

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

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DNA-BASED METHODS FOR VARIETY TESTING: ISTA APPROACH

Document prepared by an expert from the International Seed Testing Association (ISTA)

Disclaimer: this document does not represent UPOV policies or guidance

The Annex of this document contains a copy of a presentation on “DNA-based methods for variety testing: ISTA approach” to be made at the eighteenth session of the BMT.

[Annex follows]



DNA-based methods for variety testing: ISTA approach

18th BMT meeting, Hangzhou (China)
October 2019



Agenda

1. Introduction to ISTA
2. DNA based test to the Rules
3. Variety Committee present and future activities
1. Concluding remarks



International Rules for Seed Testing, Full Issue 1-19-9 (2019)
<https://doi.org/10.15258/istarules.2019.F>



International Rules for Seed Testing 2019

Introduction to the ISTA Rules
Chapters 1-19

Including changes and editorial corrections adopted at the
Ordinary General Meeting 2018, Sapporo, Japan
Effective from 1 January 2019



Introduction to ISTA

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**.

Uniformity in seed quality evaluation worldwide

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



Major achievements and services provided



DNA-based methods to the Rules

The need to include DNA-based methods in the Rules

Marker type selection (back in 2007)

Microsatellite had been successfully used for identification and genetic relationship studies in different crops.

They are multi-allelic, codominant, relatively abundant and have extensive genome coverage.

Crop experts

- To select a marker panel for each crop,
- To choose a set of commercial varieties as reference material,
- To evaluate the discrimination power of the markers selected against the reference varieties
- To test them among labs to evaluate repeatability and reproducibility: **VALIDATION**

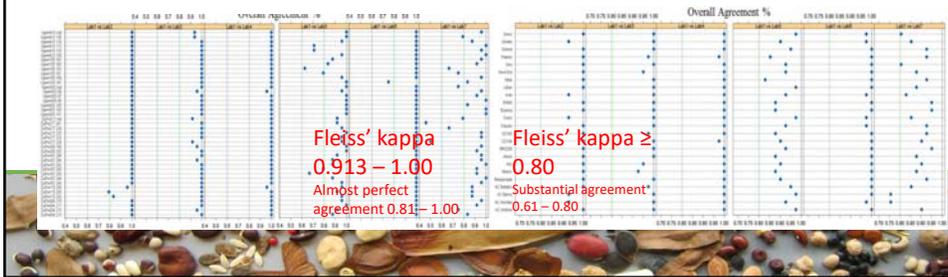


DNA-based methods to the Rules



VALIDATION: through comparative tests (CT) aiming to

- determine the influence of possible variables (e.g. different laboratory facilities and reactants)
- establish and evaluate relevant performance parameters of the method by the **evaluation of the agreement in scoring varieties/alleles across the laboratories (Fleiss' kappa, 0 - 1)**
- Overall percentage agreements considering allele results agreement for a given variety
- Overall percentage agreements considering allele results agreement across varieties for a given allele



DNA-based methods to the Rules



COMMITTEE TECHNICAL REPORT

Validation of a new method for
**“Microsatellite marker analysis for wheat
variety verification”**

COMMITTEE TECHNICAL REPORT

Validation of a new method for a **“DNA
based test on maize”**



DNA-based methods to the Rules

- The validation report has to be approved by two technical experts and one expert from the Statistics Committee.
- Then goes to the Rules Committee and the proposal is evaluated by all Technical Committees
- Then, by the Executive Committee.
- Finally has to be voted on during the ISTA Ordinary General Meeting.
- Then it is published in the ISTA Rules, valid from next January.



The image shows the table of contents for a document titled "Method Validation Reports on Rules Proposals for the International Rules for Seed Testing 2017 Edition". The document is published by the International Seed Testing Association (ISTA). The table of contents lists various sections and their corresponding page numbers.

Validation of a new method for microsatellite marker analysis for wheat variety verification	2
Preparation of the addition of Triticum aestivum as a 'rigid' Test to the new rules	44
Application of the rules proposals for the maize (Zea mays) variety verification	88
Application of the rules proposals for the maize (Zea mays) variety verification in regular testing, as applied in the ISTA test	93
Dissemination of the validation reports (VCR) for the maize (Zea mays) variety verification and the use of microsatellite markers	95
Application of the statistical methods used to verify wheat (Triticum aestivum)	102



DNA-based methods to the Rules



- The first DNA-based method, for verifying varieties of **wheat**, was approved in 2016 and included in the Rules in 2017 (8.10.2).
- A new reference DNA-based method for verifying varieties of **maize** was approved in 2017 and published in the 2018 edition (8.10.3).
- They **describe a set of prescribed microsatellite markers** required for seeking accreditation and for reporting and issuing ISTA Certificates.
- It **recommends** DNA extraction protocol, PCR procedures, reaction components and thermal cycling profile.
- **Laboratories are free to add as many markers of the same type as they need to achieve identification of a sample.**



DNA-based methods to the Rules



The strategy for including DNA-based tests into 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.



DNA-based methods to the Rules



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

- **Laboratory accreditation is to be based on proficiency tests and on-site audits** following the ISTA accreditation standard in the usual manner. At the time of the audit, **ISTA auditors will verify** that the procedures and additional sets of markers the laboratory has adopted at their discretion have been evaluated as fit for purpose.
- Once a laboratory is accredited, they will be required to use of the prescribed ISTA marker sets for variety verification testing, with supplementary markers added as needed.



Variety Committee present and future activities



The Variety Committee has members from locations spanning the globe.



Variety Committee present and future activities



- Second round of Comparative Tests (CTs) are being organized and initiated for **Oat, Pea, and Barley**.
- The **aim** for each crop species is to select a set of microsatellite markers to be introduced as new methods in Chapter 8 of the Rules.
- **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.



Variety Committee present and future activities



- The Variety Committee is endeavouring **to set up proficiency tests** (PTs) that will enable an ongoing evaluation of laboratories accredited for methods validated using the SPBA.
- Once established, the **PTs will be mandatory for laboratories that have DNA-based methods in their scope of accreditation**, but will also provide opportunity for non-accredited laboratories to benchmark themselves with accredited laboratories and prepare for future accreditation.
- Together with the Accreditation Department and the Statistic Committee we have developed a PT strategy, which includes a **test design and a rating system** for measuring laboratory performance.



Variety Committee present and future activities



- Now, our focus is on **Wheat**, the first crop to have DNA-based methods included in the Rules.
- We already have an agreement with breeders to receive a set of varieties for the PTs.
- A reference matrix will be obtained by running those varieties with the marker set selected for wheat. This will be the reference allele profile for those varieties and will be used to rate laboratories.
- As part of the introduction of new methods in Chapter 8 of the Rules, a whole revision of the chapter is planned for next triennium.



Concluding remarks



DNA-based techniques are

- developed and used by breeding companies and seed companies
- mature and available for seed testing, already used in many laboratories, in many countries

ISTA will continue to facilitate the development and use of standardised DNA-based methods in Seed Testing



Acknowledgements



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