



BMT/11/19 Add.

ORIGINAL: English

DATE: September 30, 2008

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

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

Eleventh Session
Madrid, September 16 to 18, 2008

ADDENDUM

IDENTIFICATION SYSTEM FOR SOYBEAN BASED ON THE MOST FREQUENT SSR
ALLELES

Document prepared by experts from Argentina

IDENTIFICATION SYSTEM FOR SOYBEAN BASED ON THE MOST FREQUENT SSR ALLELES



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BACKGROUND



- It is of great importance for **offices** granting plant breeders' rights to have the possibility to **adequately control the seed commerce**

- In the same way, it is also important for **quality departments** to develop variety **identification systems** which would allow a **more reliable seed commerce**

- As field trials may last two or three years, **faster and cheaper assays are needed**

- DNA-based markers** have been found to be appropriate for this kind of studies as they provide a reliable identification of genotypes.



BACKGROUND

The **DNA markers** chosen for this work are Simple Sequence Repeats (SSRs) as

- they proved to have the best fit to pedigree data while maintaining an acceptable correlation to morphological-based clustering

- are inexpensive, amenable to automation, co-dominant, independent of the environment, highly polymorphic, essentially unlimited and multiallelic, and provide coverage of the entire genome.

- Soybean**, a self-pollinated species, is a **model** to study the use of SSR markers for identification purposes.



BACKGROUND

Previous study

- We determined the **heterogeneity of Argentine soybean varieties and the number of plants to be analysed** in order to obtain a detailed allelic profile feasible to be used for identification when comparing an unknown sample with data from already characterised varieties

- We found that this characterisation has to be based on **the most frequent allele** for each variety **in order to avoid miss-classification of samples** due to the consideration of infrequent, rare alleles for the variety

- The analysis of ***100 bulked seeds*** or, alternatively, ***4 pools of 5 seeds***, will allow the development of an **identification system based only on the most frequent allele/s**



BACKGROUND



There are several **parameters** to be assessed when developing a testing method:

- **limit of detection and limit of quantification**

We are far from the **limit of detection**.

- **accuracy**

- **range and linearity**

- **specificity**

Marker **specificity** is given by the source of the marker

- **ruggedness**

- **precision**
(*reproducibility and repeatability*)

This testing method requires the assessment of **ruggedness** and **precision**.



AIMS



The aims of the present study are to test:

- **which system is more efficient for variety identification**, i.e. less time and money consuming, allows the identifying of the most frequent alleles

- **the method repeatability**, i.e. if the analysis of different samples in the same laboratory, with the same equipment and technicians will lead to the same result

- **the method ruggedness**, i.e. to test if small deviation of the method parameters will, or will not, change the results.



MATERIALS AND METHODS



- **12 commercial soybean varieties** were analysed studying **12 SSRs** selected on the basis of their capability to uniquely identify more than 230 soybean varieties from the Argentine reference collection

- Two different strategies were studied
 - 1) a global sample of 100 bulked seeds
 - 2) four small samples of 5 seeds each

Criteria

- The most frequent allele/s are those that could be amplified from both 100 bulked seed samples and from all 4 five-seed samples.

- Alleles present only in 1, 2 or 3 of the five-seed samples are considered as infrequent alleles.



MATERIALS AND METHODS



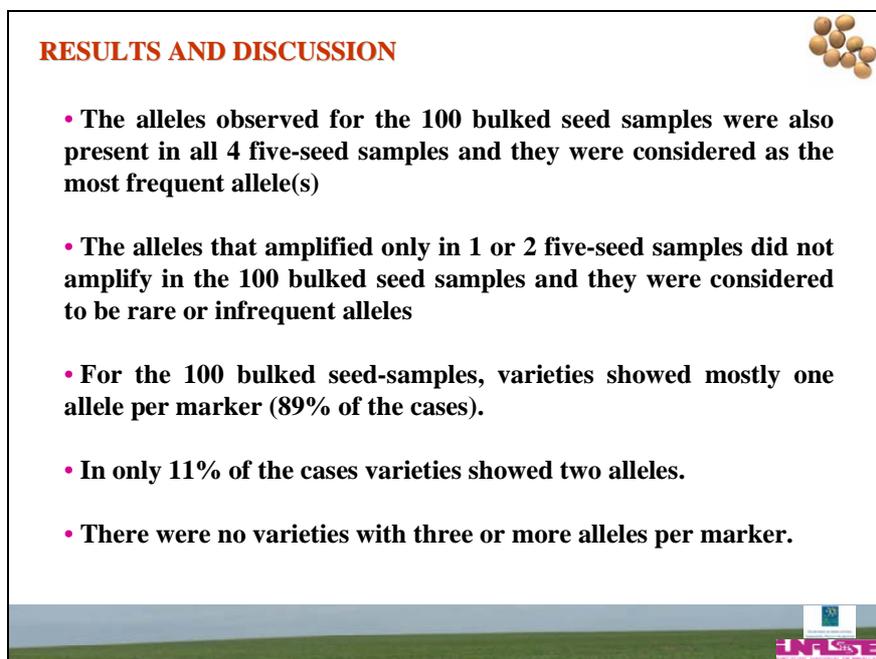
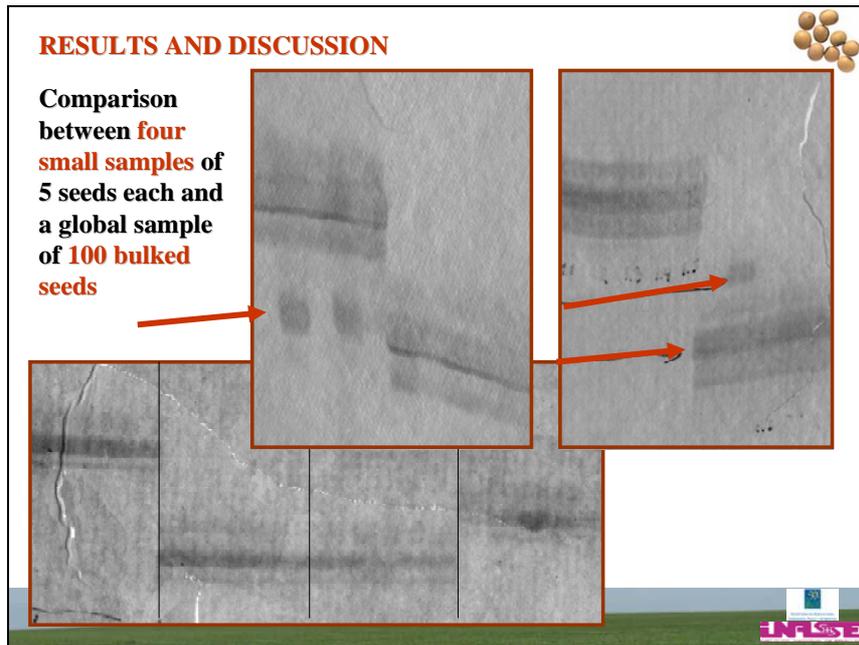
- **Repeatability** was assessed by analysing **duplicate samples of 21 varieties** of high use in Argentina that belong to six different breeding programs.

- These varieties were characterised using the same **12 SSR**.

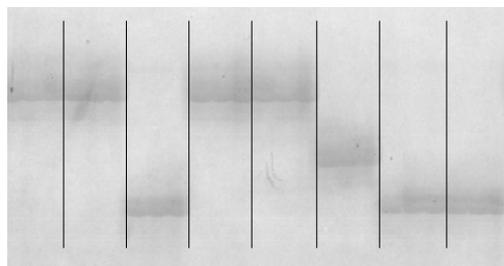
- **Ruggedness** was assessed on a **third sample**, prepared for each variety, by using different equipment and different reagents than those used for the duplicates.

- Ruggedness was tested by comparing this data with the one obtained for the duplicates.





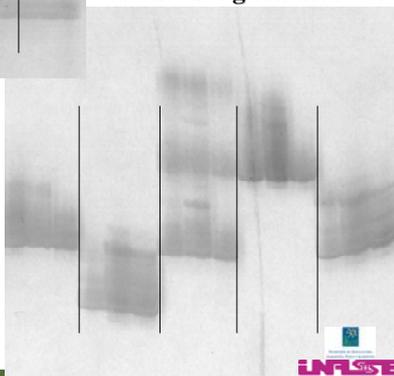
RESULTS AND DISCUSSION



In order to confirm that this method was **repeatable and robust**, three independent samples of 100 bulked seeds were generated

Duplicates for each variety gave the same results, confirming that repeatability is possible for this kind of assay.

The third independent sample gave the same results compared with the duplicates confirming the ruggedness of the method.



CONCLUSIONS AND FURTHER WORK



- The strategy based on the analysis of 100 bulked seed samples and the strategy based on 4 five-seed samples, were found to be similarly efficient in detecting the predominant major allele(s).
- Nevertheless, the 4 five-seed samples strategy was found to be less efficient when preparing the samples.
- Working with the 100 bulked seed sample is less time consuming regarding grinding and DNA extraction, which are also of best quality.
- These results confirm that the alleles obtained in this study are the major predominant or most frequent alleles and the candidates to be used to identify these varieties.



CONCLUSIONS AND FURTHER WORK



- The development of a robust identification method for soybean has two aims: 1) enforcement of breeder's rights and 2) to have a method for quality assurance and seed certification.
- Approximately two hundred soybean varieties of recent commercial introduction will be analysed using a set of 8 already inter-laboratory validated SSR markers.
- More SSR markers for soybean are now being validated through an international forum and will be also used in the near future for obtaining unique profiles for Argentine soybean varieties.
- It is also possible to adapt this method for purity purposes and as complementary information for the National Registration Office. It is now under discussion whether SSR linked to specific genes would be the appropriate markers for those purposes, or other markers such as SNPs would be better.



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