

Technical Working Party for Agricultural Crops**TWA/52/6****Fifty-Second Session****Original:** English**Virtual meeting, May 22 to 26, 2023****Date:** April 14, 2023

PRESENTATION ON THE USE OF MOLECULAR TECHNIQUES IN DUS EXAMINATION*Document prepared by experts from Argentina**Disclaimer: this document does not represent UPOV policies or guidance*

The annexes to this document contain copies of presentations to be made by experts from Argentina, at the fifty-second session of the TWA:

Annex I "Argentine experience in rice genotyping"

Annex II "Use of molecular techniques in DUS examination: Field trials details of the Argentine Soybean experience"

[Annexes follow]

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ARGENTINE EXPERIENCE IN RICE GENOTYPING



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All the cultivars that are on the National List together with those that have been granted a Property Title (PBR), were genotyped.

The samples were as follows:

- 56 varieties from different breeders
- 19 duplicates
- 23 experimental varieties (some of which would be included in the national list in the future)

- The SNP markers were ran at laboratory using the 7K chip (*The Cornell-IR LD Rice Array (C7AIR)*) Illumina platform.
- DNA was extracted from dried leaves obtained at INTA Argentina, from the second true leaf after 20 days germination of 10 seedlings.

- The laboratory submitted an allele matrix of 98 samples x 7028 SNPs.
- The data was analyzed by a group of biostatisticians from the Faculty of Exact and Natural Sciences of the University of Buenos Aires.
- As a result, 1000 SNPs were chosen. From them, two subsets of markers were identified: one for the management of reference collections and the other for trade control.

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- From the matrix of 1000 SNPs, 90 were selected for the management of reference collections and 26 for trade control.
- These 90 markers will be uploaded in a program specially designed by the National Seed Institute to obtain the distance matrix based on the 1-Jaccard Coefficient.
- The distance matrix will be included in the GAIA program developed by France, to obtain the most similar cultivars and form the aforementioned reference collection.



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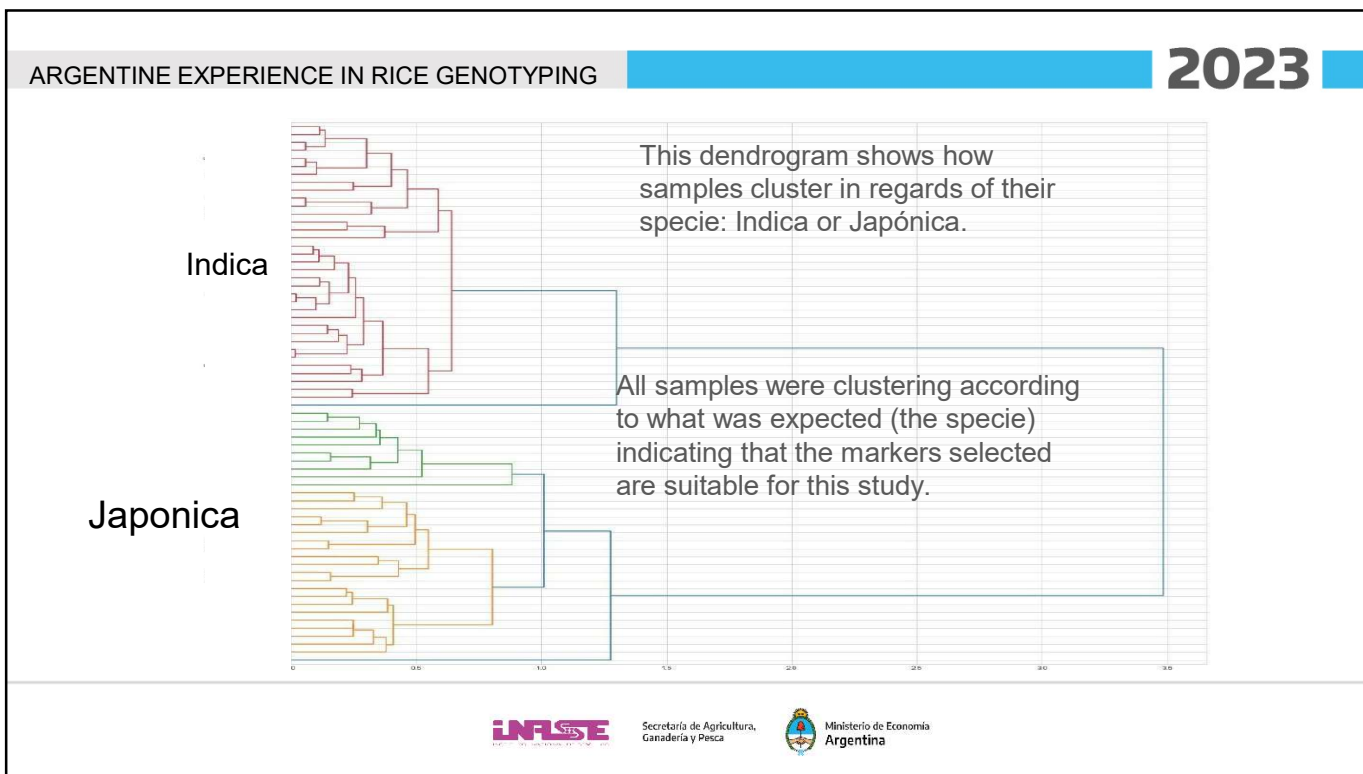
- The other marker set of 26 SNPs are being validated for being used in a real time PCR system.
- These markers will be used for the validation of the identity stated by the farmers.



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ARGENTINE EXPERIENCE IN RICE GENOTYPING **2023**

Acknowledgments

The selection of 500K and 80 SNP markers was developed by:

- Dr. Marcelo Marti and Dr. Juan Manuel Prieto (Biochemistry department, Natural and Exact Science Faculty, University of Buenos Aires).

Validation test are being carried out at the Genomic Unit, Biotechnology Institute, INTA.

All the work was done with the guidance of:

- Dra. Ana Vicario (INASE - Molecular Markers and Phytopathology lab).
- Ing. Alberto H. M. Ballesteros (INASE - Variety Registration Office).
- Lic. José Colazo (National Institute of Agricultural Technology - INTA).
- Rice Advisory Committee of the National Seed Commission.

INASE
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[Annex II follows]

Use of molecular techniques in DUS examination: Field trials details of the Argentine Soybean experience

52nd sesión of TWA
Virtual meeting
May 22 to 26, 2023

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BACKGROUND

2023

Complexity of the distinctness
process in soybean

To face these problems was
necessary to resort to

Molecular markers

Through

Project between public and private sector



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BACKGROUND

2023

- **Number of varieties in the national list: 1188**
- **Average number of new applications per year: 200**



The number of varieties to be compared with new ones is large



Large trials and high costs



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OBJETIVE

2023

Objetive

Reduce the number of varieties that should be compared in the field.



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MATERIALS AND METHODS

2023

- 858 soybean varieties analyzed coming from different breeding programs.
- DNA was extracted and samples were sent to an external genotyping service provider for genotyping with the **SoySNP6K chip**, a subset of the Illumina Infinium Beadchip SoySNP50K developed by the United States Department of Agriculture.
- 19 relevant characteristics for the distinctness of soybean varieties were selected.
- These characteristics were grouped in three levels of reliability and weightings to each combination of their expression levels were assigned.



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MATERIALS AND METHODS

2023

- They were used for comparison with molecular processed data and for the determination of the minimum phenotypic threshold.
- We select a set of 4004 SNPs markers distributed in the genome that represent the genetic background and produce efficient discrimination power.
- We determine the molecular distance threshold and the minimum phenotypical distance threshold, based on 3 years field trials.



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- Three sites (Castelar, Pergamino, Rafaela) and three years (12/13, 13/14, 14/15).
- 6 candidate varieties.
- 11 varieties to compare by candidate variety, from similar to different.
- 66 variety pairs.
- 8 variety pairs lost (one of the variety plot lost in one year or site).
- Total: 58 variety pairs.

Criteria used in the selection of Candidate Varieties

- Complexity of the distinctness process.
- Different morphological characteristics.
- Different Maturity group.
- List of varieties to compare by candidate variety: From similar to different.

58 variety pairs were classified in two groups:

- Group 1: variety pairs for which phenotypic differentiation was difficult therefore, their field evaluation was necessary.
- Group 2: variety pairs for which phenotypic differentiation was easy therefore, their field evaluation was not necessary.

Results

- No difference was found between sites and years, in the classification of the variety pairs into mentioned groups.
- No association was found between genetic distances and the classification of varieties pairs in group 1 or group 2.
- 93% of the pairs in both groups had genotypic distances above 0.36; therefore that value was taken as a molecular distance threshold.

RESULTS

2023

Validation of the molecular distance threshold (Site: Salto, Year: 2019)

- 160 variety pairs (in registration process) were evaluated at field and classified in the same two groups.
- No association was found between genetic distances and the classification of pairs of varieties in group 1 or group 2.
- 97% of the variety pairs of both groups had genotypic distances above 0.36.
- 0.36 was maintained as a molecular distance threshold.



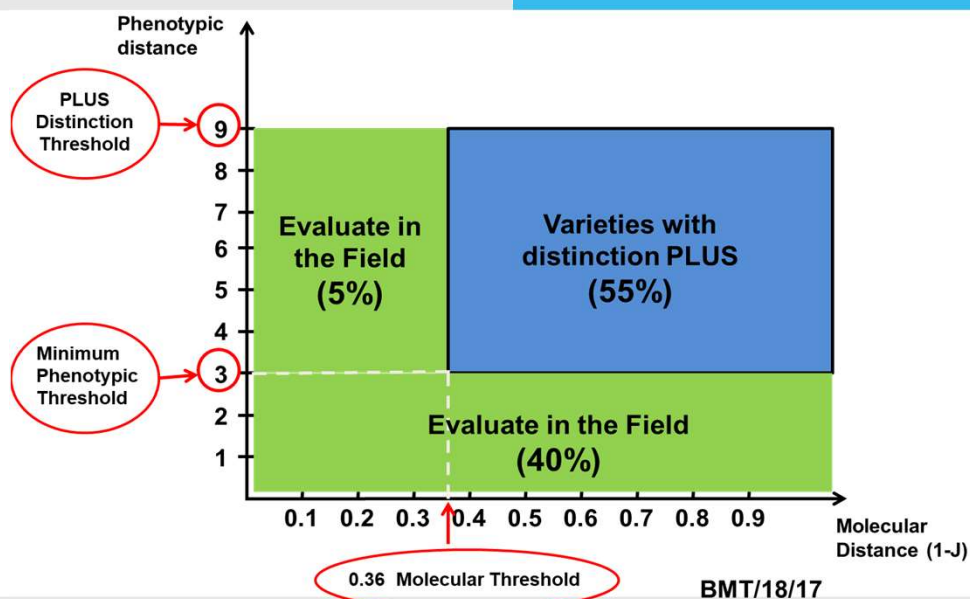
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RESULTS

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[End of Annex II and of document]