

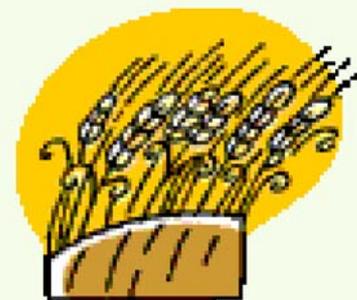


# DNA-based methods for variety testing

## Status report from the ISTA perspective

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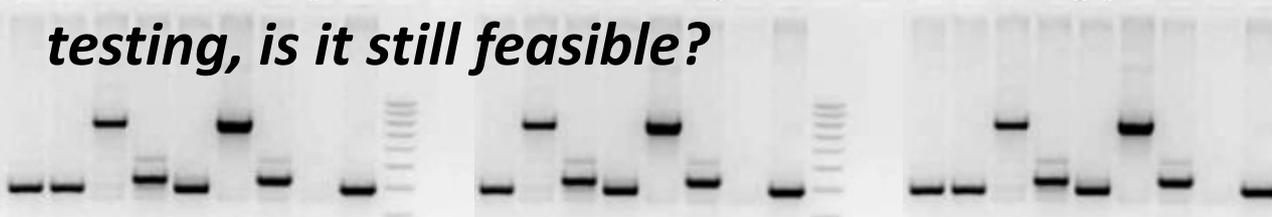


## Background

The primary purpose of ISTA is 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 in international trade.

***With the implementation of biotechnology in seed testing, is it still feasible?***





## Background

DNA-based methods are crucial in the framework of seed testing technical evolution.

ISTA laboratories have different equipment, use different reagents and protocols.

Available technologies progress quickly.

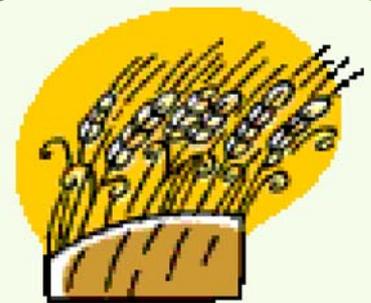
*How can we guarantee both uniformity and standardization?*



**The key factor is the reproducibility of the results.**



## Summary



Since 2009, the DNA working group of the ISTA Variety Committee has been working on variety characterization in maize, wheat, soybean and rice.

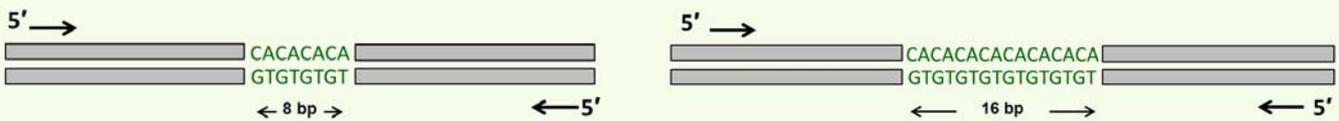
The key point was to define for each species a set of varietal markers, which make the varietal identification reliable, repeatable and reproducible.



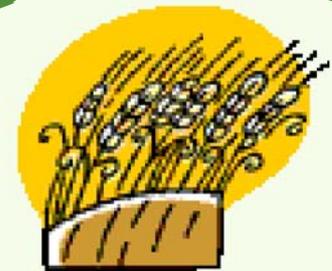
# Which markers are the most suitable for variety identification?

## MICROSATELLITES (SSRs):

- highly polymorphic, co-dominant, very informative;
- publicly available;
- technology less expensive than others (e.g. SNPs).



## Available now



- A suitable set of SSRs for each species (maize, soybean, wheat, rice) able to discriminate a number of popular varieties has been identified.
- For some crops a different number of SSRs could be selected in order to identify a larger set of varieties.
- Different visualization systems give the same bands patterns (even if allele sizes are slightly different).

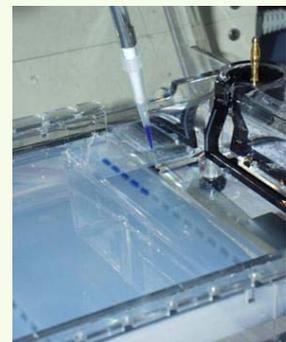


## The next steps

- ISTA could offer Proficiency Tests (PTs) for DNA based markers.
- The PTs would provide the basis to accredit ISTA laboratories wanting to carry out variety identification tests using DNA-based methods.

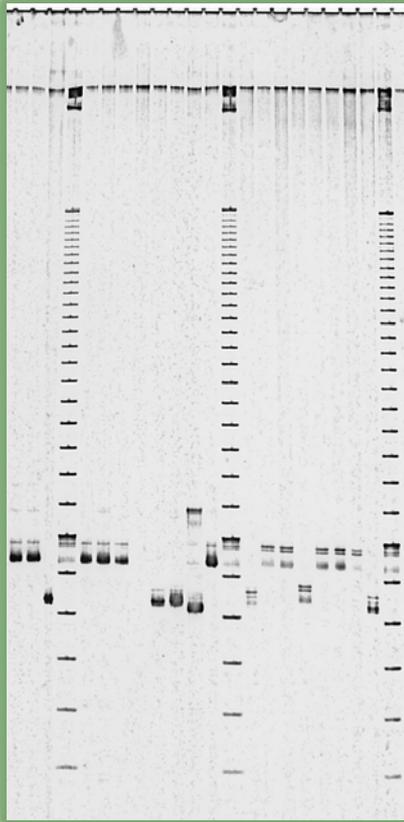


## The next steps



### The PT program would 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 the laboratory;
- evaluate the performance of laboratories;
- help in including DNA based methods in the ISTA Rules and in the Scope of ISTA Accreditation.



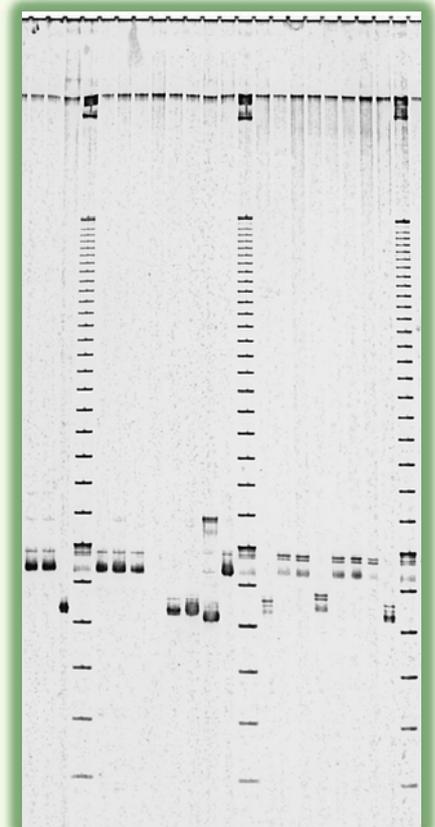
## The PT program needs:

- a set of reference varieties to be used as reference materials (RMs);
- RMs should represent the allele variability within the species (~ 30 varieties/crop);
- RMs should be pure seed (e.g. basic/pre-basic seed);
- RMs distributed to the laboratories may be represented by devitalized seeds or flour.



## The PT program would:

- distribute unknown varieties;
- provide 2 known varieties (with the description of the expected allele profiles), intended as control of performance factors (DNA extraction, PCR performance, electrophoresis conditions, comparison of allele sizes);
- provide the alleles recognition key.

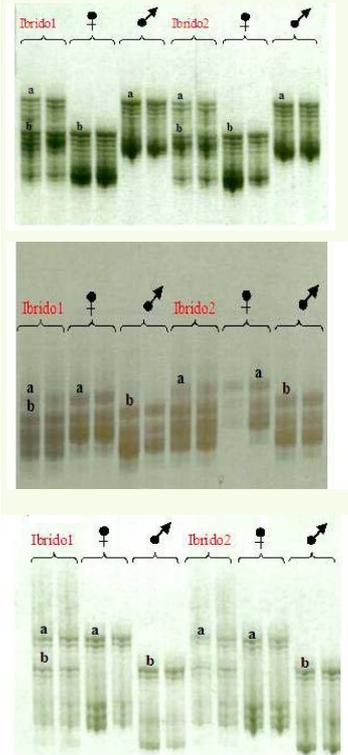




## Challenges

**Collecting RMs and organizing the PTs is not easy. Needs:**

- identifying RMs (pure seed) suppliers;
- financing RMs supply;
- storing RMs over several years;
- organizing the PTs, e.g. preparing the PT samples, sending to the participating labs.



## ISTA and UPOV

UPOV and ISTA work on the same topics (seeds!) focusing on:

UPOV - breeder rights → variety characterization

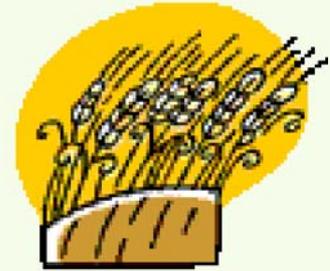
ISTA - laboratory protocols → seed testing

Laboratories are often involved in both variety characterization and seed testing.





## UPOV AND ISTA



Ought to

- synchronize the efforts in order to promote and facilitate the use of DNA based method in variety testing for the different purposes;
- co-operate in the aim to define agreed key points, hopefully (a) unique protocol/s (e.g. definition of a minimum agreed set of markers for each species);
- share information, save time and resources.



### Discussion Forum

(17<sup>th</sup> June 2013 – 13.30/14.30)



*“Discussion on the use and international standardisation of DNA technologies in the area of seeds”* (Chairs: Berta Killermann and Cheryl Dollard)

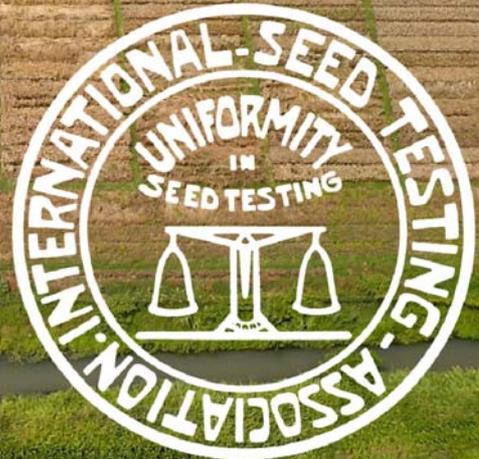
- 1) Overview of DNA technologies – current uses and applications.
- 2) Required elements of international standardization.
- 3) Use of Internationally standardized DNA technologies in seed certification/variety registration.

## Acknowledgement

A special thanks to Emanuela Casarini (leader of the DNA Working Group), Berta Killermann (chair of the VAR Committee), Chiara Delogu (member of the VAR Committee) , for their support for this presentation.

ISTA would also like to acknowledge to all private and public institutions that kindly collaborated with these tests and to ISTA colleagues who gave advise and support.

**Thank you for your attention**



[www.seedtest.org](http://www.seedtest.org)