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ADDENDUM TO: DEVELOPMENT OF MOLECULAR MARKER PANELS FOR VARIETY REGISTRATION AND TRADE CONTROL

Document prepared by an expert from Argentina

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The annex to this document contains a presentation “Development of molecular marker panels for variety registration and trade control”, made by an expert from Argentina, at the fourth session of the TWM.

[Annex follows]

Development of molecular marker panels for variety registration and trade control

Experts from Argentina, TWM 4, 2026

INASE Instituto Nacional de Semillas

Secretaría de Agricultura, Ganadería y Pesca

Ministerio de Economía
República Argentina

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General Standard Operating Procedure for developing variety identification tools for the purposes of registration and trade control

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5. **Selection of the initial marker set**
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9. Signing of the Terms of Reference and Confidentiality Agreement
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13. New budget for validation: costs and approval
14. Sample collection for DNA marker validation
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18. Regulatory drafting
19. Adjustments to the database and data analysis software, if required
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General Standard Operating Procedure for developing variety identification tools for the purposes of registration and trade control

Responsibilities of the ad hoc working group
Actively collaborate in achieving the project objectives, identify the project's weaknesses and strengths, advise on different topics, propose solutions to problems that may arise throughout the project, and establish action strategies for its progress. Get the found for the project.

4. Selection of the varieties to be analyzed

- Ideally, it is the entire known universe, all the variety list
- Alternatively, all samples that could be found or use some criteria like varieties most widely used by farmers
- It is essential that the seed be of the variety with the highest possible level of genetic purity
- Anything not included may lead to erroneous conclusions
- Duplicates from the same sample
- Duplicates of the same variety, when possible, different harvest seasons

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General Standard Operating Procedure for developing variety identification tools for the purposes of registration and trade control

5. Selection of the Initial Marker Set

- Use markers publicly available, those found in the literature or in public arrays
- Developing markers from scratch is only recommended for species for which no information is available or if the genetic pool is too specific for a region
- Initial Quantity: Be generous.

CottonSNP63K, an Illumina Infinium array containing assays for 45,104 putative intraspecific single nucleotide polymorphism (SNP) markers for use within the cultivated cotton species Gossypium hirsutum L. and 17,954 putative interspecific SNP markers for use with crosses of other cotton species with G. hirsutum.

BARCSoy6K Consortium
The BARC Soy6K panel was developed by the Soybean Genomics and Improvement Lab, Beltsville Agricultural Research Center (BARC) for the greater good of the Soybean community.

Cornell-IR LD Rice Array (C7AIR), an SNP array containing 7,098 markers

SGS TraitGenetics
5K SOYBEAN: The 5K array contains a subset (4,970) of the 6Kv2 and 50K array markers for Genomic Selection with Imputation

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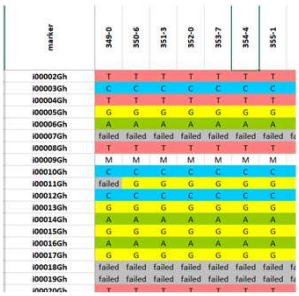
General Standard Operating Procedure for developing variety identification tools for the purposes of registration and trade control

12. Initial data analysis for panel selection

Clearly communicate to the data analyst what we want to do with the initial genomic data set, for example:

- We want to keep the markers that present:
less than 5% missing data
less than 5% heterozygote presence



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i00003Gh	C	C	C	C	C	C	C
i00004Gh	T	T	T	T	T	T	T
i00005Gh	G	G	G	G	G	G	G
i00006Gh	A	A	A	A	A	A	A
i00007Gh	failed	failed	failed	failed	failed	failed	failed
i00008Gh	T	T	T	T	T	T	T
i00009Gh	M	M	M	M	M	M	M
i00010Gh	C	C	C	C	C	C	C
i00011Gh	failed	G	G	G	G	G	G
i00012Gh	C	C	C	C	C	C	C
i00013Gh	G	G	G	G	G	G	G
i00014Gh	A	A	A	A	A	A	A
i00015Gh	G	G	G	G	G	G	G
i00016Gh	A	A	A	A	A	A	A
i00017Gh	G	G	G	G	G	G	G
i00018Gh	failed	failed	failed	failed	failed	failed	failed
i00019Gh	failed	failed	failed	failed	failed	failed	failed

- Markers that are homogeneous across all varieties will be discarded

Redundant markers will be discarded

- Keep markers with greater discriminatory power
- Select markers with homogeneous distribution in the genome
- We want to keep the MM presenting maximum similarity between replicates and maximum difference between varieties.
- Other criteria may be used with the agreement of the working group
- The data belongs to the project so can be freely managed after data generation

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General Standard Operating Procedure for developing variety identification tools for the purposes of registration and trade control

16. Interpretation of Results for the Objectives – management of RC

- There will be 1 panel for management of variety collections and 1 panel for variety identification for different purposes
- It should be considered that the panel for variety registration must be useful for several years, and therefore it is advisable to select "extra" markers for the management of variety collections in case a reselection is required in the future

17. Definition of Thresholds or Strategy for Decision-Making – management of RC

- Three thresholds for decision-making must be defined when using GAIA (management of RC)
- Genotypic data is entered as a distance in the GAIA software; it is one data point for each new variety/variety in the variety collection – select a distance coefficient

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General Standard Operating Procedure for developing variety identification tools for the purposes of registration and trade control

16. Interpretation of Results for the Objectives – var ID

- The validation data is compared with the data originally obtained from the initial genotipification
- The variation in the profile for the same variety is analyzed
- The maximum similarity between varieties is calculated.
- It is important to verify that the replicates are close and the varieties as most different as possible

17. Definition of Thresholds or Strategy for Decision-Making – var ID

- From the previous analysis, a cutoff can be established that covers the majority of cases (95%): requires at least 1 threshold
- All samples that present a similarity value greater than the defined threshold will be considered to correspond to the variety in the result
- A second threshold can be established
- Below the second threshold, it could be assumed that the sample it is not the (declared) variety.

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General Standard Operating Procedure for developing variety identification tools for the purposes of registration and trade control

20. Panel Maintenance

- It is necessary to verify (based on the volume of new registrations) that variety discrimination remains high, meeting the established thresholds.
- Remove markers that are homogeneous for new varieties due to recurrent selection on elite materials (objective: reduce similarity).
- This is why having a "generous" initial set of markers is useful.
- If new markers had to be included, it would create a problem for varieties already incorporated into the system that have not been genotyped with those new markers.
- Problem: commercial panels may not be available

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Problems and some solutions

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ddRADseq for variety registration: cotton and rice

Double Digest Restriction Site Associated DNA Sequencing

Double digest with two restriction enzymes; one cuts the DNA at common sites, and the other at rarer sites.
Cuts DNA at the same positions across different individuals.
Only the DNA pieces that were cut by both enzymes and fall within a specific size range are selected.
Sequencing and identification of genetic markers

-Modifications were made to the standardized ddRAD protocol
-Libraries were constructed and sequenced using Illumina technology
-More than 4 Gb of data were obtained for the 11 cotton samples and 10 rice samples
-They were analyzed to achieve a panel of SNPs present across all samples

Aguirre NC, Filippi CV, Zaina G, Rivas JG, Acuña CV, Villalba PV, García MN, González S, Rivarola M, Martínez MC, et al. Optimizing ddRADseq in Non-Model Species: A Case Study in *Eucalyptus dunnii* Maiden. *Agronomy*. 2019; 9(9):484. https://doi.org/10.3390/agronomy9090484

Natalia C. Aguirre, Carla Valeria Filippi, Pablo Alfredo Vera, Andrea Fabiana Puebla, Giusi Zaina, Verónica Viviana Lia, Susana Noemí Marcucci Poltri, Norma Beatriz Paniego. Double Digest Restriction-Site Associated DNA Sequencing (ddRADseq) Technology. In: Shavrukov, Y. (eds) Plant Genotyping, 2023. Methods in Molecular Biology (), vol 2638. Springer-Nature publisher. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-3024-2_4

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Soybean as an example

Started in 2012
Aim: variety registration
Two years of trials to generate field data to calibrate GAIA

2016
Formal meetings with the breeders and Ad Hoc group for MM in soy
2018
First results: Infinium Illumina BARCSoy6K soybean genotyping array
Data analysis
Calculation of distances (1-J) and application in GAIA
2019
First year of use for variety registration – management of variety collections
2021
Panel for variety identification: sample collection to validate the transfer of markers from array to PCR
2022
Completion of system validation and application
First test for variety identification of farmer's samples
2023 – 2024 – 2025
Different samples analyzed

2019
Development of the molecular marker data management software
Development of the DRV module
2021
Development of the MOL module
Development of the LAB module
2022
Development of the FISC module
First samples analyzed in real world - 2023/2024

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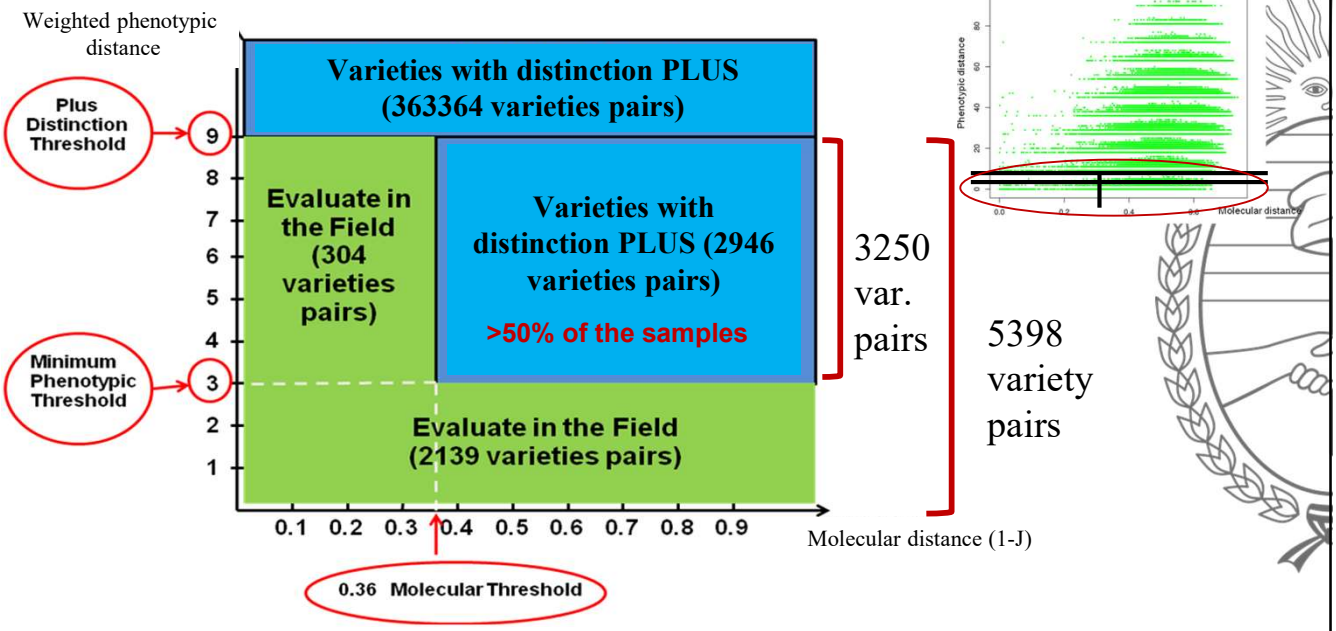
Soybean as an example



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Soybean as an example

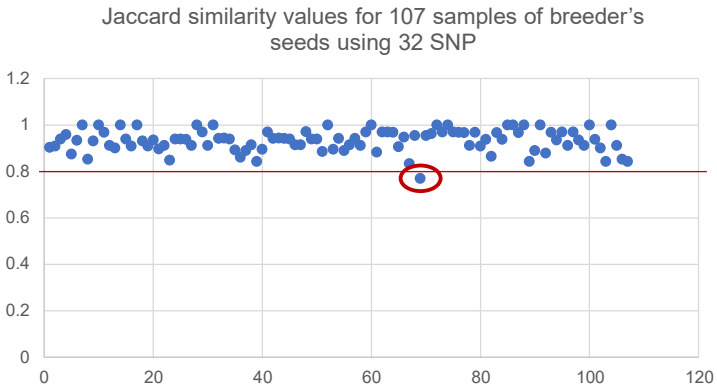
Variety identification

32SNP panel (PCR-based)

107 new seed samples from breeders

DB: genotyped varieties with 6K soy panel

99.1% agreement for the 0.8 threshold



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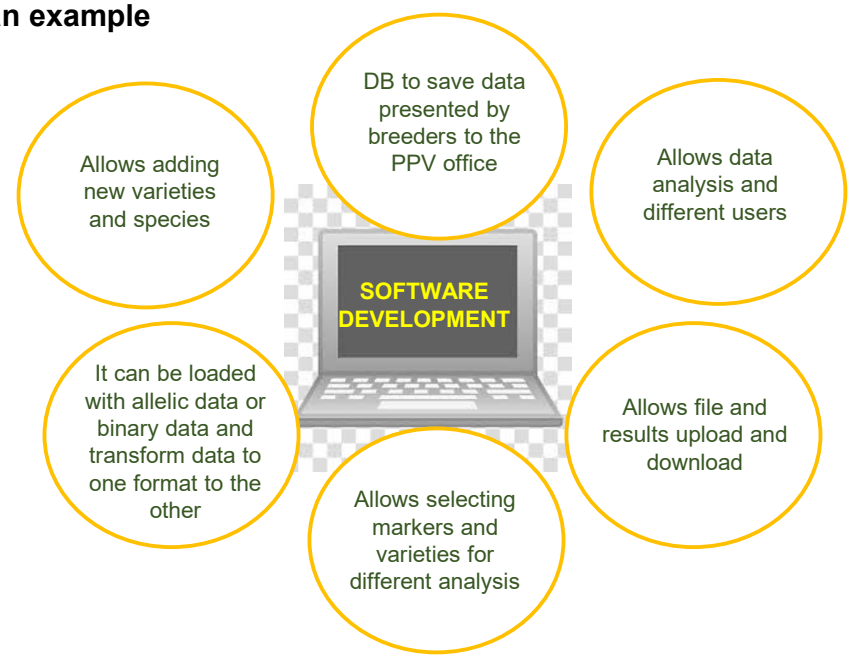
Soybean as an example



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Argentine Regulation

RESOL-2022-357-APN-INASE#MAGYP – Soybean SNP panel for variety identification (replaces RESOL-2018-228-APN-INASE#MAGYP)

RESOL-2023-370-APN-INASE#MEC – Soybean panel reduction (replaces RESOL-2019-296-APN-INASE#MAGYP)

RESOL-2022-227-APN-INASE#MAGYP – SNP panel for cotton – management of RC

RESOL-2024-186-APN-INASE#MEC – Cotton SNP panel – variety identification

RESOL-2023-459-APN-INASE#MEC – Image-based markers in wheat and barley

RESOL-2025-135-APN-SAGYP#MEC – approval of image-based tests for soybean

RESOL-2023-42-APN-INASE#MEC – authorization of molecular marker laboratories

RESOL-2019-71-APN-INASE#MAGYP – authorization of MM laboratories - addendum

RESOL-2025-512-APN-INASE#MEC – authorization of laboratories performing image-based tests

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Thanks for your attention

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