UPOV
International Union for the Protection of New Varieties of Plants

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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)

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The Annex to this document contains a copy of a presentation on "DNA-based methods for variety testing: ISTA approach", prepared by an expert from the International Seed Testing Association (ISTA), to be made at the seventeenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT).

[Annex follows]

ANNEX

DNA-BASED METHODS FOR VARIETY TESTING: ISTA APPROACH

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

DNA-based methods for variety testing: ISTA approach

Ana Laura Vicario – Chair of the ISTA Variety Committee

Instituto Nacional de Semillas – INASE

BMT meeting, Montevideo, Uruguay. September, 2018

Agenda

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

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.

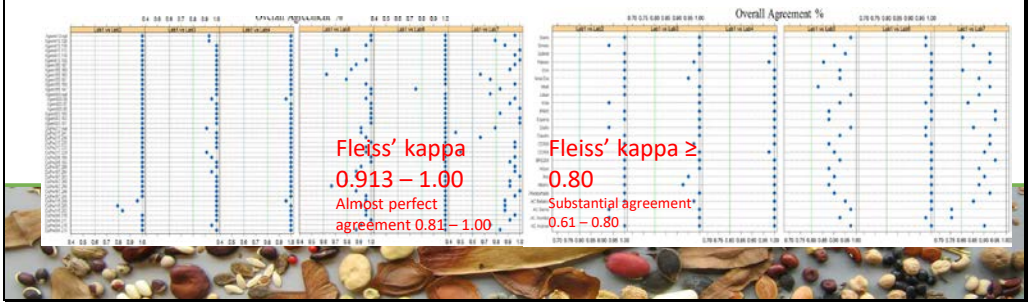
Crop experts

- To select a maker 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



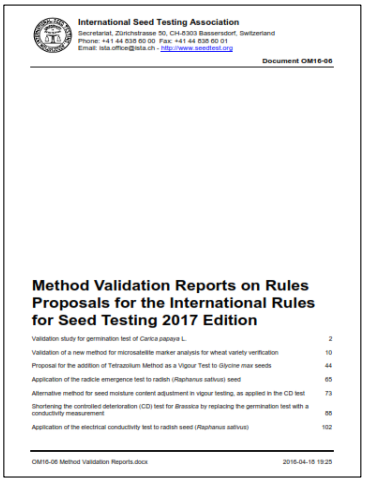
DNA-based methods to the Rules

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"



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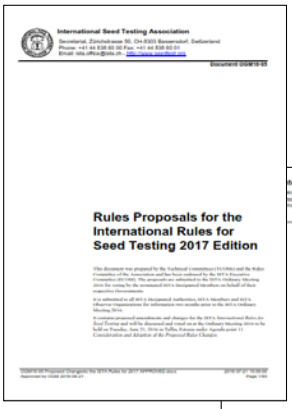
Document OM16-06

Method Validation Reports on Rules Proposals for the International Rules for Seed Testing 2017 Edition

OM16-06 Method Validation Reports.docx 2016-04-15 19:25

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.


International Seed Testing Association

Rules Proposals for the International Rules for Seed Testing 2017 Edition


International Seed Testing Association

Method Validation Reports on Rules Proposals for the International Rules for Seed Testing 2017 Edition

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.

Variety Committee present and future activities

The Variety Committee has members from locations spanning across the globe.

Variety Committee present and future activities

- New Comparative Tests (CTs) were organized and initiated for Oat, Pea, Soybean 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, Pea and Soybean 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 Austria, Canada, UK, USA, France, Italy, Serbia and Argentina.

Variety Committee present and future activities

- The Variety Committee is now 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 are developing the PT strategy, which includes a test design and a rating system for measuring laboratory performance.

Variety Committee present and future activities

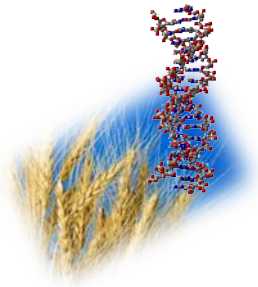
- Initially, our focus is on Wheat, the first crop to have DNA-based methods included in the Rules.
- PT participants will receive two sets of samples: one set of four reference varieties for which allele profiles will be provided, and a separate set of eight unknowns.
- Participants will analyse individual seeds from both sets using the prescribed microsatellite markers and they will submit allele profiles for each unknown, with allele sizes calibrated using the profiles of the reference samples.
- Laboratories will be rated based on the percentage of alleles correctly called and the number of varieties with correct profiles.
- Our goal is to start the first PT on DNA-based methods for wheat during 2018.
- New markers: we are thinking in SNPs (Single Nucleotide Polymorphism) as future markers to be included in the Rules.

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



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