic methods for potato viruses in seed potato production system. Now I research seed health testing methods including fungi, bacteria, and viruses, while I carry out seed testing as routine work.

My experience with ISTA is not in depth, so I would like to have more opportunities to communicate with ISTA members hereafter, in particular in Asia.

Outside of work, I enjoy doing outdoor things with my family whenever possible like hiking, fishing, and playing tennis.

(To be continued) ■

Membership requests for the Working Programme 2007–2010 (continued from page 19)

We must investigate this, before we can take any effective action.

- Is there a way to automatically be informed about updates of handbooks once you bought one?
- In the ISTA Working Sheets on Tetrazolium Testing (Vol. I and II) more detailed clarification, descriptions and more examples on non-viable seeds are needed
- We need seed collection to conduct other seed determination and we think ISTA prefers to distributed seed collection to all ISTA members.

Purchasers of ISTA Products

- If ISTA has potential purchasers, then the products should be sold to them. Business is business. There should be a price difference for ISTA members.
- To all interested persons a way for them to become interested to be members
- All interested persons and labs
- ISTA should sell to anyone who likes to be informed: spreading the word is part of our mission; a price differential should however be maintained.

- Products should have a as wide distribution as possible. Price differentiation between members and non-members (large) and membership-fee (low) should be such that interested people or organisation should encouraged to become member.
- ISTA should sell its products to all interested persons and laboratories, but the prices should differ; one price for ISTA members and one for the others
- All ISTA products should be accessed by all interested persons and laboratories, but ISTA has to give a large discount (more than now) for ISTA members only. That is one of the benefits being an ISTA member.

G. Further comments

- The number of people actively involved in ISTA work is decreasing. Organisations will accept less and less to spend time and money on ISTA.
- keep registration fees for meetings and congresses low for members
- make sure that travel expenses made for the organisations can be reimbursed (increase budget for this).
- seek ways to increase income in order to be able to keep up with increased costs, f.e. develop preferred suppliers lists, charge for validation of test kits

- The Association should consider sponsoring ISTA facilitators in regional training programmes and should intensify training programmes in seed sampling, quality assurance, seed testing etc.
- ISTA should consider to create a fund for advancement in research in seed science, which can be accessed by all association members.
- The Association should consider revising the cost of ISTA Certificates. At the moment countries that do not export so much feel the Certificate is expensive in relation to their export earnings.
- ISTA must give service (training, ISTA Certificates, Handbooks etc.), but all these services are very expensive, and Latin-American countries can not pay such a lot of money. ISTA must find a way to be more close to these countries. I do not know how (international supports, etc.) but this would be necessary. ISTA has a lot of opportunities and now ISTA has wasted the opportunity.
- If we want to translate ISTA Rules not for commercial purposes, hence be available for our analysts, should we ask permit to ISTA Secretariat, and how can we get it? ■

New faces at the ISTA Secretariat

Jonathan Taylor



**Publications
Specialist**

Jonathan Taylor was born and grew up in England, but moved to Switzerland at the age of ten years. He studied Biology at Basel University, and in 1989 joined the Animal Nutrition Department of the Vitamins Division of the Swiss pharmaceutical giant Roche, as an English-language editor. For several years he was involved in the writing, editing and production of scientific and marketing literature. These activities later also included building and managing Internet and Intranet web sites, and developing and maintaining online product documentation.

When his position was integrated into the Divisional Communications group, closer collaboration with corporate functions became more important, and he was made Divisional representative in the development of the Roche Language Style Guide.

The takeover of Roche Vitamins by the Dutch company DSM in 2003 led to the necessity for implementing widespread style changes for both print and web-based material.

Staff cutbacks forced him to leave the company in 2005, but he soon after became a proofreader and copy editor at Karger, the Basel medical and scientific publishers, thereby widening his experience of the finer points of producing high-quality technical and scientific printed material.

Jonathan Taylor joined the ISTA Secretariat in September 2007, and is a member of the ECOM Working Group on Publications.

Jette Nydam



**Accreditation
Department System
Auditor**

Jette Nydam, from Denmark, is an MSc in Agronomy from the Royal Veterinary and Agricultural University, Copenhagen. In 1983, she joined the Danish State Seed Testing Station (now the Danish Plant Directorate) at Lyngby, near Copenhagen. She worked there for almost ten years in the Department of Varietal Purity and Cleaning, before becoming Head of the Seed Testing Laboratory in 1993.

As Head, she was also responsible for the training of 6 to 12 students a year from Africa and Asia at courses in seed analysis (teaching some of the courses herself).

In addition, she performed audits at authorized seed company laboratories, and implemented quality assurance at the laboratory. ISTA accreditation was achieved in 2000.

During this period, she was also engaged in several consultancies in Africa and Asia, preparing various guidelines for seed testing laboratories.

As Vice-Chair of the ISTA Moisture Committee, she was co-editor of the recently published ISTA Handbook on Moisture Determination.

Jette Nydam remained Head of the Seed Testing Laboratory at Lyngby until joining the ISTA Secretariat in November 2007.

**Maria Francesca
Mannone**



**Event and Marketing
Specialist**

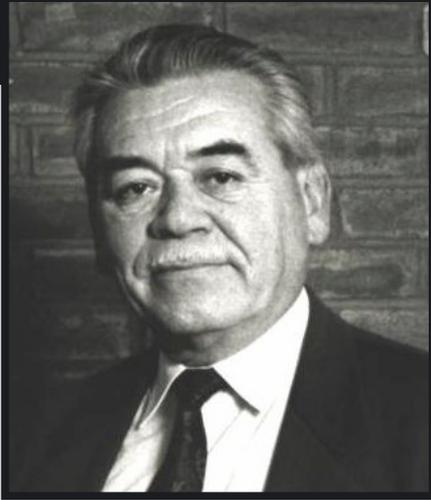
Born in Varese (Italy) in 1973, Francesca Mannone studied first at the European School and then at the Catholic University in Milan, where she obtained a degree in Political Sciences, with a specialization in international law. Her thesis was on European Community law on the "The Communitarian regime of immigration", addressing the development of immigration and the free circulation of people in Member States of the European Union.

In 2001 she did a training experience in the BMW Italy Motorcycle Division, as assistant in harmonizing and monitoring relations between national distribution outlets and the client base. In the same year, she was called to the Public Relations Unit of the European Commission's Joint Research Centre at Ispra (Italy). She was responsible for the organization of meetings and conferences, relations with the press and Italian authorities, and preparation and editing of press releases, brochures and newsletters. In 2005 she moved to the Biotechnology and GMO Unit of the European Commission's Joint Research Centre where she was responsible for communications and public relations until January 2008. She also managed the Secretariat of the European Network of GMO Laboratories, dealing with membership issues, official documents and the organization of Plenary and Steering Committee meetings.

Francesca Mannone joined the ISTA Secretariat in February 2008, where she is responsible for organization of Meetings, Workshops and Congresses.

In memory of Prof. Daniel Côme (1935–2007)

Prof. Françoise Corbineau



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<http://lpva.snv.jussieu.fr>

The Laboratoire Physiologie Végétale Appliquée (Applied Plant Physiology) at the Université Pierre et Marie Curie, Paris 6 deeply regrets to have to inform the seed science community that Prof. Daniel Côme passed away on 30 May 2007, after a long illness.

Daniel Côme was Assistant Professor from 1960 to 1965 at the University of Paris (La Sorbonne), Associate Professor from 1965 to 1975 and, later, Professor in Plant Physiology at the Université Pierre et Marie Curie, Paris 6. He was Emeritus Professor of this University since his retirement in 2004. He was head of the Laboratory of Applied Plant Physiology from 1975 to 1997 and of the CNRS (National Centre for Scientific Research) Laboratoire Physiologie des Organes Végétaux après Récolte (Plant Organ Post-harvest Physiology) at Meudon, near Paris, from 1975 to 1986.

A member of the French Academy of Agriculture from 1991 and honorary member of the Rumanian Academy of Agriculture and Forestry from 1997, he received other honours as well, among which were

the Education Gold Medal from the Society for Promotion of National Industry, Chevalier of the Mérite Agricole (Agricultural Merit) and the Knight's Cross of the Order of Merit of the Republic of Poland.

He began his own research on gas diffusion through seed coats and the involvement of their phenolic compounds in the regulation of oxygen supply to the embryo in relation to germination and dormancy. The later research of his group, which he created in 1969, covered numerous aspects of seed biology, including the control of the germination process by external factors, metabolic regulation of germination and dormancy (in particular the involvement of the pentose phosphate pathway and energy metabolism), seed aging and storability, and seed quality.

Throughout his career his research was carried out on species of horticultural, forest and agricultural importance, leading his group to occupy a unique position in France at the interfaces of basic seed biology and seed technology. He was author or co-author of 270 publications in scientific journals and proceedings, 25 chapters in books, 2 books in French (the last one *Dictionnaire de la Biologie des Semences et des Plantules* (*Dictionary of Seed and Seedling Biology*) being published in 2006), editor of 2 books on plant physiology and cold storage, and co-editor of 3 proceedings of international symposia or workshops. He was also supervisor of 26 PhD students.

Beside his research work, Daniel was responsible for numerous teaching units in plant biology and physiology at the Université Pierre et Marie Curie. He was a devoted teacher and all the students, undergraduates and graduates, and research workers that he educated will remember his scientific precision as well as his availabil-

ity, enthusiasm and friendship in informal discussions.

As head of the Plant Organ Post-harvest Physiology Laboratory, he became an expert in physiology and cold storage of plant foods. He was the President of the C2 Sciences and Food Technologies Commission (1983–1991) and of the C Section Biology and Food Science (1991–1999) of the International Institute of Refrigeration. His expertise led to his participation in numerous scientific committees of companies or societies.

Daniel Côme was a mainstay of ISTA workshops and other conferences and meetings on plant post-harvest physiology all over the world, collaborating with scientists from many countries. All who met him will remember his contributions and his open, friendly and encouraging character. Over the years, the laboratory attracted many foreign students and collaborators from numerous countries, to a large extent thanks to his enthusiasm and generous spirit. When the International Society for Seed Science (ISSS) was founded in 1999, Daniel was selected as its first President. He was also member of many scientific societies and was extremely active in the editorial work for several journals, including *Seed Science and Technology* and *Acta Physiologiae Plantarum*.

The world scientific community has lost a distinguished specialist in seed science. He will be remembered not only for his extensive research and writing on seed biology and physiology, but also for his open, warm and engaging personality, his charisma, his joy of living and his love of science. He will be greatly missed by us all, his colleagues who worked with him and all the seed scientist community throughout the world. ■

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Status 1 April 2008

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First comparative test on DNA-based methods: final report of the Variety Committee Working Group

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The Variety Committee Working Group DNA-Based Methods was formed for the purpose of developing an agreed DNA-based approach for the checking of variety identity in species of interest, to be added to the ISTA Rules.

The aim of the first comparative test was to provide a list of DNA-based markers which could be used to distinguish varieties of *Zea mays* (maize), *Oryza sativa* (rice), *Triticum durum* and *Triticum aestivum* (wheat) and *Glycine max* (soybean). The aim was also to compare results between participant laboratories, and evaluate whether it is possible to obtain the same band patterns and allele sizes even when using different reactants, equipment and working protocols. Varieties and markers were the same for all participating laboratories (table 1).

Background

Many seed testing laboratories are receiving increasing numbers of requests to apply new technologies based on molecular markers for the checking of variety identity and genetic purity. For this reason, the scope of the DNA working group is to reach a common approach in order to

obtain reproducible results among ISTA laboratories.

We consider simple sequence repeats (SSRs), also known as microsatellites, to be one of the best types of molecular markers for variety identification. SSRs are inexpensive, amenable to automation, co-dominant, independent of the environment, highly polymorphic, essentially unlimited and multiallelic, and provide coverage of the entire genome.

For each species, we aim to select a set of SSRs suitable for varieties from all parts of the world, and which, if necessary, may be complemented with additional subsets for specific regions.

In the future, a lab that wishes to perform a test using this list of SSRs will have the freedom to choose reagents, protocols and visualization methods to suit their instrumentation and other needs (tables 2, 3).

We believe that the checking of variety identity is the simplest and fastest way to start, but we also see a need, in the future, for using this method for performing purity tests as well. For purity testing it may be necessary to validate other kinds of molecular markers, such as single nucleotide polymorphisms (SNPs).

Methodology

The Working Group proposed a list of varieties and a list of markers which they believed to be appropriate (table 2).

Each laboratory was free to apply different DNA extraction protocols, visualization methods and polymerase chain

reaction (PCR) protocols, depending on the lab equipment available (table 3).

Results

After the first comparative test, seven SSRs were selected for maize, six for wheat (fig. 1), and nine for soybean. No SSRs have yet been selected for rice.

Each crop subgroup decided on a set of appropriate SSRs, based on the following criteria:

- Markers easy to score: no or little stuttering, no faint bands and sharp alleles;
- Markers giving the same clustering among labs.

SSRs not fulfilling these criteria were discarded. The remaining SSRs will be used during the second comparative test. New SSRs will be proposed in order to obtain a set of about 12 SSRs per species.

Conclusions and further steps

During this first comparative test, sets of DNA-based markers were selected that give reproducible results in laboratories around the world. These marker sets will be used during the second comparative test. It was also found that using different visualization systems may give differing results with regard to allele sizes (expressed as base pairs).

Planning of the second comparative test

The aims of the second comparative test are the following:

Table 1. Crop subgroups and collaborating laboratories

Subgroup	Group leader	Collaborating laboratories
Soybean	Ana Vicario (INASE, Argentina)	Elisa Vieira (COODETEC, Brazil), Marie-José Côté (CFIA, Canada)
Wheat	Daniel Perry (Canadian Grain Commission, Canada)	Emanuela Casarini (LaRAS, Italy), Maria José Côté (CIFA, Canada), Elisa Vieira (COODETEC, Brazil), David Zhang (Geves, France)
Maize	David Zhang (Geves, France)	Ana Vicario (INASE, Argentina), Cheryl Dollard (CFIA, Canada), Elisa Vieira (COODETEC, Brazil)
Rice	Kae-kang Hwu (National Taiwan University, Taiwan)	Cheryl Dollard (CFIA, Canada), Kunusoth Keshavulu (Seed Research & Technology Centre, India), Kalyin Brix Davis (Mid-West Seed Services, USA)

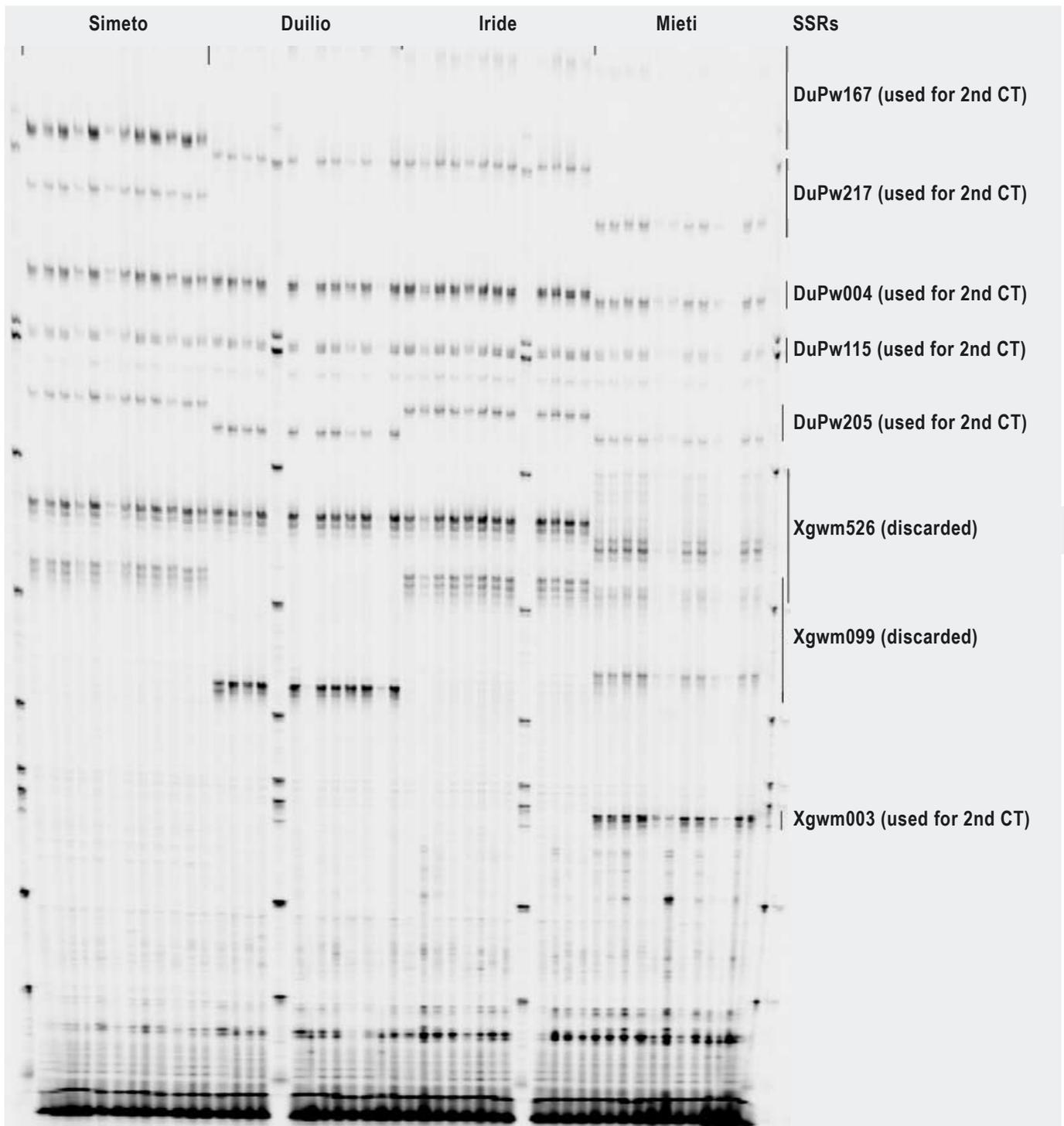


Figure 1. DNA analysis results of 8 SSRs from 4 varieties of wheat. This figure shows how it is possible to identify the variety of a species by comparing the DNA band patterns of a suitable combination of SSRs. Note how Xgwm526 and Xgwm099 did not give clear results for Mieti. CT = comparative test.

- to confirm the reliability and reproducibility of the selected SSR sets;
- to test additional replacement SSRs;
- to visualize as much as possible all allele sets for each SSR, using varieties from all over the world;
- to obtain lists of SSRs for each crop.

During the second round we will use a further 16 varieties, making a total of 24 varieties to be tested. Again, about twelve SSRs will be tested for each species. New varieties and SSRs will be chosen within each crop subgroup.

Distribution of varieties will be done as before (crushed seeds, bulked or individual will be sent to each laboratory), all samples of a given variety will be prepared from a single seed lot, whenever possible from an official reference sample or breeder seed (table 4).

RULES DEVELOPMENT

First comparative test on DNA-based methods

Table 2. SSRs and varieties used

Crop subgroup	SSRs	Varieties
Soybean	SATT114, SATT177, SATT231, SATT353, SATT534, SATT577, SATT094, SATT216, SATT307, SATT449, SAT233, SAT_001, SATT105	8 varieties
Wheat	DuPw167, DuPw217, DuPw004, DuPw115, DuPw205, Xgwm526, Xgwm099, Xgwm003 (these SSRs may be multiplexed in a single PCR)	<i>T. durum</i> : Simeto, Duilio, Iride, AC Avonlea <i>T. aestivum</i> : AC Barrie, CD104, Mieti, Onix
Maize	phi109275, phi102228, phi083, umc1122, phi452693, umc1545, umc1153, phi015, umc1061, phi032, phi093, umc1152	8 varieties
Rice	RM276, RM333, RM266, RM70, RM567, RM215, RM105, RM159	TCS10, TCSnG11, KH143, TK8, TK2, TKGlu5, KH139_1, TC191, TK9, TK17

Table 3. Methodology and equipment used by each laboratory

Crop subgroup	Laboratory	DNA extraction protocol	Thermal cycler	Visualization of amplicons
Soybean	Argentina	Adapted from Dellaporta, 1983	MJ Res. PTC100	Silver staining
	Brazil	Adapted from McDonald et al., <i>Seed Sci & Technol</i> 22:171–176, 1994	Hybaid	Silver staining
	Canada	QIAGEN DNeasy plant mini kit	MJ Res. PTC200	Li-Cor 4300
Wheat	Canada (Perry)	Adapted from McDonald et al., 1994	MJ Res. PTC200	Li-Cor 4200
	Canada (Côte)	QIAGEN DNeasy plant mini kit	MJ Res. PTC200	Li-Cor 4300
	Italy	McDonald et al., 1994	MJ Res. PTC200	Li-Cor 4300 and silver staining
	France	QIAGEN DNeasy plant mini kit	GeneAmp 9700	ABI 3130xl
	Brazil	Adapted from McDonald et al., 1994	Hybaid	Silver staining (not completed)
Maize	France	QIAGEN DNeasy plant mini kit.	GeneAmp 9700	ABI
	Argentina	Adapted from Dellaporta, 1983	MJ Res. PTC100	Silver staining
	Canada	QIAGEN DNeasy plant mini kit	ABI GeneAmp 9700	ABI 3100
	Brazil	Wizard Genomic DNA Purification Kit (Promega)	Hybaid	Silver staining
Rice	Taiwan	CTAB extraction protocol	No information	ABI 3100
	Canada	QIAGEN DNeasy plant mini kit	MJ Res. PTC200	ABI 3100
	India	Collaboration for the 2nd CT	No information	agarose gel
	USA	Dellaporte Extraction Method	GeneAmp 9700	3% MetaPhor agarose gel/ethidium bromide

Table 4. Proposed schedule for the 2nd comparative test

Date of finalization	Task
21 November 2007	Selection of new varieties and markers for the 2nd comparative test
23 November 2007	Final report on the 1st comparative test
Starting 3 December 2007	Sending varieties and lines to the laboratories
18 April 2008	Results
2 May 2008	Summary of results of the 2nd comparative test
30 May 2008	Final report on the 2nd comparative test

(Note: these deadlines are approximate)

Third step

The Working Group will meet at the Annual Meeting to present the work to other labs, communicate results and plans, debate on how far we are from a Rules proposal, discuss a possible Rules proposal, etc. ■

Comparison of oven moisture tests at 130 °C vs. 103 °C

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The Rules change proposal submitted by the ISTA Moisture Committee to the Ordinary Meeting of 2007 suggested accepting the low-temperature method for all species as the basic reference method. At the meeting, it was also decided that species currently listed for the high-temperature method should, in principle, also be listed for the low-temperature method.

Many moisture determination methods have been compared in the literature, and the findings from some of these are given as the background for including the low-

temperature method for all species. Most comparisons did not use methods that were exactly identical to the present ISTA methods, but methods close to the present ISTA methods are included in this paper. Methods comparing whole seeds with ground seeds are also included.

Results from the literature

Many laboratories have carried out comparisons between different methods of moisture determination for many years. Klitgård (1978) compared several methods for small-seeded legumes and his results (table 1) indicate that the difference between the low- and high-temperature methods is approximately 0.5% for whole *Trifolium* seeds, and 0.2–0.6% for ground seeds.

However, seed coat effects confound the results for *Trifolium* and other small-seeded Leguminosae. In a comparative test on four small-seeded legumes, the moisture content, as measured by the present ISTA high-temperature method, was 0.6 to 1.0 percentage points (p.p.) lower than that obtained by the low-temperature method for whole seeds (table 2). When the seeds were ground, the measured moisture percentage at the lower temperature was 0.8 to 1.7 points higher than by the ISTA high-temperature method. It appears that the differences are not the same for all species: *Trifolium* less, and *Medicago* and *Lotus* more, and that the differences between the high- and low-temperature methods are minimised if the seed is ground prior to oven drying. Differences between whole and ground are smallest with the low-temperature method (17 h at 103 °C), and the mean deviations between participants were considerably higher for all four species using the high-temperature method on whole seed for 1 h.

Bonner (1974) compared moisture results from various methods for *Quercus* (table 3). The results from these trials indicate hardly any differences between the low and high temperatures.

Buszewicz (1962) compared several moisture determination methods for *Abies* (table 4).

The results for ground seeds are always much higher than those for whole seeds. The discrepancy between the two 105 °C methods varies with species, being least

Table 1. Comparison of several methods for moisture determination for *Trifolium pratense* and *Trifolium repens*. The difference from that obtained by the ISTA method (1 h at 130 °C) is given (Klitgård, 1978).

	<i>T. pratense</i>			<i>T. repens</i>		
	mc (%)	difference to ISTA*	difference to whole*	mc (%)	difference to ISTA*	difference to whole*
1 h 130–133 °C, whole	8.65			8.43		
2 h 130–133 °C, whole	9.17	+0.52		9.01	+0.58	
17 h 101–105 °C, whole	9.16	+0.51		8.96	+0.53	
1 h 130–133 °C, ground	9.67	+1.02	+1.02	9.78	+1.35	+1.35
2 h 130–133 °C, ground	9.75	+1.10	+0.58	9.90	+1.47	+0.89
17 h 101–105 °C, ground	9.37	+0.72	+0.21	9.52	+1.08	+0.56

* Differences are expressed as percentage points. mc = moisture content.

Table 2. Comparative moisture determinations of *Trifolium repens*, *T. pratense*, *Medicago sativa* and *Lotus corniculatus*. Average of results obtained by 11 stations and mean deviations between the stations and the ISTA method (Klitgård, 1981)

	<i>Trifolium repens</i>		<i>Trifolium pratense</i>		<i>Medicago sativa</i>		<i>Lotus corniculatus</i>	
	mc (%)	difference to ISTA*	mc (%)	difference to ISTA*	mc (%)	difference to ISTA*	mc (%)	difference to ISTA*
No grinding, 1 h 130–133 °C (ISTA)	8.74		8.64		7.52		9.07	
No grinding, 17 h 101–105 °C	9.34	+0.60	9.26	+0.62	8.52	+1.00	10.06	+0.99
Grinding, 1 h 130–133 °C	9.87	+1.13	9.63	+0.99	9.22	+1.70	10.64	+1.57
Grinding, 17 h 101–105 °C	9.76	+1.02	9.42	+0.78	9.01	+1.49	10.43	+1.36

* Differences are expressed as percentage points. mc = moisture content.

with *A. nobilis* and considerably greater for *A. alba* and *A. grandis*. In the latter case, the results with ground seed are double those of whole seeds. These differences were ascribed to differences in the chemical composition of the seed. However, it should be noted that the difference between 24 h drying at 105 °C and 1 h drying at 130 °C was only 0.02 points for ground seeds, and 0.45 points for whole seeds.

Oxley *et al.* (1960) compared the moisture content results of ground cereal seed using a Brabender moisture tester operating at 130 °C, and those obtained after drying in a still-air oven at 105 °C for 16 h (table 5). The results obtained from the Brabender were on average 0.39 p.p. higher.

The observed differences between the low- and high-temperature methods illustrated in table 5 are probably for the greater part caused by volatiles that are lost at the high temperature of 130 °C. This is supported by Tainter *et al.* (2001), who not only published oil contents, but also percentages of volatiles for various species (table 6).

To resolve the problem of losing the volatiles in the oven test of spices, the spice trade has adopted a co-distillation moisture determination method for most spices. In this test, the spice is covered with toluene, and the toluene brought to its boiling temperature. The moisture in the spice co-distills with the toluene, and as the toluene is condensed, the moisture separates from the toluene and is measured (Tainter *et al.*, 2001).

In the literature, the magnitude of volatiles lost during the process of drying is diverse and not directly related to oil content (table 7). In general, however, the levels of volatiles lost during drying are small, generally accounting for less than 1%.

Hart and Golumbic (1962) used a vacuum distillation drying apparatus to determine whether or not some seeds give off non-aqueous volatile matter when dried at 80 °C in a vacuum (<1 mm Hg) for 5 h. Determinations were made on *Cichorium endivia*, *Allium cepa*, *Apium graveolens*, *Lepidium sativum*, *Beta vulgaris*, *Daucus carota* and *Pisum sativum* seeds. In all cases water-insoluble organic compounds, generally odorous essential oils, were obtained in the distillate along with water. No attempt was made to identify the products or to quantitatively determine them.

Table 3. Mean moisture contents as determined by three different methods for three *Quercus* species (Bonner, 1974) (time at 130 °C was not specified in the paper)

Species	Intact seed dried for 17 h at 105 °C	Pre-drying at 130 °C followed by grinding and drying at 130 °C	Seed cut in halves and dried for 15 h at 105 °C
<i>Quercus alba</i>	47.4	48.0	47.9
<i>Quercus muehlenbergii</i>	36.8	37.1	37.3
<i>Quercus nigra</i>	31.5	32.2	32.4
Mean	38.6	39.1	39.2

Table 4. Comparison of low and high temperature oven moisture determination methods for *Abies* species Buszewicz (1962)

Species	Whole, oven, 105 °C, 24 h	Ground, oven, 105 °C, 24 h	Whole, oven, 130 °C, 1 h	Ground, oven, 130 °C, 1 h
<i>Abies nobilis</i> -1	9.08	10.51	9.05	10.85
<i>Abies nobilis</i> -2	18.29	19.84	18.55	20.35
<i>Abies nobilis</i> -3	15.72	17.33	15.40	17.90
<i>Abies grandis</i> -1	7.74	13.24	7.18	12.98
<i>Abies grandis</i> -2	6.85	12.87	6.36	12.62
<i>Abies alba</i>	22.10	26.32	20.54	25.55
Mean	13.30	16.69	12.85	16.71
Difference ground-whole		+3.39		+3.86
Difference 105–130 °C			+0.45	–0.02

Table 5. Moisture content of ground cereals using a still air oven at 105 °C and a Brabender operating at semi-automatic moisture tester (average of 7 cereal samples for each moisture category) (Oxley *et al.*, 1960)

Method	Moisture category		
	dry	medium	wet
105 °C, 16 h, still air oven	12.24	14.64	18.12
130 °C, 1.5 h, Brabender semi-automatic moisture tester	12.70	14.97	18.50

Table 6. Percentages of oil and volatiles in seeds of some species (Tainter *et al.*, 2001), and the temperature of the ISTA moisture test (if available): low = 103 °C, high = 130 °C

Species	Common name	Oil content (%)	Volatiles (%)	ISTA test (°C)
<i>Pimpinella anisum</i>	Aniseed	15.9	1.5–3.5	–
<i>Carum carvi</i>	Caraway	14.6–23.1	1.5–3.5	130
<i>Elettaria cadamomum</i>	Cardamom		11	–
<i>Apium graveolens</i>	Celery	41.4–43.8	1.5–3	130
<i>Coriandrum sativum</i>	Coriander	17.8–19.6	0.1–1.5	–
<i>Cuminum cyminum</i>	Cumin	22.3–23.8	2–5	130
<i>Anethum sowa/graveolens</i>	Dill	14.5–17.9	2–5	130
<i>Foeniculum vulgare</i>	Fennel	10.0–14.9	1–3	–
<i>Trigonella foenum-graecum</i>	Fenugreek	5–7	0.2–0.5	–
<i>Origanum majorana</i>	Marjoram	6.8–7.0	0.7–3.0	–
<i>Brassica hirtaljuncea</i>	Mustard	28–36	?	103
<i>Piper nigrum</i>	Pepper	3.3–12.0	0.6–5.0	–

Table 7. Loss of volatiles during drying. Oil contents: Kew database

Species	Oil content (%)	Drying method	Duration in oven (h)	Volatiles lost (%) (>0.5% underlined)	Reference
<i>Pisum sativum</i>	1	Vacuum, 80 °C, P ₂ O ₅	5	0.9	Hart and Golumbic (1962)
<i>Triticum aestivum</i>	2	100 °C, 20 Torr	3	0.1 to 0.3	Bolling (1960)
<i>Triticum aestivum</i>	2	130 °C in N ₂	1.5	0.2 to 0.4	Bolling (1960)
<i>Triticum aestivum</i>	2	130 °C, 20 Torr, N ₂	1.5	0.2 to 0.5	Bolling (1960)
<i>Triticum aestivum</i>	2	Vacuum, 80 °C, P ₂ O ₅	5	0.0 to 0.3	Hart and Golumbic (1962)
<i>Triticum aestivum</i>	2	135 °C	1	0.0	Iden and Livingston (1970)
Several grasses	2–9	130 °C	1	0.2 to 0.7	Hart <i>et al.</i> (1959)
<i>Lolium perenne</i>	2	Vacuum, 80 °C, P ₂ O ₅	5	0.0	Hart and Golumbic (1962)
<i>Poa pratensis</i>	9	Vacuum, 80 °C, P ₂ O ₅	5	–0.5 to 0.0	Hart and Golumbic (1962)
<i>Beta vulgaris</i>	5	Vacuum, 80 °C, P ₂ O ₅	5	0.7 to 1.3	Hart and Golumbic (1962)
<i>Spinacea oleracea</i>	6	Vacuum, 80 °C, P ₂ O ₅	5	0.0	Hart and Golumbic (1962)
<i>Trifolium pratense</i>	9	Vacuum, 80 °C, P ₂ O ₅	5	–0.3	Hart and Golumbic (1962)
<i>Trifolium spp</i>	9	130 °C	1	0.7	Hart <i>et al.</i> (1959)
<i>Medicago sativa</i>	9	130 °C	1	0.9	Hart <i>et al.</i> (1959)
<i>Zea mays</i>	6	70 °C	100	0.1	Sair and Fetzer (1942)
<i>Zea mays</i>	6	100 °C	16	0.5	Sair and Fetzer (1942)
<i>Zea mays</i>	6	103 °C	72	0.3	Hunt and Pixton (1974)
<i>Zea mays</i>	6	Vacuum, 80 °C, P ₂ O ₅	5	0.1 to 0.5	Hart and Golumbic (1962)
<i>Zea mays</i> , mc 10–26%	6	103 °C	72	0.1 to 0.5	Hart (1972)
<i>Zea mays</i> , mc 35%	6	103 °C	72	0.2 to 0.9	Hart (1972)
<i>Lycopersicon esculentum</i>	15	Vacuum, 80 °C, P ₂ O ₅	5	0.1	Hart and Golumbic (1962)
<i>Allium cepa</i>	19	Vacuum, 80 °C, P ₂ O ₅	5	0.4 to 0.9	Hart and Golumbic (1962)
<i>Allium cepa</i>	19	130 °C	1	0.9	Hart <i>et al.</i> (1959)
<i>Glycine max</i>	20	130 °C	1	0.0 to 1.0	Hunt and Pixton (1974)
<i>Glycine max</i>	20	Vacuum, 80 °C, P ₂ O ₅	5	0.2 to 0.4	Hart and Golumbic (1962)
<i>Glycine max</i>	20	135 °C	1	0.1	Iden and Livingston (1970)
<i>Citrullus vulgaris</i>	20	Vacuum, 80 °C, P ₂ O ₅	5	0.3	Hart and Golumbic (1962)
<i>Petroselinum crispum</i>	27	130 °C	1	2.0	Hart <i>et al.</i> (1959)
<i>Daucus carota</i>	27	130 °C	1	1.8	Hart <i>et al.</i> (1959)
<i>Daucus carota</i>	27	Vacuum, 80 °C, P ₂ O ₅	5	0.6 to 1.2	Hart and Golumbic (1962)
<i>Cucumis sativus</i>	32	Vacuum, 80 °C, P ₂ O ₅	5	0.4	Hart and Golumbic (1962)
<i>Pastinaca sativa</i>	33	130 °C	1	0.1	Hart <i>et al.</i> (1959)
<i>Linum usitatissimum</i>	33	130 °C	1	0.6	Hunt and Pixton (1974)
<i>Linum usitatissimum</i>	33	Vacuum, 80 °C, P ₂ O ₅	5	0.1 to 0.7	Hart and Golumbic (1962)
<i>Brassica juncea</i>	35	Vacuum, 80 °C, P ₂ O ₅	5	–0.5 to 0.3	Hart and Golumbic (1962)
<i>Brassica oleracea</i>	26	Vacuum, 80 °C, P ₂ O ₅	5	–0.2	Hart and Golumbic (1962)
<i>Brassica oleracea</i>	26	130 °C	1	–0.3 to 0.2	Hart <i>et al.</i> (1959)
<i>Brassica napus</i>	42	130 °C	1	–0.2	Hart <i>et al.</i> (1959)
<i>Brassica rapa</i>	39	130 °C	1	–0.2	Hart <i>et al.</i> (1959)
<i>Lactuca sativa</i>	37	Vacuum, 80 °C, P ₂ O ₅	5	0.0 to 0.1	Hart and Golumbic (1962)
<i>Raphanus sativus</i>	42	Vacuum, 80 °C, P ₂ O ₅	5	0.0 to 0.6	Hart and Golumbic (1962)
<i>Raphanus sativus</i>	42	130 °C	1	0.5	Hart <i>et al.</i> (1959)
<i>Arachis hypogaea</i>	47	Vacuum, 80 °C, P ₂ O ₅	5	–0.4 to –0.1	Hart and Golumbic (1962)
<i>Cucurbita pepo</i>	47	Vacuum, 80 °C, P ₂ O ₅	5	–0.1	Hart and Golumbic (1962)

However, when the results of moisture determinations on these seeds by the vacuum-P₂O₅ method are compared with results from the Karl Fischer method, it appears that the amounts of the distilled volatile organic products are great enough to have a material effect on the moisture content results. For some species, such as *Arachis hypogaea*, *Poa pratensis* and *Brassica* spp., the consequence is lower moisture content results in Karl Fischer tests than in vacuum P₂O₅ tests. Furthermore, it appears that that the loss of volatiles is not related to the oil content. *Pisum sativum* appears to lose a high amount of volatiles, even though it has an oil content of only 1%.

The U.S. Department of Agriculture and the AACCC (American Association of Cereal Chemists) oven methods for grain have been subjected to critical examination by comparison with the Karl Fischer chemical titration method and by analysis of the effluents driven off by heating. For all grain except *Zea*, *Linum* and *Glycine*, the non-aqueous loss was insignificant, and the average deviation between the Karl Fischer results and the oven results was less than 0.1 p.p. A two-year study on *Zea* showed that there was an average non-aqueous loss of 0.34%. Since this loss occurred in the early heating stages, when seed temperature is heating up between 70 and 80 °C, the present method of drying whole corn for 72 h at 103 °C was deliberately chosen to leave approximately 0.3% of the moisture in the sample to compensate for this loss. Drying whole *Linum* at 130 °C results in an average non-aqueous loss of 0.6%. Drying the whole seed for 4 h at 103 °C can prevent this loss. The non-aqueous loss from *Glycine* varied from 0.0% to over 1.0%, with an average of 0.5%. The loss occurred mainly at low temperatures in the early stages of drying (Hunt and Pixton, 1974).

Zeleny (1953) mentions that *Allium cepa* and *Raphanis sativa* are species that contain non-aqueous volatile constituents that may be lost at temperatures in excess of 105 °C. He suggests that the high-temperature oven method should not be used on seed with an oil content of 25% or more, or on seed containing oils with an iodine number higher than about 150. Moreover, such seed should be tested whole. Seeds with a high oil content cannot usually be satisfactorily ground, and oils with a high iodine number are readily oxidised. Grinding accelerates

oxidation, as do increases in temperature. Such oxidation causes a weight gain that will interfere with the accuracy of the moisture determination.

Results of ISTA comparative testing

In 2005, the ISTA Moisture Committee carried out a comparative test. Ten different species were tested at two moisture levels in ten different laboratories. The results in table 8 are the average results from these ten laboratories.

The difference between 1h at 130 °C and 17 h at 103 °C is on average -0.24 p.p., and is similar for both moisture levels. As the value is negative, 17 h at 103 °C gives higher results than 1 h at 130 °C. On average, the differences between the two oven methods were limited. Differences between the methods were influenced by species and moisture level, and were both positive and negative within all comparisons. Some species show relative large differences: *Lolium*, *Spinacea*, and *Sinapis*. For *Lolium* the difference at the low moisture level was almost 1 p.p. greater than the difference at the higher moisture level. Grabe (1990) found for ryegrasses that 1 h at 130 °C was insufficient to release all water from the seeds. This behaviour may be caused by the nature of the seed coat structures, which are relatively thick. In this study it was found

that the difference between 17 h at 103 °C and 1 h at 130 °C was not correlated with the oil content of the seeds.

Summary and conclusions

From a study of published work comparing low- and high-temperature methods, we find that the difference in moisture content between the methods is always within 1.0 p.p., except for a low moisture content sample of *Lolium* in the 2005 ISTA comparative test. For most species the difference is smaller than 0.5 p.p. (table 9).

The indicated differences are considered to be limited. The greatest differences are observed in species such as *Lolium* and clovers, which have a thick seed coat, and for which evidence exists that the present ISTA method using whole seed may not be correct.

It is therefore acceptable to include the low-temperature method in the Rules for all species so far listed only under the high-temperature method. The study also demonstrates that for some species, there is a relatively large difference between the high- and low-temperature methods, and that the structure of the seed interferes with the determination. Therefore, it is necessary to carry out comparative studies before introducing the high-temperature method into the ISTA Rules. These comparative studies must include a check of the effects of cutting and grinding.

Table 8. Differences in percentage moisture content determined at two different moisture levels and using high- and low-oven temperature methods. Difference in moisture content: 1 h at 130 °C minus 17 h at 103 °C.

Species	Difference 130 °C–103 °C	
	Low moisture sample	High moisture sample
<i>Lolium perenne</i>	-1.63	-0.67
<i>Spinacea oleracea</i>	-0.53	-0.47
<i>Poa pratensis</i>	-0.02	+0.01
<i>Lycopersicum esculentum</i>	+0.08	-0.11
<i>Allium cepa</i>	-0.22	-0.29
<i>Petroselinum crispum</i>	+0.06	+0.12
<i>Sinapis alba</i>	-0.25	-0.77
<i>Camelina sativa</i>	+0.13	+0.08
<i>Brassica napus</i>	+0.29	0.00
<i>Linum usitatissimum</i>	-0.60	-0.10
Average	-0.27	-0.22

Table 9. Differences between high- and low-temperature moisture determination methods. Summary of results. 130 = 1 h 130 °C, 103 = 15–17 h 103 °C

Species	Difference 130–103 °C*	Source
<i>Trifolium pratense</i> , whole	–0.51	Klitgård, 1978
<i>Trifolium repens</i> , whole	–0.53	Klitgård, 1978
<i>Trifolium pratense</i> , ground	+0.30	Klitgård, 1978
<i>Trifolium repens</i> , ground	+0.26	Klitgård, 1978
<i>Trifolium repens</i> , whole	–0.60	Klitgård, 1981
<i>Trifolium pratense</i> , whole	–0.62	Klitgård, 1981
<i>Medicago sativa</i> , whole	–1.00	Klitgård, 1981
<i>Lotus corniculatus</i> , whole	–0.99	Klitgård, 1981
<i>Trifolium repens</i> , ground	+0.11	Klitgård, 1981
<i>Trifolium pratense</i> , ground	+0.21	Klitgård, 1981
<i>Medicago sativa</i> , ground	+0.21	Klitgård, 1981
<i>Lotus corniculatus</i> , ground	+0.21	Klitgård, 1981
<i>Quercus</i> spp, cut and ground	–0.10	Bonner, 1972
<i>Abies</i> spp, whole	–0.45	Buszewicz, 1962
<i>Abies</i> spp, ground	+0.02	Buszewicz, 1962
7 cereals, ground, low mc	+0.46	Oxley <i>et al.</i> , 1960
7 cereals, ground, intermediate mc	+0.33	Oxley <i>et al.</i> , 1960
7 cereals, ground, high mc	+0.38	Oxley <i>et al.</i> , 1960
<i>Lolium perenne</i> , low moisture level	–1.63	ISTA, 2005
<i>Spinacea oleracea</i> , low moisture level	–0.53	ISTA, 2005
<i>Poa pratensis</i> , low moisture level	–0.02	ISTA, 2005
<i>Lycopersicum esculentum</i> , low moisture level	+0.08	ISTA, 2005
<i>Allium cepa</i> , low moisture level	–0.22	ISTA, 2005
<i>Petroselinum crispum</i> , low moisture level	+0.06	ISTA, 2005
<i>Sinapis alba</i> , low moisture level	–0.25	ISTA, 2005
<i>Camelina sativa</i> , low moisture level	+0.13	ISTA, 2005
<i>Brassica napus</i> , low moisture level	+0.29	ISTA, 2005
<i>Linum usitatissimum</i> , low moisture level	–0.60	ISTA, 2005
<i>Lolium perenne</i> , high moisture level	–0.67	ISTA, 2005
<i>Spinacea oleracea</i> , high moisture level	–0.47	ISTA, 2005
<i>Poa pratensis</i> , high moisture level	+0.01	ISTA, 2005
<i>Lycopersicum esculentum</i> , high moisture level	–0.11	ISTA, 2005
<i>Allium cepa</i> , high moisture level	–0.29	ISTA, 2005
<i>Petroselinum crispum</i> , high moisture level	+0.12	ISTA, 2005
<i>Sinapis alba</i> , high moisture level	–0.77	ISTA, 2005
<i>Camelina sativa</i> , high moisture level	+0.08	ISTA, 2005
<i>Brassica napus</i> , high moisture level	0.00	ISTA, 2005
<i>Linum usitatissimum</i> , high moisture level	–0.10	ISTA, 2005

* Differences are expressed as percentage points. mc = moisture content.

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Effect of temperature and growing media on sunflower germination

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This work was carried out at the request of the ISTA Germination Committee, in order to harmonize ISTA and Association of Official Seed Analysts (AOSA) methods for sunflower (*Helianthus annuus*) germination.

AOSA recommends in its rules a germination temperature of 20 °C, whereas ISTA allows 3 temperature regimes: 20, 25 and alternating 20-30 °C. In addition, whereas AOSA recommends only Between Paper (BP) for sunflower germination, ISTA allows 3 different media: BP, Sand (S) and Organic Growing Media (O).

The objective of this study was to evaluate the effect of all of the above temperatures and growing media on sunflower germination.

Materials and methods

A comparative test was organized in order to assess the effect of various temperature regimes and media on sunflower germination.

Seed material

Four samples of sunflower seeds were used in this study. Samples 2 and 3 came from the same seed lot.

Germination methods

The 4 sunflower samples were tested using 9 different conditions. These resulted from the combination of the 3 types of growing media (BP, S and O) and the 3 temperature regimes (20, 25 and alternating 20-30 °C) that are prescribed in ISTA rules.

Each germination condition was tested using 400 seeds from each of the 4 samples.

Participants

Samples were sent to 12 laboratories. Of these, only 9 reported results, 5 using ISTA methods and 4 AOSA methods.

Statistical analysis

Repeatability and reproducibility were analysed with the statistical tool developed by S. Grégoire according to ISO 5725-2. The effect of the various factors (laboratory, growing media, temperature, ISTA vs. AOSA) were analysed by variance analysis using Statgraphics.

Results

Repeatability

Repeatability was calculated using the statistical tool developed by S. Grégoire based on ISO 5725-2. When the standard deviation is low, the repeatability of the method is high. The overall mean results for the various germination conditions are presented in figure 1.

Repeatability was similar for all the 9 combinations of conditions with no significant differences.

Reproducibility

Reproducibility is reported in the same way as repeatability.

Not all the laboratories performed the 9 conditions, and in figure 2, reproducibility was calculated from the results of the 6 laboratories which carried out the tests using all 9 germination conditions.

Figure 2 shows that reproducibility was lower using BP media, particularly at 25 °C. It was generally high for S and O, irrespective of the temperature regime.

Effect of method conditions

The results were analysed with the variance analysis module of Statgraphics in order to evaluate the influence of the various factors on germination.

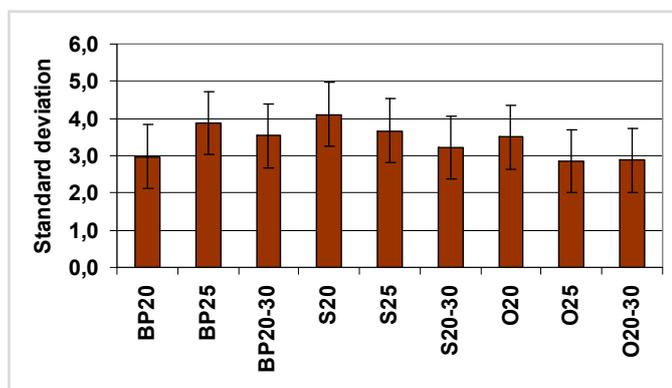


Figure 1. Repeatability (standard deviation) for all the laboratories and for the average of the 4 samples tested as a function of the germination method.

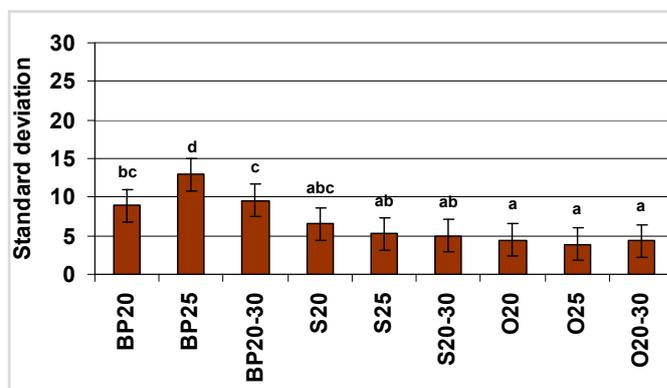


Figure 2. Reproducibility (standard deviation) for the 6 laboratories which carried out tests in all 9 germination conditions and for the average of the 4 samples tested.

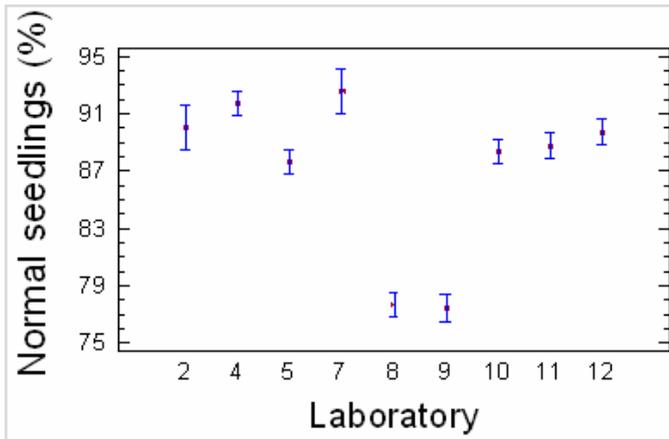


Figure 3. Percentages of normal seedlings in the various laboratories for all the germination conditions.

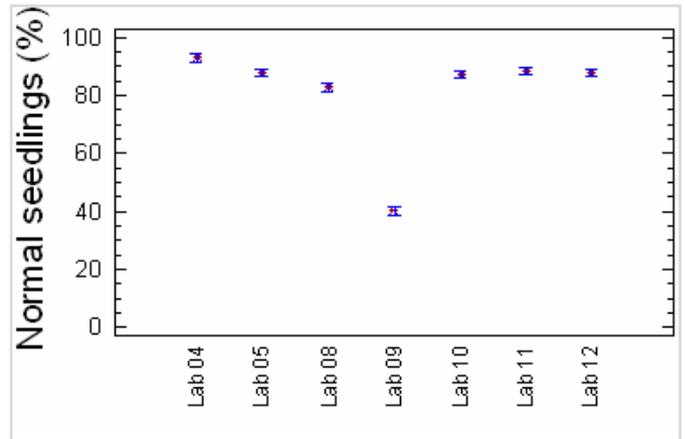


Figure 4. Percentages of normal seedlings obtained using sand at 25 °C.

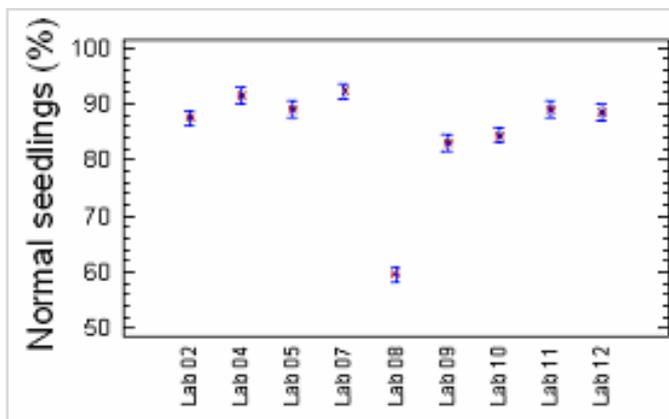


Figure 5. Percentages of normal seedlings obtained using BP at 25 °C.

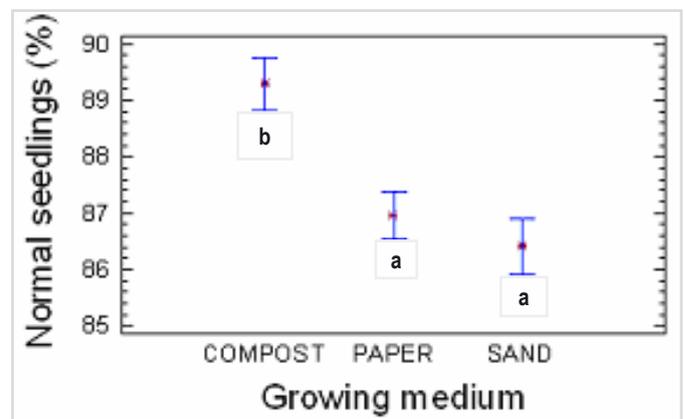


Figure 6. Overall average percentages of normal seedlings obtained using various growing media. Note: results of laboratory 8, using BP at 25 °C, and laboratory 9, using S at 25 °C were excluded from the overall average calculation. Values with different letters are significantly different (p < 0.05).

Laboratory effect

The data reported in figure 3 are the overall average results (over all samples and all germination conditions) obtained by each laboratory. Seven of the laboratories obtained results of between 87.6% (laboratory 5) and 92.6% (laboratory 7). The other 2 laboratories (laboratories 8 and 9) reported results of less than 80%. Although very experienced in testing sunflowers, laboratories 8 and 9 obtained very low germination results for specific germination conditions: S at 25 °C for laboratory 9 (fig. 4); BP at 25 °C for laboratory 8 (fig. 5).

The following results and figures are presented with the exclusion of these 2 conditions for these 2 laboratories.

Effect of growing media and temperatures

The effects of growing media and temperature on germination are presented in figures 6 and 7, respectively.

When the 2 factors growing media and temperature are separated in the variance analysis, we can see that:

- Percentages of normal seedlings are statistically different between the growing media (fig. 6). Germination results are higher with O and lower with S and BP. Results obtained with S and BP are not statistically different;
- Percentages of normal seedlings are statistically different between temperature regimes (fig. 7). Germination results are lower with 20 °C than with 25 °C or alternating 20-30 °C. Results obtained with 25 °C or alternating 20-30 °C are not statistically different.

Comparison of the 9 germination conditions

Percentages of normal seedlings for all combinations of growing media and temperatures are presented in figure 8.

Analysis of the results show that irrespective of the temperature, O gives significantly higher levels of normal seedlings than BP or S. BP and S give similar germinations at the different temperatures. For BP there are no significant differences between the levels of normal seedlings using the different temperature regimes, whereas with S, a temperature of 25 °C gives a higher level of normal seedlings than 20 °C.

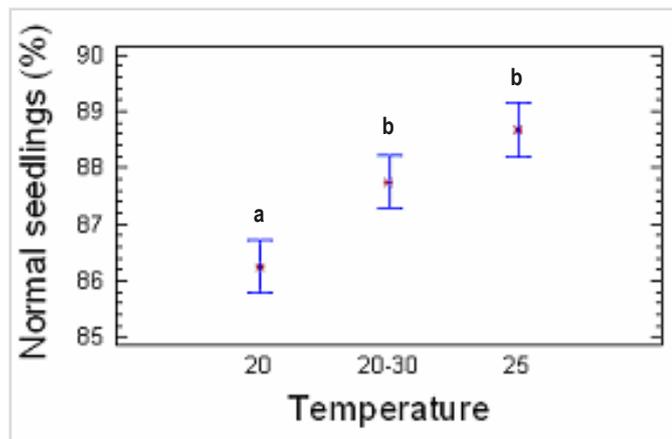


Figure 7. Overall average percentages of normal seedlings obtained using various germination temperature regimes. Note: results of laboratory 8, using BP at 25 °C, and laboratory 9, using S at 25 °C, were excluded from the overall average calculation. Values with different letters are significantly different (p <0.05).

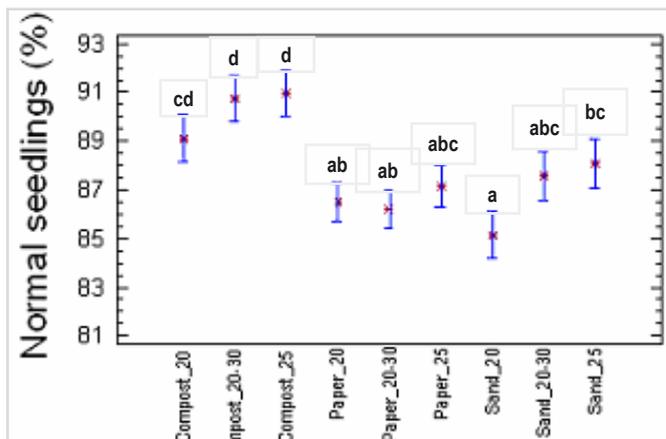


Figure 8. Percentage of normal seedlings as a function of combined conditions of growing medium and temperature regime. Note: results of laboratory 8, using BP at 25 °C, and laboratory 9, using S at 25 °C, were excluded from the calculation. Values followed by different letters are significantly different (p <0.05).

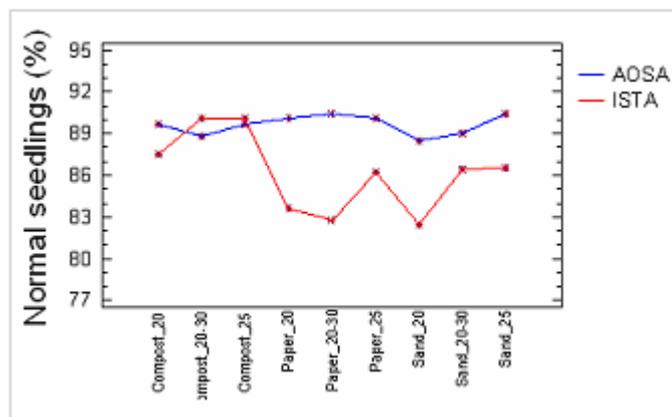


Figure 9. Percentage of normal seedlings as a function of the methods used by ISTA and AOSA laboratories, for each combination of growing media and temperature.

Comparison of results between ISTA and AOSA methods

Results of normal seedlings are globally higher for AOSA laboratories (fig. 9). The difference between ISTA and AOSA laboratories is very small with O, irrespective of temperature, but increases when germination conditions involve BP or S, especially for the conditions BP at 20 °C or 20-30 °C and S at 20 °C.

Conclusion

Combinations of all the conditions of growing media and temperatures prescribed in the ISTA Rules for sunflower germination do not give the same germination results.

Results obtained with O give the best results, irrespective of germination temperature. This growing medium also gives the most reproducible results. These results

are in accordance with the results obtained in 2005, when the effect of growing media on the germination of sunflower seeds was examined (ISTA, 2005).

In contrast, for one laboratory, S at 25 °C induced low levels of normal germination, and for another, BP at 25 °C also gives poor germination results. These results, obtained by 2 laboratories using these 2 particular conditions, contributed to the fact that S and BP were found to be less repeatable and reproducible than O in this comparative test. Although the particular laboratories are experienced in testing sunflowers, they are not familiar with these particular germination conditions, and had difficulties using them.

When these 2 conditions for these 2 laboratories were excluded from the overall data, combinations of S and BP media

with the 3 temperatures 20, 25 and 20-30 °C gave similar levels of normal germination.

The combination of BP and 20 °C recommended by AOSA gives intermediate results, lower than with all germination conditions, including O, but higher than BP at 25 °C and S at 25 °C, for all the laboratories.

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Proficiency test *Botrytis cinerea* on *Helianthus annuus* (ISTA method 07-003)

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Abstract

The first proficiency test (PT) of the ISTA Seed Health Committee was organized by the GEVES-SNES Laboratory of Pathology in 2007. Its aim was to verify the ability of laboratories to detect, using ISTA method 07-003, the percentage of *Helianthus annuus* seeds infected by *Botrytis cinerea*.

The PT was carried out with four levels of contamination (healthy, low, medium and high), obtained by mixing artificially contaminated seeds with healthy ones. Three replicates of 400 seeds were analysed by 11 accredited and 11 non-accredited laboratories in 17 countries.

This PT showed that many laboratories over- or underestimated the percentage of *B. cinerea*. This might be explained by a difficulty to identify *B. cinerea* through confusion with saprophytes (i.e. *Cladosporium*) or by a different interpretation of the term "contaminated seed". It was concluded that a workshop should be organized to harmonize the results.

We observed that the addition of malt increased the proliferation of saprophytes, a factor able to influence repeatability and reproducibility. A comparison test with and without malt in the water is planned by the SHC.

Introduction

In 2007, the ISTA Seed Health Committee and the ISTA Accreditation Department decided to organize its first proficiency test (PT). The detection of *Botrytis cinerea* on *Helianthus annuus* seeds was chosen, and the PT was organized by the GEVES-SNES Laboratory of Pathology. The aim of the PT was to verify the ability of laboratories to detect, using ISTA method 07-003, the percentage of *H. annuus* seeds infected by *B. cinerea*.

As it is difficult to obtain naturally contaminated seed lots with varying levels of contamination, it was decided to artificially contaminate healthy seeds.

Materials and methods

Various seed lots provided by seed companies were tested by the organizing laboratory using ISTA method 7-003. One healthy seed lot was selected, and artificially contaminated by soaking the seeds in a suspension of *B. cinerea* strain MAT/REF/1-2 with a concentration of 10⁶ spores per mL. Three contamination levels were obtained by spiking this seed lot with artificially contaminated seed: one low (level 1), one medium (level 2), one high (level 3). The non-contaminated seed lot was added as control (level 4).

Each lab received 3 samples of each contamination level, i.e. a total of 12 samples. Each sample contained 400 seeds. The organizing lab tested 10 further samples (i.e. 13 replicates of each contamination level) in order to obtain a mean value.

Five seeds were plated on a blotter supplemented with 3% malt solution to allow growth of *B. cinerea* as described in ISTA method 07-003. Notations were done after 5, 7 and 9 days of incubation of plates at 20 °C in darkness.

All results were reported as the number of seeds contaminated with *B. cinerea* by plate in the notation sheets provided and the percentage of contaminated seeds was calculated.

Repeatability and reproducibility were analysed with the statistical tool developed by S. Grégoire according to ISO 5725-2.

Results

Several challenges were linked to this PT. The use of artificially contaminated seed lots allowed only a short period between contamination and dispatch. Samples had to be analysed within 15 days after receipt. This delay was too short for some laboratories. Obtaining phytosanitary certificates

was difficult because contaminated seeds were sent. Eight laboratories in six countries were not able to participate for this reason.

The final result of an analysis corresponds to the last notation, so the final values, after 9 days, were analysed first.

Analysis of ten extra samples showed that as expected, three contamination levels were obtained (fig. 1): one low (3.78%), one medium (21.46%) and one high (36.71%). The non-contaminated seed lot showed a very low contamination level (0.15%) and we decided to include it in the analysis (level 4).

All laboratory results are presented in figure 2. The values of eight laboratories (2, 4, 12, 13, 14, 16, 19, and 22) were always lower than the expected mean, and those of two (9, 17) always higher. The values obtained by laboratory 8 were very low, many being zero. These values were not fit for use. Therefore the results of laboratory 8 were not included in the further analysis.

Overall repeatability and reproducibility values

The repeatability and reproducibility values increased with the contamination level. This corresponded to the expected result. The values were very similar for the low contamination levels (data not shown). As this is the first PT, we do not know whether this result is typical or not. This fact must therefore be checked against the next tests.

Reproducibility and repeatability analysis a) Repeatability

Repeatability expressed as the h value is shown in figure 3. Laboratory 17 significantly overestimated results, and the results of this laboratory were therefore not included in the further analysis or calculation of h values (fig. 4).

Figure 4 shows that laboratory 19 obtained an overestimation for the levels 1 and 4, i.e. for the low contamination levels. Laboratory 22 also obtained also an overestimation for level 4. Laboratory 9

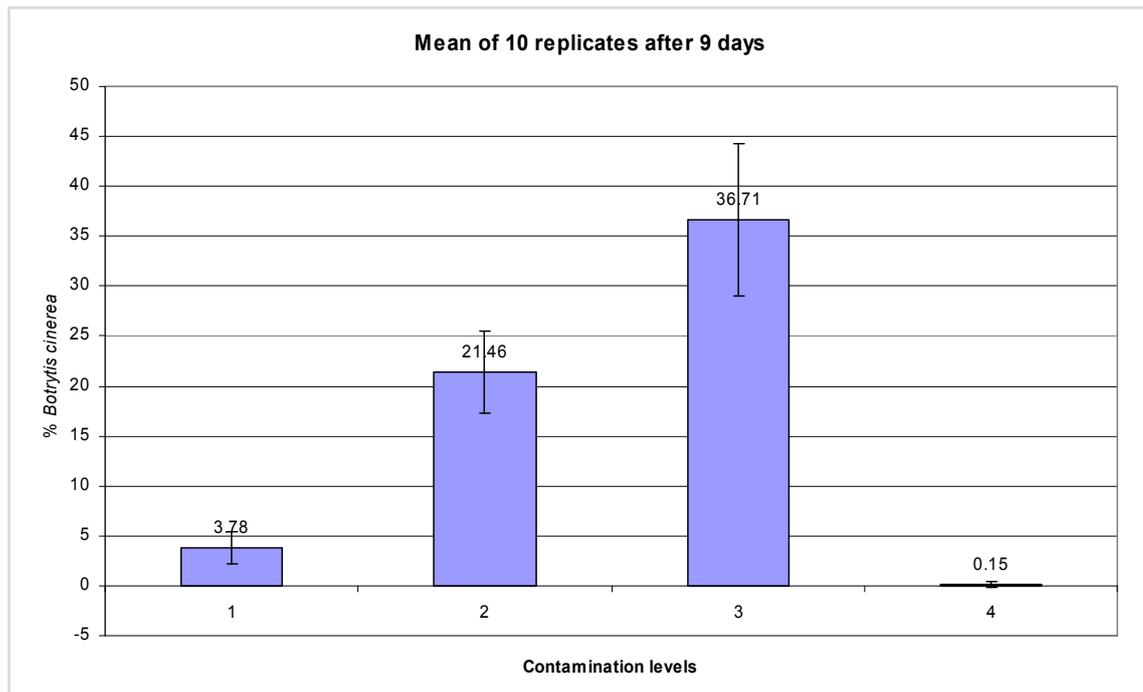


Figure 1. Contamination levels.

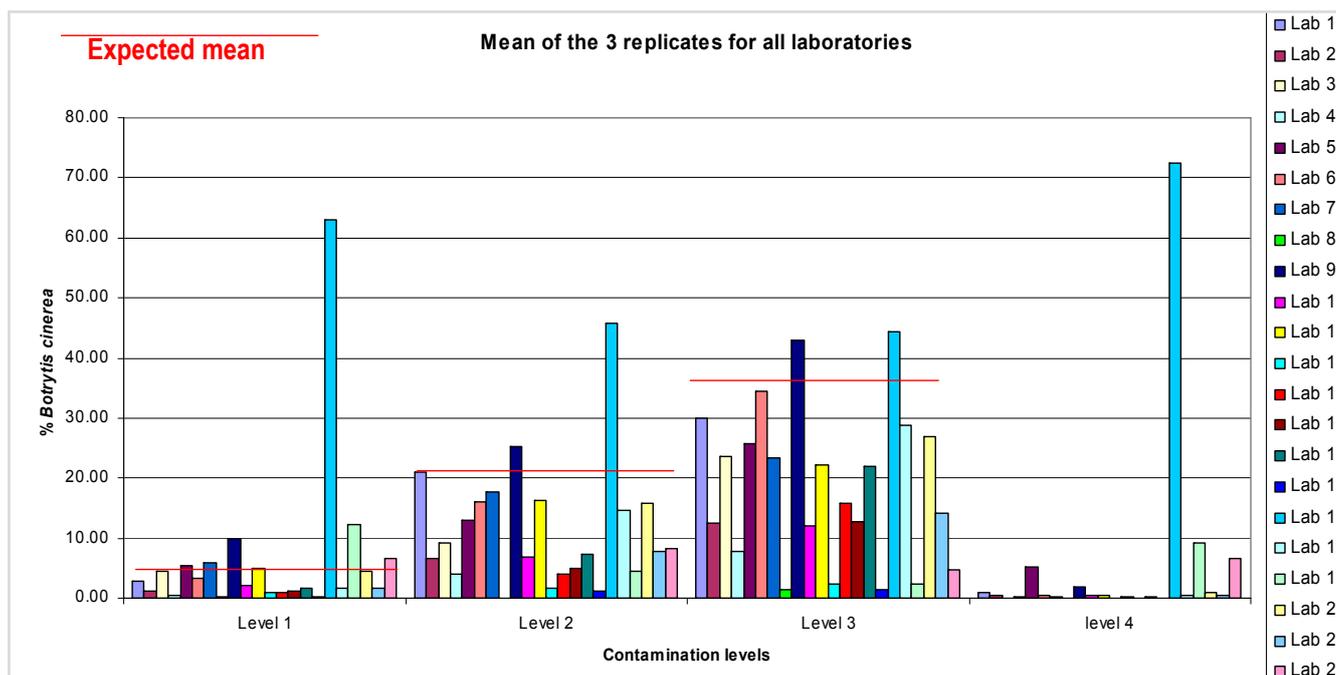


Figure 2. All laboratory results.

obtained significant overestimations for levels 1, 2 and 3 (data not shown).

Laboratories 2, 4, 12, 13, 14 and 16 did not obtain significant h values, but they did have a tendency to underestimate. This was confirmed by comparison with the expected values.

b) Reproducibility

Figure 5 shows that the k values of laboratory 17 are different from those of the

others, and results of this laboratory were therefore not included in the further analysis or calculation of k values (fig. 6).

Figure 6 shows that laboratories 19 and 22 obtained the higher variability of the repeats for the levels 1 and 4, i.e. for the low contamination rates. Laboratories 1, 5 and 15 showed a variability of the repeats for levels 2 and 3, i.e. for the high contamination rates.

Results after 5 and 7 days

Several laboratories did not give results or gave negative results after 5 or 7 days.

Due to the negative results and lack of values, it was not possible to make a statistical analysis of 5- and 7-day notations. The tendency to under- or overestimate was the same at 5 and 7 days versus 9 days (data not shown).

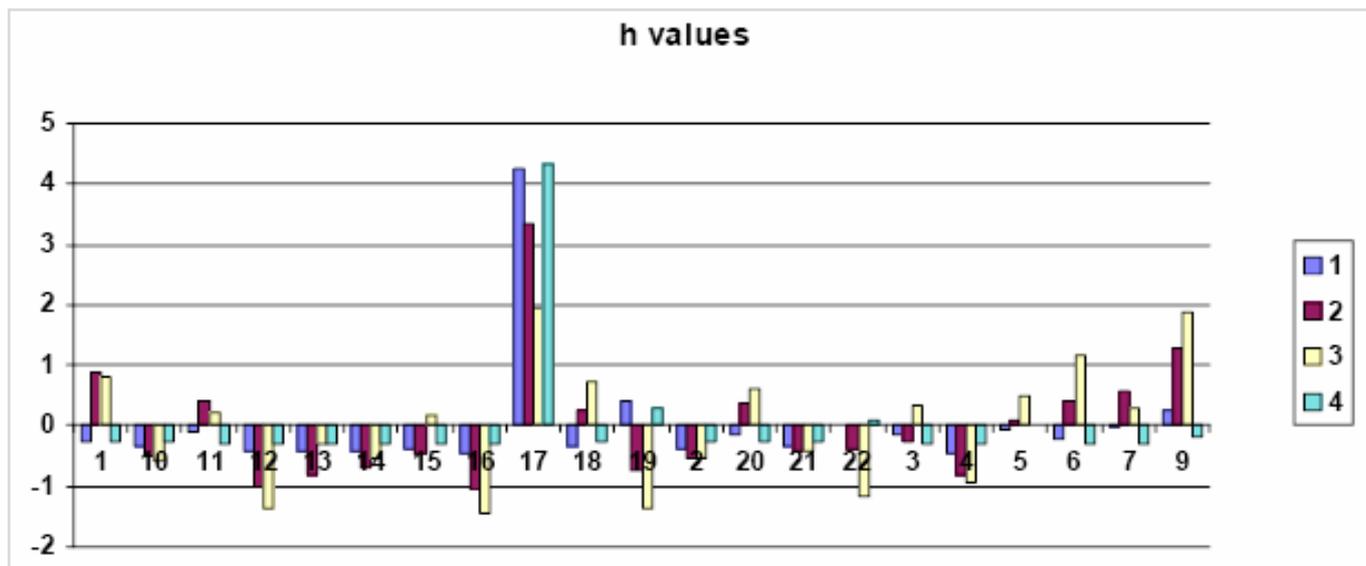


Figure 3. Repeatability expressed as h values.

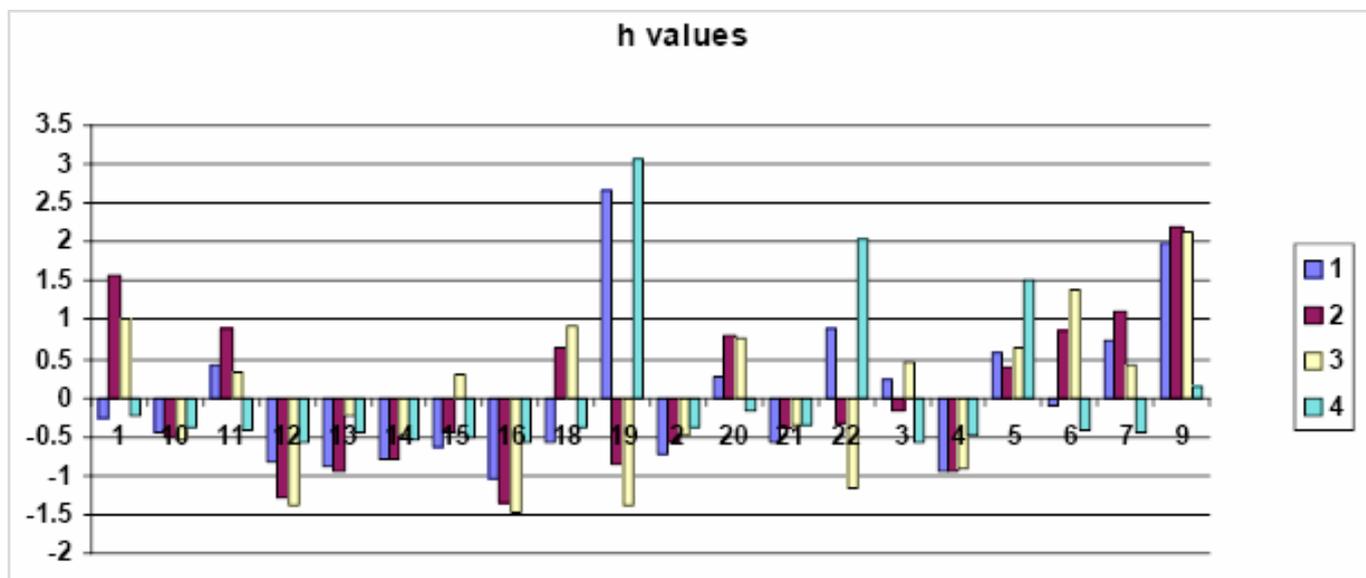


Figure 4. Repeatability expressed as h values, calculated without laboratory 17 results.

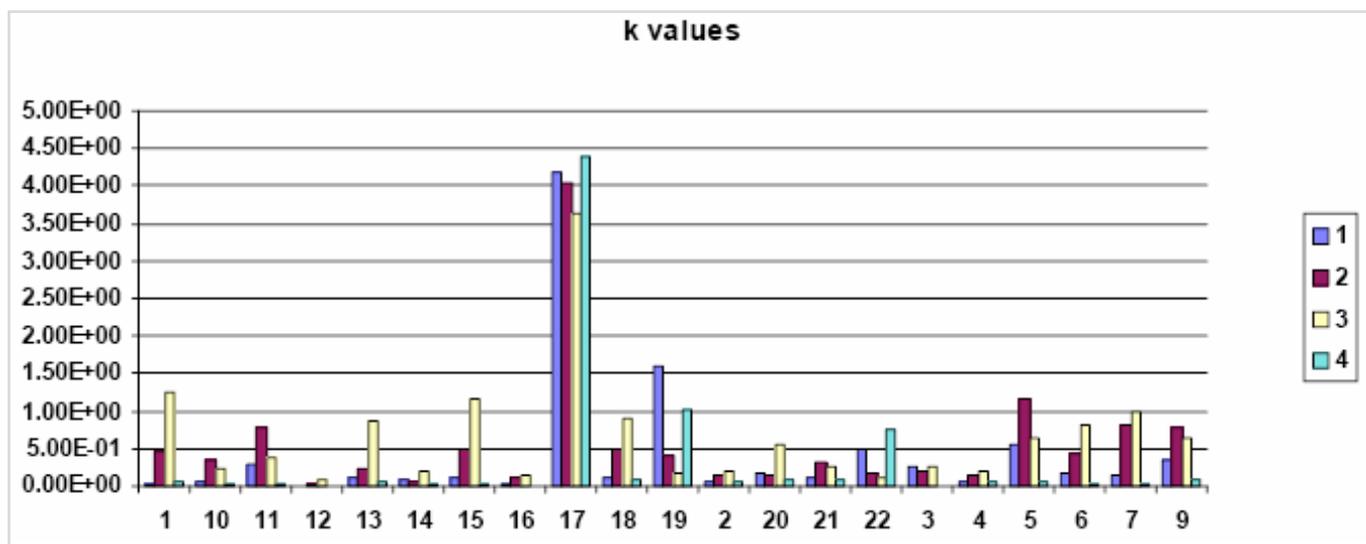


Figure 5. Reproducibility expressed as k values.

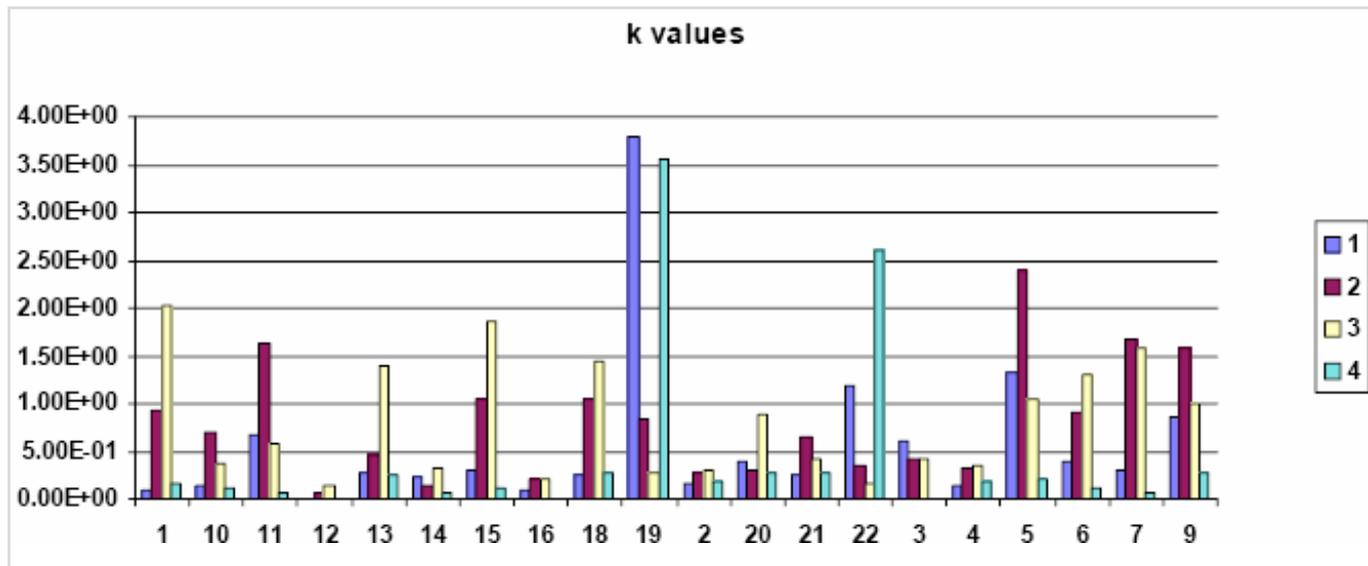


Figure 6. Reproducibility expressed as k values, calculated without laboratory 17 results.

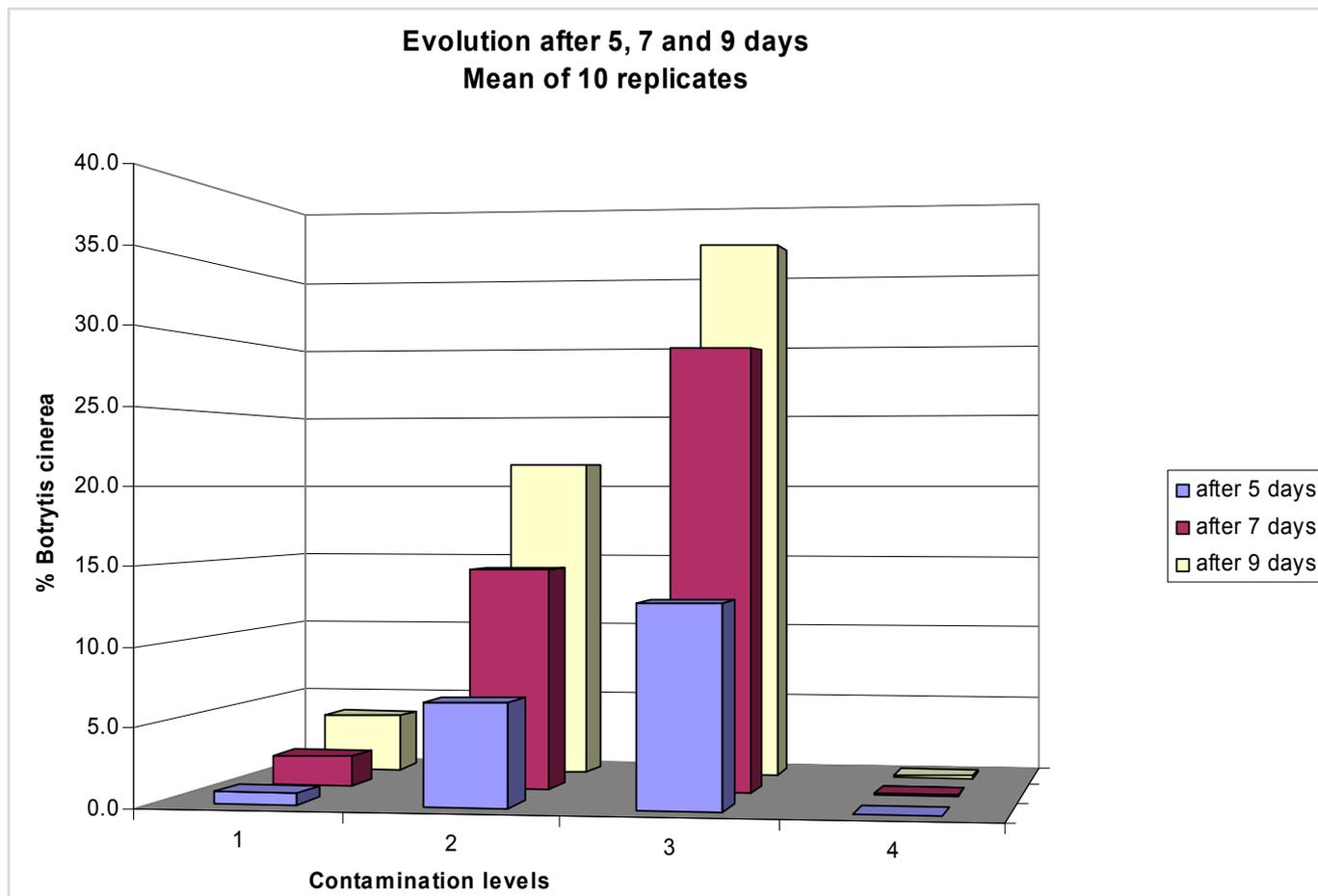


Figure 7. Comparison between the three notations.

Comparison between the three notations

The comparison of results on the 10 further samples for the three notations showed that the percentage of *B. cinerea* increased progressively, between the first and the last notation for the four contamination levels studied (fig. 7), even after 7 days. The notation at 9 days seems to be necessary.

Conclusion

This PT was the first of the Seed Health Committee. It allowed us to compare results between laboratories and the ten further samples. It also showed whether laboratories obtained too high or too low results, compared to expected contamination levels.

Many laboratories over- or underestimated the percentage of *B. cinerea*. Future proficiency tests will allow us to know whether laboratories improve their results.

High or low results given by several laboratories could be explained by a difficulty to identify *B. cinerea*. Overestimation can be explained by confusion with saprophytes (i.e. *Cladosporium*). Underestimation can be explained by a different interpretation of the term “contaminated seed” which can explain the low results. Some laboratories consider a seed contaminated

when the roots show soft rot and not when just one conidiophore is present (laboratory 16, personal communication). Their values showed an underestimation.

We observed that the addition of malt, as described in the ISTA method, increased the proliferation of saprophytes (fig. 8a, b). This may be a factor influencing repeatability and reproducibility, due to difficulty of notation, confusion of saprophytes with *B. cinerea*, or less proliferation of *B. cinerea* compared to saprophytes.

This fact was discussed by the SHC in May 2007, and a comparison test with and without malt in water is planned.

The percentage of *B. cinerea* increased progressively between the three notations. The first notation showed a very low level of contamination. It may be questionable whether the first notation is necessary or could be optional. The comparison between the three notations showed that notation at 9 days seems to be necessary, so the last notation (9 days) could possibly be sufficient. Notation at 5 or 7 days seems to be necessary only to check whether saprophytes are present which could disturb the last notation. New tests should be carried out to determine whether the three notations are useful.

To obtain contaminated seeds, we used an artificial contamination by soaking in a suspension of spores. The spores are on the surface of the seeds, and secondary contamination could occur from one seed to another, before the test. This technique does not allow the exact rate of contamination to be predicted. This method allowed us to obtain four different levels, even if they were different from the theoretical levels. Another technique more representative of natural contamination should be used to obtain an internal artificial contamination.

Due to this high variability of results, a workshop should be organized to harmonize the results.

This proficiency test leads to the following perspectives:

- Comparison of conditions with and without malt;
- Verification of the necessity of notation at 5 and 7 days;
- Natural versus artificial contamination, and which method for artificial contamination;
- Organization of a workshop. ■



Figure 8a. Incubation culture with malt.



Figure 8b. Incubation culture without malt.

Should the rules regarding the evaluation of ungerminated seeds be changed?

Ronald Don¹ and Günter Müller²

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Following Proficiency Test Round 07-1 on *Panicum maximum*, the Germination Committee sent a questionnaire to participating laboratories regarding the evaluation of ungerminated seed. The results of the proficiency test indicated that labs had difficulty in evaluating ungerminated seeds at the end of a germination test, and the responses on completed questionnaires would seem to confirm this conclusion (fig. 1).

Six percent of respondents classified all ungerminated seed as dead without any detailed examination of the ungerminated seed. They concluded that any seed that had not germinated after the prescribed testing period had to be dead, since dormancy breaking procedures had been employed. Forty percent of respondents limited their assessment to a visual examination of the ungerminated seed, with those looking mouldy and those soft to the touch being classified as dead. The remaining 54% removed the hard seed coat of the ungerminated *P. maximum* seed, and classified seed without caryopsis as dead. Those with caryopsis were then assessed as dead or fresh, based on either a visual examination or a tetrazolium test. Thirty-one percent of labs made a visual assessment, whereas 24% made a tetrazolium assessment (fig. 2).

How laboratories evaluated ungerminated seed makes a significant difference to whether fresh seed were reported. Those who classified all ungerminated seed as dead did not report any fresh seed. For those who based their assessment on purely a visual assessment and touch, only 15% reported any fresh seed. The proportion of labs reporting fresh seed increased to 48% with labs who carried out a visual assessment of the naked caryopses of

ungerminated seed. Seventy five percent of labs who carried out a tetrazolium assessment of the caryopses of ungerminated seeds reported fresh seed.

The *Panicum* proficiency test was extremely challenging. There were large numbers of empty seed in the pure seed fraction, and dormancy and saprophytic infection led to problems in the assessment of germination. Visual examination alone

was unlikely to give an accurate assessment of ungerminated seed, and it might be argued that only those labs that subjected ungerminated seeds to a tetrazolium evaluation obtained a true representation of the level of fresh seed in the sample. However, only 24% of laboratories who responded to this questionnaire carried out a tetrazolium evaluation of the ungerminated seed.

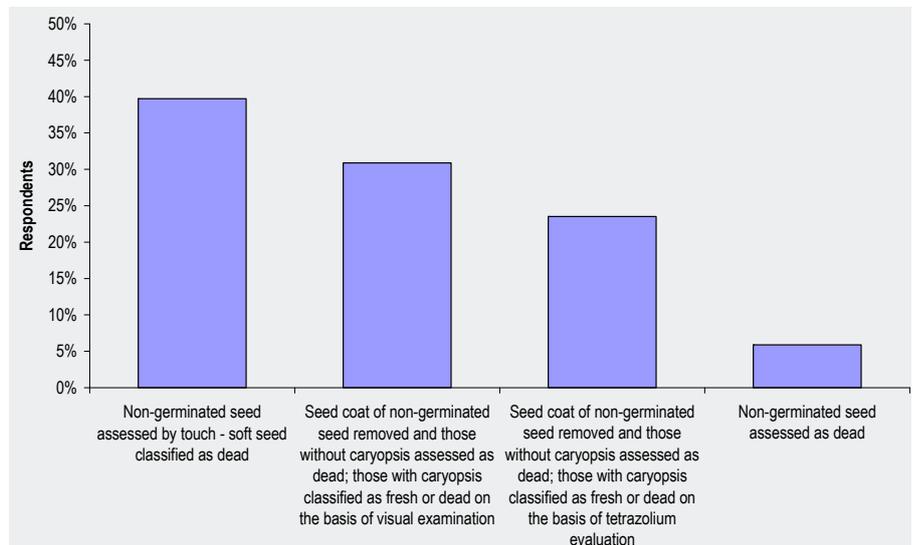


Figure 1. Assessment of ungerminated seed.

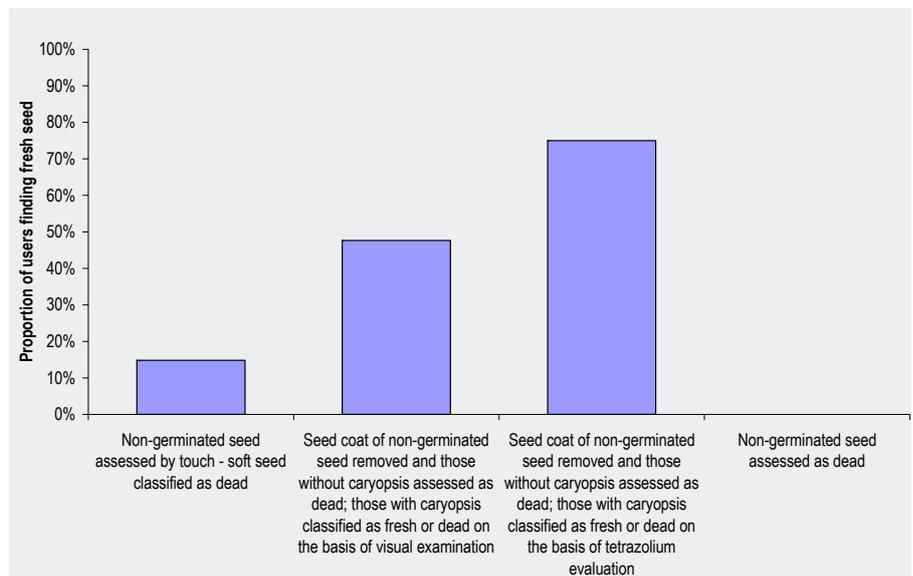


Figure 2. Method of evaluating ungerminated seed.

Perhaps they are the only labs who carried out the analysis correctly.

Laboratories need guidance on the evaluation of ungerminated seed. The Germination Committee have produced a flowchart, giving guidance of how ungerminated seed should be assessed (fig. 3). This flowchart will be published as an appendix to the Seedling Evaluation Handbook, together with another which will indicate how laboratories should report the result of a germination test where fresh seed have been found.

As well as producing guidance on the evaluation of ungerminated seed, the Germination Committee have been considering the reasons why laboratories may have had difficulties. Many laboratories indicated that they do not have the experience necessary to carry out tetrazolium testing, whereas others felt that tetrazolium analysis of such a small species was too difficult to attempt. For many inexperienced laboratories the tetrazolium test is also very time consuming, and laboratories may not have enough manpower during the peak

season to carry out large numbers of tetrazolium examinations. In addition, many laboratories indicated that their customers would not be prepared to pay for a tetrazolium evaluation of ungerminated seed at the end of a germination test.

The germination committee realise that TZ testing can not become a routine component of germination testing and that dormancy breaking methods — pre-chilling, pre-drying, KNO_3 , gibberellic acid, acid scarification, pre-washing etc., depending on species — should, in the vast majority of samples, result in dormancy being overcome. Where there are large numbers of ungerminated seeds at the end of a germination test that appear to be dormant, laboratories are encouraged to retest using different or a combination of different dormancy-breaking pretreatments. Some committee members feel that customers are not interested in ungerminated seed and that it should be possible for labs to be able to report “ungerminated seed”, rather than have to try and distinguish between fresh and dead seed. If a customer required information on the different categories of ungerminated seed, this could be the subject of an additional test.

The germination committee would like to open this debate to the ISTA community and readers of Seed Testing International. Should customers be provided with information on different categories of ungerminated seed, or should this be the subject of an additional test? E-mail your views to tcom@ista.ch. ■

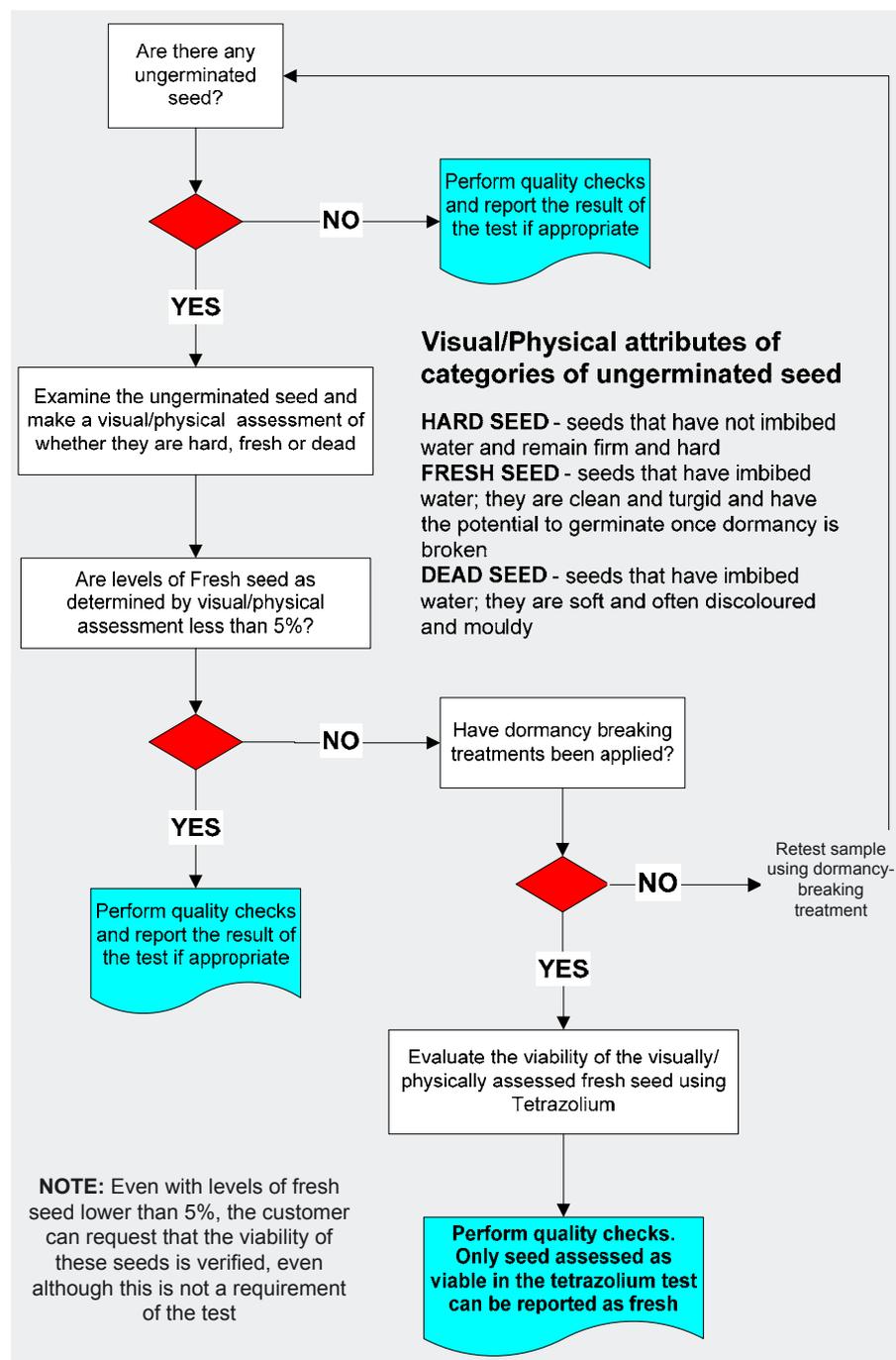


Figure 3. Assessment of fresh seed at the end of a germination test.

Seedcalc8
now available
More information and
free download from
the ISTA web site

http://www.seedtest.org/en/stats_tool_box_content---1--1143.html

<http://www.seedtest.org/upload/cms/user/Seedcalc8.zip>

ISTA GMO Proficiency Test PT09

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In August 2007 the ninth GMO Proficiency Test was launched with the shipping of the samples. A total of 64 laboratories took this opportunity to demonstrate proficiency in detecting and quantifying the presence of GM seeds, and registered as participants.

The laboratories received a set of 12 maize samples, spiked with GM seeds at various levels. Upon request, two additional samples consisting of GM seeds spiked with conventional seeds could be obtained.

Evaluation

Fifty-six participants submitted their results; four provided qualitative results only.

Qualitative results: detection

More than 85% (48 laboratories) of the participants reported correct results for all 12 samples, and achieved an A rating. Two laboratories with one misclassification and three laboratories with two were given a B rating. Two laboratories reported three false results (C rating), and one had a total of four misclassified samples (BMP rating) (fig. 1).

All false-negative results occurred in samples with a 0.1% spiking level, to equal proportions in MON863 and NK603 samples.

False-positive results were reported by three laboratories, two of which had additional false negative results, resulting in C or BMP ratings, accordingly.

Quantitative results

For the rating of the quantitative results, only the totals reported by participants are taken into consideration. It is based on the quantification results for the 11 positive samples and their reference value. The reference value is either the number of GM seeds in percent, or the mass of the GM seeds in percent, or the median of the results reported by the participants in the unit ‘%number DNA copies’.

Which of the reference values is chosen is determined by the participant’s unit of results:

- Subsampling quantification and results reported in number percentage (9 laboratories);
- results reported in mass percentage (22 laboratories);
- results reported in any other unit (e.g. number percentage of DNA copies): median (21 laboratories).

The distribution of the results reported by a total of 52 participants is as follows:

- A rating: 27 laboratories;
- B rating: 11 laboratories;
- C rating: 1 laboratory;
- BMP rating: 13 laboratories.

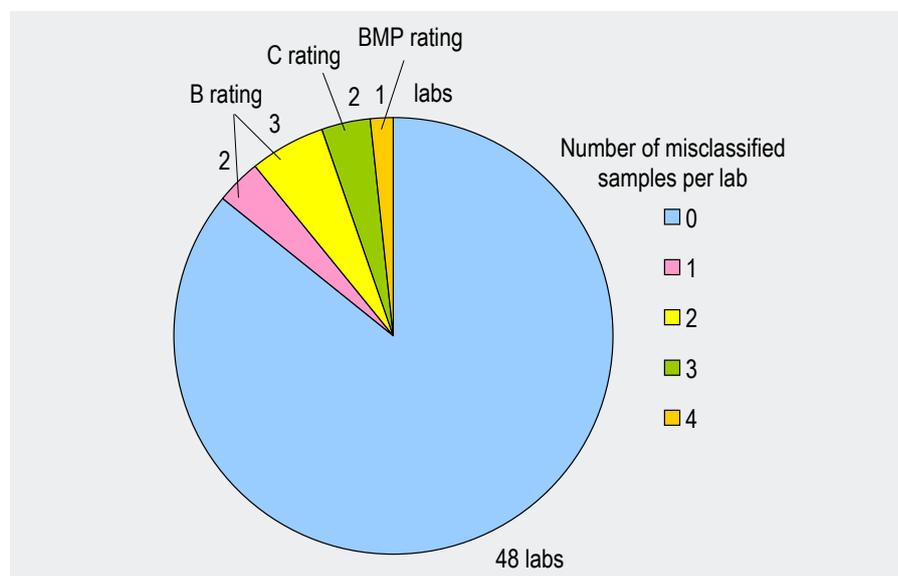


Figure 1. PT09 detection results.

Table 1. Sample details

Spiking level	0%	0.1%	0.1%	0.8%	0.8%	0.8%	90%	96%
Event		Mon863	NK603	MON863 + NK603	MON863 + NK603	MON863 + NK603	MON863	MON863
Lot No.	1	2-4	5, 6	7, 8	9, 10	11, 12	13	14
Number of samples	1	3	2	2	2	2	1	1
Number of non-GM seeds	2000	1998	1998	1984	1984	1984	5	2
Number of GM seeds	0	2	2	8 + 8	4 + 12	12 + 4	45	48

The determinant for a BMP rating is a proportion of more than 50% outside the accepted range of [0.5 (reference value); 2 (reference value)]. Most laboratories rated BMP had actually more than 75 % of their results outside this range. Only two participants were close to the threshold and could be considered borderline cases.

The number of deviating results (i.e. outside the accepted range) for the samples with the 0.1% spiking level is considerably higher than for the 0.8% samples: 37% of the sample results compared to 24%. A total of 15 participants indicated that the GM content was below their quantification level, and did not provide quantitative results for the corresponding samples (figs. 2, 3).

Identification

Participants were allowed to indicate the presence or absence of specific events or provide event-specific quantitative results, and 34 laboratories used this opportunity. This information was not evaluated for the rating.

GM purity

Upon request, two additional samples of GM seeds spiked with conventional seeds

were included in the sample sets (90% and 96% GM purity based on the number of seeds). 31 participants had requested the samples for testing genetic purity in their registration forms. 15 participants reported quantitative results for this voluntary part of the proficiency test.

Irrespective of whether the number of seeds or the mass of seeds was used as a reference unit, 12 laboratories reported results deviating less than 10% from the true value, and 9 laboratories even less than 5%.

Conclusion

By participating in this 9th round of proficiency testing in GMO analysis, laboratories were able to demonstrate whether they were able to detect adventitious presence and achieve quantification results consistently close to the samples' reference values. In addition, the opportunity for evaluating performance in identifying individual events and quantifying GM purity was provided.

Within the ISTA GMO PT, the unit of measurement for reporting quantification results is at the participants' discretion. The evaluation process is identical, irrespective of the unit chosen; only the

reference values differ to some extent. For mass percentage and number percentage of seeds, the sample preparation process provides individual records for each of the samples according to the numeric or mass proportion of the GM seeds in the samples. Consequently the true values for each sample are known.

For the unit percentage of DNA copy numbers, the reference value is calculated as the median value of all results reported in percentage of DNA copy numbers for each spiking level (i.e. identical value for a number of samples).

It is assumed that a majority of participants opting for this unit report results expressed on the basis of haploid genome. Due to the fact that the information provided does not always include a reference to the haploid genome, other approaches may equally be adopted. This leads to a level of confidence in the reliability of the reference value for percentage of DNA copy results that is inferior to the confidence associated with the reference value for the units mass percentage and number percentage of seeds. ■

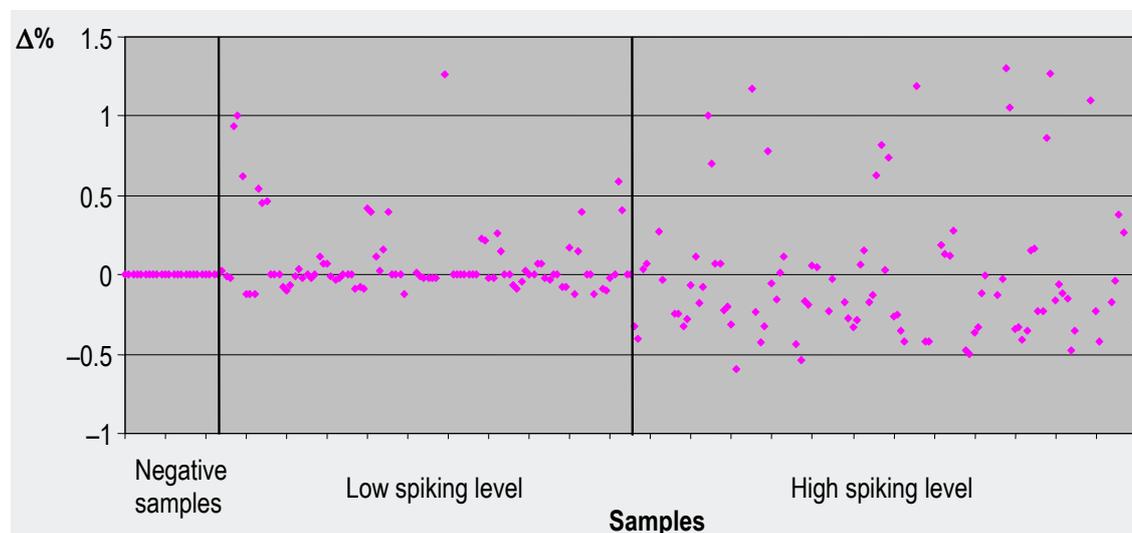


Figure 2. DNA percentage reported (reference value: percentage of DNA copy numbers).

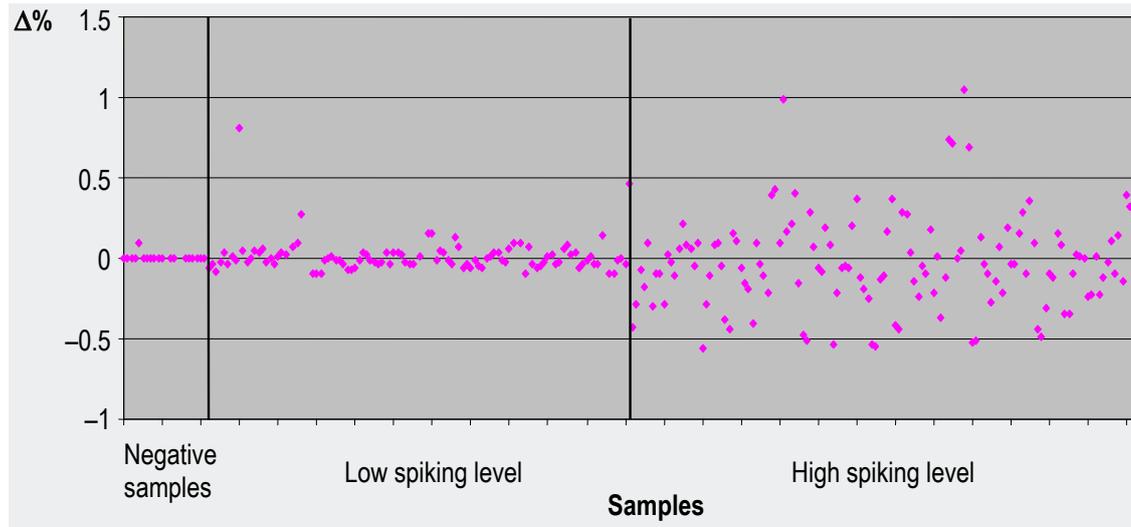


Figure 3. DNA percentage reported (reference value: seed mass).

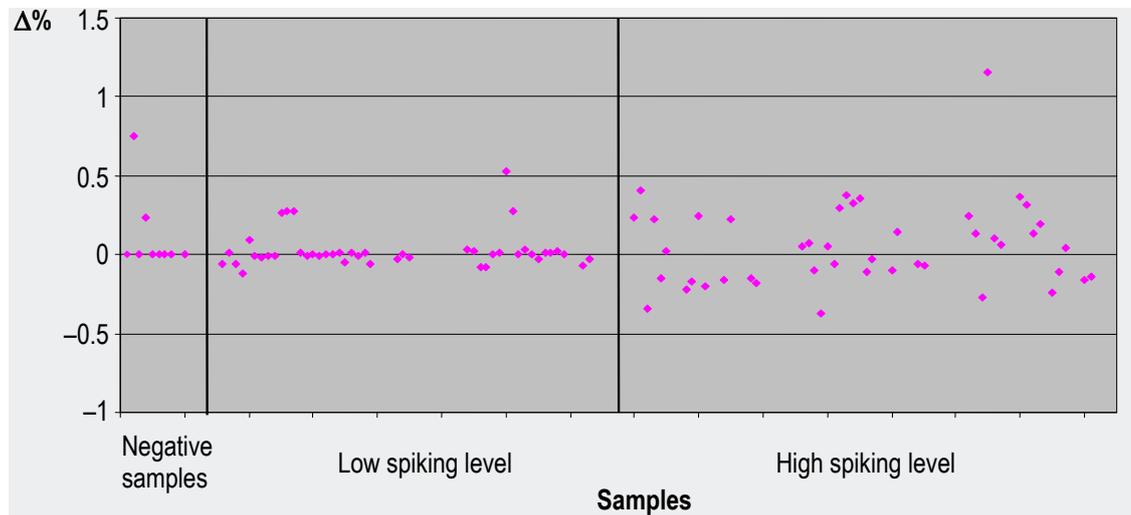


Figure 4. DNA percentage reported (reference value: number of seeds).

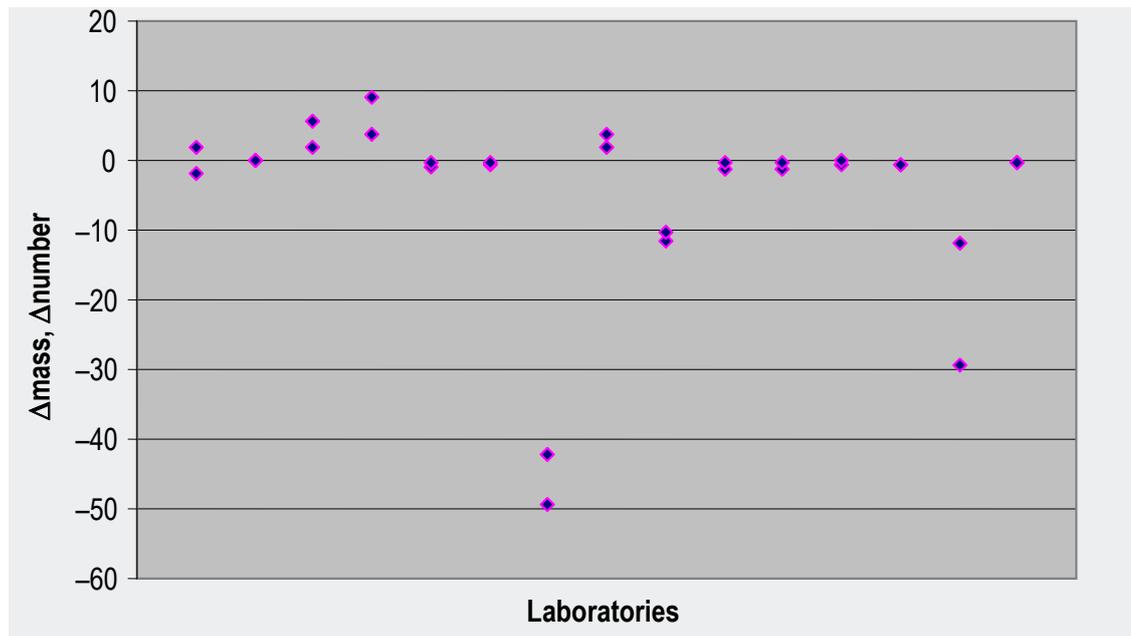


Figure 5. Purity (reported value - reference value)

Accreditation documents – update

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The ISTA Executive Committee approved the new version of the Scope of Accreditation Policy document at their meeting in February 2008. The changes reflect the 2008 version of the ISTA Rules, in particular with regard to moisture determination and seed health. Moisture determination is now divided into the following activities, in accordance with the Table 9A of Chapter 9:

- Moisture determination on species which do not require grinding;
- Moisture determination on species which require fine grinding;
- Moisture determination on species which require coarse grinding;
- Moisture determination on species which require cutting;
- Moisture determination on species of Table 9A by using a moisture meter.

The new seed health methods that were voted into the ISTA Rules at the Ordinary Meeting in 2007 were added to the list of methods for which a laboratory may

become accredited. The Executive Committee also followed the proposal to combine purity determination and counting of other seeds into one test for which accreditation may be sought. It was decided that both activities require competence in identification of other seeds, and that there is no need to distinguish between the two tests in terms of competence required.

The latest version of the Scope of Accreditation Policy document is available for download on the ISTA website under: http://www.seedtest.org/en/related_documents_content---1--1253.html.

Now in stock...

ISTA Handbook on Moisture Determination Edition 2007

By the ISTA Moisture Committee; editors H. Nijënstein, J. Nydam and R. Don

ISBN 978-3-906549-49-1

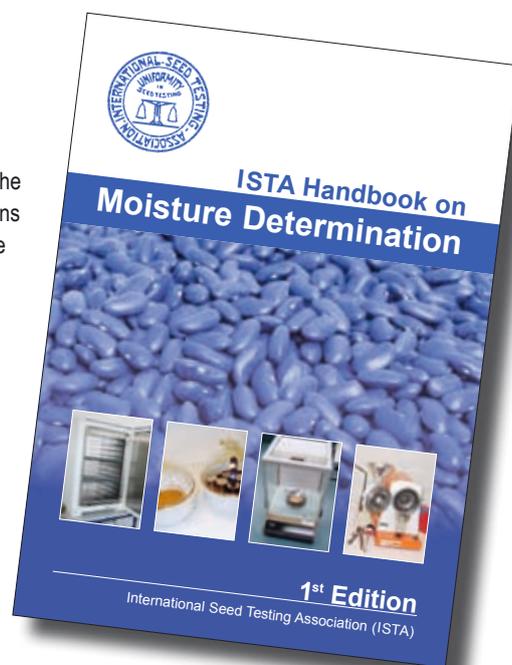
This first version of the *ISTA Handbook on Moisture Determination* provides additional help to the *International Rules for Seed Testing*, Chapter 9: Moisture. Its detailed instructions, interpretations of Rules sections, and examples of how to calculate results in detail should prove to be of value to those concerned with seed moisture testing.

The main chapters are:

- Moisture in seed (seed physiology, moisture testing during several stages in a seed's life, non-orthodox seeds);
- Methods of moisture testing (Karl Fischer, phosphorus pentoxide, ovens, capacitance and conductance type meters, NIR, NIT, NMR, RH);
- Sampling and sample preparation (examples, quality assurance);
- Reference method (including detailed procedure and examples of how to do the calculations);
- Constant temperature oven method (around 50% of the Handbook; contains detailed instructions, photographs, flow charts, examples of SOPs etc.);
- Moisture meters (detailed information, including monitoring and calibration);
- Reporting of results (calculations, tolerances, validation of new methods);
- References.

The chapters 'Moisture in seed' and 'Methods of moisture testing' are aimed mainly at trainee seed analysts, trainers and students, and contain background information on moisture in seed.

Price: CHF 267.00 (approx. USD 270.00/EUR 170.00) from the ISTA Secretariat (for contact details, see back cover)



Laboratory accreditation changes

Status 20 March 2008

<p>Re-accreditations</p> <p>Canada</p> <p>CADL0400</p> <p>Ontario Plant Laboratories Plant Pathology Laboratory Canadian Food Inspection Agency Ottawa Laboratory Fallowfield Ottawa, Ontario K2H 8P9 Phone: +1 613 7591292 Fax: +1 613 759 1260 Email: devernol@inspection.gc.ca</p>	<p>United Kingdom</p> <p>GBDL0400</p> <p>Official Seed Testing Station Scottish Agricultural Science Agency Headquarters 1 Roddinglaw Road Edinburgh EH12 9FJ Phone: +44 131 556 8400 Fax: +44 131 244 8940 Email: ronald.don@sasa.gsi.gov.uk</p>	<p>SEDL0700</p> <p>Frökontrollen Mellansverige AB Section Örebro P.O. Box 22014 702 02 Örebro Phone: +46 19 6114607 Fax: +46 19 135082 Email: frokontrollen@hush.se</p>
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<p>Czech Republic</p> <p>CZDL0200</p> <p>FGMRI Strnady 136, 252 02 Jiloviste Research Station Kunovice Na Záhonech 601 686 04 Kunovice Phone: +420 572 420920 Fax: +420 572 549119 Email: semkon@vullmuh.cz</p>	<p>Romania</p> <p>RODL0200</p> <p>Institutul de Cercetari si Amenajari Silvice (ICAS) Laboratorul de Seminte Foresti Sos. Stefanesti 128, sector 2 077190 Bucuresti Phone: +40 21 350 32 45 Fax: +40 21 350 32 45 Email: genetica@icas.ro</p>	<p>Zambia</p> <p>ZMDL0100</p> <p>Seed Control and Certification Institute Official Seed Testing Station Mount Makulu P.O. Box 350199 13201 Chilanga Phone: +260 1 278170 Fax: +260 1 278112 Email: scci@zamnet.zm</p>
<p>Germany</p> <p>DEDL1000</p> <p>Landwirtschaftliche Untersuchungs- und Forschungsanstalt Referat Saatgutprüfung Obere Langgasse 40 67346 Speyer am Rhein Phone: +49 623 21360 Fax: +49 623 2136110 Email: seibert@lufa-speyer.de</p>	<p>Sweden</p> <p>SEDL0200</p> <p>Statens Jordbruksverk Utsäsesenheten (Swedish Board of Agriculture) Onsjövägen 83 268 81 Svalöv Phone: +46 36 1550 00 Fax: +46 36 1583 08 Email: utsadeskontroll@sjv.se</p>	<p>Termination of accreditation</p> <p>Denmark</p> <p>DKML0700</p> <p>L. Daehnfeldt A/S Quality Laboratory Fraugde-Kaervevej 94 A 5220 Odense</p>

Establishing quality control in UK wildflower seed production

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Over the past 20 years or so there has been a great increase in the use of seeds of native wildflower species in the United Kingdom, as in other European countries and North America. Wildflowers have been increasingly sown in urban regeneration and civil engineering projects for their attractive appearance and low maintenance requirements (fig. 1). Wild flowers also attract wildlife, thereby increasing biodiversity compared with plantings of traditional amenity grass species. Environmental restoration initiatives have also created a demand for seed, and there is a burgeoning use of wildflower species in gardens and private estates (Laverack et al., 2007).

The increased use of wildflowers has been followed by an increase in the commercial production of seeds and a new trade in approximately 200 species of plants, most of which have not previously been traded. Many of them are now being grown regularly as crops (fig. 2), while some are gathered directly from the wild. The plants

range from meadow species (including native grasses) to heath and wetland plants and woodland species, and cover a wide range of families and plant types.

Despite the increase in commercial production, in the UK, the trade remains largely unregulated. Agricultural seed legislation, in the form of the Fodder Seeds Regulations (HMSO, 1993), coincidentally covers some native species which are also traded as 'wild' material (e.g. *Lotus corniculatus* L.), but the requirement for registered cultivars (tested for distinctness, uniformity and stability) means that natural populations cannot possibly conform to the varietal standards. Neither have the standards for purity and germination in these regulations been applied to wildflower seeds. A recent attempt has been made in England to amend the regulations to include wild material, but this applies to the origin of the seed and not to other quality factors.

There has been considerable debate over the last twenty years about the importance of origin (or provenance) of the material of native species, especially as much of the seed has been imported to the UK from other European countries. Even here there is no regulation, but a voluntary code of

practice operates (Flora Locale, 2001). In contrast, little work seems to have been done to establish the fundamental requirement for germination testing to determine the planting value of wildflower seeds, and there are neither regulatory levels nor industry standards. Seed is generally traded without germination or purity test results being available.

For some species, the conditions for germination testing are quite straightforward, and a few of the species have germination conditions described in the ISTA Rules, e.g. *Leucanthemum vulgare* (ISTA, 2006). However, these have been developed mainly for cultivated material, and wild populations do not always behave in the same way. A major factor inhibiting the development of germination testing amongst producers, traders and users of wildflower seeds is the presence and range of types of dormancy found in native populations (Baskin and Baskin, 2001), which has inhibited the adoption of routine testing. Dormancy affects wildflower seeds in field and nursery conditions as well as laboratory testing, and in cases of seed failure, the lack of established testing methods often leads to the acceptance that, as the seed quality and behaviour is unknown, the cause just



Figure 1. Annual wildflowers sown on a traffic junction in Scotland in 2007. Species: *Papaver rhoeas*, *Centaurea cyanus*, *Chrysanthemum segetum* and *Triplospermum inodorum* (© F. Guest and G. Laverack).



Figure 2. Wildflower seed production plots in Scotland. (© F. Guest and G. Laverack)

cannot be determined. Valuable information on testing conditions and breaking dormancy is now becoming available from the Millennium Seedbank database, and this forms a useful basis for the development of protocols in many species.

The results summarised here are part of a comprehensive study of wildflower seed quality at Scotia Seeds, funded by the Scottish Executive under research and development grants. The work has concentrated on four topics: a) breaking dormancy for germination testing and improvement of field establishment, b) developing germination testing protocols for a wide range of species, c) surveying the quality of seed in the market to determine current standards and d) testing the repeatability of the protocols developed at the Scotia Seeds laboratory with ISTA-accredited laboratories. The results of the survey (topic c) and repeatability work (topic d) are presented here.

Methods

Seed quality survey

The study tested nine wildflower species from eight seed companies (producers and merchants). Seeds were purchased anonymously from the internet. The samples obtained were first examined for purity and then put through germination tests using the protocols developed in the earlier stages of the project (topic b above). Germination tests were set up on germination paper and held at a controlled temperature and

light regime. Germination was assessed as total germination, which includes all seeds that have achieved at least physiological germination, i.e. production of a radicle at least 2 mm long.

Comparative germination study

This trial was carried out in collaboration with the ISTA Germination Committee as a comparative germination study of five wildflower species across seven ISTA-accredited seed testing laboratories.

Each of the laboratories was sent samples and instructions for storage prior to

test, the period of test and germination protocols (table 1). For two species, two protocols were tested, one that already existed in the ISTA Rules for cultivated species and one proposed by Scotia Seeds for wildflower species. Guidelines for assessment of physiological germination, normal and abnormal seedlings and recording of results were also provided. All data were returned to Scotia Seeds for statistical analysis.

The results from this trial were subjected to an ANOVA, Z score analysis and ISO-5725 analysis (tables 3, 4; figs. 1, 2).

Table 1. Germination protocols for wildflower seeds

Species	Pretreatment	Method of germination	Temperature of germination (°C)	Light regime
<i>Achillea millefolium</i> (proposed protocol*)	None	TP, H ₂ O	25	12/12
<i>Achillea millefolium</i> (existing protocol*)	None	TP, H ₂ O	20–30	8/16
<i>Leucanthemum vulgare</i> (proposed protocol*)	None	TP, H ₂ O	20	12/12
<i>Leucanthemum vulgare</i> (existing protocol*)	None	TP, H ₂ O	20–30	8/16
<i>Chenopodium album</i>	None	TP, KNO ₃	15	12/12
<i>Vicia cracca</i>	Scarify: chip seed coat with scalpel (approx. 2 mm)	PP, H ₂ O	20	12/12
<i>Hypochaeris radicata</i>	None	TP, H ₂ O	20	12/12

* Proposed protocol for testing wild flower species; Existing protocol in the ISTA Rules for cultivated species.

Table 2. Comparison of total germination* and purity of wildflower seeds from different online sources**a) Germination**

Company source	Species*								
	A	B	C	D	E	F	G	H	I
1	49	64	3	20	93	22	–	45	95
2	61	61	58	3	92	74	–	81	76
3	48	86	52	28	–	88	–	–	67
4	0	20	20	–	88	30	44	–	13
5	90	87	60	23	85	92	–	83	78
6	34	69	65	–	3	57	–	88	75
7	51	7	–	9	98	–	37	92	56
8	81	78	77	50	52	74	9	100	–

b) Purity

Company source	Species								
	A	B	C	D	E	F	G	H	I
1	100	97.4	99.2	99.2	97.4	100	–	96.5	99.8
2	100	98.7	99.4	98.8	96.3	99.4	–	97.2	99.5
3	98.7	91.5	97.3	88.3	–	97.9	–	–	95.4
4	99.1	99.2	82.6	–	95.6	100	85	–	99.2
5	100	86.3	97.0	82.3	93.7	97.7	–	91.9	99.4
6	92.4	95.2	99.7	–	93.5	98.8	–	98.3	98.7
7	92.4	95.4	–	100	97.7	–	100	100	95.5
8	100	100	99.3	100	100	100	100	99.3	–

* Total germination = all seeds that achieve at least physiological germination, i.e. a 2 mm radicle. A = *Primula veris*; B = *Leucanthemum vulgare*; C = *Ranunculus acris*; D = *Papaver rhoeas*; E = *Prunella vulgaris*; F = *Silene dioica*; G = *Ajuga reptans*; H = *Achillea millefolium*; I = *Galium verum*.

The individual species were analysed by a one-way ANOVA, and the laboratory and species means were analysed by two-way ANOVA. The Z score analysis was used to give an indication of the performance of each of the participating laboratories in comparison to all others. The ISO 5725 was used to assess the tendencies of laboratories to over- or underestimate results, and further to assess the variation between repeats and reproducibility across laboratories.

Results

Seed quality survey

Large differences in the percentage of total germination were found between samples of the same species (table 2). For example, in the case of *Primula veris*, germination ranged from 0 to 90%, and for *Leucanthemum vulgare* germination ranged from 7 to 87%. Of the 60 samples tested, 13 had germination below 25%. One sample of *Achillea millefolium* reached the maximum 100% total germination (i.e. all seeds achieved physiological germination or more), whereas in *Papaver rhoeas* the maximum was only 50%. Since all the germination data were based on total germination, it is likely that the normal germination percentage of the samples would be lower than shown here.

There were also differences in the overall quality of seed from different companies with, for example, company 5 supplying seeds with better overall germination rates than company 4.

Purity testing also showed differences in the proportion of inert matter found in some of the samples. In the case of *Leucanthemum vulgare* purity ranged from 86.3 to 100% and for *Ranunculus acris* and *Papaver rhoeas* 82.6 to 100% and 82.3 to 100%, respectively.

Table 3. ANOVA on normal germination assessed by 7 different laboratories (%)

Species	Laboratory							Mean ¹
	1	2	3	4	5	6	7	
<i>Achillea millefolium</i> (Proposed protocol*)	92 ^a	93 ^a	95 ^a	78 ^b	69 ^c	92 ^a	94 ^a	88 ^a
<i>Achillea millefolium</i> (Existing protocol*)	90 ^a	93 ^a	91 ^a	90 ^a	64 ^b	94 ^a	90 ^a	87 ^a
<i>Leucanthemum vulgare</i> (Proposed protocol*)	59 ^{ab}	57 ^b	65 ^a	61 ^{ab}	59 ^{ab}	61 ^{ab}	66 ^a	61 ^b
<i>Leucanthemum vulgare</i> (Existing protocol*)	58 ^a	61 ^a	60 ^a	48 ^b	61 ^a	61 ^a	57 ^a	58 ^b
<i>Chenopodium album</i>	47 ^c	19 ^d	76 ^a	27 ^d	63 ^b	48 ^c	54 ^c	48
<i>Vicia cracca</i>	75 ^{abc}	80 ^{ab}	71 ^c	77 ^{abc}	74 ^{abc}	74 ^{bc}	80 ^a	76
<i>Hypochaeris radicata</i>	57 ^a	64 ^a	59 ^a	44 ^b	58 ^a	62 ^a	50 ^b	56
Mean ¹	68 ^{BC}	67 ^C	74 ^A	61 ^E	64 ^{DE}	70 ^B	70 ^B	

Values with different superscripts are significantly different.

* Proposed protocol for testing wild flower species; existing protocol in the ISTA Rules for cultivated species.

¹ Means of species and laboratories analysed by 2-way ANOVA; lab means for single species analysed by 1-way ANOVA.

Comparative germination tests

The overall means of the seven laboratories involved in the comparative test revealed small, but significant differences in normal germination between laboratories (table 3). However, analysis of the total germination data reported by the laboratories (not shown) showed fewer differences. This suggested that some of the differences seen in the overall means of normal seedlings may be attributable to assessment of normal seedlings in often unfamiliar species.

For the individual species tested, comparative germination test results (table 3) for *Achillea millefolium* and *Leucanthemum vulgare* showed no significant differences between the existing protocol for cultivated species and the proposed protocol for wild species. Similarly, the results for *Vicia cracca* and *Hypochaeris radicata* showed no significant differences across laboratories. Only *Chenopodium album* gave unacceptable differences across laboratories.

The Z scores (table 4) revealed that in only 2 out of 49 instances were the data outside acceptable limits, and these were for existing protocols.

The H values (fig. 3) showed small, and acceptable over- and underestimations across all laboratories and all species. One laboratory (Lab 5, fig. 3) showed significant underestimation of germination for *Achillea millefolium* (existing) and another (Lab 4) did so for *Leucanthemum vulgare* (existing). The K values (fig. 4) showed small, acceptable variations across all laboratories and species. One laboratory (Lab 3; fig. 4) showed significant variation for *Hypochaeris radicata*.

Discussion

The survey results revealed quality problems in a high proportion of the wildflower seed lots being sold in the UK market, with some lots being clearly unsuitable for planting because of very low (or no) germination. This was the case even though the less demanding criterion of total germination

Table 4. Z score analysis of normal germination results (%)

Species	Laboratory						
	1	2	3	4	5	6	7
<i>Achillea millefolium</i> (Proposed protocol*)	0.47	0.52	0.7	-0.95	-1.86	0.45	0.67
<i>Achillea millefolium</i> (Existing protocol*)	0.03	0.55	0.33	0.24	-2.24*	0.62	0.24
<i>Leucanthemum vulgare</i> (Proposed protocol*)	-0.56	-1.32	1.18	-0.18	-0.56	-0.03	1.48
<i>Leucanthemum vulgare</i> (Existing protocol*)	0.01	0.59	0.43	-2.11*	0.7	0.64	-0.26
<i>Chenopodium album</i>	-0.02	-1.45	1.44	1.07	0.75	0.03	0.31
<i>Vicia cracca</i>	-0.02	1.21	-1.47	0.27	-0.53	-0.59	1.28
<i>Hypochaeris radicata</i>	0.09	1.09	0.38	-1.74	0.24	0.85	-0.91

* Proposed protocol for testing wild flower species; existing protocol in the ISTA Rules for cultivated species.

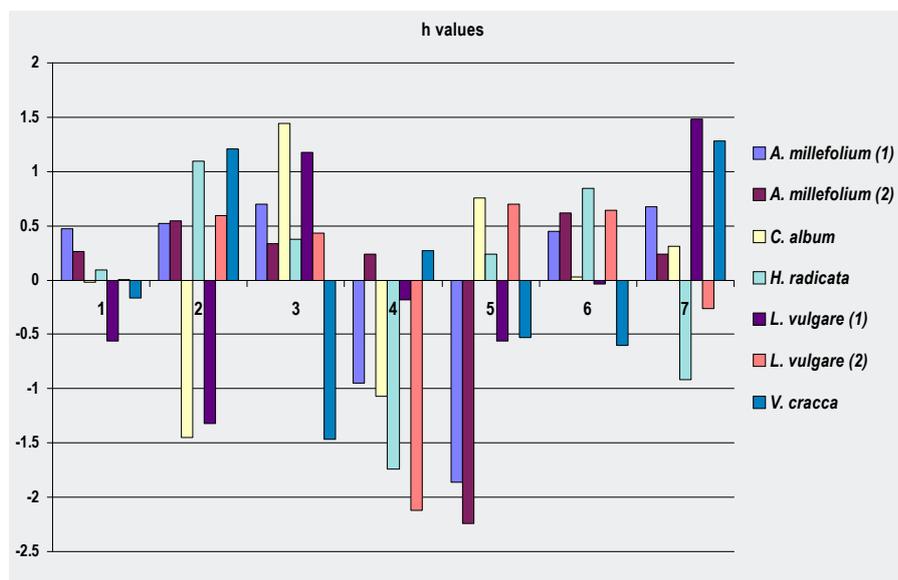


Figure 3. Results of ISO 5725 analysis (H values) for normal germination of 5 species (2 alternative protocols) tested in 7 laboratories. Note: species followed by number 1 denotes proposed protocol, number 2 denotes existing protocol.

(production of at least a 2 mm radicle) was used in the germination test. In some species, even the highest levels of germination found were quite low, and work to establish whether there is potential to improve on these levels may be worthwhile. Most of the suppliers provided seed with poor germination in at least one species, suggesting that they could all improve quality control, and some offer seed which is inferior to other companies overall. The variability within species may be due to differences in field, processing or storage factors, and there is a clear need to identify the causes of the problems. Variation in purity may also be partly due to field factors, but is also

strongly affected by processing, and there is clearly potential for improvement in the case of some suppliers. This data suggests that there is a strong case for the introduction of more regular quality control in the wildflower seed trade in the UK to prevent inferior seeds being sold.

The comparative germination trial demonstrated that reliable and repeatable methods for testing the germination of wildflower seeds can be developed. For *Achillea millefolium* and *Leucanthemum vulgare*, the results showed that the proposed protocols could be used as an alternative to the existing protocols in the ISTA Rules. Similarly, the protocols developed

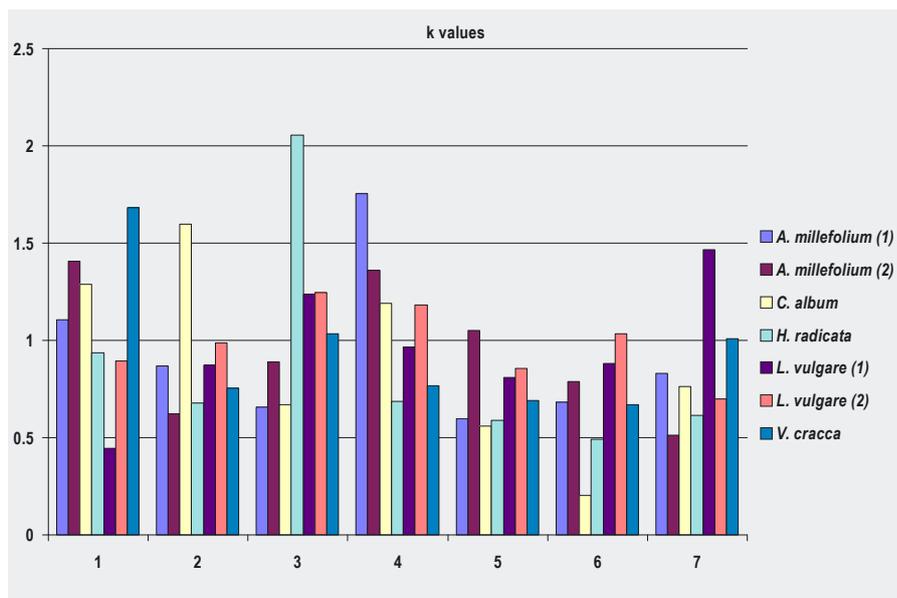


Figure 4. Results of ISO 5725 analysis (K values) for normal germination of 5 species (2 alternative protocols) tested in 7 laboratories. Note: species followed by number 1 denotes proposed protocol, number 2 denotes existing protocol.

for *Vicia cracca* and *Hypochaeris radicata* could be adopted for the testing of these species. The tests for *Chenopodium album* showed unacceptable differences between laboratories, and further work is necessary. It may be that the dormancy was not consistently broken in this species, or that small differences in the germination testing procedure used by different laboratories (e.g. germination paper, moisture regime) may have influenced germination. In species such as these, for which there is little testing experience, the inclusion of total germination, which includes any seed that achieves physiological germination (2 mm radicle), is particularly useful. Thus, where there are differences between laboratories in the percentage of normal germination, the observation of similar total germination results from the same laboratories suggests that the laboratories differ in their evaluation of normal and abnormal seedlings.

The establishment of appropriate and repeatable germination protocols for wildflower species is the first step towards quality control in these species. In our work to date we have developed protocols for germination testing of 150 UK wildflower species. This could form the basis for establishing quality control in UK wildflower seed production.

Acknowledgments

We are very grateful to Ronald Don at the Scottish Agricultural Science Agency (SASA) for his help in organising the comparative test. Thanks are also due to: Linda Maile, OSTS Cambridge, UK; Gillian McLaren, SASA, Edinburgh, UK; Lea Mazor, Official Seed Testing Station, Bet Dagan, Israel; Zita Ripka, OMMI, Budapest, Hungary; Rita Zecchinelli, ENSE, Tavazzano, Italy; and Sylvie Ducournau, SNES, GEVES, Beaucouzé, France, for participating in the tests. We would also like to thank Dr. S. Matthews for useful discussions, and the Scottish Executive for funding.

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New edition...

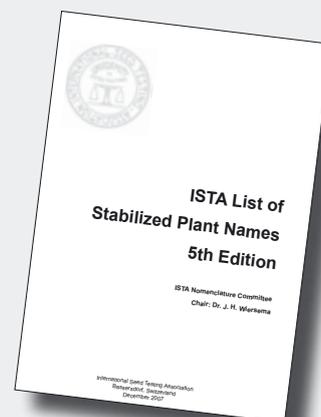
ISTA List of Stabilized Plant Names 5th Edition, 2007

By the ISTA Nomenclature Committee
Compiled by J.H. Wiersema
ISBN-13 978-3-906549-45-3

The new edition includes a total of 2915 entries, with 63 new species names and 264 changes, including 62 changes to family names, and many other changes of spelling, author names, synonym names and other nomenclatural or taxonomic changes, bringing the List up to date with current taxonomy.

These changes will be consistent with the *International Rules for Seed Testing* for the next six years.

Free internet download at www.seedtest.org



ISTA Quality Management Training Course

Bangalore, India, 12–16 May 2008

ANNOUNCEMENT

Location	Participation fee
Hotel 'The Chancery', Lavelle Road	ISTA Members: EUR 560 (USD 820) Non-members: EUR 840 (USD 1230) The fee includes participation in the workshop, all literature and supporting material for the workshop, lunches and coffee breaks, excursion and workshop dinner.
Local organizer	Payment details
Indo-American Hybrid Seeds (India) Pvt., Ltd., Bangalore, India Contact person: Dr. G.V. Jagadish jagadish@indamseeds.com ISTA Accredited Seed Testing Laboratory	Registration fee must be paid correctly and as soon as possible to confirm your attendance. Bank details: Payments should be made in USD. To: Chase Manhattan Bank, New York (Payment should be routed either via CHIPS ABA 0002 OR FED ABA 021000021) SWIFT: CHASUS33 For Credit to: 001-1-407376 UTI Bank Ltd, Bangalore SWIFT: UTIBINBB009 Ultimate beneficiary: A/c. No.102010200000426 (with UTI Bank Ltd, BSK II stage Branch, Bangalore) Indo-American Hybrid Seeds (INDIA) Pvt. Ltd., 7th Kilometer Banashankari-Kengeri Link Road, Channasandra Village, Subramanyapura Post, Uttarahalli Hobli, Bangalore-560061; Tel: +91-80-28604499 Fax: +91-80-28602912 Please stress that your reference number (or name) must be mentioned to facilitate quick identification.
Lecturers	Participants
Martina Rösch (Head of ISTA Accreditation Department) Anny van Pijlen (ISTA Technical Auditor)	Maximum 20 participants
Aim of the Workshop	Accommodation
This workshop aims at presenting and discussing basic principles of quality management and it focuses on the needs of seed testing laboratories that wish to comply with the ISTA Accreditation Standard and prepare for attaining ISTA Accreditation. Successful participants should be able to: – know about the ISTA Accreditation Scheme; – understand the requirements of the ISTA Accreditation Standard; – evaluate the situation of their laboratory with regard to conformity with the ISTA Accreditation standard; – document the quality management system in a manual and related documents for their laboratory.	There are pre-reserved rooms in Hotel 'The Chancery' where the workshop will take place. Hotel 'The Chancery', 10/6 Lavelle Road, Bangalore 5600 001, India www.chanceryhotel.net Single room rate is approx. EUR 125 per night. Price includes complimentary breakfast buffet (Indian/Continental). Participants who wish to stay in another hotel may be accommodated in Hotel Hoysala. The rate per night will be EUR 25–30. Please contact the local organiser for booking.
Workshop content	Target group
– Basic principles of quality management – How to create quality documentation – Interpretation of the ISTA Accreditation Standard – Quality management in seed testing Participants of this workshop will be actively involved through group work, discussions, presentations and visiting of the sampling site at the organising laboratory. The theoretical background will be given through lectures. The workshop language is English.	Quality managers, laboratory managers, participants with or without experience in quality management may register.
Preliminary programme	Registration form
Day 1: Inauguration, introduction, the ISTA Accreditation Scheme, basic principles of quality management, quality documentation, group work Day 2: Laboratory visit, interpretation of the ISTA Accreditation Standard, group work, workshop dinner Day 3: Excursion including a visit in a seed quality centre & production facility Day 4: Quality management in seed testing, technical aspects, group work Day 5: Quality management in seed testing, technical aspects, workshop evaluation, adjournment	The registration form can be downloaded from the workshop detail page at: https://www.seedtest.org/en/workshop.html

ISTA Moisture, Forest Tree and Shrub Seed and Tetrazolium Workshop

Peri, Verona, Italy, 12–14 June 2008

Objectives	Registration deadline
The workshop will deal with practical problems related to seed testing of forest and shrub species. The goal of the meeting is to intensify an exchange of information in this area. The topics to be discussed are Sampling, Moisture and Tetrazolium Testing.	30 April 2008
Location	Accommodation
Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale	Accommodation will be available at the Agriturismo al Castello (single: EUR 30, double: EUR 50 per night) which is at a distance of 4 km and at Albergo Olivo (single: EUR 40, double: EUR 60 per night) which is just a short walking distance from the Workshop venue. All rooms include breakfast. Please indicate the dates for which you require accommodation on the registration form and/or to the local organiser.
Preliminary programme	Travel information
<p>Thursday, 12 June: Tetrazolium testing: theory and sample preparation</p> <p>Friday, 13 June: Sampling: theory and practice – Tetrazolium testing: sample evaluation Official dinner</p> <p>Saturday, 14 June: Moisture testing: theory and practice</p> <p>If required, transfer to Verona main railway station for trains to Bologna, the venue of the ISTA Annual Meeting (16-19 June 2008).</p>	Regular scheduled flights from every continent, most countries, and major cities of the world land at “Valerio Catullo” international airport, Verona (www.aeroporto.verona.it). Also, Verona can be reached by train and bus from most European countries. Details for transportation to the hotels will follow later.
Participants	Contact
Maximum 20 participants	For workshop registration and hotel reservation please fill in the registration form and return it to the local organiser:
Participation fee	Fabio Gorian Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale Via del Ponte 256 37020 Peri (VR) ITALY
<p>ISTA Members: EUR 150 Non-members: EUR 225</p> <p>This includes workshop documentation and other material, coffee breaks and lunches, official dinner and transfer to the railway station on Saturday afternoon.</p> <p>Accompanying persons: EUR 60 This includes official dinner and transfer to the railway station.</p> <p>Payment must be made to: RAMBLER VIAGGI srl BANCO POPOLARE DI VERONA E NOVARA AGENZIA DI PARONA- c/c n° 3191 CHECK DIGIT IT 63 B05188 11714 000000003191 -(CIN B ABI 5188 CAB 11714)</p>	<p>E-mail: f.gorian@corpoforestale.it Phone: +39 045 6284071 Fax: +39 045 6284089</p>
	Registration form
	The registration form can be downloaded from the workshop detail page at: https://www.seedtest.org/en/workshop.html

ISTA Workshop on Purity, Germination and Tetrazolium Testing on Tropical and Subtropical Seeds

Córdoba, Argentina, 28–31 July 2008

The ISTA Purity Committee and the National Seed Institute (INASE-Argentina) invite you to their Workshop on Tropical and Subtropical Seeds, to be held in Córdoba (Argentina) from 28-31 July 2008.

Workshop content

- Physiology of tropical species
- Morphology of tropical grasses
- Purity test
- Germination test
- Tetrazolium test

Participants

Maximum: 20

Registration fee

ISTA members: USD 250

Non-members: USD 375

The registration fee includes participation, supporting material and literature, lunches and refreshments, daily travel hotel/laboratory, and official dinner.

Registration deadline

28 April 2008

Further information

Further notice about hotels and other activities, and a more comprehensive programme will be announced soon.

Contact

For more information please contact:
Monica Inés Moreno mimoreno@inase.gov.ar

Registration form

The registration form can be downloaded from the workshop detail page at:

<https://www.seedtest.org/en/workshop.html>

8th ISTA Seminar on Statistics in Seed Testing

Aussonne, France, 6–10 April 2009

(rescheduled from Roelofarendsveen, NL, September 2008)

Date

To be arranged

Location

Pioneer Génétique
Chemin de l'Enseigne
31840 Aussonne
France

Nearest airport: Toulouse Blagnac, France

Participants

Maximum 20–25

Contact

Please contact Charlotte Philip for further information.
email: charlotte.philip@pioneer.com

Preliminary programme

The planning of the programme for this seminar is currently under way. We envisage alternating theoretical presentations with practical presentations around statistical aspects of seed testing. Concerning the theoretical aspects, we envisage lectures on the Linear Model, the Generalized Linear Model and Bayesian statistics. For the more practically oriented presentations, there will be readings on the analysis of proficiency tests, statistical aspects of GMO detection, repeatability/reproducibility computations, detection of outliers and tools for visualizing data. Also, participants are welcome to suggest any topics for consideration or for a presentation by themselves.

Registration form

The registration form can be downloaded from the workshop detail page at:

<https://www.seedtest.org/en/workshop.html>

ISTA Workshop on Seed Moisture, Germination and Vigour Nakuru, Kenya, 3–7 November 2008 (postponed from June 2008)

The ISTA Moisture, Germination and Vigour Committees and the National Seed Quality Control Service Seed Testing Laboratory, Nakuru, Kenya, invite you to their workshop on moisture, germination and vigour testing in Nakuru (Kenya). The workshop will cover lectures and practical work on seed moisture, germination and vigour testing.

Location
National Seed Quality Control Service Seed Testing Laboratory, Nakuru, Kenya

Local organizer
Joseph Ahenda
National Seed Quality Control Service Seed Testing Laboratory, P.O. Box 1679, Nakuru 20100, Kenya
Phone: +254 051 850106 Fax: +254 051851268
E-mail: kephissq@africaonline.co.ke

Workshop content

- Moisture: principles and methods of seed moisture determination, including the oven moisture test and grinding and tolerances (lecture/discussion and practical work)
- ISTA Rules Chapter 9 Moisture (lecture/discussion)
- Quality assurance in moisture determination (lecture/discussion and practical work)
- New species (incl. tropical and subtropical species) for the Rules (lecture/discussion and practical work)
- Work of the Moisture Committee (discussion)
- Germination: ISTA Rules Chapter 5 Germination (lecture/discussion)
- Quality assurance in germination testing (lecture/discussion and practical work)
- Practicals and theory on selected species (*Phaseolus* spp., *Allium cepa*, *Desmondium* spp., *Arachis hypogaea*, *Daucus carota*, *Helianthus annuus*) for germination testing
- Principles and methods of germination testing (lecture/discussion)
- Work of the Germination Committee (discussion)
- Seedling evaluation handbook (lecture/discussion)
- Vigour: The concept of vigour (lecture)
- Vigour tests (lecture)

Lecturers
Craig McGill, Massey University (ISTA Moisture Committee Member)
Anny van Pijlen, General Dutch Inspection Service (NAK) (ISTA Germination Committee, Tetrazolium Committee and Training Committee Member)
Gillian McLaren, Official Seed Testing Station, Scottish Agricultural Science Agency (ISTA Germination Committee and Vigour Committee Member)

Participants
Maximum 30
There will be a minimum number of participants required for this workshop to take place.

Registration fees
ISTA Members: USD 430
Non-members: USD 645
The registration fee includes all literature and supporting material for the workshop, lunches and coffee breaks, workshop dinner and transfer between the workshop venue and hotels, but not between Nairobi and Nakuru (see below). Details for payment will be provided on receipt of your registration.

Accommodation (bed and breakfast)
Details for payment will be provided upon reception of your registration.

Hotel	Merica	Midland	Kunste	Waterbuck
Single room	75	75	30	45
Double room	120	95	50	60
Triple room	165	–	50	90
Junior suite	140	–	50	–
Executive suite	150	–	60	–

Accommodation prices are in USD. The approximate exchange rate is USD 1 = KES 65.

Additional information
Minibuses from Jomo Kenyatta International Airport and return at the conclusion of the workshop may be arranged. Participants who wish to travel by minibus are asked to advise their arrival and departure times at the airport. Cost of the minibus will be based on the number of participants using the service and will be advised closer to the Workshop. Should you not wish to utilize the minibus, a taxi from the airport, Nairobi, to the Seed Testing Station, Nakuru, 155 km away, is approximately 230 USD. The Seed Testing Station is about 10 km from Nakuru town. Transport during the workshop and from the hotels to the seed testing station will be organized. A one-day post-workshop tour of Lake Nakuru National Park can be arranged at an extra cost of USD 55. Applicants interested in the post-workshop tour should indicate it in the application form.

Registration form
The registration form can be downloaded from the workshop detail page at:
<https://www.seedtest.org/en/workshop.html>

ISTA Workshop on Seed Sampling of Agricultural Species NAK Emmeloord, Netherlands, 11–14 September 2007

Max Soepboer, NAK, Netherlands

Vice-Chair, ISTA Bulking and Sampling Committee

From 11 to 14 September, the NAK¹ organized an ISTA Workshop on Seed Sampling of Agricultural Species in Emmeloord, the Netherlands. Participants from various European countries attended the workshop, which consisted of lectures and practicals. The subjects dealt with were:

- general principles of seed sampling;
- statistics in seed sampling;
- the ISTA Handbook on Seed Sampling;
- sampling containers (bags, boxes, big bags etc.);
- dividing the samples into submitted samples;
- automatic sampling;
- training, licensing and monitoring of samplers.

The lecturers were Michael Kruse (Professor of Seed Science and Seed

Technology, University of Hohenheim, Germany), Gerry Hall [Head of Cereal Certification for Scotland (SASA) and Member of the ISTA Bulking and Sampling Committee (BSC)] and Max Soepboer (Senior Specialist Seed Policy of the NAK and Vice-Chair of the BSC). The Chair of the BSC, Mrs. Leena Pietilä (Senior Inspector of the Finnish food safety authority Evira) was also present.

The practicals were given at Agrifirm (a co-operative cereal seed company) at Emmeloord, and at Barenbrug Holland (a grass seed company) at Oosterhout. During the practicals, the participants carried out sampling of seed in various types of containers. Practical aspects of automatic sampling were also dealt with. During the lecturers and practicals there were many lively discussions and valuable contributions from the participants.

The social programme consisted of a visit to the former island of Urk, which is now part of the Noordoost-Polder, and to Giethoorn, which with its canals and high bridges is called the Dutch Venice. Here a boat trip was made during which the old Giethoorn houses and farms with beautifully thatched roofs could be admired. The boat trip also gave an impression of the system of transportation by water, which is still operated in Giethoorn.

On the last day of the workshop, Mrs. Leena Pietilä gave a presentation of the present work and experiments of the BSC, as well as its plans for the future. After that she evaluated the workshop together with the participants, and last but not least presented the certificates. ■

¹ General Dutch Inspection Service for Agricultural Seeds and Seed Potatoes

Tree Seeds 2008 – Trees, Seeds and a Changing Climate



On behalf of the organising committee, we are delighted to invite you to the biennial meeting of the International Union of Forestry Research Organisations' Seed Physiology Working group entitled, 'Trees, Seeds and a Changing Climate'. This meeting will be held on 22–25 September 2008 at the Royal Botanic Gardens Kew Millennium Seed Bank Project, Sussex and the conference centre of the University of Sussex.

Forests are disappearing at an alarming rate, and with them are lost a wide range of goods and services, such as carbon capture, fuel wood, medicines, biodiversity, timber, water catchment, food security and cultural values. In addition, trees support ecological niches for other species. Many tree species also have high use value as food (mainly oilseeds and nuts). However, in general the seeds of trees are under-researched. Moreover, seed quality at harvest is linked to environmental conditions, making seeds valuable 'sensors' of climate change.

The impact of climate change on tree seed quality, tree distribution, pathogens and conservation was a major concern at the last meeting held in Canada (2006). Consequently, the purpose of this meeting is to showcase recent advances

in tree seed biology, in particular those aspects of relevance to understanding, predicting and coping with climate change impacts.

We look forward to seeing you in Sussex in September 2008.

Conference Organisers:

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ISTA Moisture, Purity and Germination Workshop

GEVES-SNES, Angers, France, 8–12 October 2007

Sylvie Ducournau¹, Maria Rosaria Mannino³ and Joël Léchappé²

¹Vice-Chair and ²Member, ISTA Germination Committee, and ³Chair, ISTA Purity Committee

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The ISTA Moisture, Purity and Germination Workshop was held at the French National Seed Testing Station (SNES) in Angers, France, and was attended by 33 seed analysts from 20 different countries: Belgium, Cyprus, Denmark, France, Germany, Hungary, India, Ireland, Israel, Italy, Latvia, Luxembourg, Netherlands, Norway, Poland, South Africa, Scotland, Spain, Switzerland and Tanzania.

The first day was dedicated to Moisture, the two following days to Purity and the two final days to Germination.

Six lecturers were involved in the preparation of the sessions: Jette Nydam (Vice-Chair of the Moisture Committee), Elizabeth Jane Taylor (Vice-Chair of the Purity Committee), Ronald Don (Chair of the Germination Committee), Maria Rosaria Mannino (Chair of the Purity Committee), Joël Léchappé (Member of the Executive Committee) and Sylvie Ducournau (Vice-Chair of the Germination Committee).

The practical organization of the Workshop, including preparation of documents, equipment for the practical, samples and tests was done by the Seed Testing Station analysts. Overall coordination was managed by Bénédicte Brangeon.

Moisture

The Moisture programme included lectures by Jette Nydam, and demonstration sessions that saw the staff of the moisture laboratory of the SNES very actively involved. One of the theoretical presentations concerned a general presentation of seed moisture and the various methods of moisture determination. In a special session, particular attention was given to the principles of the oven moisture test: items such as equipment, the process step by step and calculation of results were illustrated and discussed. Another session was dedicated to moisture determination by moisture meters; this session included a practical demonstration on the use of a moisture meter by FOSS France, and also some examples of moisture meter calibration. An important item presented by Jette Nydam concerned quality assurance.

The demonstrations covered three subjects, also presented during the theoretical sessions, but with practical examples of the procedure to follow: oven moisture determination and grinding with examples of the process, and quality assurance in moisture with exercises in internal quality control. The participants were split into three groups in order to promote the contribution of everyone and a fruitful discussion.

Purity

The two-day Purity workshop was run by Maria Rosaria Mannino and Jane Taylor. Jette Nydam also participated very actively in the meeting, presenting one session on blowing and contributing to the discussion. The purity programme was prepared with the aim of covering the whole process of a purity analysis by theoretical presentations, followed by practical exercises on pure seed definitions, identification of inert matter and other seed, calculation of results and reporting. With regard to pure seed definitions, PSD 33 was covered by an exercise on *Triticum spelta*, PSD 36 by an exercise and a discussion of the referee test results obtained on *Panicum maximum*,



Grinding in preparation for moisture testing



Purity analysis



Seedling evaluation

PSDs 1 and 2 with a discussion on the most appropriate PSD for *Spinacia*, and PSD 25 for *Valerianella*. We also spoke about the purity analysis of coated seeds and the difficulties of interpretation of the relevant ISTA Rule chapter.

Two sessions covered the assessment of pure, broken and empty seeds and the distinction between pure seeds and inert matter for small or immature seeds (e.g. *Avena* spp., *Papaver* spp., *Malva* spp. and *Juncus* spp.).

A presentation on the development of the Universal List of Species opened the part of the workshop dedicated to identification of other seed. We prepared and distributed samples and documents with seed descriptions and pictures of genera and species included in the Universal List, in particular *Echinochloa* spp., *Setaria* spp., *Panicum* spp. and *Avena* spp. As with the moisture workshop, one session during the purity workshop was dedicated to quality assurance, a very important subject for laboratory management.

This purity workshop allowed the participants to enlarge their knowledge on purity analysis, and also to have an interesting exchange of experiences between laboratories in different regions of the world. The discussion we had also helped to put forward the work that the Purity Committee was carrying out for the preparation of the Rules Proposal 2008.

Germination

The programme of the Germination part of the Workshop was a mix of lectures and practical sessions. Lectures were given on the use of the new Handbook on Seedling Evaluation, on some principals for seedling evaluation (evaluation of the coleoptile of maize, evaluation of the root system of *Lolium*, evaluation of cotyledons) and on the Growing Media Specification Checks. Practical sessions were related to the lectures, and participants had working sessions on maize coleoptile evaluation, *Lolium* root system evaluation, and sunflower, cucumber and lettuce cotyledon evaluation, and during the last day demonstrations were made on how to apply the standard operating procedures for

checking pH, conductivity, water retention capacity and the innocuity of various media used in germination tests. A lot of work was done by the analysts of the laboratory in co-ordination with Ronald Don, Joël Léchappé and Sylvie Ducournau.

These five days of the Workshop were very rich in exchange and very formative, since explanations and demonstrations of a lot of new documents and rules were given during the sessions. It was a very busy and intense workshop, and very little free time was available for social events. Nevertheless, we took the time for a guided and wet walk in the old city centre of Angers, before a friendly dinner near the Museum of Art. ■

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

publications and products catalogue 2008

rules
science
handbooks
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ISTA publications and products catalogue 2008
Available from the ISTA Secretariat or online at www.seedtest.org

2008

- 7–11 April **ISTA Seed Health Workshop, Pretoria, South Africa** www.seedtest.org
- 13–18 April 5th International Crop Science Congress 2008, ICC Jeju, Korea
www.cropscience2008.com
- 14–18 April **6th ISTA Seed Health Symposium, Kruger National Park, South Africa**
www.up.ac.za/conferences/ielc/
- 15–18 April **ISTA Seed Vigour Workshop, Bologna, Italy** www.seedtest.org
- 21–25 April **ISTA Workshop on Species and Variety Testing, Freising-Munich, Germany**
www.seedtest.org
- 12–16 May **ISTA Quality Management Training Course, Bangalore, India** www.seedtest.org
- 26–28 May ISF Congress, Prague, Czech Republic www.worldseed.org
- 12–14 June **ISTA Moisture, Forest Tree & Shrub Seed and Tetrazolium Workshop, Peri, Verona, Italy** www.seedtest.org
- 16 June **ISTA Seminar on Specified Trait Seed Testing, Bologna, Italy** www.seedtest.org
- 16–19 June **ISTA Annual Meeting 2008, Bologna, Italy** www.seedtest.org
- 24–27 June 1st Global Conference on GMO Analysis, Como, Italy <http://gmoglobalconference.jrc.it/>
- 28–31 July **ISTA Purity, Germination, and Tetrazolium Test on Tropical and Subtropical Seeds, Córdoba, Argentina** www.seedtest.org
- 22–25 September Tree Seeds 2008 – Trees, Seeds and a Changing Climate, Wakehurst Place, West Sussex, UK <http://kewgardens.org/msbp/tree-seeds-2008/index.htm>
- 3–7 November **ISTA Moisture, Germination and Vigour Workshop, Nakuru, Kenya**
www.seedtest.org

2009

- 6–10 April **8th ISTA Seminar on Statistics in Seed Testing, Ausonne, France** www.seedtest.org
- 15–18 June **ISTA Annual Meeting, Zurich, Switzerland**

2010

- 16–22 June **29th ISTA Congress, Cologne, Germany**





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