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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

Fifteenth Session

Moscow, Russian Federation, May 24 to 27, 2016

DNA-BASED METHOD FOR VARIETY TESTING: ISTA APPROACH

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

The Annex to this document contains a copy of a presentation “DNA-based method for variety testing: ISTA approach” to be made at its fifteenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in particular (BMT).

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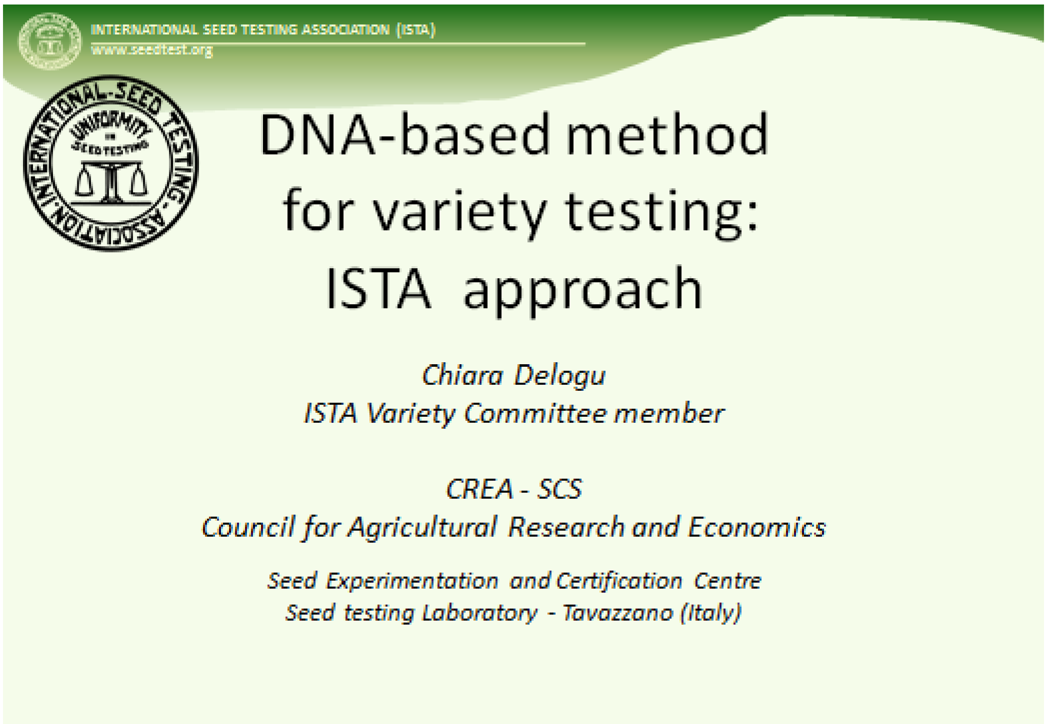
[Annex follows]

ANNEX

DNA-BASED METHOD FOR VARIETY TESTING: ISTA APPROACH



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DNA-based method for variety testing: ISTA approach

Chiara Delogu
ISTA Variety Committee member

CREA - SCS
Council for Agricultural Research and Economics

Seed Experimentation and Certification Centre
Seed testing Laboratory - Tavazzano (Italy)

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- ➢Introduction to ISTA
- ➢ISTA Variety Technical Committee (Variety TCOM), DNA Working Group Activities
- ➢What is coming next

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Following the Articles of ISTA (International Seed Testing Association) the objectives of the Association are:

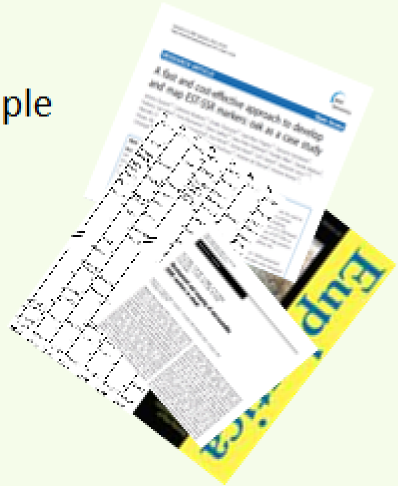
(a) **to develop**, adopt and publish standard procedures for sampling and testing seeds, and **to promote** uniform application of these procedures for evaluation of seeds moving across international trade.

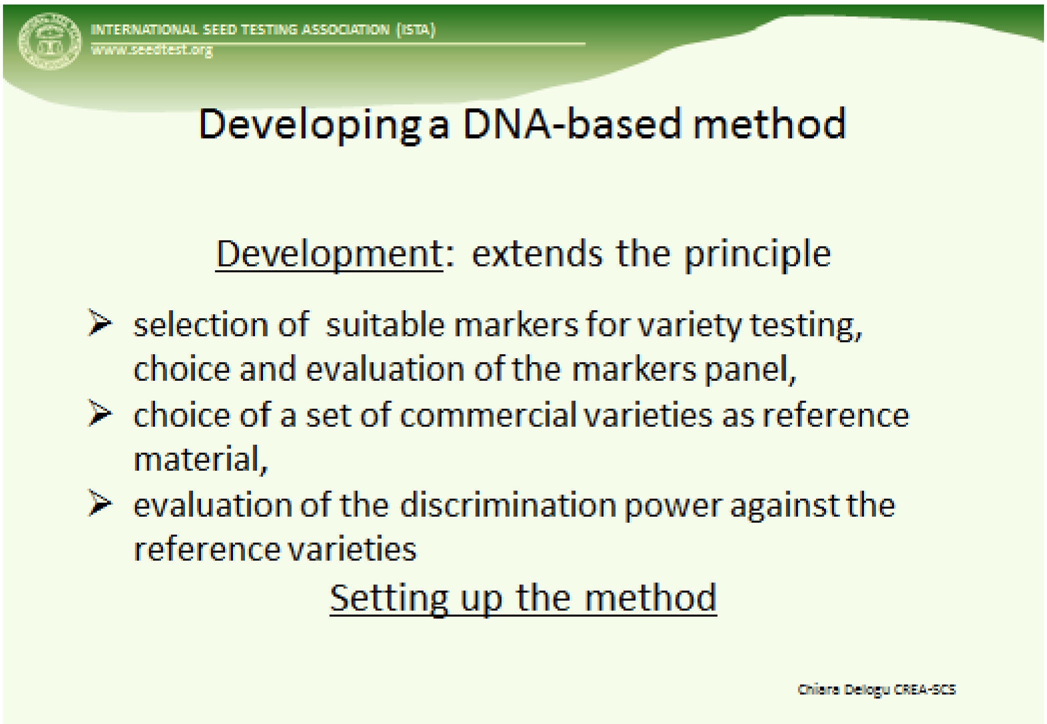
(b) **to promote** research in all areas of seed science and technology (source: ISTA web site)

Developing a DNA-based method

<ins>Research</ins>: establishes the principle

marker development,
phylogenetic studies,
genetic map construction





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Developing a DNA-based method

Development: extends the principle

- selection of suitable markers for variety testing, choice and evaluation of the markers panel,
- choice of a set of commercial varieties as reference material,
- evaluation of the discrimination power against the reference varieties

Setting up the method

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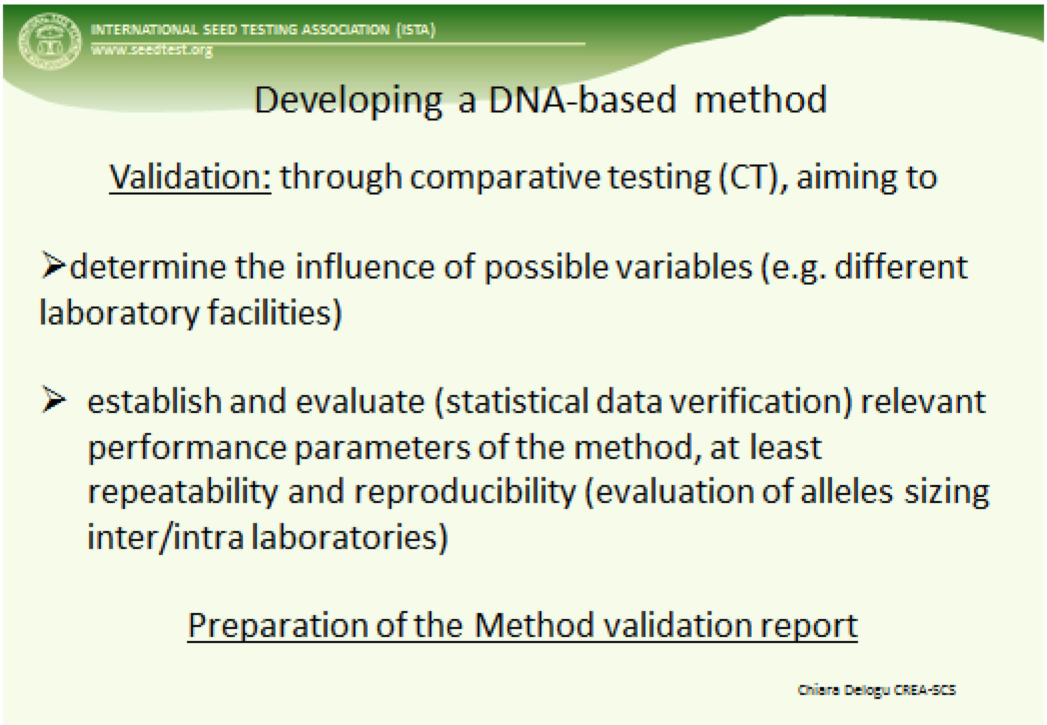

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Developing a DNA-based method

Validation: through comparative testing (CT), aiming to

- determine the influence of possible variables (e.g. different laboratory facilities)
- establish and evaluate (statistical data verification) relevant performance parameters of the method, at least repeatability and reproducibility (evaluation of alleles sizing inter/intra laboratories)

Preparation of the Method validation report

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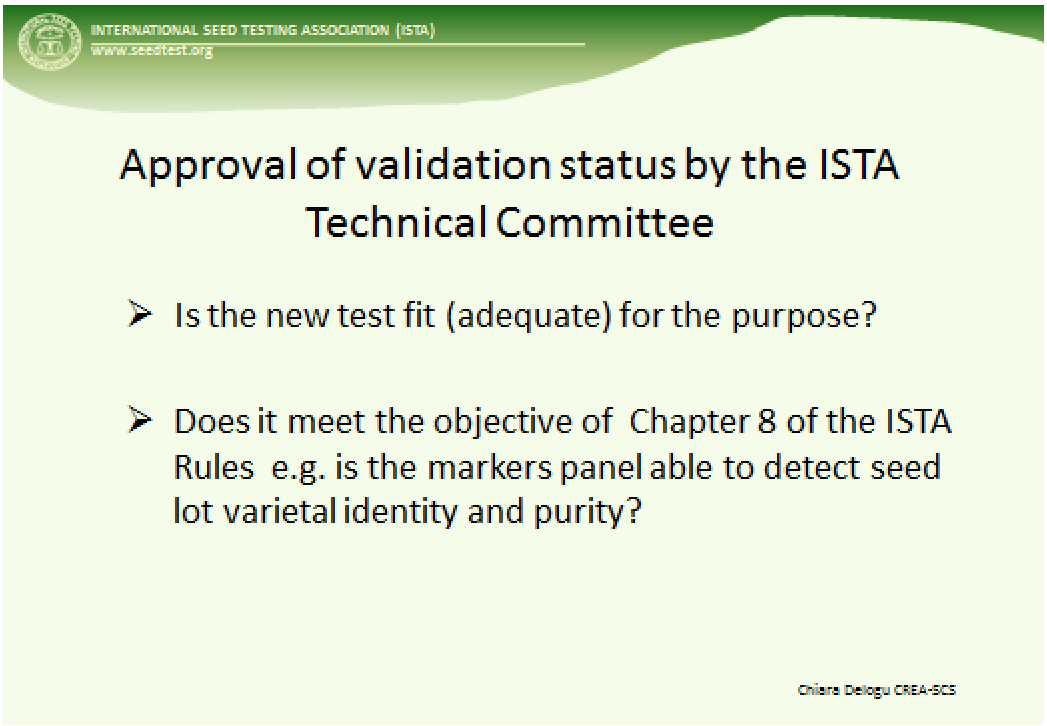

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Approval of validation status by the ISTA Technical Committee

- Is the new test fit (adequate) for the purpose?
- Does it meet the objective of Chapter 8 of the ISTA Rules e.g. is the markers panel able to detect seed lot varietal identity and purity?

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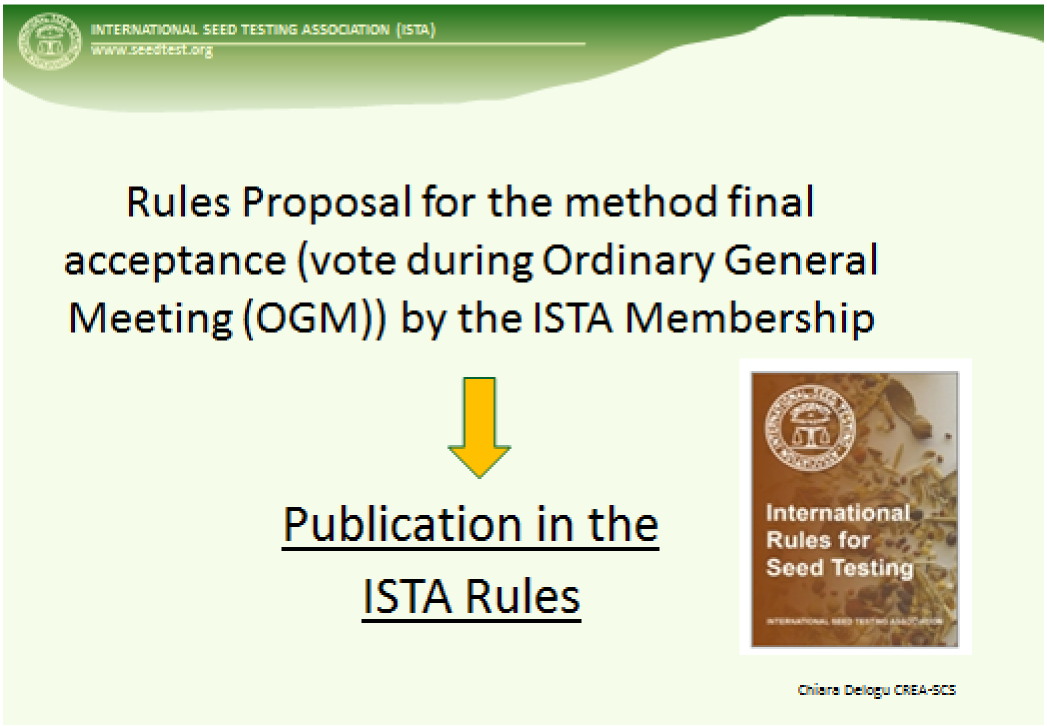

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Rules Proposal for the method final acceptance (vote during Ordinary General Meeting (OGM)) by the ISTA Membership

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<u>Publication in the ISTA Rules</u>

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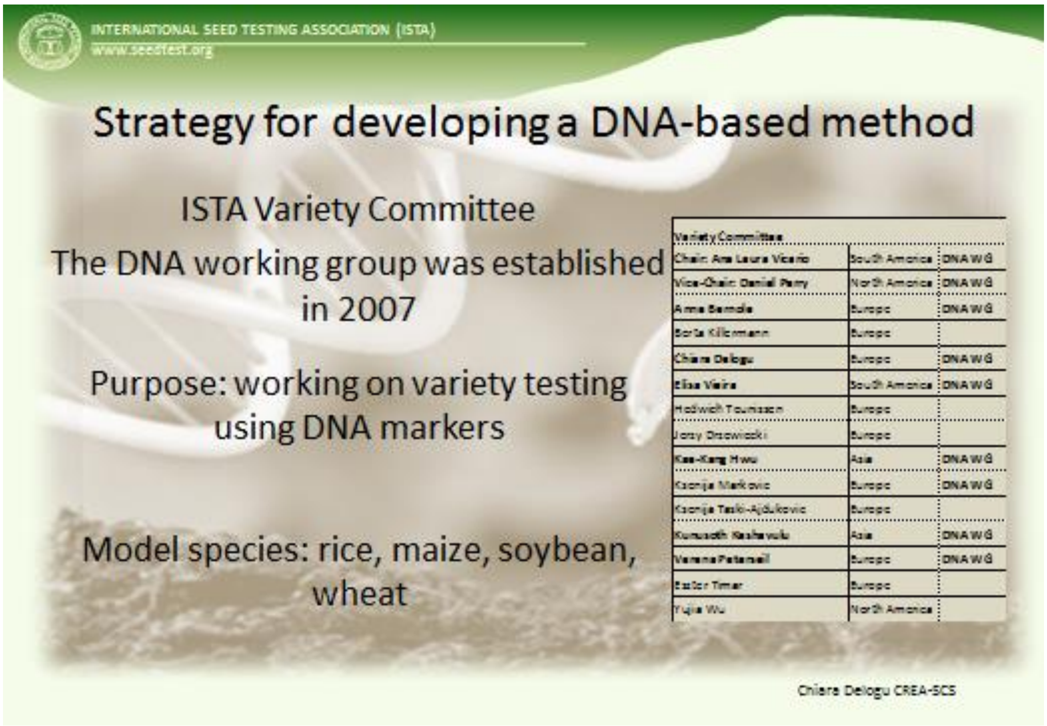

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Strategy for developing a DNA-based method

ISTA Variety Committee

The DNA working group was established in 2007

Purpose: working on variety testing using DNA markers

Model species: rice, maize, soybean, wheat

Variety Committee		
Chair: Ana Laura Vicario	South America	DNA WG
Vice-Chair: Daniel Parry	North America	DNA WG
Anne Bernole	Europe	DNA WG
Berta Killermann	Europe	
Chiara Delogu	Europe	DNA WG
Elisa Vieira	South America	DNA WG
Hedwich Teunissen	Europe	
Jerzy Drozwiecki	Europe	
Kae-Kang Hwu	Asia	DNA WG
Ksenija Markovic	Europe	DNA WG
Ksenija Taski-Ajdukovic	Europe	
Kunusoth Keshavulu	Asia	DNA WG
Verena Peterseil	Europe	DNA WG
Eszter Timar	Europe	
Yujia Wu	North America	

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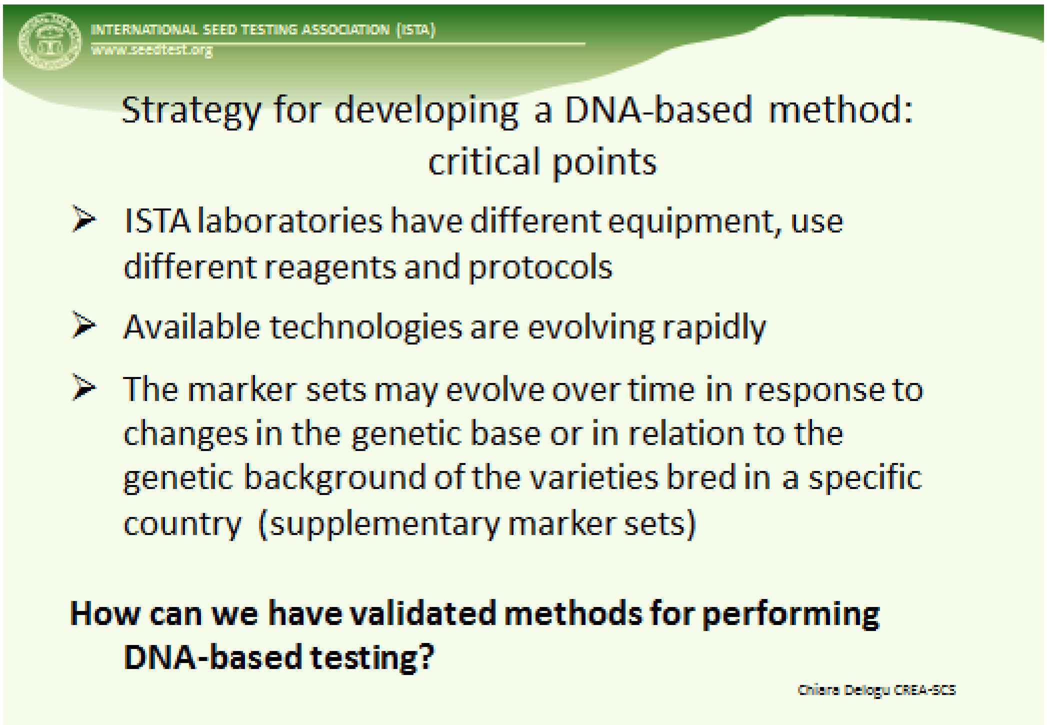

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Strategy for developing a DNA-based method: critical points

- ISTA laboratories have different equipment, use different reagents and protocols
- Available technologies are evolving rapidly
- The marker sets may evolve over time in response to changes in the genetic base or in relation to the genetic background of the varieties bred in a specific country (supplementary marker sets)

How can we have validated methods for performing DNA-based testing?

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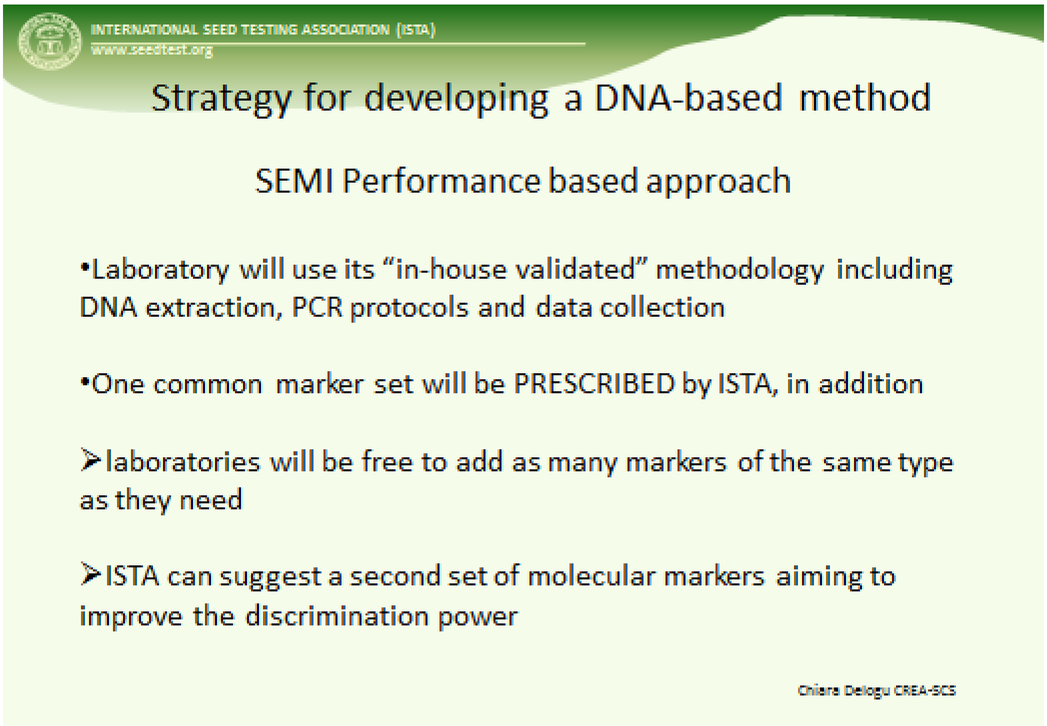

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Strategy for developing a DNA-based method

SEMI Performance based approach

- Laboratory will use its “in-house validated” methodology including DNA extraction, PCR protocols and data collection

- One common marker set will be PRESCRIBED by ISTA, in addition

➢ laboratories will be free to add as many markers of the same type as they need

➢ ISTA can suggest a second set of molecular markers aiming to improve the discrimination power

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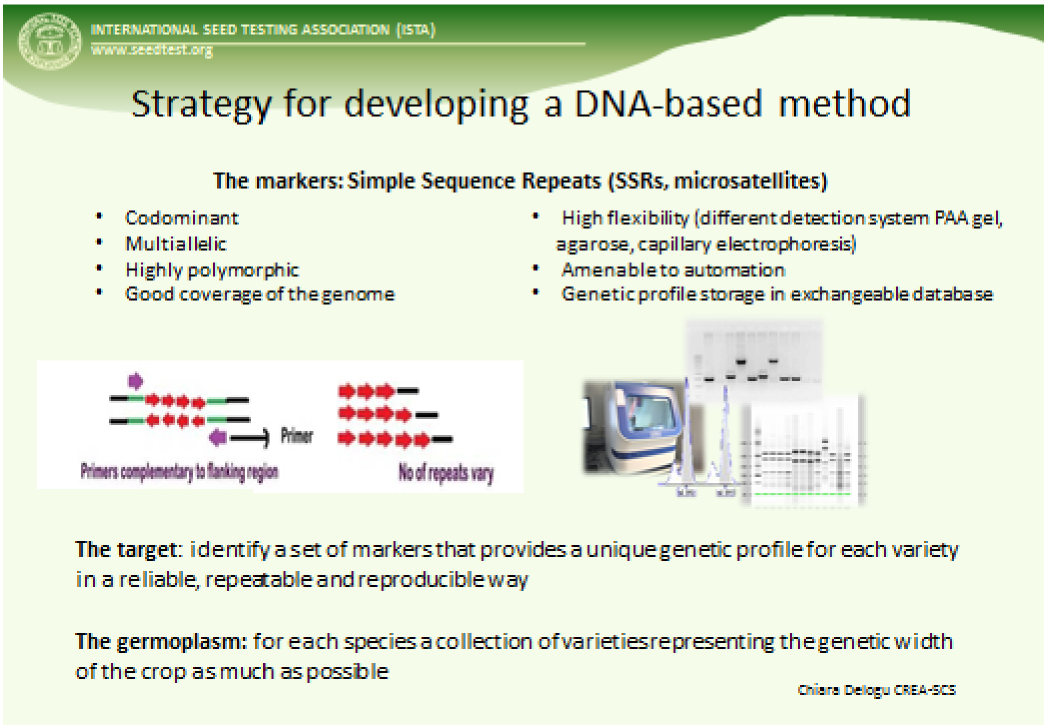

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Strategy for developing a DNA-based method

The markers: Simple Sequence Repeats (SSRs, microsatellites)

- Codominant
- Multiallelic
- Highly polymorphic
- Good coverage of the genome
- High flexibility (different detection system PAA gel, agarose, capillary electrophoresis)
- Amenable to automation
- Genetic profile storage in exchangeable database

The target: identify a set of markers that provides a unique genetic profile for each variety in a reliable, repeatable and reproducible way

The germoplasm: for each species a collection of varieties representing the genetic width of the crop as much as possible

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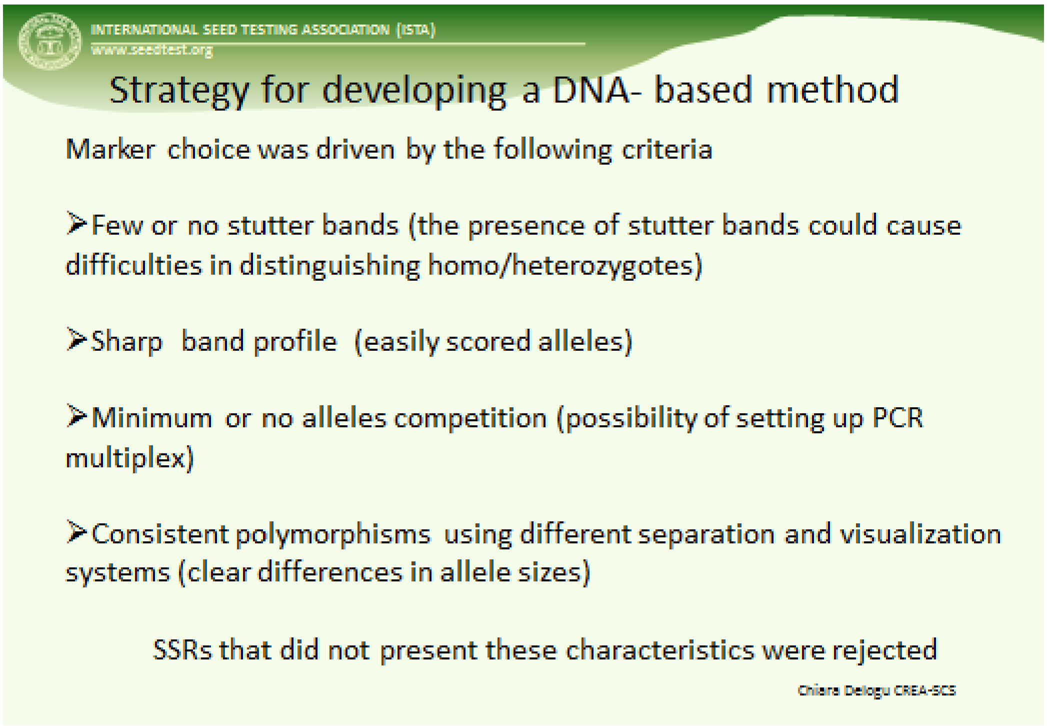

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Strategy for developing a DNA- based method

Marker choice was driven by the following criteria

- Few or no stutter bands (the presence of stutter bands could cause difficulties in distinguishing homo/heterozygotes)
- Sharp band profile (easily scored alleles)
- Minimum or no alleles competition (possibility of setting up PCR multiplex)
- Consistent polymorphisms using different separation and visualization systems (clear differences in allele sizes)

SSRs that did not present these characteristics were rejected

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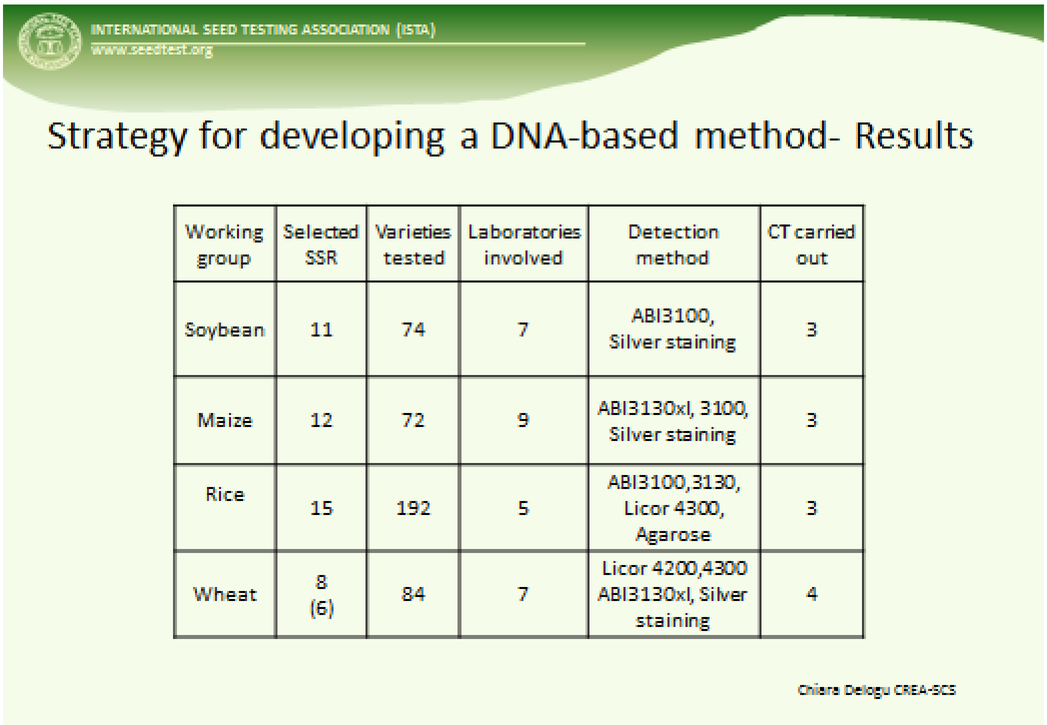

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Strategy for developing a DNA-based method- Results

Working group	Selected SSR	Varieties tested	Laboratories involved	Detection method	CT carried out
Soybean	11	74	7	ABI3100, Silver staining	3
Maize	12	72	9	ABI3130xl, 3100, Silver staining	3
Rice	15	192	5	ABI3100,3130, Licor 4300, Agarose	3
Wheat	8 (6)	84	7	Licor 4200,4300 ABI3130xl, Silver staining	4

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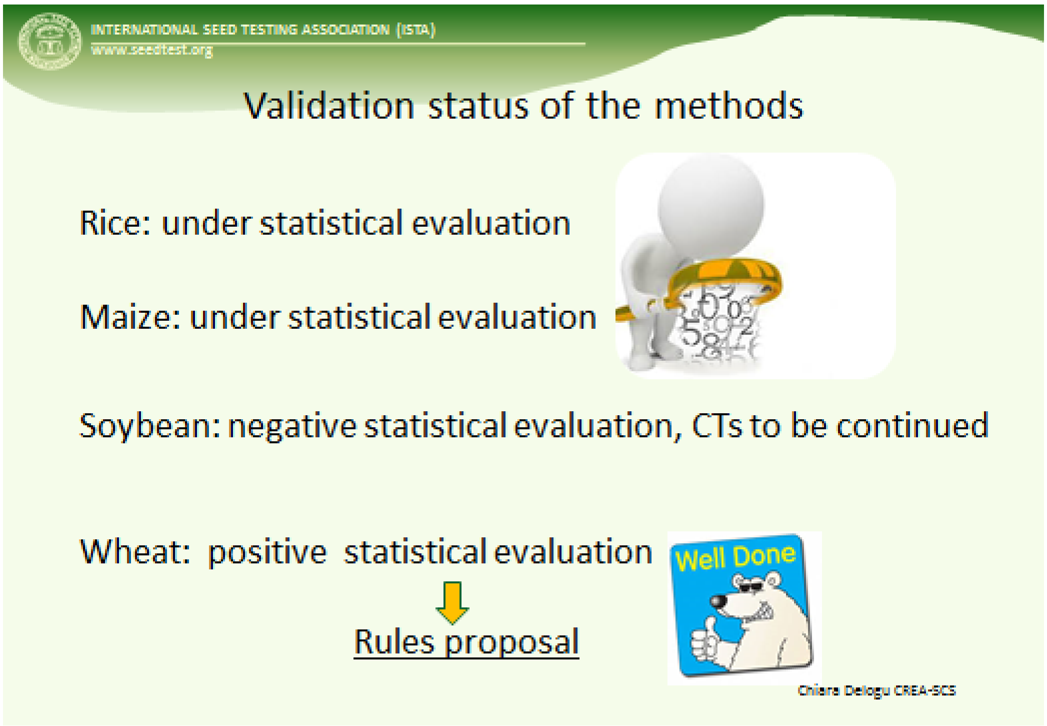

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Validation status of the methods

Rice: under statistical evaluation

Maize: under statistical evaluation

Soybean: negative statistical evaluation, CTs to be continued

Wheat: positive statistical evaluation

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Rules proposal

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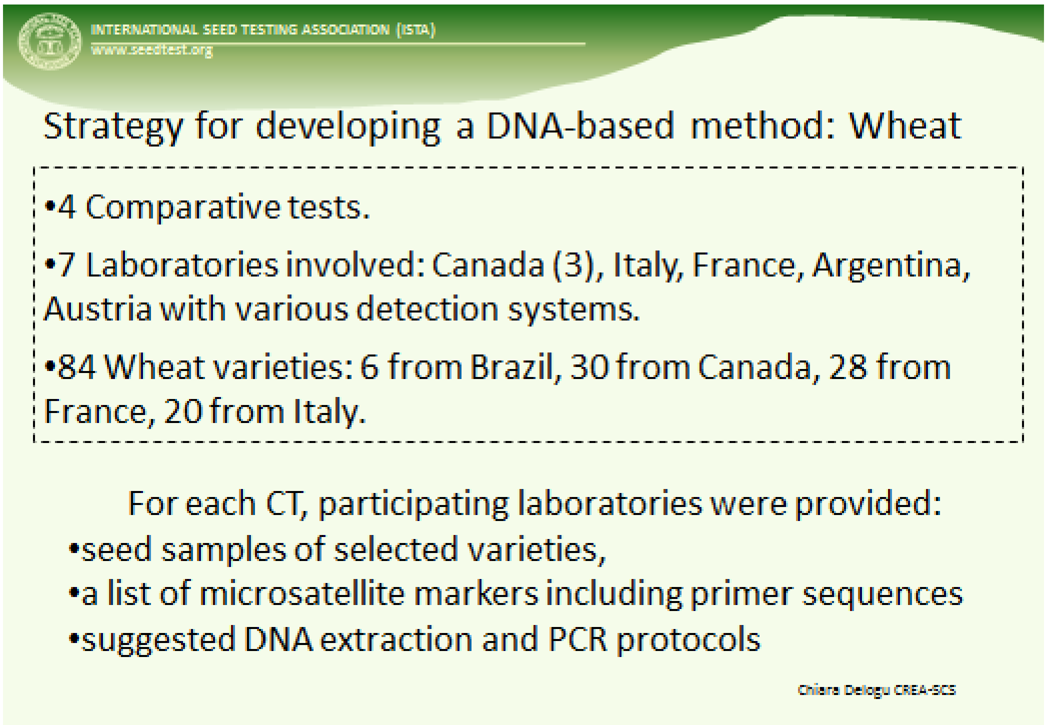

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Strategy for developing a DNA-based method: Wheat

- 4 Comparative tests.
- 7 Laboratories involved: Canada (3), Italy, France, Argentina, Austria with various detection systems.
- 84 Wheat varieties: 6 from Brazil, 30 from Canada, 28 from France, 20 from Italy.

For each CT, participating laboratories were provided:

- seed samples of selected varieties,
- a list of microsatellite markers including primer sequences
- suggested DNA extraction and PCR protocols

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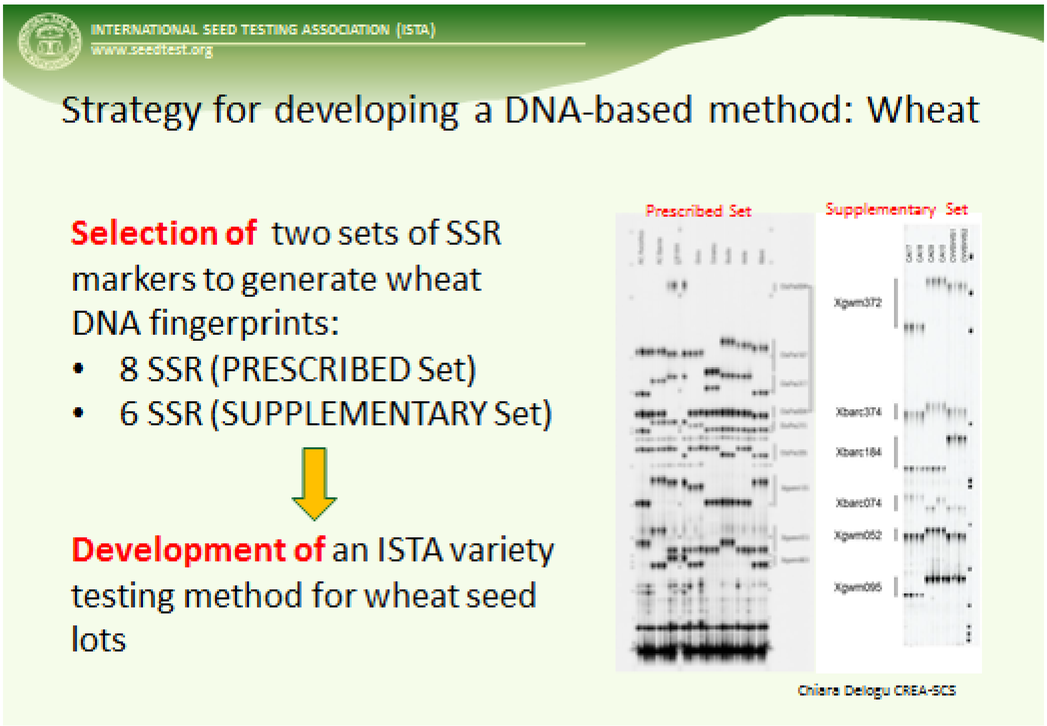

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Strategy for developing a DNA-based method: Wheat

Selection of two sets of SSR markers to generate wheat DNA fingerprints:

- 8 SSR (PRESCRIBED Set)
- 6 SSR (SUPPLEMENTARY Set)

Development of an ISTA variety testing method for wheat seed lots

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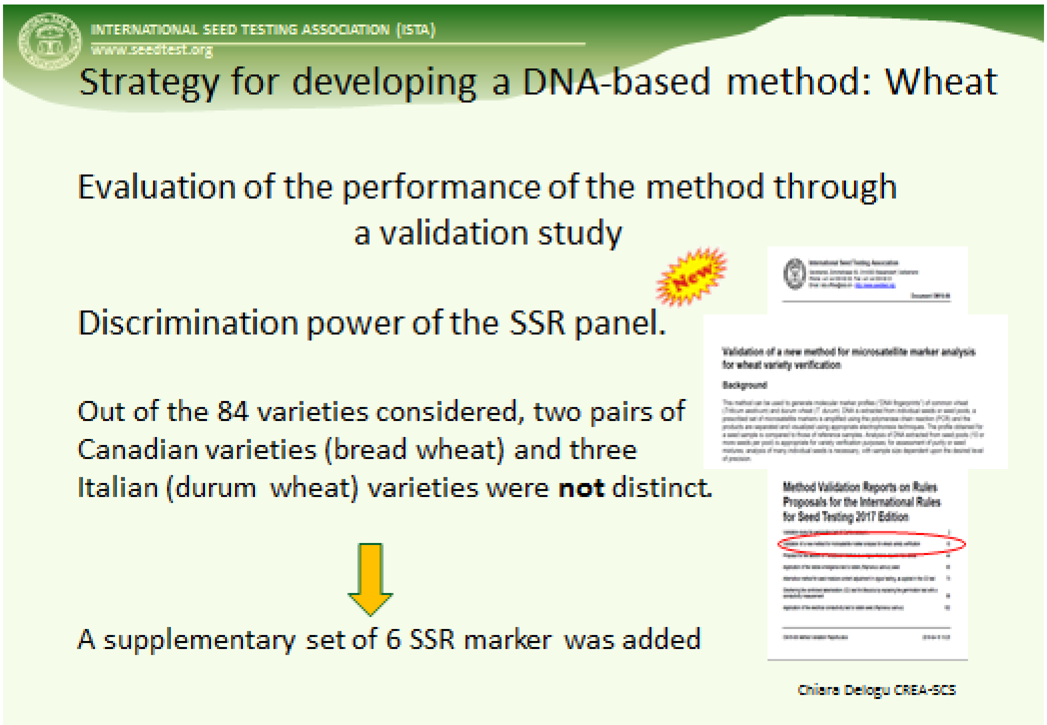

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Strategy for developing a DNA-based method: Wheat

Evaluation of the performance of the method through a validation study

Discrimination power of the SSR panel.

Out of the 84 varieties considered, two pairs of Canadian varieties (bread wheat) and three Italian (durum wheat) varieties were **not** distinct.

A supplementary set of 6 SSR marker was added

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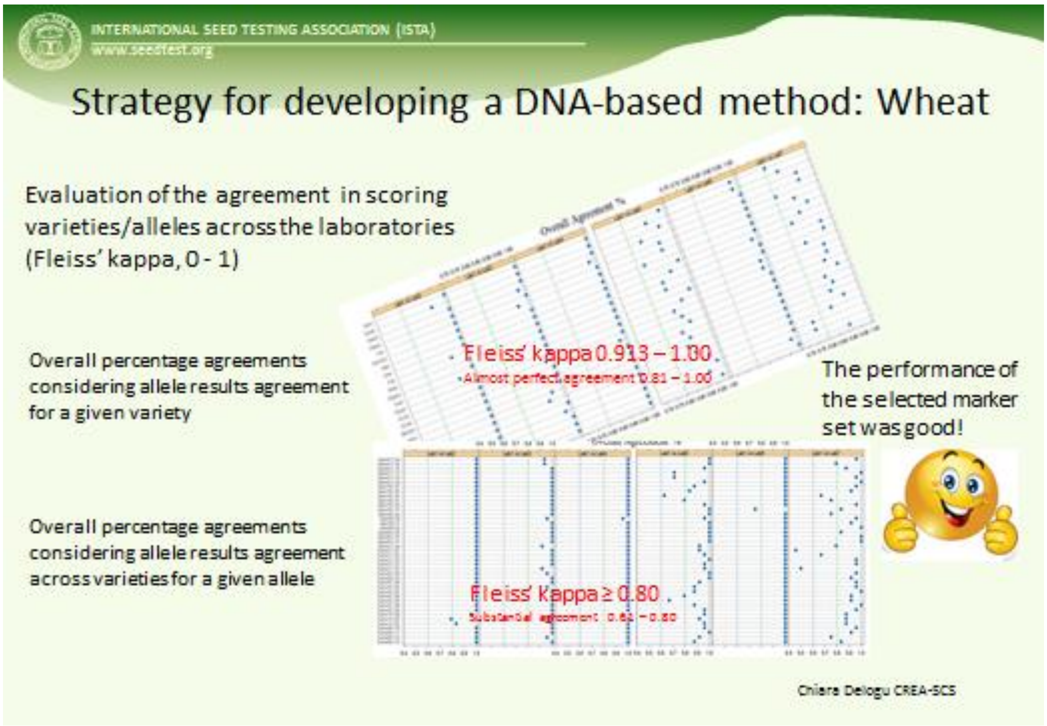

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Strategy for developing a DNA-based method: Wheat

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

Fleiss' kappa 0.913 – 1.00
Almost perfect agreement 0.81 – 1.00

Overall percentage agreements considering allele results agreement across varieties for a given allele

Fleiss' Kappa ≥ 0.80
Substantial agreement 0.61 – 0.80

The performance of the selected marker set was good!

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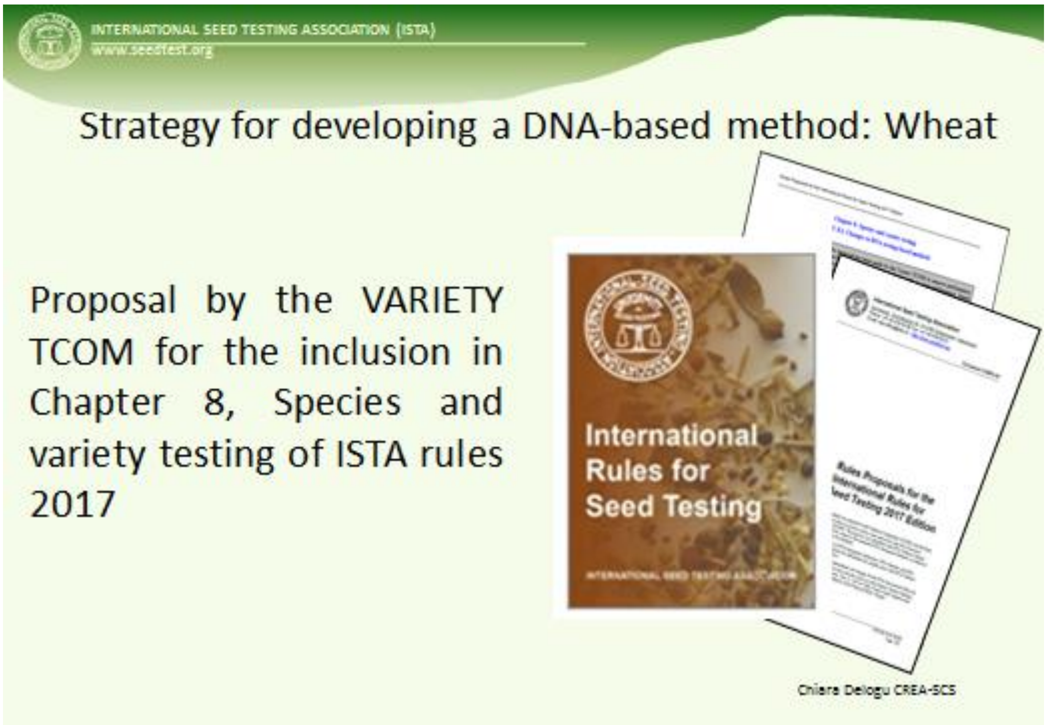

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Strategy for developing a DNA-based method: Wheat

Proposal by the VARIETY TCOM for the inclusion in Chapter 8, Species and variety testing of ISTA rules 2017

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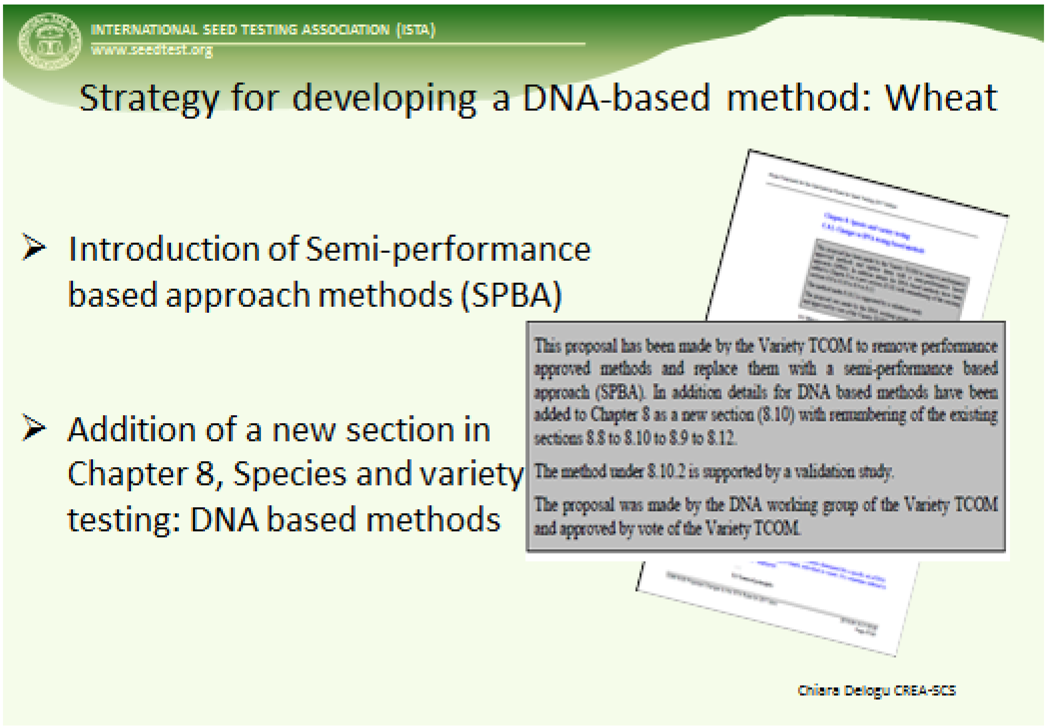

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Strategy for developing a DNA-based method: Wheat

- Introduction of Semi-performance based approach methods (SPBA)
- Addition of a new section in Chapter 8, Species and variety testing: DNA based methods

This proposal has been made by the Variety TCOM to remove performance approved methods and replace them with a semi-performance based approach (SPBA). In addition details for DNA based methods have been added to Chapter 8 as a new section (8.10) with renumbering of the existing sections 8.8 to 8.10 to 8.9 to 8.12.

The method under 8.10.2 is supported by a validation study.

The proposal was made by the DNA working group of the Variety TCOM and approved by vote of the Variety TCOM.

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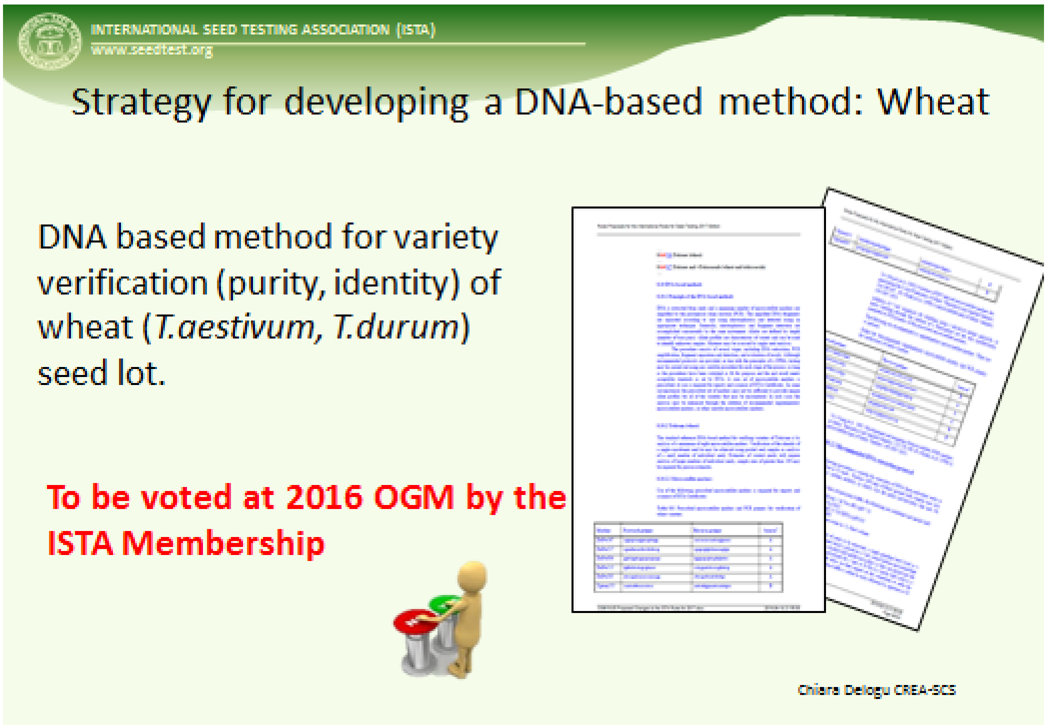

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Strategy for developing a DNA-based method: Wheat

DNA based method for variety verification (purity, identity) of wheat (*T.aestivum, T.durum*) seed lot.

To be voted at 2016 OGM by the ISTA Membership

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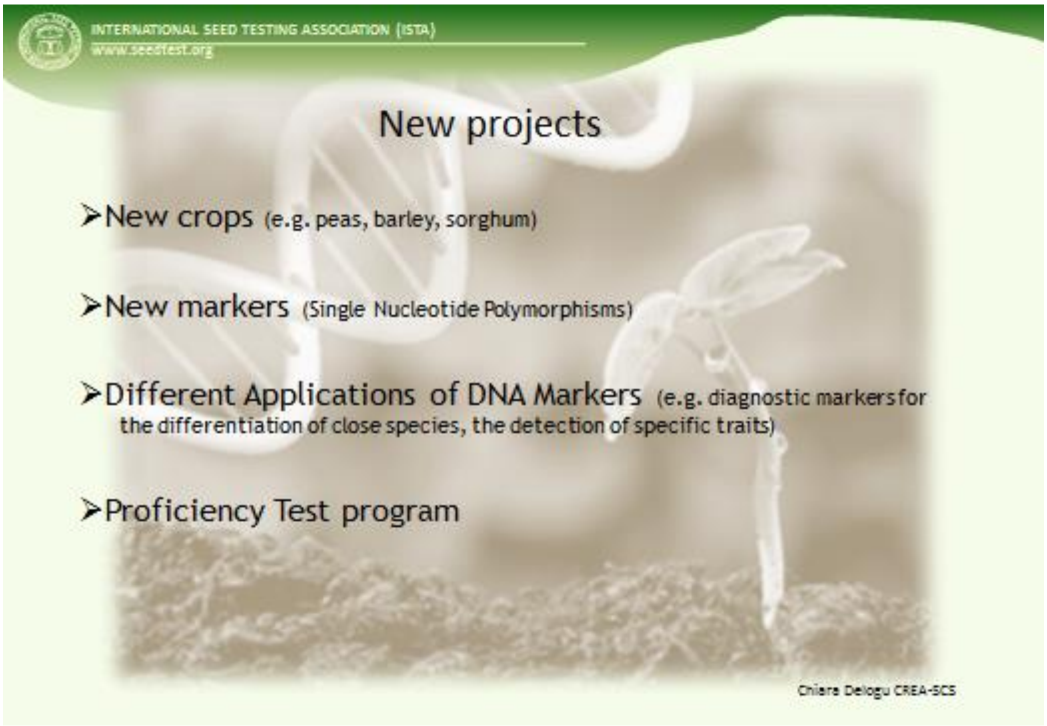

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New projects

- New crops (e.g. peas, barley, sorghum)
- New markers (Single Nucleotide Polymorphisms)
- Different Applications of DNA Markers (e.g. diagnostic markers for the differentiation of close species, the detection of specific traits)
- Proficiency Test program

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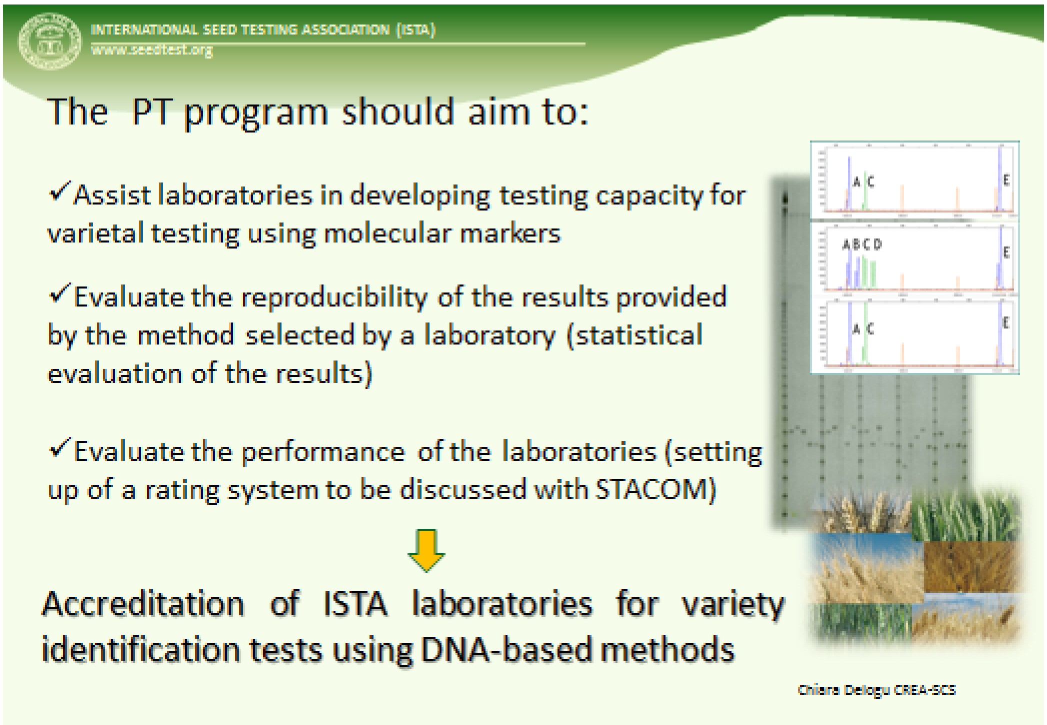

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The PT program should aim to:

✓Assist laboratories in developing testing capacity for varietal testing using molecular markers

✓Evaluate the reproducibility of the results provided by the method selected by a laboratory (statistical evaluation of the results)

✓Evaluate the performance of the laboratories (setting up of a rating system to be discussed with STACOM)

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Accreditation of ISTA laboratories for variety identification tests using DNA-based methods

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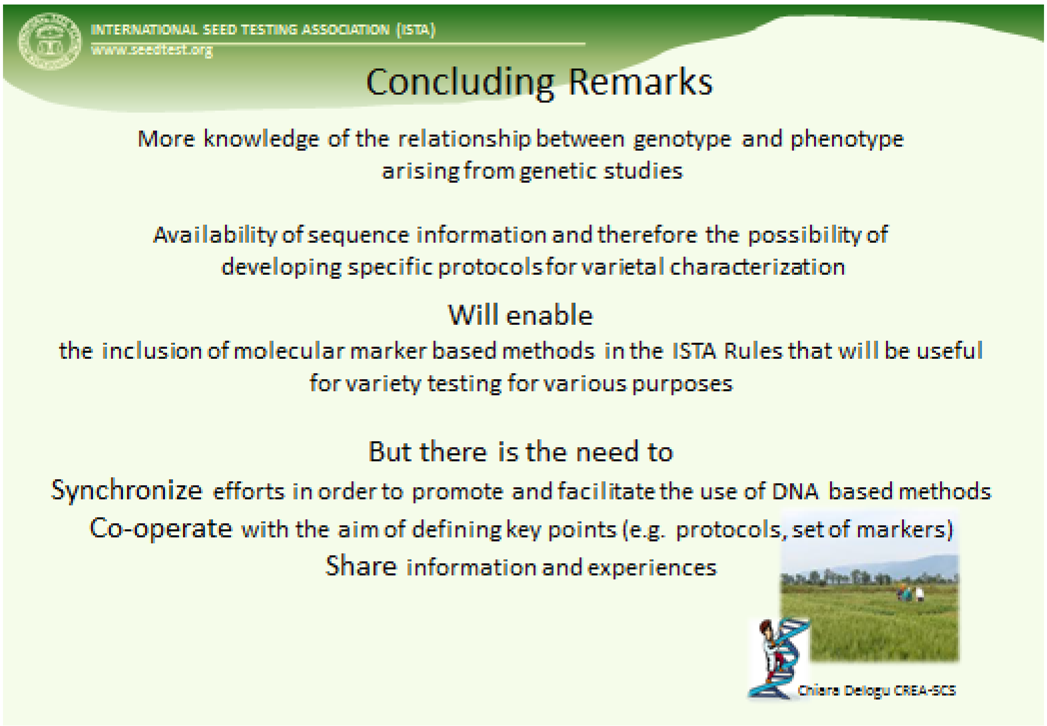

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Concluding Remarks

More knowledge of the relationship between genotype and phenotype arising from genetic studies

Availability of sequence information and therefore the possibility of developing specific protocols for varietal characterization

Will enable

the inclusion of molecular marker based methods in the ISTA Rules that will be useful for variety testing for various purposes

But there is the need to

Synchronize efforts in order to promote and facilitate the use of DNA based methods

Co-operate with the aim of defining key points (e.g. protocols, set of markers)

Share information and experiences

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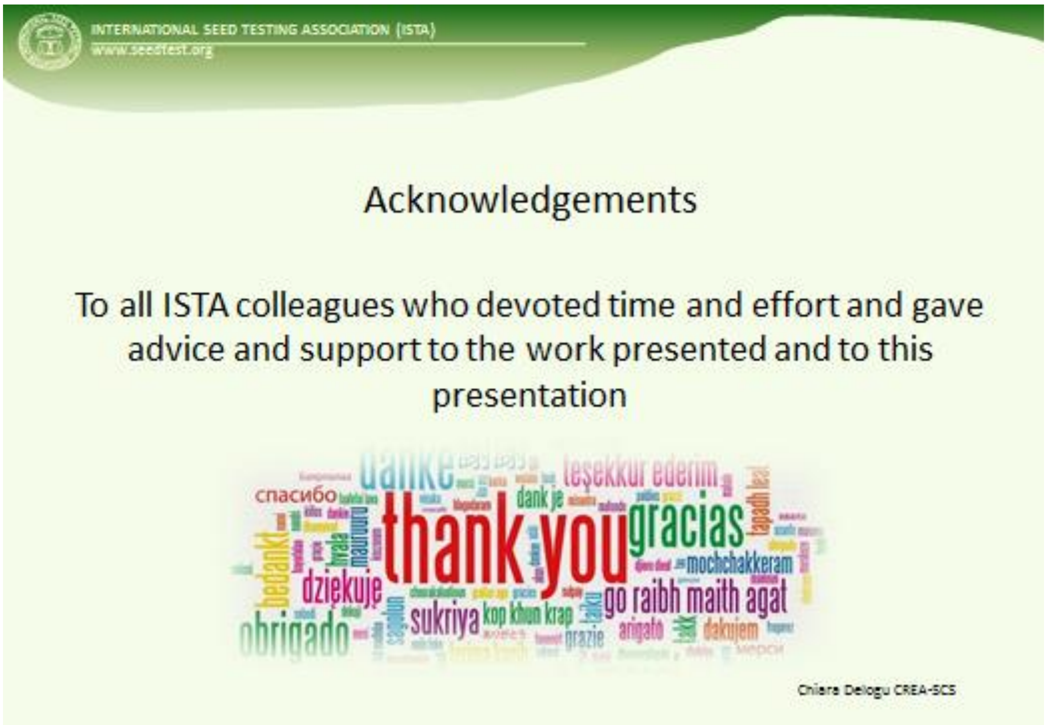

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Acknowledgements

To all ISTA colleagues who devoted time and effort and gave advice and support to the work presented and to this presentation

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