UPOV
International Union for the Protection of New Varieties of Plants

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Technical Working Party on Testing Methods and Techniques **TWM/4/23 Add.**

Fourth Session
Cambridge, United Kingdom, June 2 to 5, 2026

Original: English
Date: June 15, 2026

ADDENDUM TO:
UPDATE ON THE ACTIVITIES OF THE ISTA VARIETY COMMITTEE (VARCOM)

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 presentation “ISTA update on the use of methods for variety identification and verification”, made by an expert from the International Seed Testing Association (ISTA), at the fourth session of the TWM.

[Annex follows]

ISTA update on the use of methods for variety identification and verification

Marie-Claude Gagnon – VARCOM Chair
TWM – UPOV
June, 2026

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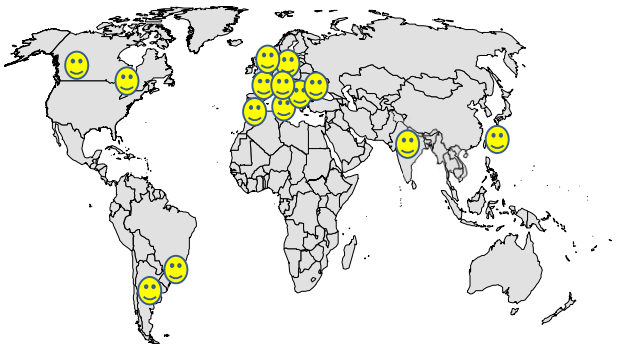
ISTA VARCOM members

1	Chair: Marie-Claude Gagnon	Member since 2020
2	Vice-Chair: Ana Laura Vicario	Member since 2007
3	Vice-Chair: Sean Walkowiak	Member since 2022
4	Anne Bernole	Member since 2016
5	Berta Killermann	Member since 2004
6	Chiara Delogu	Member since 2007
7	Kae-Kang Hwu	Member since 2007
8	Ksenija Markovic	Member since 2013
9	Ksenija Taski-Ajdukovic	Member since 2010
10	Keshavulu Kunusoth	Member since 2010
11	Ana Patricia Fernandez Getino	Member since 2021
12	Mariana Menoni	Member since 2021
13	Umashankar Bellan	Member since 2023
14	Lorella Andreani	Member since 2023
15	Beni Kaufman	Member since 2024



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Presentation Outline

- DNA- based method for *Hordeum vulgare* (barley).
- Update on the validation of newly developed *Lolium* markers for determination of annual types in perennial ryegrass varieties.
- Update on the use of AI (neuronal network) for variety identification.
- Handbook for DNA-based markers validation and utilization.

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DNA-based method for *Hordeum vulgare* (barley)

- Leader of comparative study Doris Kaiser from Austria, in collaboration with Verena Peterseil, Ana Laura Vicario and Marie-Claude Gagnon.
- Validations documents and rules proposal have been reviewed and approved by VAR COM members with 14 approbations and 1 abstention. They have been submitted for inclusion in the 2027 ISTA Rules.
- If accepted by delegates, this proposal will be the fifth DNA-based method to be included in the ISTA Rules since 2017 after *Triticum aestivum*, *Zea mays*, *Pisum sativum* and *Avena sativa*.

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DNA-based method – Proficiency Testing

- Proficiency testing (PT) for DNA-based method is available for *Triticum aestivum.* DNA-PT 2 is now open for registration (deadline June 2026).
- The objective of the PT is to check the ability of individual laboratories to obtain DNA profiles using the marker set in the Rules.
- Participation is mandatory for ISTA accredited laboratories and open to non accredited laboratories willing to test their performance
- The reference collection samples are prepared and shipped by the Canadian Grain Commission in Winnipeg (team of Dr. Sean Walkowiak)

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Validation of new *Lolium* markers

Collaborative work
Project leader: Giovanny López (ATC)
Collaboration with Shaun Bushman from USDA who developed the markers, Daniel Curry from Oregon State University who provided seeds samples for the test and technical support, and Ingo Lenk from DLF providing technical support.

- First round of marker development for qPCR (real-time PCR). Markers were often, but not always, able to distinguish ARG contamination in PRG down to 1%. Italian ryegrass varieties were particularly challenging.
- In a second round of marker optimization, we adapted the same primers/probes to be used with dPCR (digital PCR). Digital PCR offers a more precision and quantification over real-time PCR.

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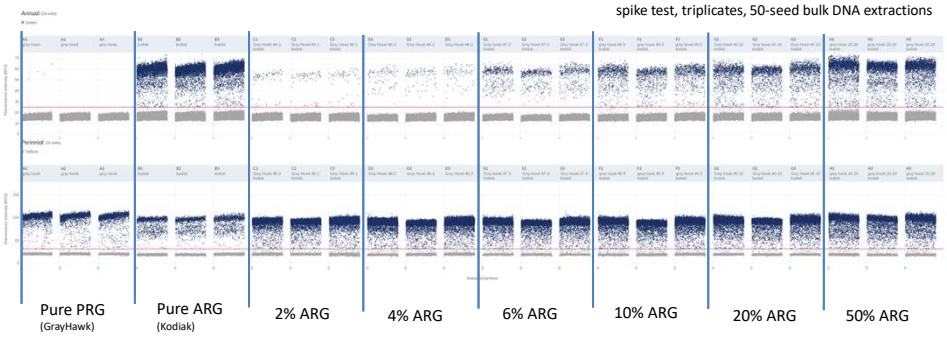
Validation of new *Lolium* markers

spike test, triplicates, 50-seed bulk DNA extractions

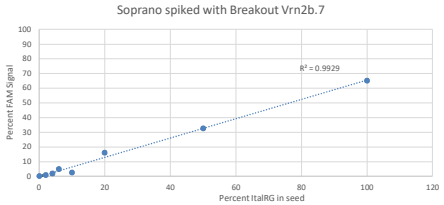


- Pure PRG has no FAM signal, but pure ARG has about a 20% SUN signal. None of the 3 markers tested could achieve an absence of signal in pure ARG.

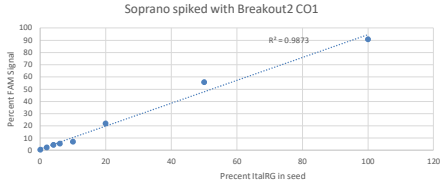
Validation of new *Lolium* markers

- However, it is still possible to get good correlations in spike tests, even with Italian varieties.

Entry	% ARG	% FAM Signal	CI
Soprano	0	0.031	1.08
Soprano 49-1 Breakout2	2	0.802	0.28
Soprano 48-2 Breakout2	4	1.774	0.21
Soprano 47-3 Breakout2	6	4.863	0.12
Soprano 45-5 Breakout2	10	2.541	0.19
Soprano 40-10 Breakout2	20	16.040	0.06
Soprano 20-20 Breakout2	50	32.676	0.04
Breakout2	100	65.129	0.02



Entry	% ARG	% FAM Signal	CI
Soprano	0	0.484	0.248
Soprano 49-1 Breakout2	2	2.439	0.158
Soprano 48-2 Breakout2	4	4.440	0.124
Soprano 47-3 Breakout2	6	5.486	0.095
Soprano 45-5 Breakout2	10	7.024	0.109
Soprano 40-10 Breakout2	20	21.952	0.052
Soprano 20-20 Breakout2	50	55.670	0.035
Breakout2	100	90.666	0.026



Validation of new *Lolium* markers

Next steps:

- Test the dPCR markers on “real” samples and compare results.
- Develop a reference library of pure-type expected results.
- Find other labs using dPCR to perform a validation study.
- Prepare validation report and proposal for ISTA Rules.

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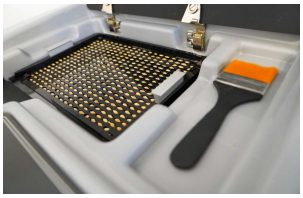
Use of AI for variety identification

Collaborative work on the ZoomAgri technology for variety identification
Project leader: Ana Laura Vicario (VAR)
Collaboration with Mailen Martinez (ATC)



- Method used in 3 countries for wheat and 15 countries for barley variety identification from seeds. Other models are currently being developed (ex. soybean variety ID, GMO detection, etc.).
- Spiking tests were performed in two different laboratories for barley and wheat.
- A pure seeds test was performed in one laboratory for wheat.
- Datasets were analyzed by STACOM in early 2026 to determine what type of experimental set-up would be best to use in a comparative trial.

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Use of AI for variety identification

- The test gives a % of identification of the sample compared with the varieties already learned by the software. This % of identification is based on the total number of seeds tested.
- Technology performs well, but not uniform across varieties. Over 42 wheat varieties tested, 4 failed a homogeneity test.
- This could be explained by the seed source, which sometimes differed across variety (ex. breeder, 1st multiplication, damaged seeds, etc.). How this affects the final result and how to manage this will be discussed further.

variety	replicates	test_stat	pval
WRS 1	5	9.2598425	0
WRS 2	1	0	0
WRS 3	6	4.1967213	0
WRS 5	3	9.8292683	0
WRS 6	3	27.35	0
WRS 7	4	15.9386503	0
WRS 8	8	211.8872786	0.0000266
WRS 9	3	68.3768116	0.0812696
WRS 10	6	18.7066667	0
WRS 11	3	50.8310249	0.0009109
WRS 12	2	0.2025316	0
WRS 13	4	9.483871	0
WRS 14	2	4.1390728	0
WRS 15	4	114.0582751	0.1770016
WRS 16	4	4.814433	0
WRS 17	3	43.992126	0.0000478
WRS 18	4	8.512476	0
WRS 19	7	86.8476821	0
WRS 20	5	400.459605	1
WRS 21	6	54.4659686	0
WRS 22	5	92.386921	0.0000001
WRS 23	4	36.1149425	0
WRS 24	4	3.3308271	0
WRS 25	1	0	0
WRS 26	3	8	0
WRS 27	6	37.7137546	0
WRS 28	4	10.9882353	0

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Update on DNA Handbook

Collaborative work with 8 members of the VARCOM

- Kae-Kang Hwu
- Ksenija Taski Ajdukovic
- Lorella Andreani
- Chiara Delogu
- Marie-Claude Gagnon
- Ana Vicario
- Sean Walkowiak
- Umashankar Bellan

- Topics include guidelines for the organization of comparative tests, validation of DNA based markers, the organization of proficiency testing, and statistical approches to analyze results.
- Constructive comments were received from ISTA accreditation department and STACOM.
- Can be used in the collaboration between ISTA, UPOV and OECD.

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Thanks for your attention

Engage with the Variety Committee by contacting the ISTA Secretariat ista.office@ista.ch or online https://www.seedtest.org/en/technical-committees/variety-committee.html

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[End of Annex and of document]