

**Working Group on Biochemical and Molecular Techniques
and DNA-Profiling in Particular**

BMT/19/13

**Nineteenth Session
Alexandria, United States of America, September 23 to 25, 2020**

Original: English
Date: September 23, 2020

ISTA APPROACH FOR DNA BASED MARKERS

Document prepared by an expert from the 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 on “ISTA approach for DNA based markers”, prepared by an expert from the International Seed Testing Association (ISTA), to be made at the nineteenth session of the BMT.

[Annex follows]

ISTA approach for DNA based markers

Presenter: Ana Laura Vicario
Location: 19th BMT Meeting
Date: September 23-25, 2020



AGENDA

Introduction to ISTA

Update on VARCOM activities related to molecular markers

ISTA/UPOV joint activities



Introduction to ISTA



Objectives of the Association

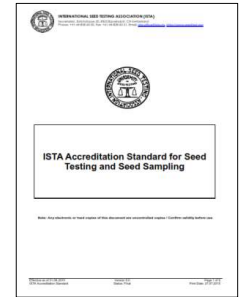
This facilitates seed trading nationally and internationally, and also contributes to food security.

(a) **develop, adopt and publish standard procedures for sampling and testing seeds, and to promote uniform application of these procedures** for evaluation of seeds moving in international trade.

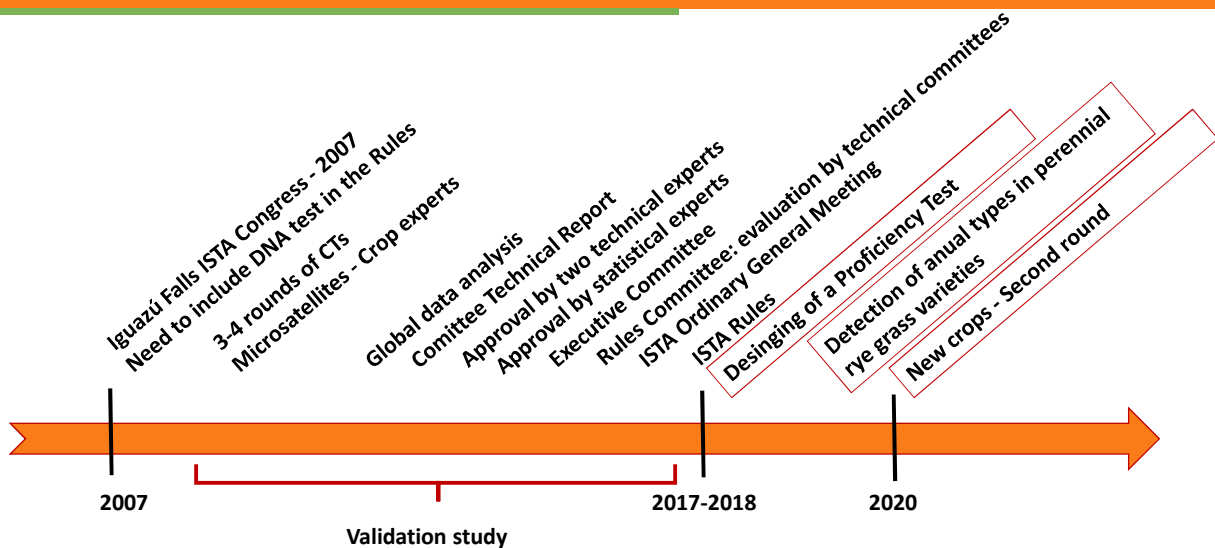
(b) **to promote research in all areas of seed science and technology, to participate in conferences and training courses and to establish and maintain liaison with other organisations.**



Uniformity in seed quality evaluation worldwide




Update on VARCOM activities related to molecular markers



Update on VARCOM activities related to molecular markers



Comparative tests: NEW CROPS

- First round finished during 2019
- Second round organized and sent out to laboratories in 2019
- Results were planned to be ready by May-June 2020 

CTs Leaders:

- The CTs for **Oat** and **Pea** are being led by Marie-José Côté from the Ottawa Plant Laboratory of the **Canadian Food Inspection Agency**.
- Verena Peterseil from the **Austrian Agency for Health and Food Safety**, AGES, is leading the **Barley** CT.
- **Participating laboratories** are located in a wide range of countries including: South Africa, Spain, Canada, Austria, France and Italy.

Update on VARCOM activities related to molecular markers



Design of a Proficiency Test

- Samples of breeder seed were received and the **Reference Matrix** was prepared at CGC Canada for Wheat.
- These are the reference allele profiles for testing laboratory performance.
- The laboratory **rating system** defined before as a general approach, was discussed based on the results obtained for the Reference Matrix
- A **new proposal** for a rating system that takes into account single seed testing and bulk seed testing is under discussion.

Update on VARCOM activities related to molecular markers



Rating system

Change any value in a yellow cell

Rating for microsatellite analysis PTs			
Number of alleles:	33		
Number of varieties:	6		
Minimum requirements for A rating :			
% of varieties wrongly rated:	0%	and	% of alleles with a wrong result: 0%
Minimum requirements for B rating :			
% of varieties wrongly rated:	20%	and	% of alleles with a wrong result: 10%
Minimum requirements for C rating :			
% of varieties wrongly rated:	40%	and	% of alleles with a wrong result: 20%

Rating	Lab	# of varieties wrongly rated	(%)	# of alleles with a wrong result	(%)
A	1	0	0.0%	0	0.0%
B	2	1	16.7%	1	3.0%
B	3	2	33.3%	0	0.0%
C	4	0	0.0%	2	6.1%
BMP	5	5	83.3%	6	18.2%
A	6	0	0.0%	0	0.0%
C	7	2	33.3%	2	6.1%

		# of varieties with at least one error	# of alleles with at least one error	Variety 1					
				Xgwm003	Xgwm413	Xgwm155	DuPw205	DuPw115	DuPw115
Reference A									
Reference B									
Lab 1	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8	0	0						
Lab 2	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8	0	0						
Lab 3	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8	0	0						

Update on VARCOM activities related to molecular markers



The strategy for including DNA-based tests in the ISTA Rules

- Specific PCR primers that define a set of microsatellite **markers are prescribed**
- The **analytical procedures used to interrogate those markers is left to the discretion of individual laboratories**, so long as those procedures have been evaluated as fit for purpose and the end result meets acceptable standards as set by ISTA.

This **SPBA** provides guidance to laboratories and will facilitate processes for laboratories seeking accreditation for these types of tests.

Update on VARCOM activities related to molecular markers



Three laboratories already accredited for DNA-based tests



INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)
Secretariat, Zürcherstrasse 50, 8303 Bassersdorf, CH-Schweizland
Phone: +41-44-838 60 00, Fax: +41-44-838 60 01, Email: ista.office@ista.ch, http://www.seedtest.org

Scope of ISTA Accreditation

Annex to the Accreditation Certificate - ISTA Standard Methods

ISTA code IT01
Address Dipartimento di Scienze e Tecnologie
Agricoltura (DSTAL)
Viale F.lli, 40
40127 Bologna
IT

Issued on 05.11.2018
Valid to 07.05.2021



INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)
Secretariat, Zürcherstrasse 50, 8303 Bassersdorf, CH-Schweizland
Phone: +41-44-838 60 00, Fax: +41-44-838 60 01, Email: ista.office@ista.ch, http://www.seedtest.org

Scope of ISTA Accreditation

Annex to the Accreditation Certificate - ISTA Standard Methods

ISTA code ES01
Address Estación de Ensayos INIA
Carretera de la Corona, Km. 7,500
28049 Madrid
ES

Issued on 19.02.2020
Valid to 24.09.2022



INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)
Secretariat, Zürcherstrasse 50, 8303 Bassersdorf, CH-Schweizland
Phone: +41-44-838 60 00, Fax: +41-44-838 60 01, Email: ista.office@ista.ch, http://www.seedtest.org

Scope of ISTA Accreditation

Annex to the Accreditation Certificate - ISTA Standard Methods

ISTA code IT03
Address CREA-DC, Sede di Tavazzano - Laboratorio
Via Emilia km 307
28038 Tavazzano (LO)
IT

Issued on 09.07.2018
Valid to 10.05.2021

Verification of species and variety: Verify if species/variety corresponds to the species/variety as requested - DNA – based method	Triticum	8.10.2
	Zea mays	8.10.3

Update on VARCOM activities related to molecular markers



Single marker approach: lolium test

UPOV **Model I** is focused on following single traits using molecular markers

In 2018 VARCOM tested a marker set for determination of annual types in perennial ryegrass varieties.

We concluded from the GOT and from the results obtained in two laboratories, that the variable background levels of annual marker detected in perennial varieties are due to **incomplete coupling of the annual marker and the annual trait**, and that estimates vary depending upon which varieties are used in the PCR calibrator.

Now ISTA will provide financial support for a validation of a larger markers set which is currently under development.



ISTA/UPOV joint activities

UPOV codes from **GENIE database** were included in the **ISTA List of Stabilized Plant Names 7th edition**

The current edition thus includes 3044 entries, comprising 2388 accepted names and 656 synonyms, and representing 185 different vascular plant families. For the first time, the approved list includes 16 names that are accepted at the rank of subspecies and six at the rank of variety. At the request of attendees at the side meeting of the Nomenclature Committee in Hyderabad, corresponding codes from the International Union for the Protection of New Varieties of Plants (UPOV) are also included. In the *Stabilised List* two different methods are employed in linking synonyms to



rank of variety. At the request of attendees at the side meeting of the Nomenclature Committee in Hyderabad, corresponding codes from the International Union for the Protection of New Varieties of Plants (UPOV) are also included. In the *Stabilised List* two different

[Trisetum flavescens \(L.\) P. Beauv.](#)

[Tristania conferta R. Br.](#)

= [Lophostemon confertus \(R. Br.\) Peter G. Wilson & J. T. Waterh.](#)

× [Triticosecale](#)

[*Secale* × *Triticum*]

[Triticum aestivum L. subsp. aestivum](#)

(Synonym: *Triticum sativum* Lam.)

4

Poaceae
[Graminae]

[TRISE FLA](#)

Myrtaceae

[LPHST CON](#)

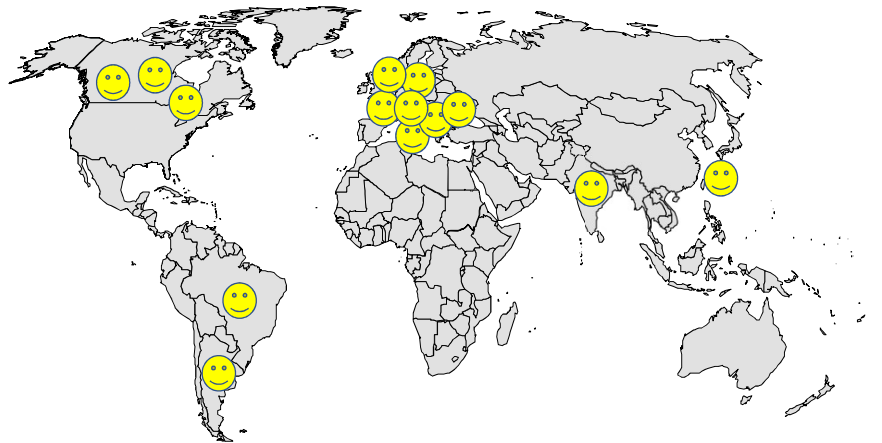
Poaceae
[Graminae]

[TRITL](#)

Poaceae
[Graminae]

[TRITI AES AES](#)

<https://www.upov.int/genie/details.xhtml?cropId=5691>



ISTA VARCOM
We have members
spanning the world

ISTA VARCOM members



Thanks to members, CT leaders and vice-chair for the great work done so far to achieve VARCOM and ISTA goals

	Member Since
1 Chair: Ana Laura Vicario	2007
2 Vice-Chair: Daniel Perry	2013
3 Anne Bernole	2016
4 Berta Killermann	2004
5 Chiara Delogu	2007
6 Elisa Vieira	2007
7 Hedwich Teunissen	2007
8 Kae-Kang Hwu	2007
9 Ksenija Markovic	2013
10 KsenijaTaski-Ajdukovic	2010
11 Keshavulu Kunusoth	2010
12 Verena Peterseil	2016
13 Marie José Côté	2016
14 Marie Claude Gagnon	2020

Thank you!

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