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COTTON GENOTYPING USING THE TAMU 63KSNPSARRAY

Document prepared by an expert from Argentina

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The annex to this document contains a copy of a presentation on "Cotton genotyping using the TAMU 63KSNPsArray", prepared by an expert from Argentina, to be made at the first session of the TWM.

[Annex follows]

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Argentina

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Genotyping cotton varieties using the TAMU 63KSNPsArray

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- 88 samples were sequenced.
- Each sample consisted of:
  - a duplicate of the same variety
  - a replicate from different years
  - a replicate from different locations

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- Through a bio-informatic analysis, the most polymorphic 3K SNPs were draw out from the TAMU 63KSNPsArray
- This set of 3000 SNPs will be used for the management of reference collections, to aid in differentiation process.
- A second set of 103 SNPs was selected from the 3K set with the purpose of variety verification and identification for market control and law enforcement.

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Markers indicate possible alleles for samples
ACTG nucleotides indicate that the sample is homozygous for that marker.
The letters M, K, Y and R indicate heterozygous samples.
The first column are the markers and the following ones are the samples
In the cells are the alleles found for each sample by marker.

i33921Gh_C_A	C	C	A	M	failed	C	M
i33584Gh_C_T	C	C	T	T	C	T	T
i09119Gh_T_C	Y	T	Y	Y	C	Y	T
i33388Gh_G_A	R	G	G	G	G	G	A
i32821Gh_T_C	T	C	Y	Y	T	T	T
i22734Gh_G_A	R	G	G	G	G	G	A
i32465Gh_C_T	Y	C	C	C	C	C	T
i08991Gh_G_A	G	G	R	R	G	A	G
i23904Gh_A_G	R	A	A	A	A	A	G
i31809Gh_C_T	C	C	C	Y	T	T	C
i05593Gh_A_G	A	G	R	G	G	R	G
i31433Gh_C_T	C	T	C	Y	C	C	T
i23737Gh_A_G	A	G	G	R	A	A	A
i23790Gh_T_C	T	C	C	C	T	T	C
i30764Gh_G_A	R	G	G	G	G	G	A
i23856Gh_G_T	G	G	K	G	K	G	G
i05844Gh_G_A	R	G	G	G	G	G	G
i30175Gh_T_G	G	T	T	K	G	G	T

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- INASE developed a software (INASE platform for Molecular Markers) with the capacity for: - storing the basic matrix (all genotypic data), - transform data from allelic to binary, calculating the Jaccard association coefficient, 1-J distance used in the GAIA program.

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- The 3000 SNPs matrix is transformed from allelic data to binary data.
- 1-Jaccard distance was calculated using a platform developed by INASE.
- The distance values obtained are uploaded in the GAIA software to combine molecular and morphological data for detecting the most different varieties that will not require field test.

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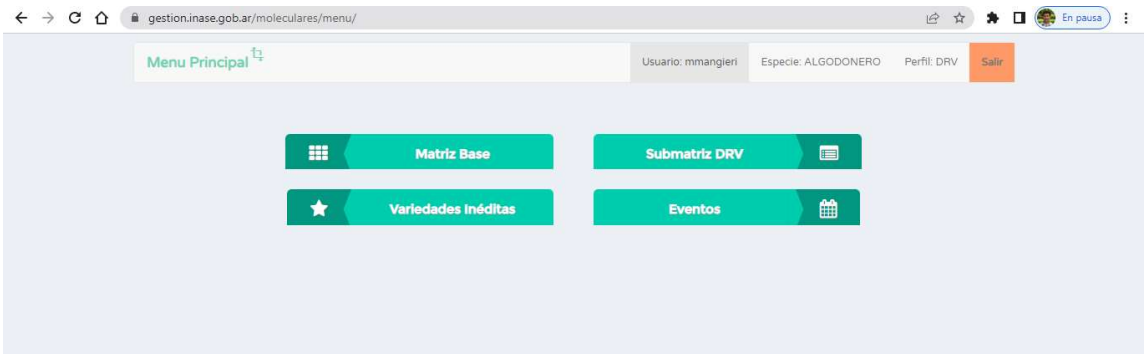

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Display distances coefficiet (based on 1-Jaccard) between catalogue varieties and candidate variety

Var. Number	Var. Number	Distance*	Value of Distance
VAR 1	VAR C	JAC	0.59
VAR 2	VAR C	JAC	0.67
VAR 3	VAR C	JAC	0.57
VAR 4	VAR C	JAC	0.68
VAR 5	VAR C	JAC	0.65
VAR 6	VAR C	JAC	0.60
VAR 7	VAR C	JAC	0.52
VAR 8	VAR C	JAC	0.57
VAR 9	VAR C	JAC	0.65
VAR 10	VAR C	JAC	0.53

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- The set of 103 SNPs were bioinformatically analyzed in the aim to select those most suitable for developing a PCR markers set.
- The first trials are already done and now final validation with a smaller marker set is ongoing.

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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 Biotechnology Genomic Unit of 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).
Ing. Mauricio Tcach - Ing. Iván Bonacic Cresic (National Institute of Agricultural Technology - INTA)
Argentine Seed Association (ASA) members and technical coordination, Ing. Juan Erdmann

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