E

BMT/13/22 Add.
ORIGINAL: English
DATE: December 8, 2011

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

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

Thirteenth Session
Brasilia, November 22 to 24, 2011

ADDENDUM

AN OVERVIEW OF DNA-BASED METHODS FOR VARIETY IDENTIFICATION AT INRAN-ENSE (ITALIAN SEED CERTIFICATION AGENCY)

Document prepared by experts from Italy

AN OVERVIEW OF DNA- BASED METHOD IN VARIETY IDENTIFICATION AT INRAN-ENSE THE ITALIAN SEED CERTIFICATION AGENCY

C. Delogu*, L. Andreani * , P.G. Bianchi**,R. Zecchinelli*
INRAN-ENSE-Seed Testing Laboratory*,
INRAN-ENSE- General Affairs Office**

Italian Ministry of Agriculture

INRAN-ENSE SEED DEPARTMENT

INRAN-ENSE is the governmental seed certification agency
•Variety registration
•Seed certification of agricultural and horticultural species
•Science-based technical and commercial services to government, the seeds industry and farmers
•Independent science-based research

The Seed Testing Laboratory located in Tavazzano, carries out

•TRADITIONAL SEED TESTING
•GMO TESTING
•VARIETAL TESTING

Accredited by the International Seed Testing Association (ISTA) and member of European Network of GMO Laboratories (ENGL)

Use of molecular markers in seed testing

- Support to traditional analysis;
- Evaluation of varietal identity;
- Evaluation of purity of seed lots;
- Variety characterization;

C. Delogu INRAN-ENSE

Advantages of MM in seed testing

- Flexibility of PCR technique → different kind of markers
- Wide range of species
- DNA extraction from different kind of tissue
  → analysis in different step of seed production and seed testing
- Published sources are available to collect technical information about markers, sequences and level of polymorphism in different species

C. Delogu INRAN-ENSE

But it is necessary to define

- Purpose of each analysis;
- Genetic characteristic of the crop (hybrid, inbred line, self o cross pollinated variety);
- Standard varieties;
- Type of molecular marker;
- Number of markers/their distribution throughout the genome;
- Number of individuals;
- Threshold of polymorphism.

EXPERIMENTAL PLAN

C. Delogu INRAN-ENSE

Our experiences

The laboratory has tested some MM, with different purposes:

✓**Support to traditional analysis**

✓Identity and Purity

✓Variety characterization

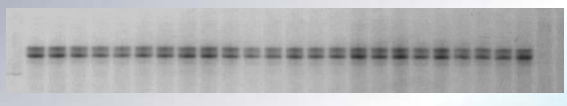
C. Delogu INRAN-ENSE

Support to traditional analysis - 1

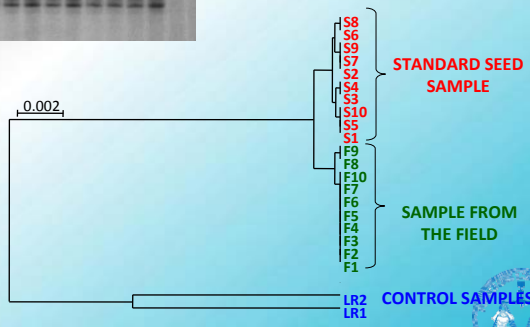
MAIZE INBRED LINE IDENTITY

Discrepancy between official description and some morphological characteristics influenced by the enviroment

12 SSR NO POLYMORPHISM



4 AFLP PRIMER COMBINATIONS: 191 BANDS, 6% POLYMORPHICS



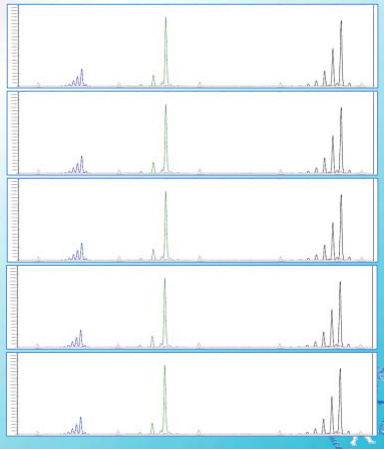
C. Delogu INRAN-ENSE

Support to traditional analysis - 2

RICE GENETIC UNIFORMITY

During the certification controls a different level of seed pigmentation was observed.

11 SSR not discriminate for pigmentation in rice seed



After a visual analysis of representative samples, the different state of expression was confirmed. Microsatellite analysis to evaluate the genetic uniformity was done.

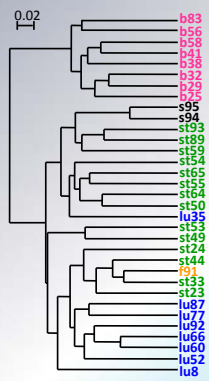
C. Delogu INRAN-ENSE

Support to traditional analysis – 4

OAT SPECIES IDENTIFICATION

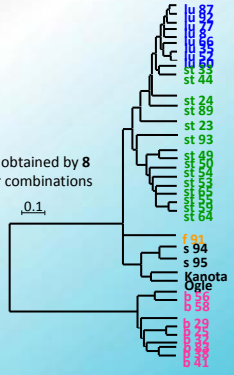
In seed testing, identification of the different *Avena* species is often a major issue and the laboratory is asked to identify the different species.

Dendogram obtained by **28 SSRs**



A. Ludoviciana
A. sterilis
A. Barbata
A. Fatua
A. sativa

Dendogram obtained by **8 AFLP** primer combinations



AFLP dendrogram grouped the species in good agreement with morphological data

C. Delogu INRAN-ENSE

Our experiences

The laboratory has tested some MM, with different purposes:

✓Support to traditional analysis

✓**Identity and Purity**

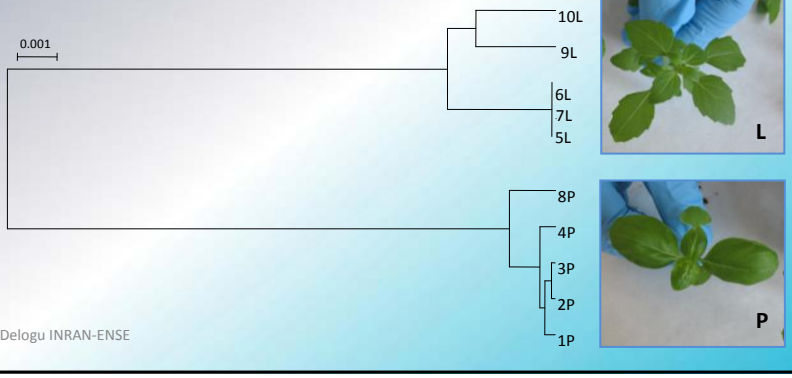
✓Variety characterization

C. Delogu INRAN-ENSE

Identity and Purity – 1

BASIL VARIETY IDENTIFICATION

Verification of the genetic difference among individual plantlets from a nursery with different leaf morphological characteristics and aroma by using AFLP marker.

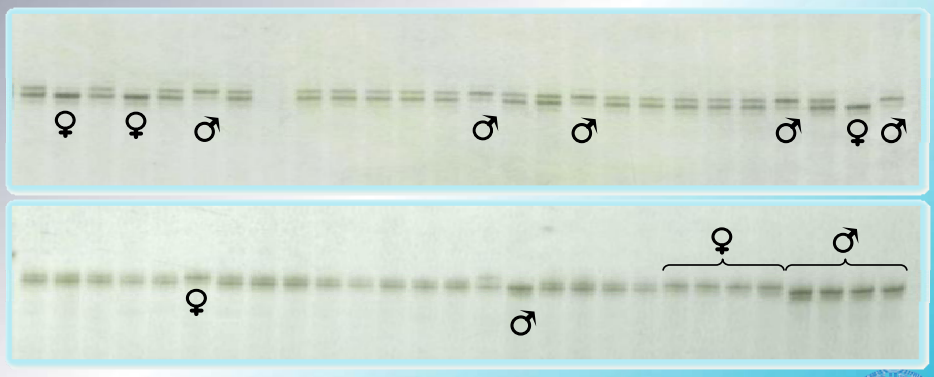


C. Delogu INRAN-ENSE

Identity and Purity – 1

TOMATO CONTROL OF HYBRID SEED LOTS

SSR markers have been used to describe parental lines and to evaluate the genetic quality of hybrid seed production by evaluating the presence of self-pollinated individuals in the seed lots before marketing.



C. Delogu INRAN-ENSE

Our experiences

The laboratory has tested some MM, with different purposes:

- ✓Support to traditional analysis
- ✓Identity and Purity
- ✓**Variety characterization**

C. Delogu INRAN-ENSE

Variety characterization -1

CHARACTERIZATION OF LOCAL VEGETABLES LANDRACES

The heterogeneity of environmental and climatic conditions favoured the selection of a great number of vegetables landraces in Italy. Due to the reduced cultivation, many of them are facing the risk of extinction.

The laboratory is involved with local groups and other research organizations in recovering of some of these old varieties with the end goal of their inclusion in the conservation varieties catalogue.

- ✓ Berretta da prete" (pumpkin)
- ✓ "Peperone di Voghera" (pepper)
- ✓ "Cipolla di Breme" (onion)

C. Delogu INRAN-ENSE

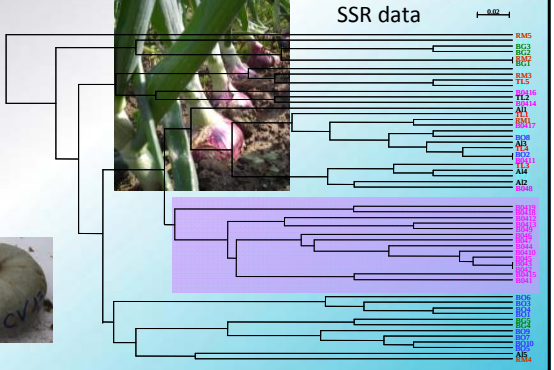
Variety characterization -1

CHARACTERIZATION OF LOCAL VEGETABLES LANDRACES

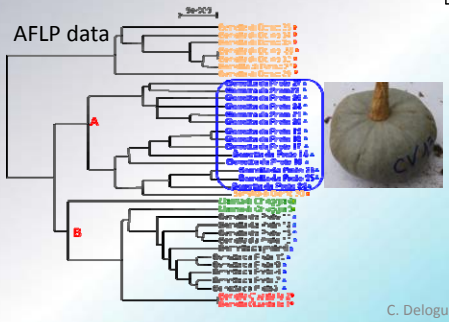
Morphological, biochemical and molecular data were used to characterize the traditional cultivar.

AFLP and SSR data are an effective tool to support their recovery and maintenance.

SSR data



AFLP data



C. Delogu INRAN-ENSE

Variety characterization -2

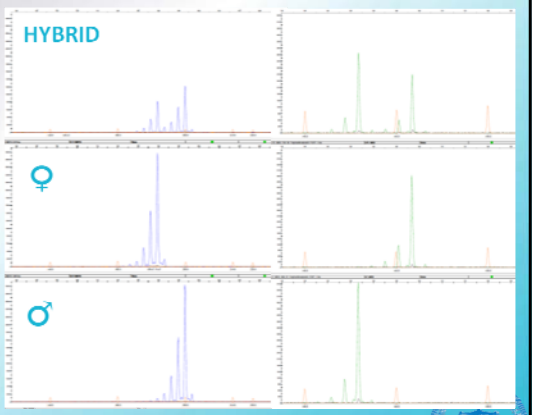
Hybrid rice characterization

In the framework of the revision of the national protocol for new rice variety listing, hybrid rice SSR profiling was proposed. Microsatellite markers are very suitable for controlling the hybrid formula.

12 SSRs (1/chromosome) were selected.

A protocol with forward primer M13 tailed was considered to reduce dye labelled primer costs.

As a future development, this SSR panel will be used to test all the varieties of the rice reference collection. The final purpose is to use it to characterize rice new varieties and include DNA-profiles as complementary information in the official description for the National rice catalogue.



C. Delogu INRAN-ENSE

Conclusion

Molecular markers would present a valuable addition to the DUS and seed testing procedures. However, their implementation still depends upon resolving the issues of:

✓defining the required number and

✓selecting the appropriate set of markers,

✓setting the threshold values for distinctness and uniformity.

Some technical instrument would be very useful to standardize the procedures and to evaluate the results:

✓Official protocols;

✓Molecular markers database;

✓Methods for statistical evaluation.

C. Delogu INRAN-ENSE

Thank you!

C. Delogu INRAN-ENSE

[End of document]