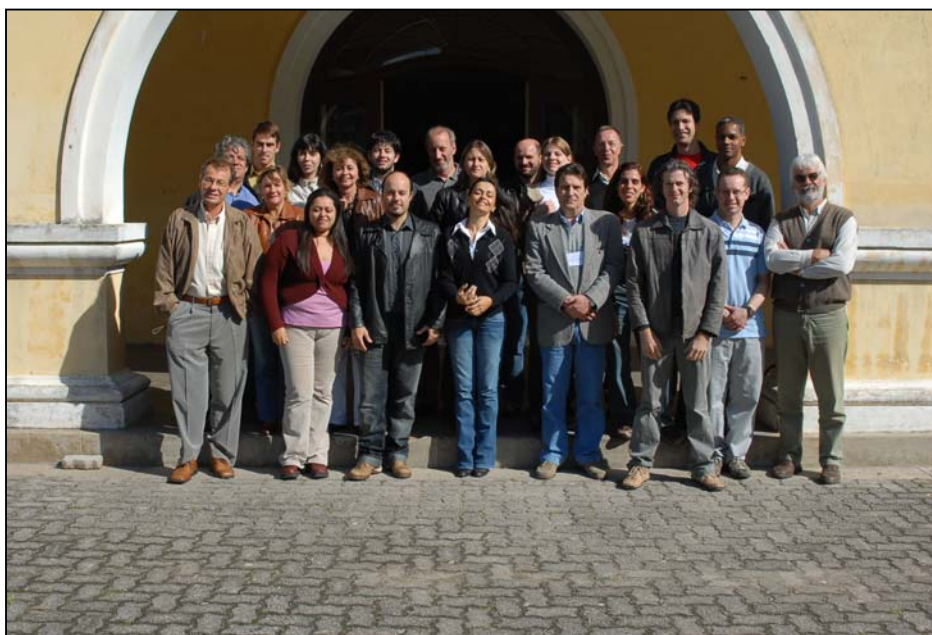




ISTA VARIETY TESTING WORKSHOP

25 – 27 April 2007, Pelotas, Brazil

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Participants of the ISTA Variety Testing Workshop, Pelotas Brazil, 25-27 April 2007

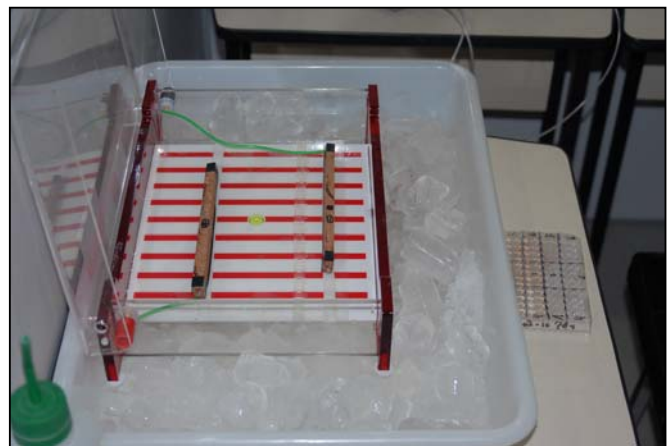
The 2007 ISTA Variety Testing Workshop was held from 25 – 27 April at the Universidade Federal de Pelotas (UFPEL) in Pelotas, in the South of Brazil. Attendants included graduate students from UFPEL in the Seed Science and Technology Department, technicians from the Brazilian Ministry of Agriculture, representatives of FUNDACEP and a variety of technicians from BioVision Seed Labs in Edmonton, Alberta, Canada. The practical aspects of the workshop were performed in the laboratory of Professor Paulo Dejalma Zimmer. Professor Dr. Norbert Leist, ISTA GMO Chairman, presented the theory portions of the workshop. Senior Analyst Rainer Knoblauch, ISTA Variety Chairman, instructed the practical portions, which included chemical and physical methods (*Lupinus*, *Brassica*) as well as electrophoretic work with storage proteins of *Zea mays*, *Triticum aestivum*, *Oryza sativa*, *Helianthus annuus* and *Lycopersicon esculentum*.

The first day began with welcome speeches by Professor Dr. Silmar Peske, 2.Vice President of ISTA and Dejalma Zimmer, and an outline of the topics to be covered during the workshop. Since the majority of the attendees were learning about ISTA for the first time, Norbert Leist started the workshop with a presentation on the history and formation of the International Seed Testing Association. This was followed by presentation and discussion of the various methods for determining the variety of a sample, from morphological to biochemical.

The first practical session included a demonstration of the Fluorescence test for *Lolium spp.* and *Avena spp.*, the Lupine alkaloid test for *Lupinus spp.* and the Formic acid test for *Sinapsis arvensis* in *Brassica*. Especially germinated *Lolium perenne* and *multiflorum* seeds were placed under ultra-violet light to observe the fluorescence of the root traces of *multiflorum* and no fluorescence of *perenne*. Also seeds of a white and a yellow *Avena* variety were tested under ultra-violet light and we could see that the white variety produces bluewhite fluorescence, while the yellow variety showed no fluorescence. The next was a Lupine alkaloid test, using Lugol's solution, for determining sweet lupines (low alkaloid content) and bitter lupines (high alkaloid content). When Lugol's solution was applied to the water that the *Lupinus* seeds were soaked in, the sweet lupines showed a clear solution but the bitter lupines a reddish-brown outfall within the solution. The final non-biochemical test was with Formic acid to determine *Sinapsis arvensis* in *Brassica* samples. When a solution of 98% Formic acid was applied to the seeds, rapidly the *Sinapsis arvensis* seed solutions stained red.

There was one problem for the workshop; it was not possible to get the chemicals needed (supported by the Company Sinus) through Customs. The lecturers - aware of the problem- took some chemicals and some ready gels with them, to allow the workshop to become a success also in practical work. It was also not possible to receive the electrophoresis chambers (supported by the Company Sarsted) which were waiting at the customs in the next airport.

The lecturers could manage these deficiencies by a sophisticated arrangement of lectures and practical work, so that Rainer Knoblauch could build a very special chamber for electrophoresis by hand. He succeeded to set up a "Pelotas chamber" which was improved day by day and at the end delivered perfect results. Besides this, the participants learned how to prepare home made equipment!



The "Pelotas Chamber"

Still on the first day, following lunch, lectures on the theory associated with Ultrathin Layer Isoelectric Focusing (UTLIEF) of seed storage proteins were started. We also discussed how to evaluate the IEF gels after the separation of the proteins. This was followed by another practical session in which the preparation of samples of *Oryza sativa* and *Triticum aestivum* were presented. Also during this session, a sample of *Zea mays* was run by IEF. The workshop participants were asked to prepare and load the gel in order to understand how to use the procedure. After the IEF run was finished, the *Zea mays* gel was fixed, stained, destained and dried. We were then able to see the bands of the male, female and hybrid seeds that were loaded onto the gel.

Day two started with a short review of the previous day by the workshop participants and then went right into the practical aspects starting an IEF run for *Oryza sativa*. After that the theory of other electrophoresis methods was presented and how to develop a test for an unknown species using IEF. At this time, the IEF run was complete and we were able to go through the steps for fixing, staining, destaining and drying the IEF gel which was then ready for interpretation. We could observe the protein bands for the *Oryza sativa* well defined. After lunch, the practical session started with an IEF run for *Triticum aestivum*, followed by lecture how to

troubleshoot an IEF run, and how to report the results. The latter depends on what type of test is performed: Hybrid determination is the look for presence of hybrid seed after comparison with male and female standards. Varietal confirmation is a test to confirm the variety of the sample by comparing to the known standard. Varietal identification is a test to identify the unknown sample by comparison to a library of known varieties. Day Two ended with a practical session, in which the *Triticum* IEF gel was fixed, stained and destained, so it was possible to observe the bands for the *Triticum* samples.



The Electrophoretic Run was successful.

The final day of the workshop started again with a short review of the day before followed by practical IEF run of *Helianthus annuus*. While the gel was running, we had a theoretical session discussing the IEF method. Dr. Norbert Leist also gave a presentation on the ISTA GMO Task Force and their activities. Having finish the *Helianthus* gel we could evaluate the protein bands of the samples. After lunch, the workshop participants started an IEF run of *Lycopersicon esculentum* followed by a final discussion regarding variety identification. When the gel for the *Lycopersicon* sample was ready, we brought all of the gels back to the lecture room and did one final evaluation of all of the gels. Looking at the gels, we noticed that the best gel produced was the last one. Rainer Knoblauch attributed this to the ease of use of the IEF system and the quick learning ability of the workshop participants.

The workshop was closed with a small ceremony where Norbert Leist, Rainer Knoblauch, Paulo Dejalma Zimmer and Silmar Peske handed out certificates to all of the workshop participants. Silmar Peske also had some kind words to say to the group. He stated how proud he was of the students and their ability to understand and participate in the workshop, as it was presented in English. He also praised Desalma Zimmer for his excellent work in organizing the workshop. That night, the workshop participants and organizers ended the week with a typical nice brasilian dinner at one of the famous local barbeque restaurants.



Participants enjoying the Workshop Dinner.



Visiting the President of Pelotas University



Lectures

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