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**THE USE OF MOLECULAR TECHNIQUES FOR THE MANAGEMENT  
OF SOYBEAN REFERENCE COLLECTIONS**

*Document prepared by experts from Argentina*

## THE USE OF MOLECULAR TECHNIQUES FOR THE MANAGEMENT OF SOYBEAN REFERENCE COLLECTIONS

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### Introduction

1. The importance of soybean for Argentina results from the steady increase in production and cultivated area during the last 12 years. Based on the UPOV system, conventional morphological, phenological and physiological traits are used to examine the distinctness of new varieties in the legal framework of granting breeders' rights. As the number of varieties in the reference collections becomes larger, it is more costly and difficult to carry out field trials for all new varieties undergoing DUS testing. In order to minimize the workload and lower the cost of field trials, in 2009 the Variety Registration Office and the Molecular Markers and Phytopatology Laboratory (Quality Department) started a project based on the UPOV "Option 2"<sup>1</sup> approach for the management of soybean reference collections using DNA-based markers.

### Background

#### *The Argentine DUS System for Soybean*

2. The Variety Registration Office at the National Seed Institute (INASE) carries out the analysis required for variety registration and for the granting of plants breeder's rights. Breeders are responsible for the DUS trials and the preparation of the technical report. INASE verifies the report and examines whether the requirements for protection are met. The Variety Office is composed of crop examiners that perform the examination of denomination and stability, fill out the variety description in a specific computer program ("Distinctness Program") and analyze the technical results. The Distinctness Program was first developed in 1993 and revised in 1998. It allows the inclusion of the complete description of the variety under study and is flexible according to the species. The Distinctness Program produces a report for the candidate variety in comparison to all the registered, protected varieties or varieties under examination, showing the different expressions between them. Afterwards, the technical analysis and interpretation by the examiner is required.

3. According to the Distinctness Program results, the candidate variety could be classified to be:

1. Similar or very close to others
3. Distinct but close to others
5. Distinct
7. Very distinct
9. Totally distinct

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<sup>1</sup> Option 2 "Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characters" (see documents TC/38/14-CAJ/45/5 "Ad Hoc Subgroup of Technical and Legal Experts on Biochemical and Molecular Techniques" and TC/38/14 Add-CAJ/45/5 Add.

In accordance with these results, the examiner may, in case of a result 5, 7 or 9, provide a positive distinctness for the candidate variety or, in case of a result 1 or 3, ask the breeder to submit additional information regarding the variety. This new information will be necessary to verify distinctness (based on morphological, physiological, phenological or disease resistance characteristics) between the candidate variety and the similar or very similar varieties in the reference collection. Nevertheless, INASE examiners may take into account the results from their own field trials or other trials conducted to verify the fulfillment of DUS requirements and/or verify the breeder's DUS trials in the field.

4. In the case of soybean, there are around 150 applications per year and a total of 495 varieties have been registered. Currently, approximately 95% of the new candidate varieties are ranked 1 or 3 when compared to the reference collection after the analysis using the Distinctness Program.

5. Since 1995, INASE has managed its own DUS field trial in addition to a replication managed by the breeders at the same time. These INASE DUS Trials includes all the varieties in the reference collection. INASE notifies the list of similar or close varieties to the breeders, and requests more information. INASE examiners verify the variety description at the breeder's trials (verification of description) and at INASE DUS trials. The breeders visit the INASE DUS trials and take notes for data they need (principally from candidate varieties applied by other breeders). Then, they submit the results to INASE. The new information recorded by breeders allows them to compare their candidate variety against the candidate varieties from other breeders (ALL are included in the INASE DUS Trial). This leads to more technical exchange between examiners and breeders, greater knowledge of the UPOV system by the breeders and less time needed for examination concerning the new information supplied by breeders. This also leads to greater transparency in the PBR system.

#### *The Molecular Markers System*

6. During the 1990's, INASE decided to determine the ability of DNA-based markers for variety identification and also for uniformity and stability testing using *Glycine max* as a model.

7. The aims of the study were to:

1. Study soybean varieties, using 3 different types of molecular marker (SSR, AFLP and RAPD), comparing them with 16 characteristics widely used for conventional DUS testing and also with pedigree data;
2. Establish minimal genetic distance between varieties;
3. Test the level of uniformity of commercial soybean varieties;
4. Assess stability;
5. Analyze the similarity based on SSR for those varieties that seemed to be very similar in morphological field trials.

This work allowed us to select a set of 12 SSR markers capable of identifying more than 250 soybean varieties that fulfill the requirements of high level of polymorphism, repeatability, a known distribution throughout the genome and the avoidance of null alleles. These results showed that SSRs are adequate for variety identification and, after establishing a distinctness threshold of 0.8, also for supporting the work of the national Variety Office. However, if these markers are to be implemented to assess uniformity and stability, values of off-type tolerances have to be established and microsatellites have to be carefully selected to discard highly hypervariable loci (Giancola, 2002; Vicario, 2002; Loray 2003).

8. It was also agreed that the variety characterization needs to be based on the most frequent allele for each variety in order to avoid mis-classification of samples due to the consideration of infrequent, rare alleles for the varieties. Appearance of low frequency rare alleles due to evolution is not surprising, even when the varieties under study have been tested for uniformity of morphological characteristics, because a large proportion of the background genome may still be segregating. Other factors like purity maintenance or cross-pollination may also affect the appearance of new alleles (Loray, 2006). In order to obtain a profile based on the most frequent allele(s), the identification system and the management of the soybean reference collection will be carried out using 100 bulked seeds.

#### Varieties and markers used

9. Using the DUS scheme explained above, Argentina has more than 495 plant variety protection titles in force for soybean varieties, and the collection continues to grow. In this context, Argentina is evaluating the possibility of using DNA-based markers for reducing the number of soybean varieties in the reference collection which need to be included in DUS field trials. This project is based on the UPOV “Option 2” approach for the management of reference collections by means of molecular markers.

10. In 2009, the Variety Registration Office and the Molecular Markers and Phytopathology Laboratory (Quality Department) started this project in order to minimize the workload and lower the cost of field trials. A total of 132 soybean varieties selected from the maturity groups V and IV (American standard) are now being studied using the set of microsatellite (SSR) markers under validation within ISTA (International Seed Testing Association). These varieties are also being analyzed by the National Variety Office using a set of 8 characteristics usually evaluated during the field trials. These characteristics are: Growth type; Plant type; Pubescence color; Leaf shape; Flower color; Peroxidase reaction; Hilum color and Maturity group.

11. By the end of April 2010 a researcher from INASE will be hosted at the *Groupe d'Etude et de contrôle des Variétés et des Semences* (GEVES) in France for data analysis using the GAÏA Software. After this analysis, a larger set of varieties will be analyzed using both morphological and DNA-based traits in order to calibrate both systems so as to manage the soybean reference collections, in order to minimize the workload and lower the cost of field trials.

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