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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

**WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES,
AND DNA-PROFILING IN PARTICULAR**

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ADDENDUM

DEVELOPMENT OF AN INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)
DNA-BASED APPROACH FOR TESTING VARIETY IDENTITY


Document prepared by an expert from VARCOM

 INTERNATIONAL SEED TESTING ASSOCIATION (ISTA)
www.seedtest.org



**DEVELOPMENT OF AN
INTERNATIONAL SEED TESTING
ASSOCIATION (ISTA) DNA-BASED
APPROACH FOR TESTING VARIETY
IDENTITY**

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The standardization of methods on the international level

Standardized Methods for the international level need to provide reproducible results.

- This means, independently of the purpose, the result achieved in laboratory 1 in country 1 need to be the same (within a given tolerance) in laboratory 2 in country 2.
- If this can not be achieved the value of the method is questionable on the international level and might create a barrier for the trade.

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DNA Methodologies are complex from many viewpoints

- A DNA method is a series of different methodologies (Extraction of DNA, Purification of DNA, selection of Primers, PCR protocols, differences in PCR machines, visualization systems, protocols and procedures.)
- DNA methods are extremely sensitive for contaminations.
- Repeatability within the same laboratory (through the same analyst or through a different analyst) is not easily achieved.



For the time being no DNA-Methodology is standardized in the ISTA International Rules for Seed Testing


- Methods being included within the ISTA International Rules for Seed Testing are being validated internationally.
- This means, at a minimum an acceptable level of accuracy, repeatability and reproducibility has been demonstrated in comparative tests.

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The strategy for incorporating DNA-based tests into the ISTA Rules.

- Establishing the DNA WG in 2007
- Selection of microsatellites (SSR) markers as they have proven to be one of the most appropriate for variety identity and genetic relationship studies.
- Selection of crops based on those representing the most important ones in terms of cultivated area and world production.
- Conducting comparative tests (CTs) for the selection of the most reliable markers per crop.
- These CTs were performed initially by laboratories having experience with each crop and/or technique.


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The strategy for incorporating DNA-based tests into the ISTA Rules.

- AIMS:
 - to compare results between participant laboratories, and evaluate whether it is possible to obtain the same band patterns and allele sizes even when using different reactants, equipment and working protocols.
 - to select the most suitable SSRs for variety identification
 - to verify if the marker set was polymorphic enough to provide unique DNA-based patterns for a larger variety set.
 - to prove robustness of the method by including additional laboratories that would run the selected SSRs and the same varieties tested by the original laboratories.


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The strategy for incorporating DNA-based tests into the ISTA Rules.

- **Characteristics of the SSR:**
 - Repeatable between laboratories and between CTs
 - Sharp band profile
 - Without faint bands or null alleles
 - Without stuttering
 - Polymorphic with the most common technical equipments
- **SSR that did not present these characteristics were rejected and substituted in the following CT**


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Summary of results

Crop group	Participating laboratories per CT			Visualization methods used
	CT1	CT2	CT3	
Wheat	Canada x2 France Italy	Canada x2 France Italy	Austria Argentina Canada x3 France, Italy	Licor 4200 and 4300, ABI 3130xl and Silver stain
Rice	Canada Italy Taiwan	Canada Italy Taiwan	Canada India Italy, USAx2, Taiwan	ABI3100 and 3130, Licor 4300 and Agarose
Soy	Argentina Brazil Canada	Argentina Brazil Canada, USA	Argentina x3 Brazil Canada, USA	Silver stain and ABI 3100
Maize	Argentina Brazil Canada France	Argentina Brazil Canada France	Brazil Canada China, France x2, Italy, USA x2	ABI3130xl, Silver stain, ABI 3100 and Silver Stain


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Summary of results

Working group	Number of selected SSR	Amount of tested Varieties	Tested Areas
Soybean	11	74	Argentina Brazil
Wheat	8	84	Canada Brazil France Italy
Maize	12	72	France Canada
Rice	15	192	Asia Italy USA

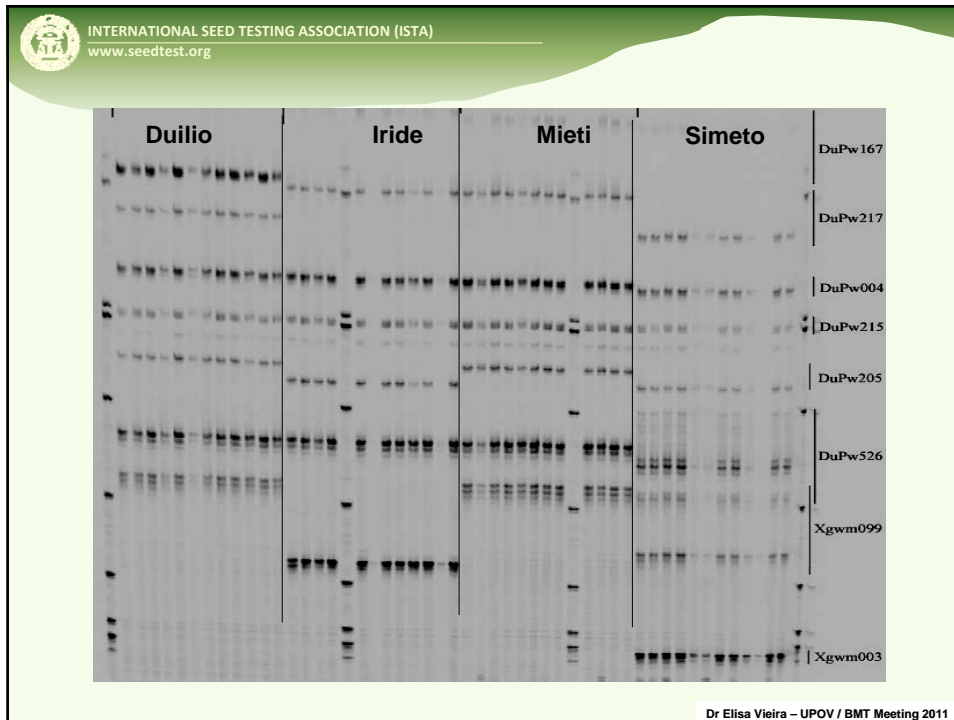
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Summary of results

- SSR were qualified as “good” and “very good”, meaning that repeatable and reproducible results were obtained for that marker during 3 rounds of comparative tests.
- For all crops different number of SSR could be selected
- For most crops, more SSR should be selected so as to identify large sets of varieties.
- Different visualization systems prove to give the same bands patterns (even when the allele sizes may not have been the same).

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Summary of results

- The maize group has established an SSR set that is ready to be applied for the organization of a Performance Test.
- For the Performance Test it is necessary to have a core set of varieties to be used as reference material (RM).
- ISTA is working on a strategy for the development of RM suitable for these tests.
- The PT will allow the development of an appropriate approach for the accreditation of laboratories willing to carry out variety identification tests.

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Further steps

- Once the marker sets have been established, a defined set of “reference varieties” will be identified for each crop that can be used by laboratories that wish to establish protocols for variety verification.
- Following this, the group will work to define a strategy together with ISTA's Statistics Committee for the accreditation of laboratories performing DNA-based variety verification tests.
- Finally, a proficiency testing (PT) program will be established in the future to assist laboratories develop testing capacity for varietal verification for these crops using molecular markers and to promote use of these internationally standardized methods.



Acknowledgement

We would like to acknowledge to all private and public institutions that kindly collaborated with these tests and to ISTA who gave technical and administrative support.

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Thank you for your attention



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