

Technical Working Party on Testing Methods and Techniques**TWM/2/18****Second Session****Virtual meeting, April 8 to 11, 2024****Original:** English**Date:** April 8, 2024

ISTA REPORT ON THE USE OF TECHNIQUES FOR VARIETY IDENTIFICATION AND VERIFICATION*Document prepared by an expert from International Seed Testing Association (ISTA)**Disclaimer: this document does not represent UPOV policies or guidance*

The annex to this document contains a copy of a presentation “ISTA report on the use of techniques for variety identification and verification”, to be made by an expert from International Seed Testing Association (ISTA), at the second session of the Technical Working Party on Testing Methods and Techniques (TWM).

[Annex follows]

ISTA report on the use of techniques for variety identification and verification

Presenter: Ana Vicario – VARCOM Chair
Location: TWM - UPOV
Date: April, 2024

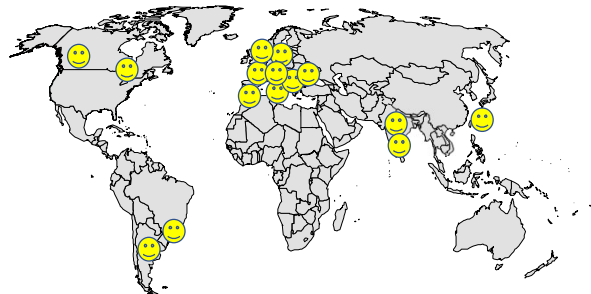
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ISTA VARCOM members



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Agenda



DNA-based markers for variety identification /
verification and laboratory accreditation

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

Use of neuronal networks for variety identification

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DNA-based markers for variety identification /
verification and laboratory accreditation



2016
position paper

The **objective:**
to have laboratories accredited to
perform DNA-based variety verification
testing by means of molecular markers.

semi-performance-based approach - SPBA

**various aspects of the laboratory are
performance- based**

the **markers sets to be used are "prescribed"**

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**DNA-based markers for variety identification /
verification and laboratory accreditation**



Validation of the marker set will follow the **ISTA Method Validation for Seed Testing** procedure

Identify informative, repeatable and reproducible markers

Markers should be **selected based on the literature and the knowledge and experience** of the crop group regarding marker quality and levels of polymorphism.

Selected molecular markers **must be publicly available** such that primer sequences could be disclosed to laboratories seeking accreditation.

Varieties used **should represent as much as possible the global variability of the cultivated species.**



**ISTA Method Validation
for Seed Testing**

Note: Any electronic or hard copies of this document are uncontrolled copies. Confirm validity before use.

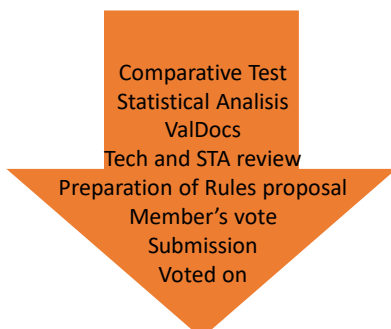
INTERNATIONAL SEED TESTING ASSOCIATION | 2019

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**DNA-based markers for variety identification /
verification and laboratory accreditation**



1- Validation Procedure



If positive, published on ISTA Rules

2- Guarantee future Proficiency Test (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.

Guarantee sample preparation and shipment.

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**DNA-based markers for variety identification /
verification and laboratory accreditation**



3- Design a way for results evaluation

Specially for DNA-based tests

4- Test the whole rating system in a PT "0"

1. Prepare the PT (select samples and references)
2. Prepare and ship samples
3. Evaluation of results (using the new spreadsheet)
4. Reports for laboratories
5. General report for ISTA

Two PT ran already
Laboratories were required to
demonstrate their ability to
reproduce the marker profile
(genotype) of each variety
Laboratory performed with A and B.

5-Writing a Handbook

Special WG

From the preparation of a CT to criteria
for accepting PT results

HANDBOOK ON DNA BASED TESTS – Table of Contents

- i. Preface
- ii. Health and Safety Information
- iii. Acknowledgments
- iv. Contributors

Content

1. Introduction
- 1.1 Summary
- 1.2 History of DNA-based varietal identification and ISTA
- 1.3 The purpose of DNA-based testing in the ISTA rules
- 1.4 Goal and scope of the ISTA "handbook on DNA testing"
2. Development: guidelines for Comparative Tests (CT) organization
 1. Considerations on the varieties
 2. Considerations on the sample size
 3. Considerations on the markers
 4. Other considerations
3. Validation: validation of DNA based markers
 1. Adoption as an official method
 2. Reference material collection (RMC)
4. ISTA accreditation: evaluation of performance method and proficiency tests
5. Statistical approaches for results analysis
6. ISTA accreditation for DNA based testing
7. Auditing laboratories for DNA-based Testing
8. Appendices

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

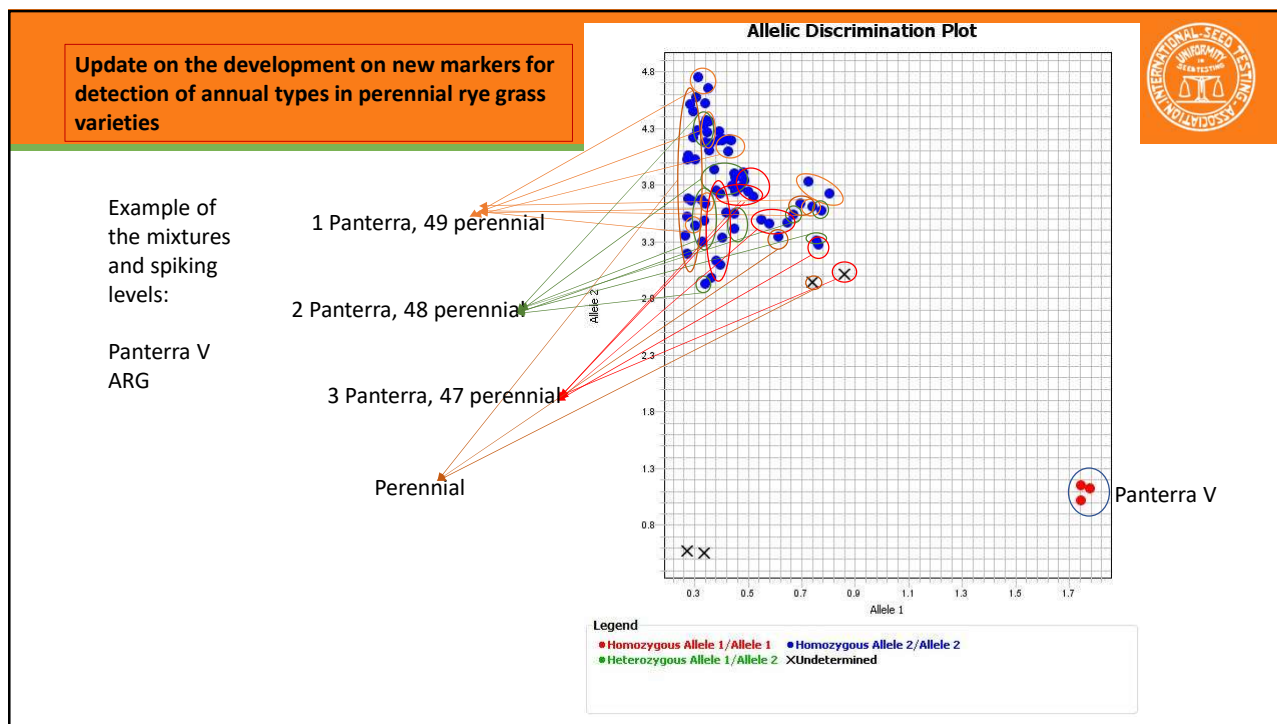


Project leader: Giovanni López

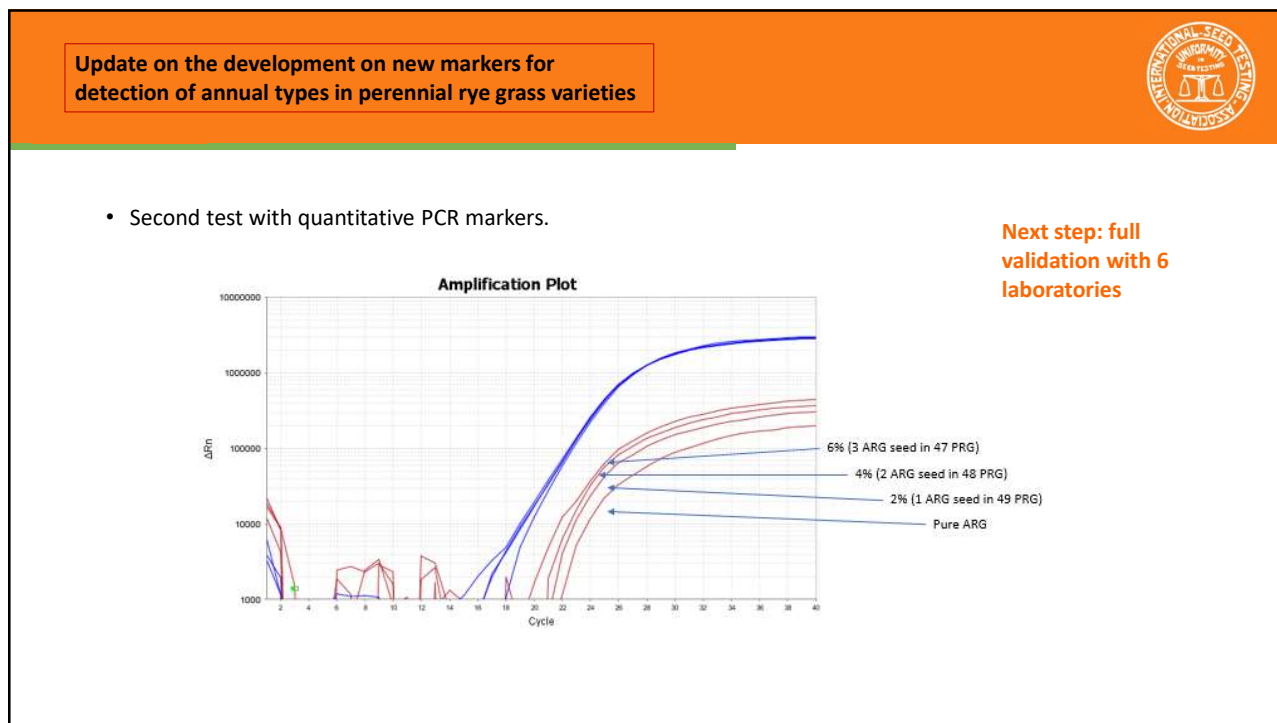
Collaboration with Shaun Bushman from USDA who developed the markers and Daniel Curry from Oregon State University who provided seeds samples for the test and technical support.

- Next-Generation sequencing of the genomes
- Selection of a set of 8 to 10 initial markers for testing
- Preliminary test for selecting a subset of primers using KASP: 6 variety combinations (Perennial Rye Grass varieties spiked with Annual Rye Grass varieties) on seed samples to define the sensitivity of the method (1/50, 2/50, 3/50).

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Use of neuronal networks for variety identification



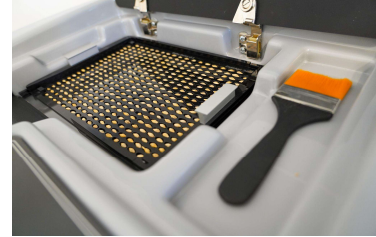
VARCOM is working on a special project together with ISTA Advanced Technologies Committee (ATC)

The software is able to discriminate varieties within a “universe” of varieties already learned by the software.

The equipment consist of a scanner (for taking the seed image) and a PC (for image analysis and results output)

The software learns, through convolutional neural networks, the morphological profile of thousands of seeds from the same variety that gather most variability as possible (i.a. harvesting years and climate and soil conditions)

The outcome of the software in an excel file indicating the % of identity of the sample with a stated variety or with the whole set of varieties previously learned by the software



Tests were ran at INASE Argentina and now a repetition of the test is ongoing in a different facility to analyze reproducibility.

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Thank you!

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