UPOV
International Union for the Protection of New Varieties of Plants

E

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

BMT/20/11

Twentieth Session
Alexandria, United States of America, September 22 to 24, 2021

Original: English
Date: September 17, 2021

ISTA REPORT ON BIOCHEMICAL AND MOLECULAR TECHNIQUES

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 report on biochemical and molecular techniques”, prepared by an expert from the International Seed Testing Association (ISTA), to be made at the twentieth session of the BMT.

[Annex follows]

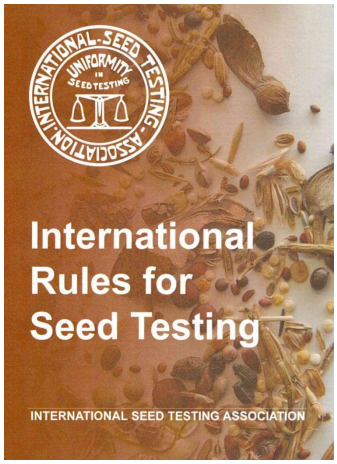
ISTA report on biochemical and molecular techniques

Presenter: Ana Vicario
Location: Buenos Aires, Argentina
Date: UPOV – BMT meeting. September 22nd, 2021

AGENDA

Introduction to ISTA

Update on VARCOM activities related to molecular markers



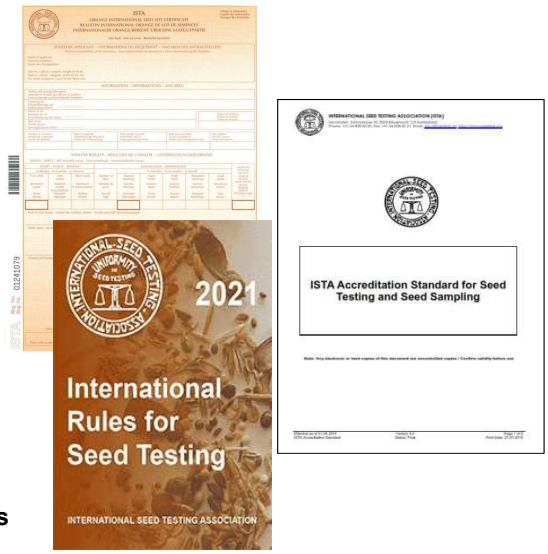
Introduction to ISTA

ISTA Mission
Uniformity in seed quality evaluation worldwide

Objectives of the Association

(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.



ISTA 3 main products

Introduction to ISTA

Other ISTA publications

ISTA Newsletter
Your Association weekly updates
Subscribe to the ISTA Newsletter
https://www.seedtest.org/en/newsletterabo.html

ISTA Handbooks
The ISTA Technical Committees are actively involved in writing technical handbooks which give valuable background information to seed testing procedures.

1. Flower Seed
2. Germination
3. Moisture
4. Nomenclature
5. Purity
6. Laboratory Equipment
7. Sampling
8. Seed Health
9. Statistics
10. Tetrazolium
11. Tree and Shrub Seed
12. Variety
13. Vigour
14. Replacement Binder

Update on VARCOM activities related to molecular markers

PT "0" – First Proficiency Test for DNA based tests

Detection of annual types in perennial rye grass varieties

New crops – final report

2018

2021

UPDATE

PT "0" – First Proficiency Test for DNA based tests

Three laboratories are accredited for DNA-based tests

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

PT "0" has finalized

- 6 participating laboratories: 3 accredited and 3 willing to evaluate their performance.
- Mandatory for accredited laboratories.
- Set of 6 blind samples and 2 varieties sent out as reference together with their SSR profiles.
- 8 SSR as in ISTA Rules Table 8.B.

- Data analysis revealed that two labs had problems with allele calling.
- Some improvement should be done in relation with the communication with laboratories.

- The PT "0" experience will help improve laboratory performance as well as some organization details.

PT "0" – First Proficiency Test for DNA based tests

Project leader: Daniel Perry (CGC – Canada) former VAR vice-chair

Rating for microsatellite analysis PTs — Change any value in a yellow cell

Number of alleles:	48					
Number of varieties:	6					
Number of markers:	8					
		wrong var				
Rating system						
Minimum requirements for A rating :						
% of varieties wrongly rated:	0%	0	and	% of markers with a wrong result:	0%	
Minimum requirements for B rating :						
% of varieties wrongly rated:	20%	1	and	% of markers with a wrong result:	25%	
Minimum requirements for C rating :						
% of varieties wrongly rated:	40%	2	and	% of markers with a wrong result:	50%	

Rating	Lab	# of varieties wrongly rated	(%)		# of markers with a wrong result	(%)	
B	1	1	16,7%	B	1	12,5%	B
B	2	1	16,7%	B	1	12,5%	B
C	3	1	16,7%	B	3	37,5%	C
A	4	0	0,0%	A	0	0,0%	A
BMP	5	4	66,7%	BMP	1	12,5%	B
BMP	6	2	33,3%	C	5	62,5%	BMP

Reference A / Reference B		# of varieties with at least one error	# of alleles with at least one error	Variety 1						
				Xgwm003	Xgwm413	Xgwm155	DuPw205	DuPw115	DuP	
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							

Detection of annual types in perennial rye grass varieties

Project leader: Giovanny López (DLF – Netherlands)

The previous test did not work…the association between the annual marker and the annual growth habit is incomplete and that estimates vary depending upon which varieties are used in the calibrator.

The new proposal - progress:

1 Sequence the L. perenne and L. multiflorum genomes.
2 Search through those genomes for target genes.

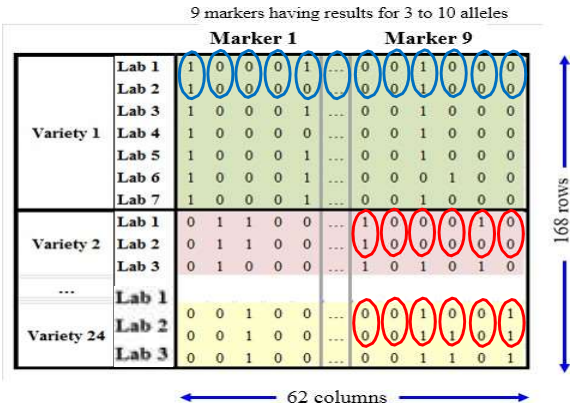
Shaun Bushman, USDA-ARS Forage and Range Research Laboratory, Logan, UT USA

3-Create full-gene primers from each gene, amplify in Lolium entries and sequence.
4-Align sequences from samples and associate with known perenniality.
5 Create Taqman primers.
6 Send out to seed testing labs internationally for validation.
7 Prepare de MVD for sending it out for RP.

The product of the project will be a validation document in accordance with the ISTA validation procedure.

New crops – final report

This is how the data is analyzed and looks like - ISTA format for presenting data



Overall percentage agreements considering **allele results agreement for a given variety**.

Cohen's kappas considering allele results agreement for a given variety.

Overall percentage agreements considering **allele results agreement across varieties for a given allele**.

Cohen's kappas considering allele results agreement across varieties for a given allele.

New crops – final report

EXAMPLE

There is a total of 41 alleles and the two laboratories provide same results on 30 + 6 = 36 alleles leading to an overall percent agreement of 36 / 41 = 87.8%.

		Lab 2		
		0	1	Total
Lab 1	0	**30**	2	32
	1	3	**6**	9
	Total	33	8	**41**

		Lab 2		
		0	1	Total
Lab 1	0	n_{00}	n_{01}	n_{0+}
	1	n_{10}	n_{11}	n_{1+}
	Total	n_{+0}	n_{+1}	N

However, this percent agreement is overestimated as agreement between the two laboratories can be due **by chance only**.

New crops – final report

The most popular measure taking into account the possible chance agreement for two laboratories is the Cohen's kappa coefficient (Cohen, 1960). It is computed as:

$$\hat{\kappa}_C = \frac{p_a - p_e}{1 - p_e}$$

0.63

$$p_a = \frac{n_{00} + n_{11}}{n}$$

36 / 41 = 87.8%

$$p_e = \frac{32}{41} \times \frac{33}{41} + \frac{9}{41} \times \frac{8}{41} = 0.671$$

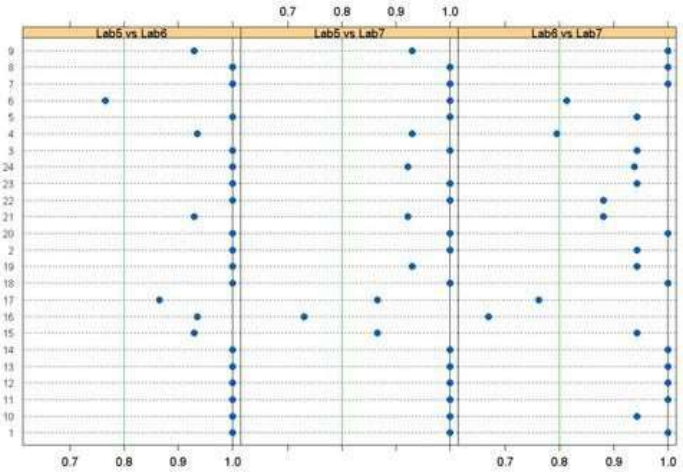
		Lab 2						Lab 2		
		0	1	Total			0	1	Total
Lab 1	0	30	2	**32**		Lab 1	0	n_{00}	n_{01}	n_{0+}
	1	3	6	**9**			1	n_{10}	n_{11}	n_{1+}
	Total	**33**	**8**	**41**			Total	n_{+0}	n_{+1}	N

New crops – final report

This is how the results look like

$\hat{\kappa}_C$	Interpretation
< 0	No agreement
0.0 — 0.20	Slight agreement
0.21 — 0.40	Fair agreement
0.41 — 0.60	Moderate agreement
0.61 — 0.80	Substantial agreement
0.81 — 1.00	Almost perfect agreement

categories defined by Landis and Koch



allele results agreement for a given variety

New crops – final report

Conclusions for PEA and OAT

Project Leader **Marie-José Cotê**

Given these results, there is enough evidence for validating the methods.

Most results (Lab/Lab comparisons) fall in a range of 0.8-1, which indicates that there is good agreement among laboratories when analyzing these markers, and that these agreements are not due to random but because of the possibility of reproducing data from these markers by participating laboratories.

This MVD and all related documents will be sent for RP this year.

Conclusions for BARLEY

Project Leader **Verena Peterseil**

Data is already obtained but data analysis is under revision. Final report still pending.

Thanks to all **members** that actively participated to achieve these goals.

I Specially would like to thank our former vice-chair **Daniel Perry** who made most, not to say all, of our goals come true since 2007 when the Variety Committee started to work again.

I wish him all the best for his new moment in life.

[End of Annex and of document]