Proficiency Testing Program for Variety Verification at ISTA

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Outline

1) DNA-based methods included in ISTA Rules
2) Semi-performance based approach
3) Accreditation and testing
4) Proficiency Testing
5) Rating System
6) DNA PT 00 and PT 01 - Wheat
7) Challenges
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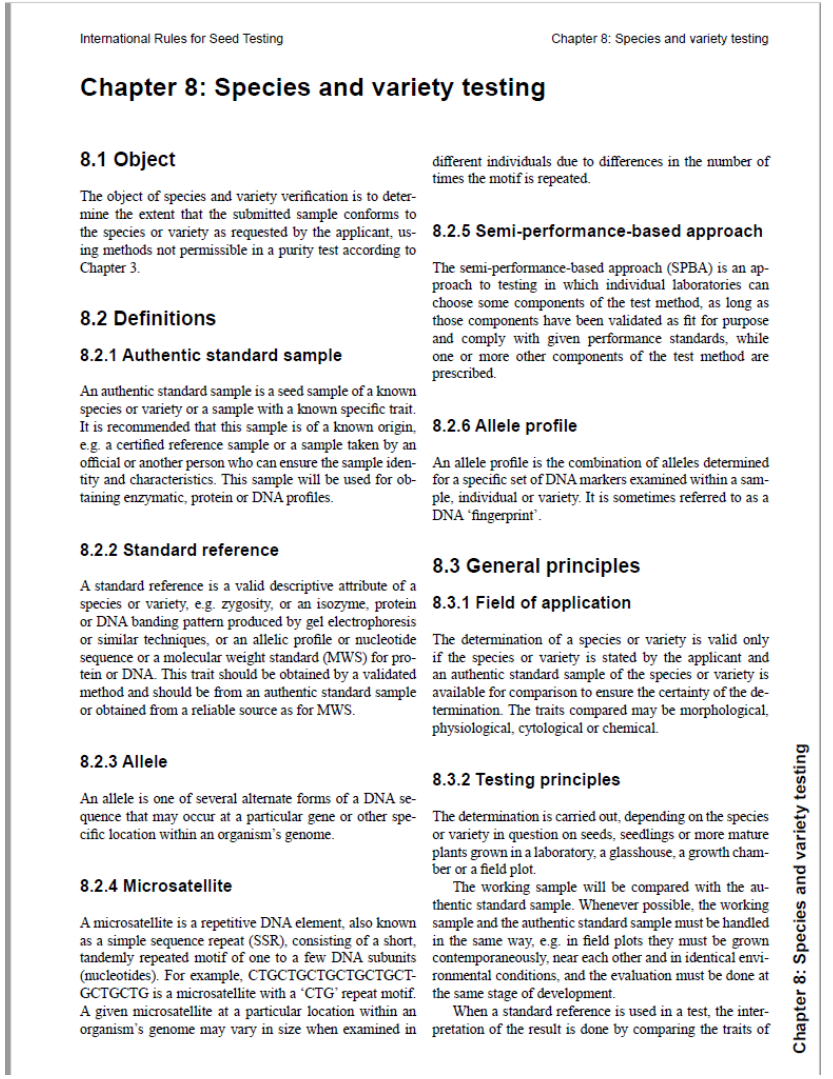
DNA-based methods included in ISTA Rules

- DNA-based methods are included in Chapter 8 of the ISTA Rules since 2017.
- The purpose of including DNA-based tests in the Rules is for laboratory accreditation.
- The goal is to have a strategy for testing laboratory performance with the objective of having accredited labs for variety testing by means of DNA markers.



Chapter 8: Species and variety testing

8.1 Object

The object 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.

8.2 Definitions

8.2.1 Authentic standard sample

An authentic standard sample is a seed sample of a known species or variety or a sample with a known specific trait. It is recommended that this sample is of a known origin, e.g. a certified reference sample or a sample taken by an official or another person who can ensure the sample identity and characteristics. This sample will be used for obtaining enzymatic, protein or DNA profiles.

8.2.2 Standard reference

A standard reference is a valid descriptive attribute of a species or variety, e.g. zygosity, or an isozyme, protein or DNA banding pattern produced by gel electrophoresis or similar techniques, or an allelic profile or nucleotide sequence or a molecular weight standard (MWS) for protein or DNA. This trait should be obtained by a validated method and should be from an authentic standard sample or obtained from a reliable source as for MWS.

8.2.3 Allele

An allele is one of several alternate forms of a DNA sequence that may occur at a particular gene or other specific location within an organism's genome.

8.2.4 Microsatellite

A microsatellite is a repetitive DNA element, also known as a simple sequence repeat (SSR), consisting of a short, tandemly repeated motif of one to a few DNA subunits (nucleotides). For example, CTGCTGCTGCTGCTGCTGCTG is a microsatellite with a 'CTG' repeat motif. A given microsatellite at a particular location within an organism's genome may vary in size when examined in different individuals due to differences in the number of times the motif is repeated.

8.2.5 Semi-performance-based approach

The semi-performance-based approach (SPBA) is an approach to testing in which individual laboratories can choose some components of the test method, as long as those components have been validated as fit for purpose and comply with given performance standards, while one or more other components of the test method are prescribed.

8.2.6 Allele profile

An allele profile is the combination of alleles determined for a specific set of DNA markers examined within a sample, individual or variety. It is sometimes referred to as a DNA 'fingerprint'.

8.3 General principles

8.3.1 Field of application

The determination of a species or variety is valid only if the species or variety is stated by the applicant and an authentic standard sample of the species or variety is available for comparison to ensure the certainty of the determination. The traits compared may be morphological, physiological, cytological or chemical.

8.3.2 Testing principles

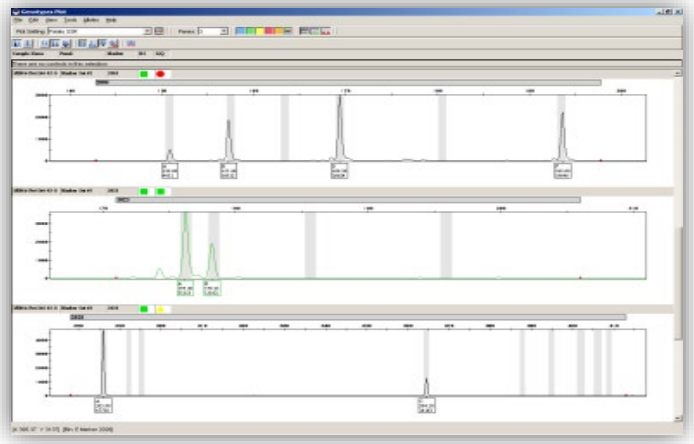
The determination is carried out, depending on the species or variety in question on seeds, seedlings or more mature plants grown in a laboratory, a glasshouse, a growth chamber or a field plot.

The working sample will be compared with the authentic standard sample. Whenever possible, the working sample and the authentic standard sample must be handled in the same way, e.g. in field plots they must be grown contemporaneously, near each other and in identical environmental conditions, and the evaluation must be done at the same stage of development.

When a standard reference is used in a test, the interpretation of the result is done by comparing the traits of

DNA-based methods included in ISTA Rules

- Standard reference methods are available for maize, wheat, peas and oats.
- All DNA-based methods included in the Rules are based on the use of microsatellites (or Simple Sequence Repeats – SSRs) as marker type.
- The use of other marker types (i.e. Single Nucleotide Polymorphisms – SNPs) is under study.



Semi-performance based approach

- The strategy proposed for laboratory accreditation is a semi-performance based approach.
- The strategy is considered to be semi-performance based because various aspects of the laboratory methodology such as DNA extraction, quantification, PCR conditions and visualization will be performance-based.
- For these aspects, the laboratory may choose to use in-house validated methodology as long as the end results is acceptable.
- However, the markers sets to be used will be “prescribed”.

Crop	Marker set prescribed
Wheat	8 microsatellite markers
Maize	8 microsatellite markers
Peas	11 microsatellite markers
Oat	9 microsatellite markers

Accreditation and testing

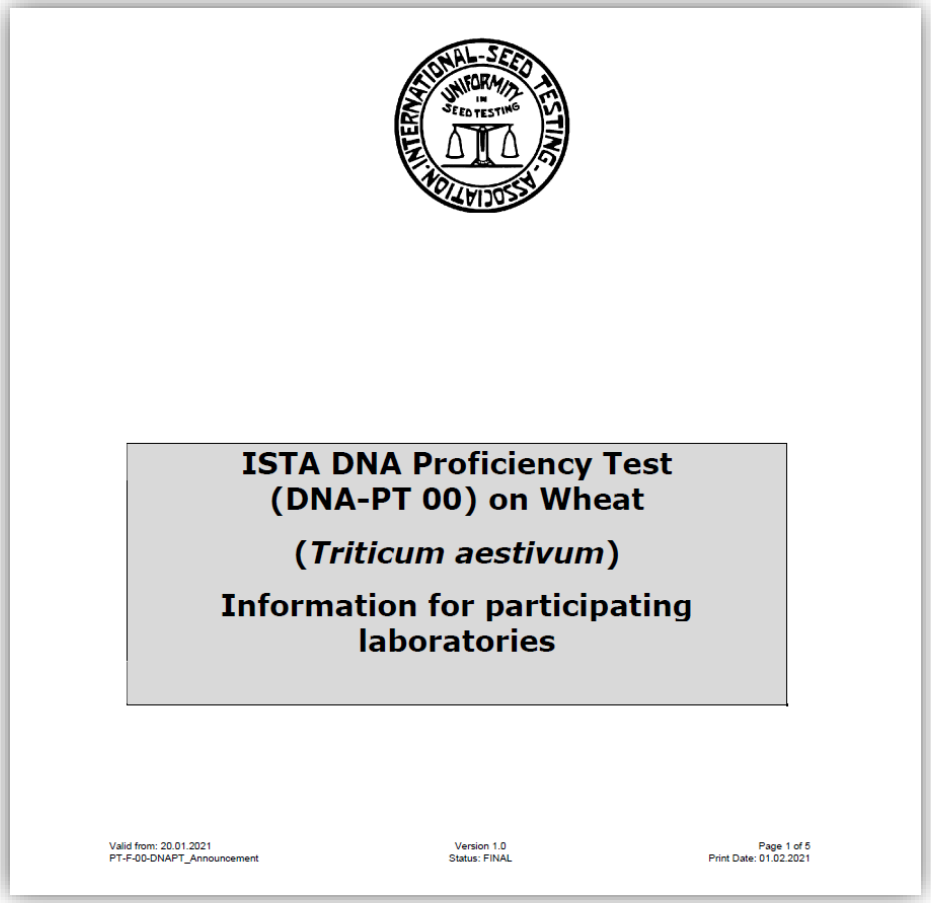
- Laboratories are required to demonstrate their ability to reproduce the marker profile (genotype) of each variety. The number of alleles present in the variety set provided in a given PT need to be sufficient to permit an evaluation of the ability of a laboratory to differentiate and identify multiple alleles at each marker.

- Following accreditation, when a request for a variety verification test is made to the accredited laboratory, use of the prescribed ISTA marker set is 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.

- Recommended supplementary microsatellite markers are available for wheat in Chapter 8 of the Rules.

Proficiency Testing

- Proficiency Testing for variety testing using DNA-based method is available for wheat.
- A collection of reference varieties was obtained from breeders, and a matrix of reference profiles was established.
- Seed samples are kept, prepared and shipped for each PT by Sean Walkowiak, VARCOM member from the Canadian Grain Commission (CGC).
- Two rounds of proficiency testing (PT 0 and PT 1) were performed in 2021 and 2023 respectively.



ISTA DNA Proficiency Test (DNA-PT 00) on Wheat

(*Triticum aestivum*)

Information for participating laboratories

Valid from: 20.01.2021
PT-F-00-DNAPT_Announcement

Version 1.0
Status: FINAL

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Print Date: 01.02.2021

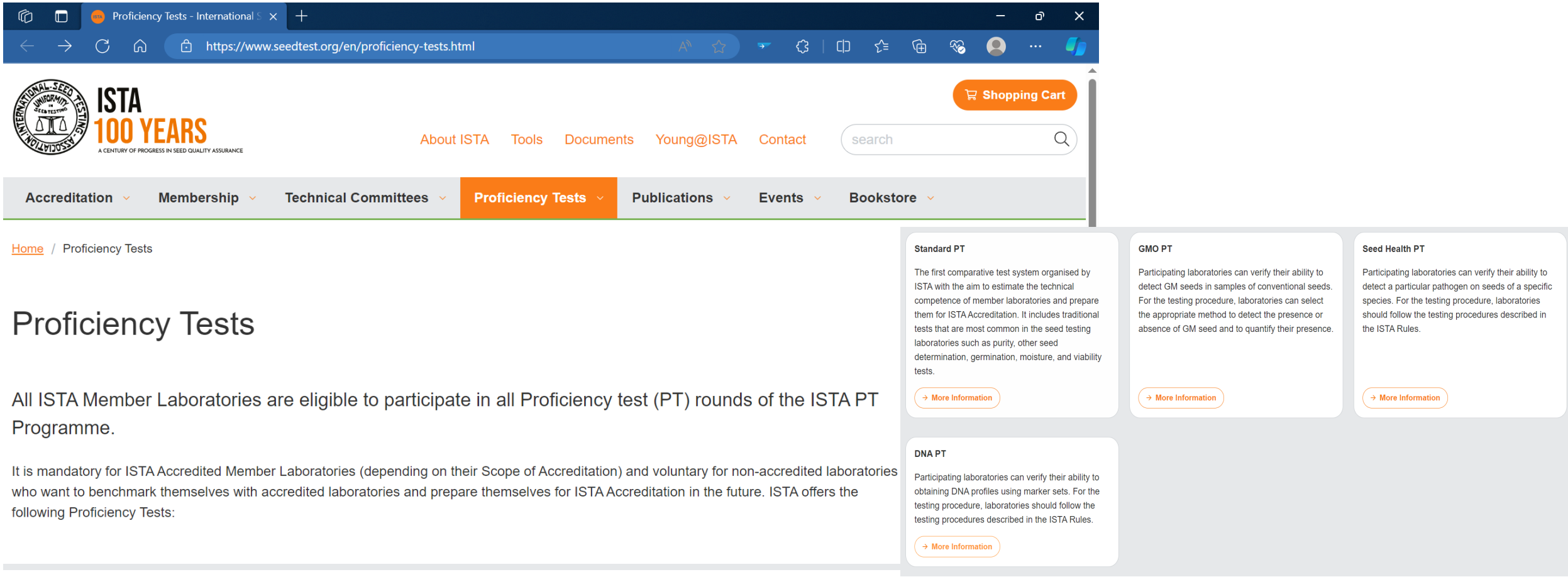
Proficiency Testing

- Each participating laboratory is receiving a sample set made of 6 numbered wheat seed samples, each containing 12 crushed individual single seed.
- DNA profiles have to be reported for 8 seeds per sample, the remaining 4 seeds are sent as backup.
- In addition, participants are receiving 2 samples (bulks of 10 seeds) along with their allelic profiles to be used as a reference to calibrate allele calls.

Table of Results																	
Laboratory Code																	
		Sample 1								Sample 2							
		Xgwm003	Xgwm413	Xgwm155	DuPw205	DuPw115	DuPw004	DuPw217	DuPw167	Xgwm003	Xgwm413	Xgwm155	DuPw205	DuPw115	DuPw004	DuPw217	DuPw167
Reference A		97	110	165	188	208	310	238	260	97	110	165	188	208	310	238	260
Reference B		95	122	163	183	205	213	229	246	95	122	163	183	205	213	229	246
KERNELS	1	103	110	163	183	208	310	NULL	262	101	110	161	188	205	213	229	246
	2	103	110	163	183	208	310	NULL	262	101	110	161	188	205	213	229	246
	3	103	110	163	183	208	310	NULL	262	101	110	161	188	205	213	229	246
	4	103	110	163	183	208	310	NULL	262	101	110	161	188	205	213	229	246
	5	103	110	163	183	208	310	NULL	262	101	110	161	188	205	213	229	246
	6	103	110	163	183	208	310	NULL	262	101	110	161	188	205	213	229	246
	7	103	110	163	183	208	310	NULL	262	101	110	161	188	205	213	229	246
	8	103	110	163	183	208	310	NULL	262	101	110	161	188	205	213	229	246

Proficiency Testing

- It is possible to verify on ISTA website if there is a DNA PT under preparation: https://www.seedtest.org/en/proficiency-tests.html



Rating System

- Laboratory performance and rating is based on an Excel spreadsheet developed with the help of ISTA STATCOM.
- Rating system is based on the comparison of the allele profile obtained by each participant, per variety and per maker, to the reference matrix obtained by the CGC.
- Participants may report up to two off-types per variety and marker without impact on the final rating, to account for the finding of off-type seeds or new alleles.
- Allele sizes differing from the reference matrix in +1/-1 bp from the expected allele size are accepted. This required an adjustment of the matrix template used to calculate lab performance.

Rating System

- Final rating is based on the % of varieties wrongly rated and % of markers with wrong results.
- Rating established as follows:

		Wrong varieties				worng markers
Minimum requirements for A rating :						
% of varieties wrongly rated:	0%	0	and	% of markers with a wrong result:	0%	0
Minimum requirements for B rating :						
% of varieties wrongly rated:	20%	1	and	% of markers with a wrong result:	25%	1 or 2
Minimum requirements for C rating :						
% of varieties wrongly rated:	40%	2	and	% of markers with a wrong result:	50%	3 or 4

- If a laboratory reports more than 40% of wrongly rated varieties and more than 50% of markers with a wrong results, the rating for the laboratory will be BMP.
- For each PT, the PT leader prepares the reports for each participating lab as well as an overall report.

DNA PT 00 - Wheat

- 6 participating laboratories: 3 accredited and 3 willing to evaluate their performance.
- PT 00 was 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.

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						wrong markers
Minimum requirements for A rating :								
% of varieties wrongly rated:	0%	0	and	% of markers with a wrong result:	0%		0	
Minimum requirements for B rating :								
% of varieties wrongly rated:	20%	1	and	% of markers with a wrong result:	25%		1 or 2	
Minimum requirements for C rating :								
% of varieties wrongly rated:	40%	2	and	% of markers with a wrong result:	50%		3 or 4	

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

DNA PT 00 - Wheat

- Ratings awarded for PT 00 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.

Rating	Number of laboratories
A	3
B	1
C	0
BMP	2

DNA PT 01 - Wheat

- 5 participating laboratories: 3 accredited and 2 willing to evaluate their performance.
- PT 01 was 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 in August 2023.
- Data from one laboratory were not included in the report.

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						wrong markers
Minimum requirements for A rating :								
% of varieties wrongly rated:	0%	0	and	% of markers with a wrong result:	0%		0	
Minimum requirements for B rating :								
% of varieties wrongly rated:	20%	1	and	% of markers with a wrong result:	25%		1 or 2	
Minimum requirements for C rating :								
% of varieties wrongly rated:	40%	2	and	% of markers with a wrong result:	50%		3 or 4	

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

DNA PT 01 - Wheat

- Ratings awarded for PT 01 were as follows:
- One laboratory failed in running 3 of the prescribed markers and presented results for 3 other markers not in the Rules.
- The full data set could not be analyzed using the established rating system and results were not included in the final report.

Rating	Number of laboratories
A	3
B	1
C	0
BMP	0

Challenges

- Goal is to establish proficiency tests for variety testing using DNA-based method for other crops (i.e. maize).
- A challenge is to find pure public lines of many different varieties to be used in proficiency test, and to obtain enough seeds for each of them (i.e. 2 – 5 kilograms).
- Another challenge is to find laboratories willing to store the PT samples, genotype them and ship them to PT participants.
  - For wheat: PT samples are stored, genotyped and shipped by the Canadian Grain Commission.
  - For other crops: Public or private laboratories have to be found, in addition to storing the seeds. Funding is necessary to pay private laboratories for storage.

Conclusion

- VARCOM is open to include new species in Chapter 8 of the Rules for DNA-based tests.
- If someone has a particular interest in validating new markers sets, please contact us (https://www.seedtest.org/en/technical-committees/variety-committee.html).

Variety Committee

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Vice	Marie-Claude Gagnon
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CAMBRIDGE, UNITED KINGDOM

Thank you

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