



Annual Meeting 2022

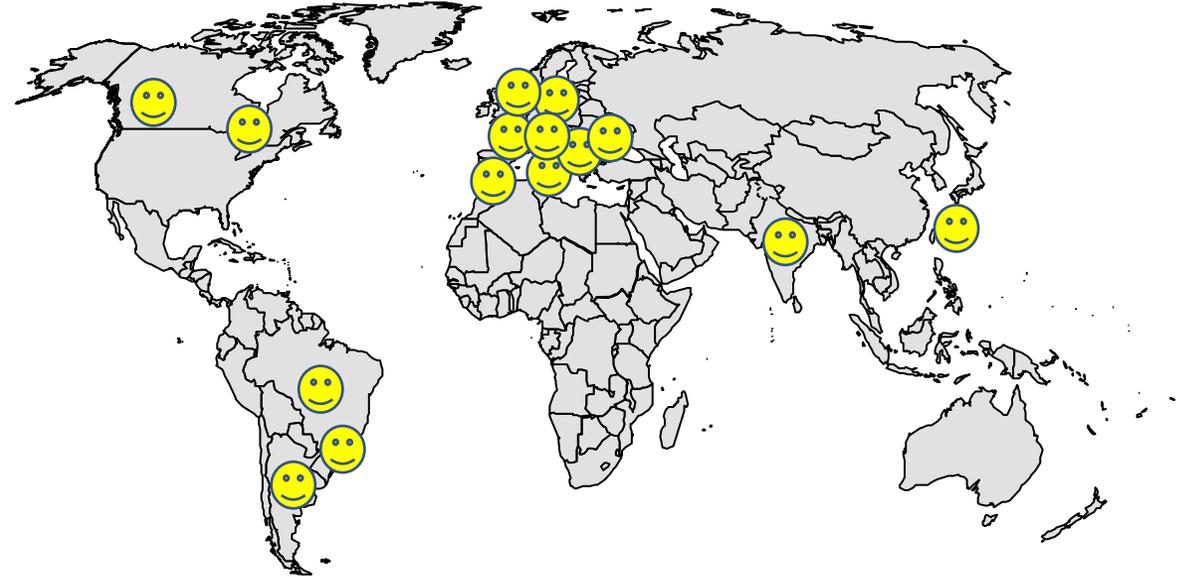
Variety Committee report

2021-2022

Presenter: Ana Vicario
Location: Buenos Aires, Argentina
Date: May, 2022

ISTA VARCOM members

	Member Since
1 Chair: Ana Laura Vicario AR	2007
2 Vice-Chair: Marie Claude Gagnon CA	2020
3 Anne Bernole FR	2016
4 Berta Killermann GR	2004
5 Chiara Delogu IT	2007
6 Elisa Vieira BR	2007
7 Hedwich Teunissen NL	2007
8 Kae-Kang Hwu TW	2007
9 Ksenija Markovic RS	2013
10 KsenijaTaski-Ajdukovic RS	2010
11 Keshavulu Kunusoth IN	2010
12 Verena Peterseil AT	2016
13 Mariana Menoni UY	2021
14 Ana Patricia Fernández Getino SP	2021
15 Sean Walkowiak CA	2022





Agenda



How ISTA included DNA-based markers in the Rules

Proficiency Test (wheat) PT "0" – Results

**Pea and Oat to the Rules
Progress with Barley**

**Update on the development on new markers for
detection of annual types in perennial rye grass varieties**

How ISTA included DNA-based markers in the Rules

In 2016 the Variety Committee wrote a position paper regarding the use of DNA-based markers in ISTA.

The **objective** of including DNA-based test in the Rules was to have laboratories accredited to perform DNA-based variety verification testing by means of molecular markers.

The strategy proposed to achieve this goal was a **semi-performance-based approach**.

The strategy proposed is considered to be semi-performance-based because **various aspects of the laboratory methodology** such as DNA extraction, quantification, PCR conditions and electrophoresis will be performance-based; laboratories may choose to use in-house validated methodology for these purposes so long as the end result is acceptable.

However, **the markers sets to be used are “prescribed”**.

How ISTA included DNA-based markers in the Rules

Validation of the marker set will follow the **ISTA Validation procedure**

The goal of comparative test is to **identify informative markers** that provide consistent results among laboratories and also within the lab.

Markers should be **selected based on the literature and the knowledge and experience** of the crop leader and the crop group regarding marker quality and levels of polymorphism. Selected molecular markers **must be publicly available** such that primer sequences may be disclosed to laboratories seeking accreditation.

The varieties used **should represent as much as possible the global variability of the cultivated species**. The number of varieties used should be sufficient to allow the detection of multiple alleles per marker, but not so many as to make the comparative test economically difficult to achieve.



How ISTA included DNA-based markers in the Rules



Laboratories will be required to demonstrate their ability to reproduce the marker profile (genotype) of each variety.

The number of alleles present in the variety set provided in a given PT will need to be sufficient to permit an **evaluation of the ability of a laboratory to differentiate and identify multiple alleles at each marker.**

Following accreditation, when a request for a variety verification test is made to the accredited laboratory, **use of the recommended ISTA marker set will be mandatory.** If these markers are not sufficient to provide a unique DNA profile for each variety in the pool of varieties that are likely to be encountered in the particular region or circumstance, the **laboratory will be free to add as many markers of the same type as needed.**

How ISTA included DNA-based markers in the Rules

ISTA validation Procedure

- Comparative tests (at least 2)
- Statistical analysis
- Preparation of validation documents
- Technical and statistical reviews
- Preparation of Rules proposal
- Member's vote and comments
- Submission of Rules proposal

Guarantee future PT

- Request reference varieties from breeders and find a place to keep them for a long period so they could be available for future PT
- Obtain the matrix of reference profiles

Sample preparation and shipment

Design a specific way for results evaluation

Specially for DNA-based tests consisting of a spreadsheet containing the criteria for rating the labs (number of varieties wrongly rated and number of alleles with wrong results)

Test the whole rating system in a PT (PT"0")

- Prepare the PT (select samples and references)
- Prepare and ship samples
- Evaluation of results (using the new spreadsheet)
- Reports for laboratories
- General report for ISTA



Proficiency Test (wheat) PT “0” – Results



PT “0” already finalized and evaluated

6 participating laboratories: 3 accredited and 3 willing to evaluate their performance.

Mandatory for accredited laboratories.

Set of 6 varieties, 8 SSR wheat markers, 2 varieties sent as control and also their SSR profiles.

Final report sent on October 2021.

One laboratory required to repeat the test.

Samples are kept and prepared and shipped for each PT by Sean Walkowiak at CGC.

Proficiency Test (wheat) PT “0” – Results

The ratings awarded in this round were as follows:

Laboratories awarded with BMP failed in calibrating the alleles obtained for the blind samples with the reference sample profiles provided.

A second sample was sent with perfect results.

Rate	Number of laboratories
A	3
B	1
C	-
BMP	2

Pea and Oat to the Rules – Progress with Barley

Part C. Rules changes and new methods requiring a vote

Chapter 8

C.8.10.4. New method on *Pisum*

C.8.10.5. New method on *Avena sativa*

Identification and verification of varieties or testing genetic purity by means of DNA-based techniques is of extended use in many laboratories nowadays. In 2017, the first DNA-based method was included in the Rules for testing wheat. The interest for including such a technical procedure increased over time. The Variety Committee propose the inclusion of a DNA-based test for testing *Pisum* and *Avena* varieties.

The following proposal has been developed by a working group of the ISTA Variety Committee and approved by majority of votes by committee members.

For barley

The statistical analysis is finalized. Leaders are writing the validation documents and Rules proposal for this year.



Update on the development of new markers for detection of annual types in perennial rye grass varieties



Project leader: Giovanni López

Collaboration with Shaun Bushman from USDA who is developing the markers and Daniel Curry from Oregon State University who is providing seeds samples and technical support.

Next-Generation sequencing of the genomes

Selection of a set of 10 markers.

Development of the primers and probes.

Oregon Seed Lab and Utah Lab are testing the markers on seed samples to define the LOD (different seed bulks of various contamination levels).

Results will come out soon and we will be able to assess the selected markers in more laboratories (ISTA validation procedure).

Plans for next triennium

New crops and marker type.

Inclusion of statistical consideration in Chapter 8.

Plan a WS with STACOM.

Work with ATC in optical markers validation.

Set up a working group to continue writing the DNA handbook.

Define a strategy for setting up and organizing PT on new crops.

**Thanks to members, CT leaders, Lolium Project leader, PT Leader and vice-chair, for the great work done so far to achieve VARCOM and ISTA goals.
To STACOM chair for data analysis and help in thinking.**

Thanks to Elisa Vieira from Brazil who is leaving the Committee.



Thank you!

Follow us on social media:

