Variety Committee Report

ISTA Annual Meeting 2024

AGENDA

Members

Markers for variety identification / verification and laboratory accreditation

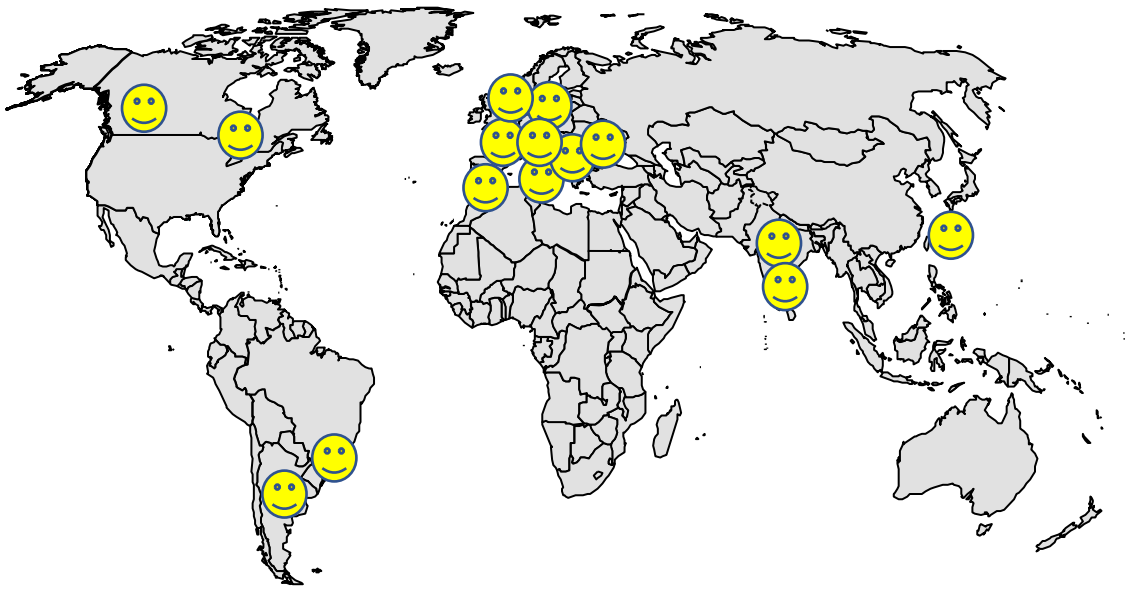
Update on the development on new markers for detection of annual types in perennial rye grass varieties

Use of neuronal networks for variety identification

Handbook

Acknowledgments

Members



	COMMITTEE MEMBERSHIP LIST	
1	Chair: Ana Laura Vicario	Member since 2007
2	Vice-Chair: Marie-Claude Gagnon	Member since 2020
3	Anne Bernole	Member since 2016
4	Berta Killermann	Member since 2004
5	Chiara Delogu	Member since 2007
6	Kae-Kang Hwu	Member since 2007
7	Ksenija Markovic	Member since 2013
8	Ksenija Taski-Ajdukovic	Member since 2010
9	Keshavulu Kunusoth	Member since 2010
10	Ana Patricia Fernandez Getino	Member since 2021
11	Mariana Menoni	Member since 2021
12	Sean Walkowiak	Member since 2022
13	Umashankar Bellan	Member since 2023
14	Stephanie Guillet	Member since 2023
15	Lorella Andreani	Member since 2023

Markers for variety identification / verification and laboratory accreditation

VARCOM SCOPE

The purpose of species and variety verification is to determine the extent that the submitted sample conforms to the species or variety as requested by the applicant, using methods not permissible in a purity test according to Chapter 3.

WHAT WE HAVE NOW

CONVENTIONAL METHODS: morphological traits, possible to see under daylight or UV light, by naked eye or using some magnifier, with or without previous treatment.

PROTEIN-BASED: refer to standard methods for obtaining storage proteins band patterns that allow variety verification.

DNA-BASED: refer to a semi-performance based methods for obtaining DNA patterns that provide a tool for assessing laboratory performance aimed to laboratory accreditation.

WHAT IS UNDER DEVELOPMENT AND NEW TO COME

DNA-based test for specific traits for variety identification:
Lolium test to discriminate ARG from PRG

Morphological based test using New Technologies Equivalent to the Human Analyst for Seed Analysis:
Use of neuronal networks for variety identification

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

Project leader: Giovanny López from ATC

Collaboration with Shaun Bushman from USDA who sequenced ARG and PRG varieties and developed about 10 markers.

Daniel Curry from Oregon State University who provided seeds samples for the test and technical support.

1st test done using KASP system (results presented last year).

2nd test with quantitative PCR markers aimed to specific SNP: 4 markers tested.

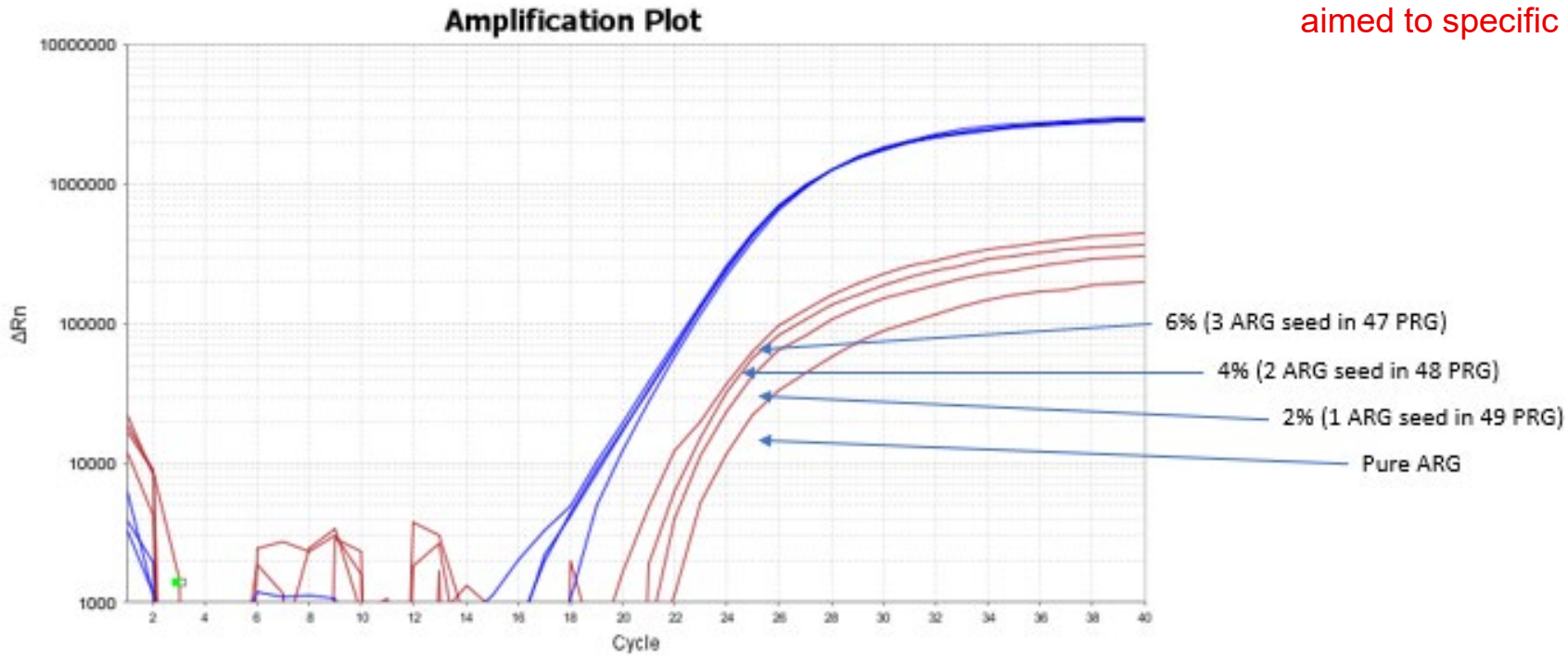
3rd test on a larger set of annual, perennial and hybrid seed samples.

4th test on spiked samples (undergoing).

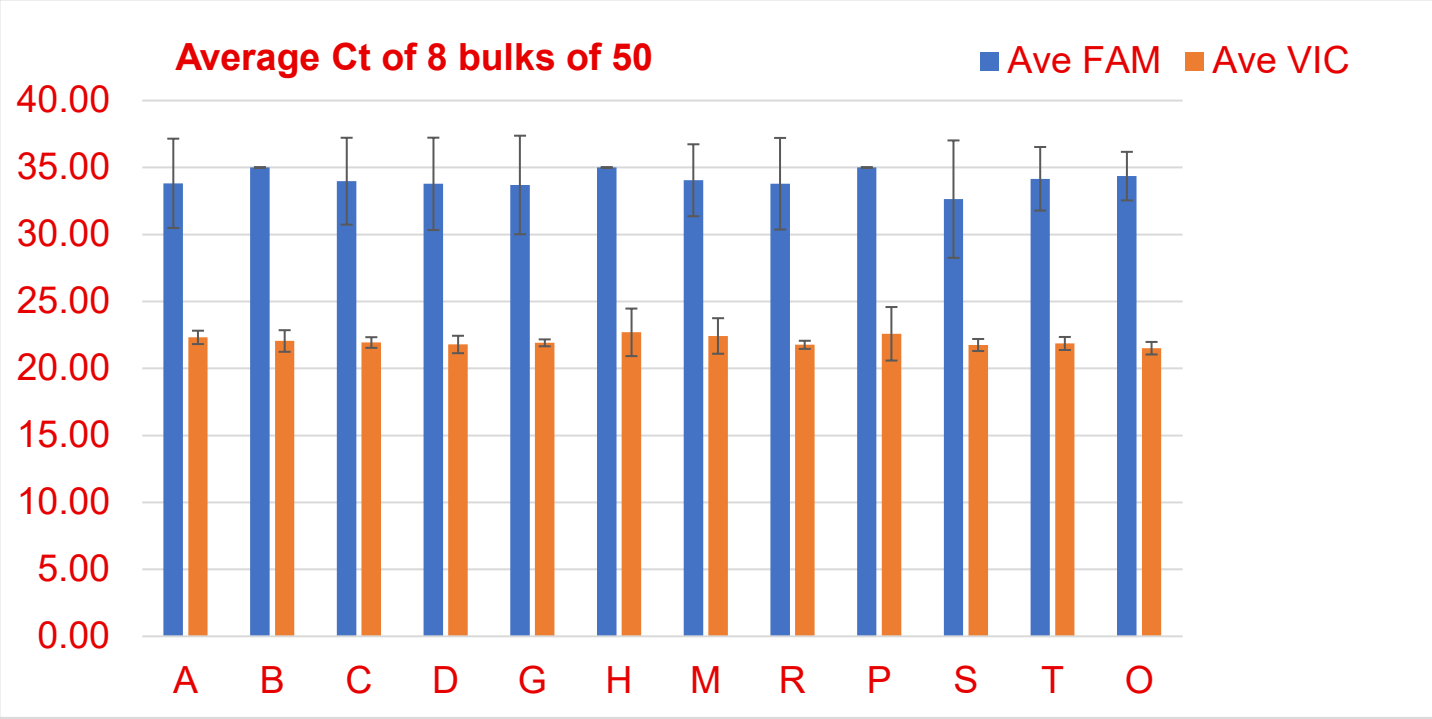
5th test interlaboratory trial with best markers (next year).

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

2nd test with quantitative PCR markers aimed to specific SNP: 4 markers tested



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



3rd test on a larger set of annual, perennial and hybrid seed samples

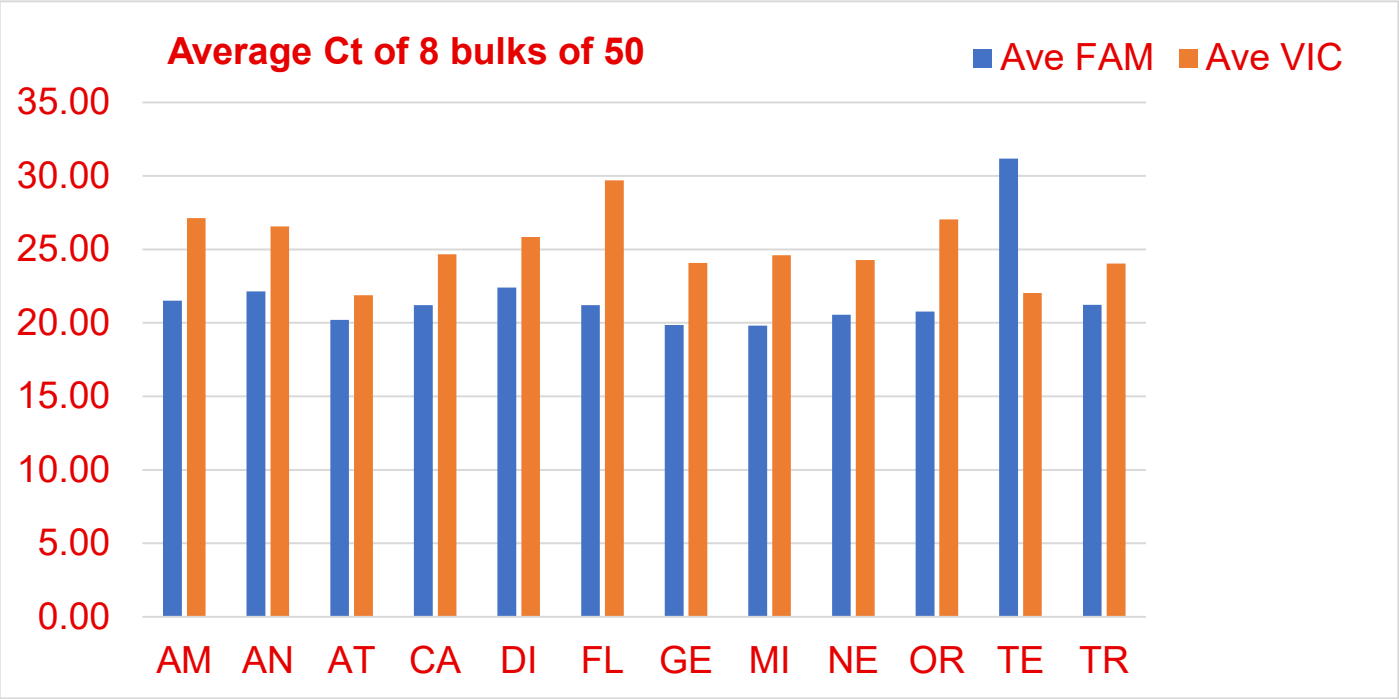
Test on 12 perennial varieties

FAM fluorophore is for the annual ryegrass

VIC fluorophore is for the perennial ryegrass

Example for 1 of the 3 markers tested

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



3rd test on a larger set of annual, perennial and hybrid seed samples

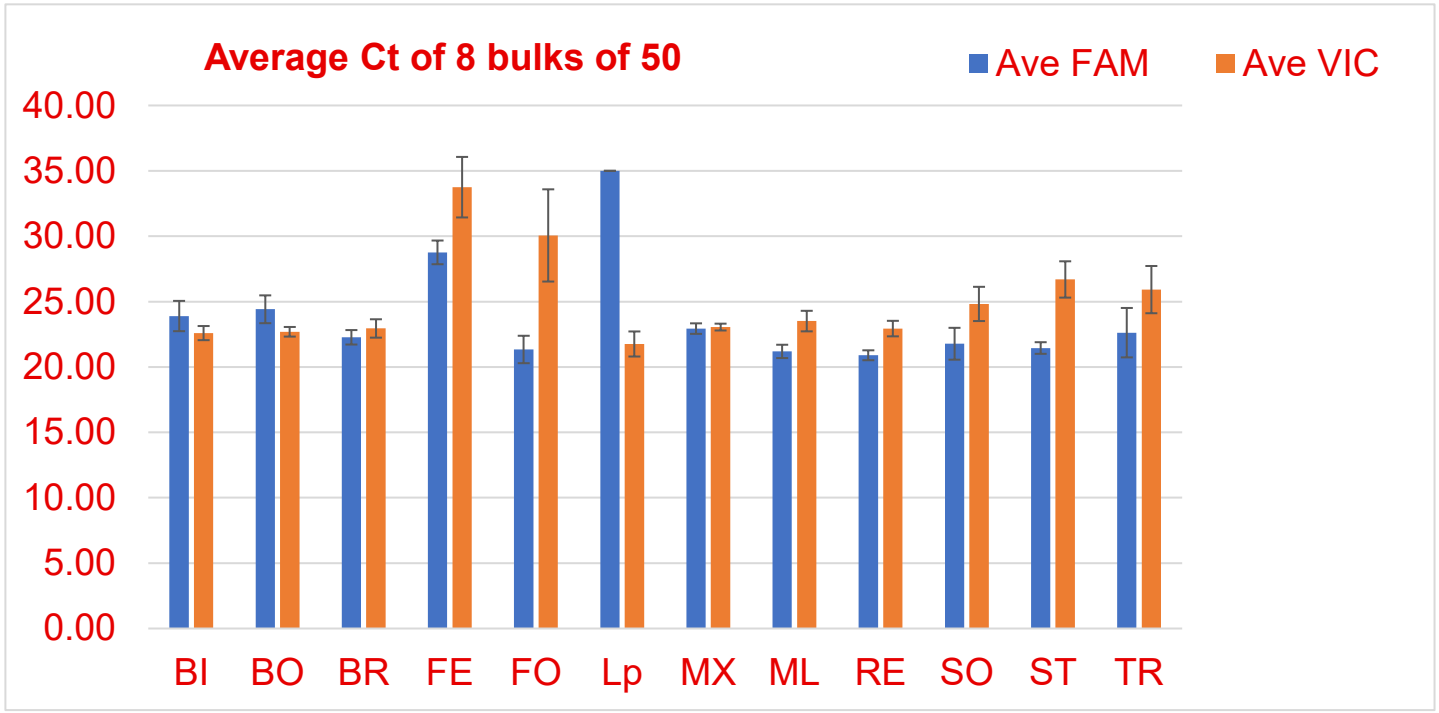
Test on 10 annual varieties + last two hybrids

FAM fluorophore is for the annual ryegrass

VIC fluorophore is for the perennial ryegrass

Example for 1 of the 3 markers tested

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



3rd test on a larger set of annual, perennial and hybrid seed samples

Test on 11 hybrids varieties + 1 perennial (Lp)

FAM fluorophore is for the annual ryegrass

VIC fluorophore is for the perennial ryegrass

Example for 1 of the 3 markers tested

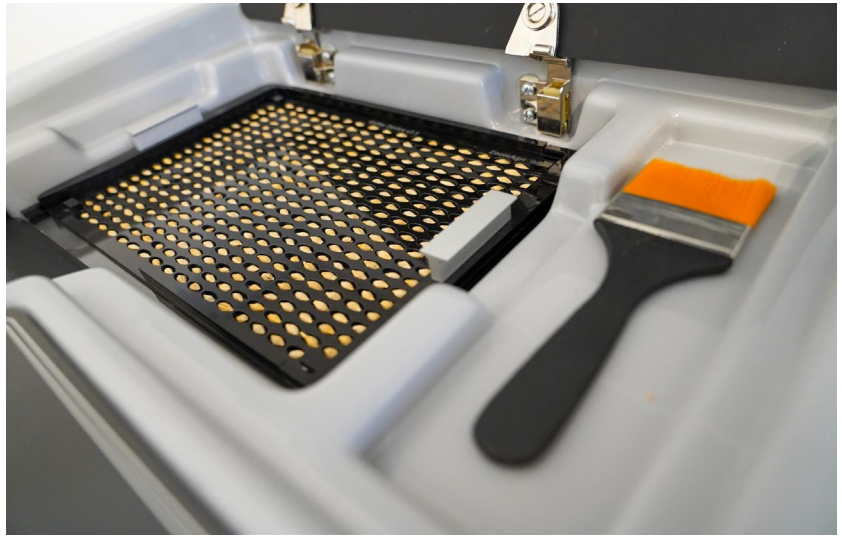
Use of neuronal networks for variety identification

Joint work with ATCOM – Mailen Martinez

Method used in 3 countries for wheat and 15 countries for barley

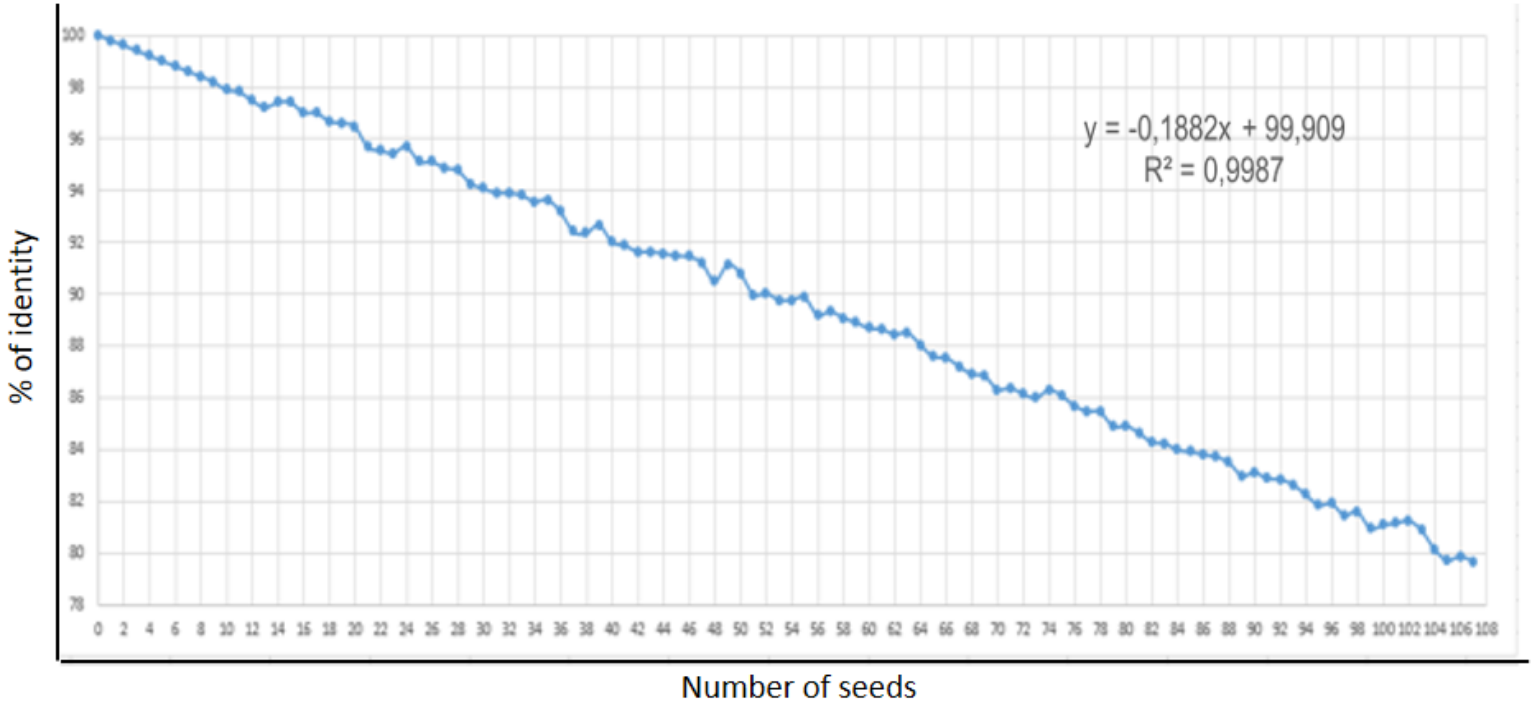
We ran a spiking test and a repeatability test.

Advice from STACOM: run again the spiking test in a different laboratory for checking on robustness, repeatability and reproducibility. This second test is undergoing.



Use of neuronal networks for variety identification

Spiking test: testing the impact of 1 single seed on the % of identity

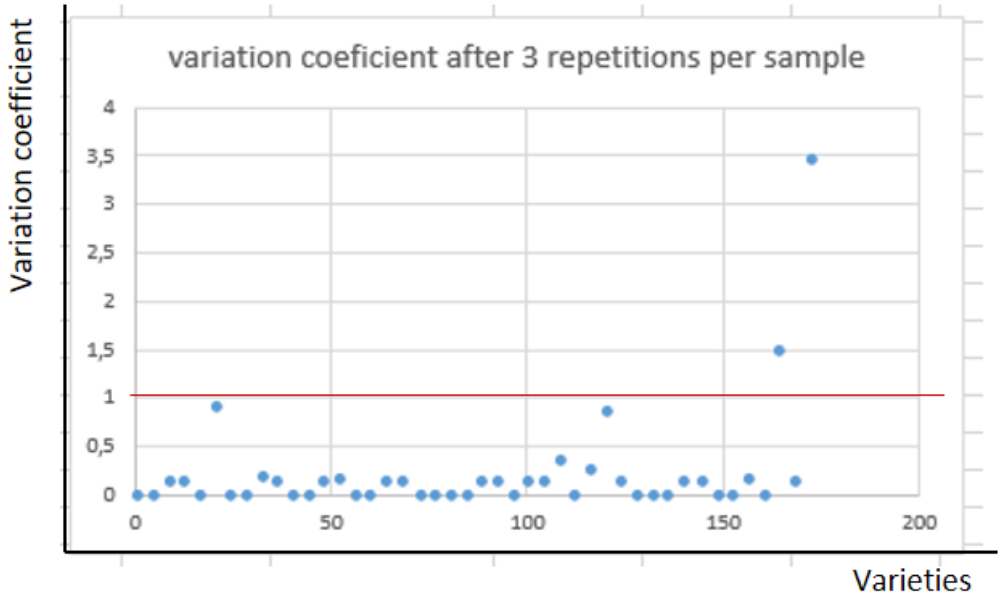


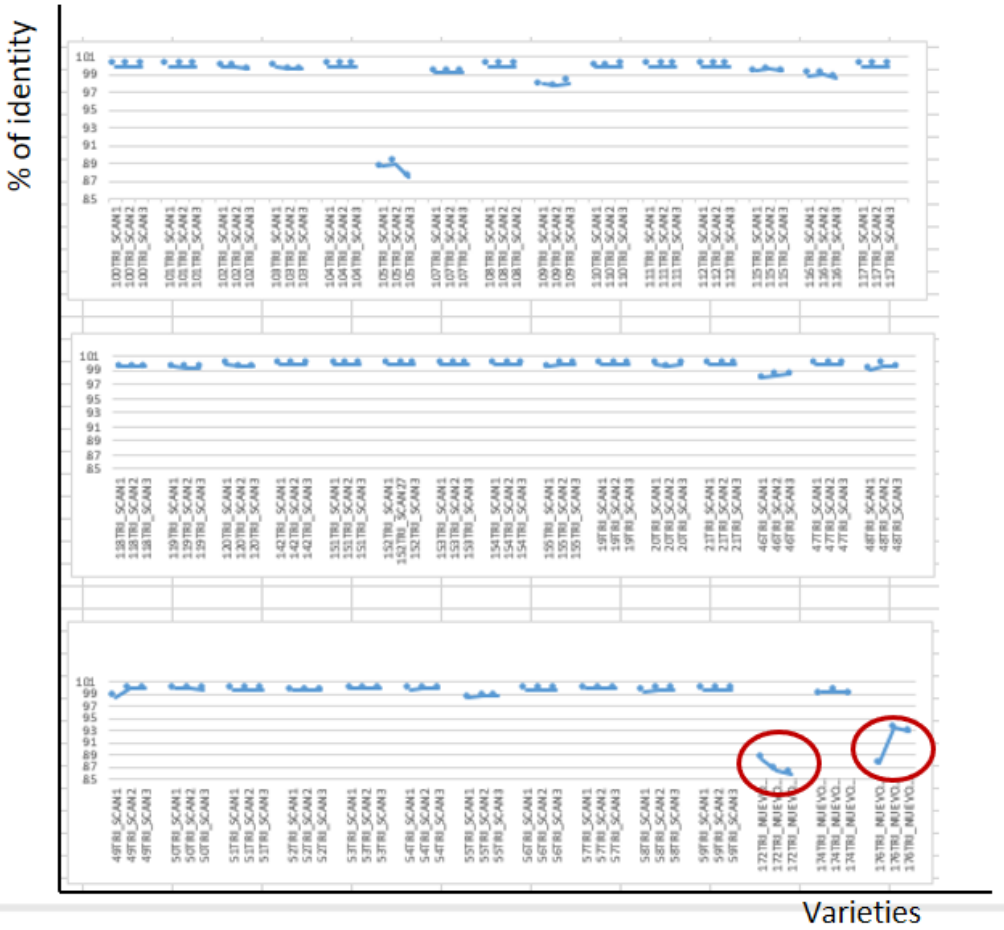
Each dot correspond to the identity value obtained after taken out one seed from the grid and replace it by a seed from another variety.
The "intercept" value is closed to the expected 0.2% value, which is the % of each seed in a 500 seeds grid (1/500).

Use of neuronal networks for variety identification

Repeatability test

Each group correspond to a variety, tested 3 times. Less than 5% of the samples presented variation of results higher than 1.





Handbook

6 members in the working group

HANDBOOK ON DNA BASED TESTS – Table of Contents

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

Content
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”
Development: guidelines for Comparative Tests (CTs) organization
Considerations on the varieties
Considerations on the sample size
Considerations the on markers
Other considerations

HANDBOOK ON DNA BASED TESTS – Table of Contents

Validation: validation of DNA based markers (guide to following ISTA SOP for validation of methods)
Adoption as an official method
Reference material collection (RMC)
Statistical approaches for results analysis (including : evaluation of performance method and proficiency tests) - draft to be presented to STATCOM
ISTA accreditation: evaluation of performance method and proficiency tests
Statistical approaches for results analysis
ISTA accreditation for DNA based testing (to be discussed with Acc department)
Auditing laboratories for DNA-based Testing (to be discussed with Acc department)
Appendices

Acknowledgments

We would like to thank all VARCOM members and collaborators for their contribution to the committee work aimed to achieving ISTA goals

ISTA ANNUAL MEETING 2024
01-04 JULY
CAMBRIDGE, UNITED KINGDOM

Thank you for your attention