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International Union for the Protection of New Varieties of Plants

Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular

BMT/17/13 Add.

Seventeenth Session
Montevideo, Uruguay, September 10 to 13, 2018

Original: English
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ADDENDUM TO IMPLEMENTATION OF SNP MARKERS TO IDENTIFY SOYBEAN VARIETIES COMMERCIALIZED IN URUGUAY

Document prepared by an expert from Uruguay

The Annex to this document contains a copy of a presentation on “Implementation of SNP markers to identify soybean varieties commercialized in Uruguay”, prepared by an expert from Uruguay, which was made at the seventeenth session of the Working Group on Biochemical and Molecular Techniques and DNA-Profiling in Particular (BMT).

[Annex follows]

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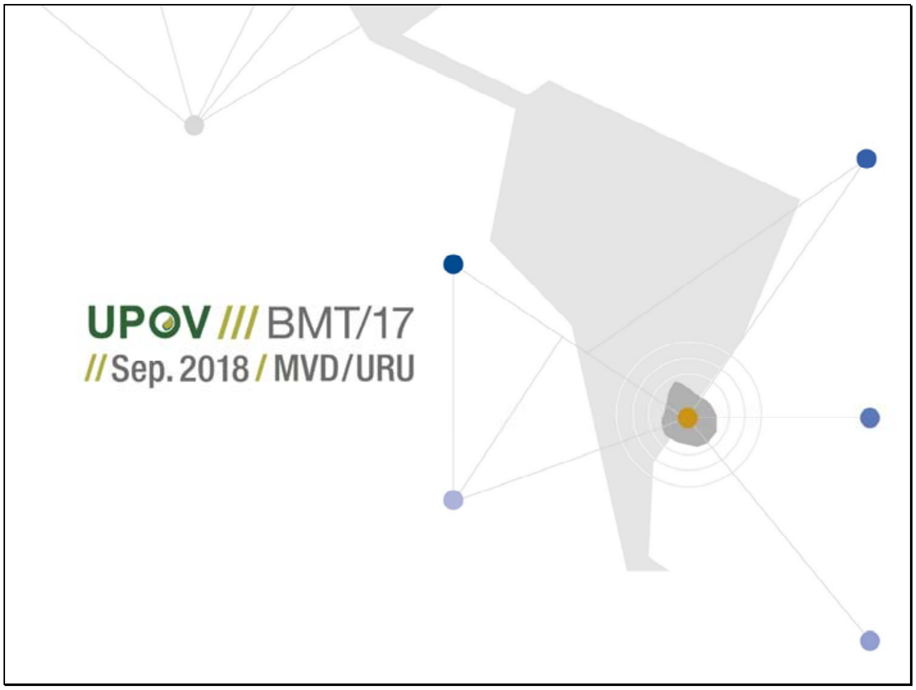
ANNEX

IMPLEMENTATION OF SNP MARKERS TO IDENTIFY SOYBEAN VARIETIES COMMERCIALIZED IN URUGUAY

Presentation prepared by an expert from Uruguay



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INASE URUGUAY

"Implementation of SNPs markers to identify soybean varieties commercialized in Uruguay"

Mariana Menoni

LABORATORIO DE CALIDAD DE SEMILLAS

Some objectives and goals of INASE…

- ✓ To encourage the production and use of seed with high quality and proven identity
- ✓ To protect new varieties
- ✓ Promote seeds export
- ✓ To monitor compliance with legal regulations

URUPOV – INASE Project

The Uruguayan Breeders Association (URUPOV) and INASE signed a cooperation agreement to implement the use of SNPs markers for soybean variety identification.

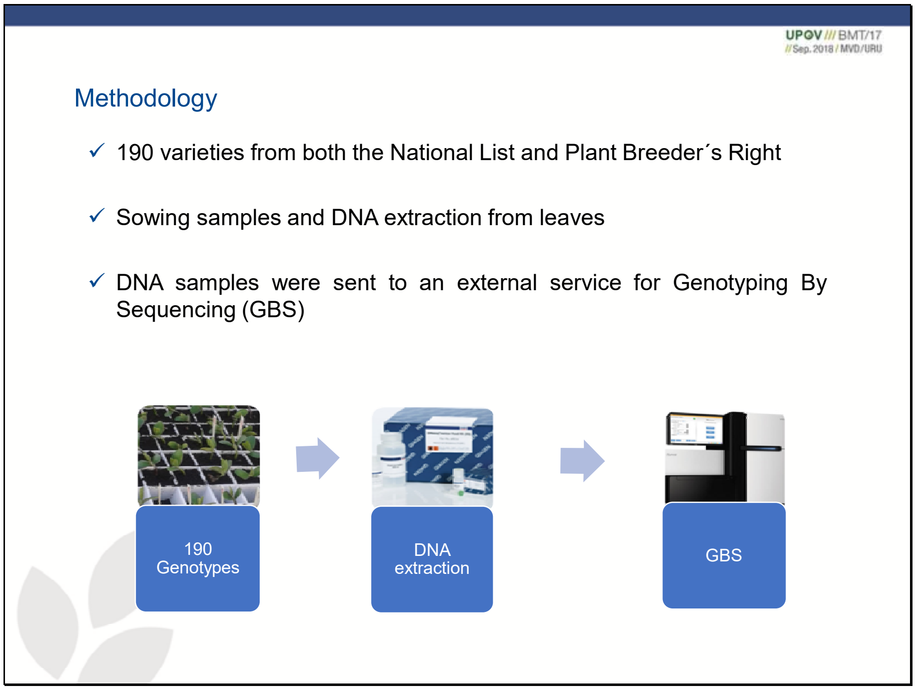
The principal purpose of this project is to protect breeders´ rights.

- ✓ It has been shown that breeders rights are frequently violated in farm saved seeds
- ✓ Monitor seed trade control

Objective

To select a set of SNPs markers to identify soybean varieties registered in Uruguay:

- ✓ For plant breeders rights enforcement mainly in farm saved seeds
- ✓ To apply in seed trade control (avoiding field trials for variety identification)



Methodology

- ✓ 190 varieties from both the National List and Plant Breeder´s Right
- ✓ Sowing samples and DNA extraction from leaves
- ✓ DNA samples were sent to an external service for Genotyping By Sequencing (GBS)

190 Genotypes → DNA extraction → GBS

Methodology

Why do we used GBS instead of 6K chip?

- ✓ More affordable technology
- ✓ A priori, we did not know if 6K chip would be polymorphic for our population of genotypes
- ✓ We were running another project with GBS so we had the experience to deal with GBS sequence data

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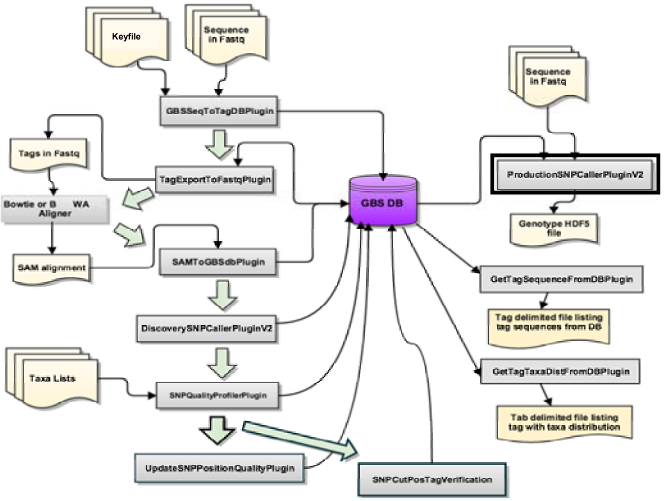
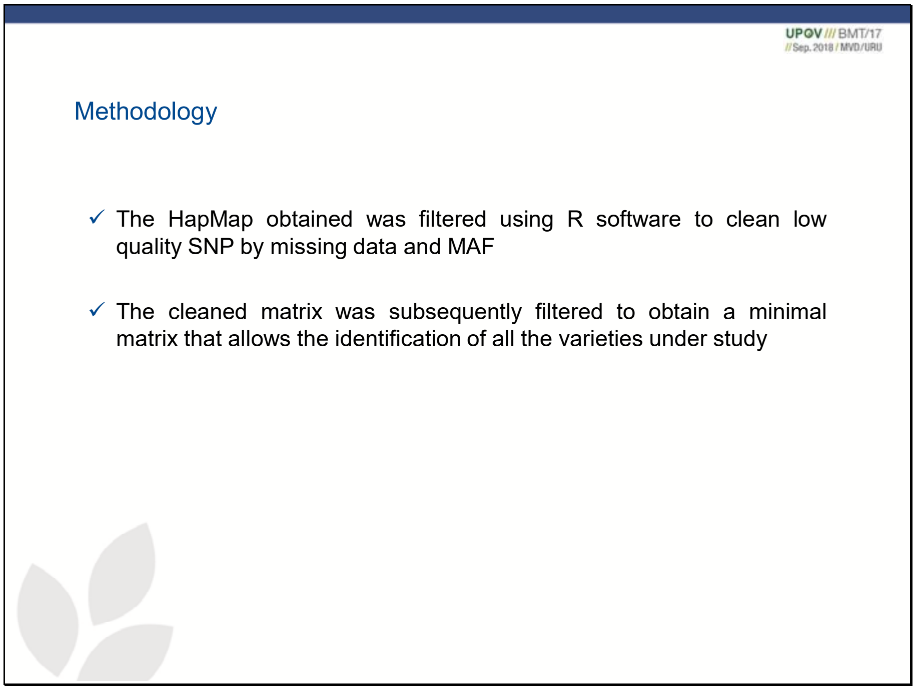
Methodology

- ✓ Trimming using FastQC
- ✓ SNP calling using Tassel Pipeline 5v2



https://bitbucket.org/tasseladmin/tassel-5-source/wiki/Tassel5GBSv2Pipeline



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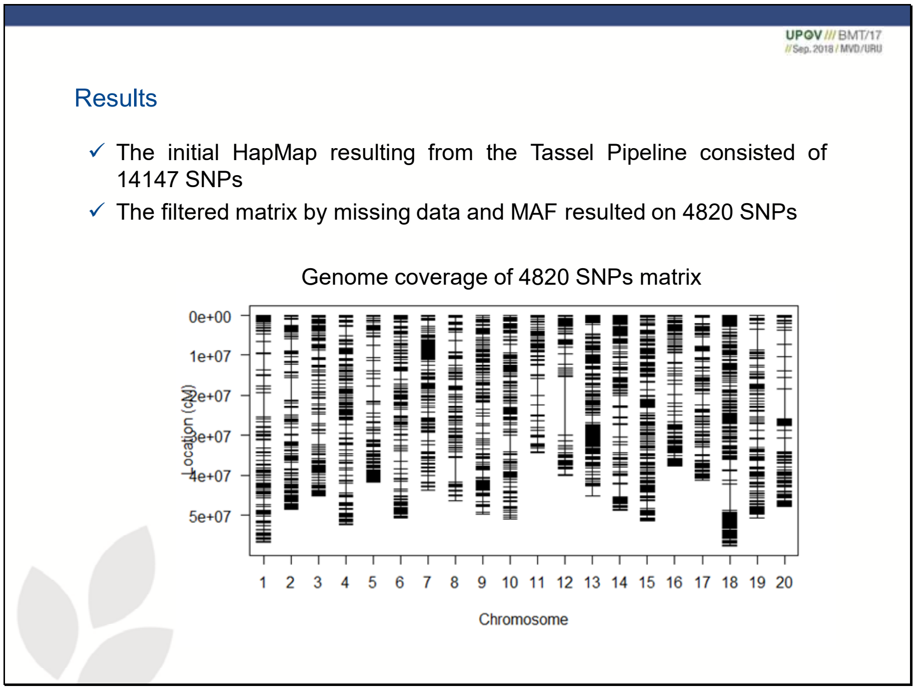
Methodology

- ✓ The HapMap obtained was filtered using R software to clean low quality SNP by missing data and MAF
- ✓ The cleaned matrix was subsequently filtered to obtain a minimal matrix that allows the identification of all the varieties under study



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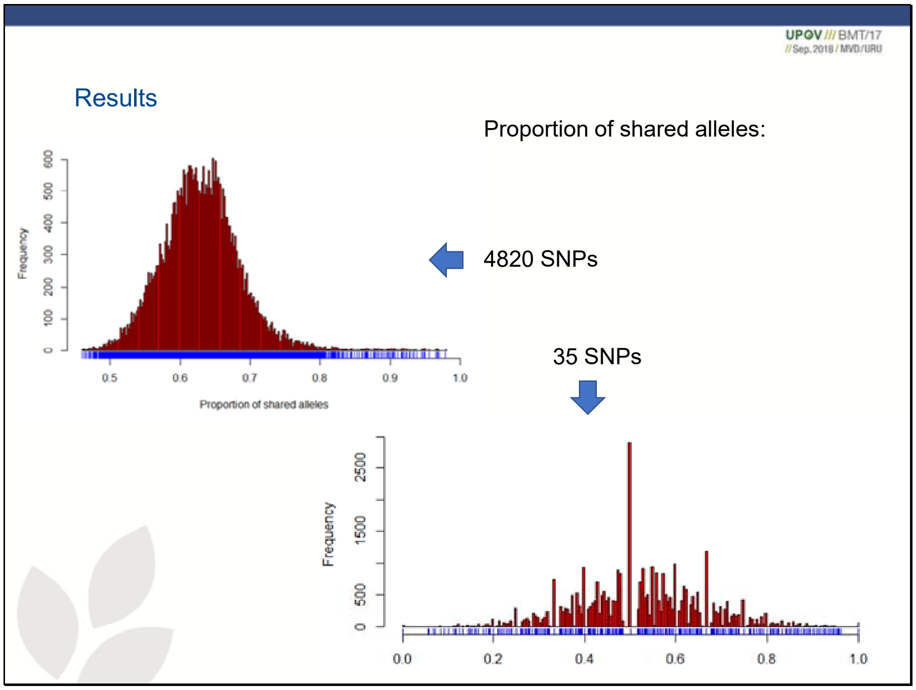
Results

- ✓ The initial HapMap resulting from the Tassel Pipeline consisted of 14147 SNPs
- ✓ The filtered matrix by missing data and MAF resulted on 4820 SNPs

Genome coverage of 4820 SNPs matrix



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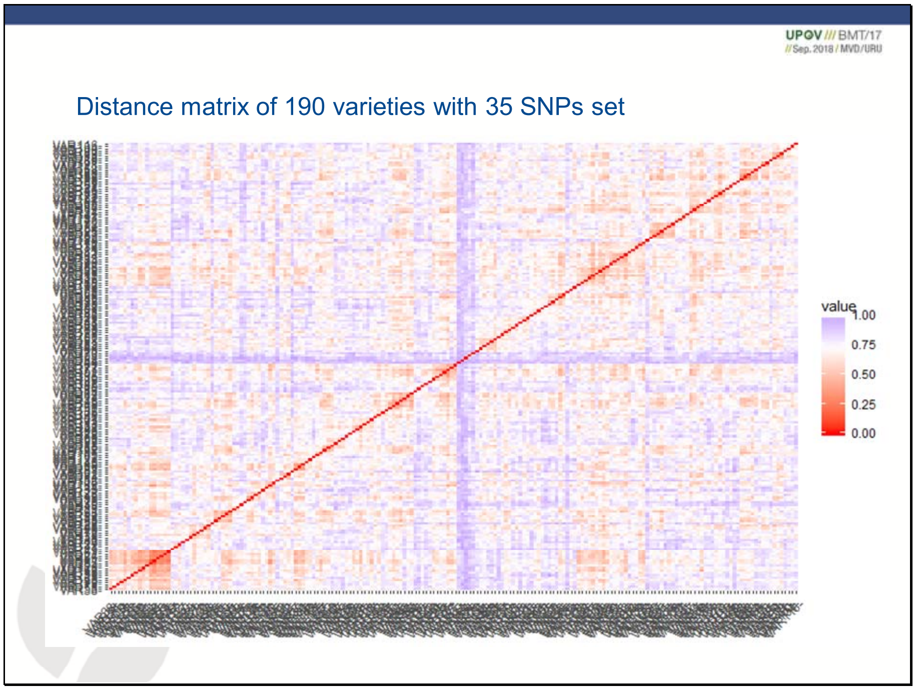
Results

Proportion of shared alleles:

4820 SNPs

35 SNPs



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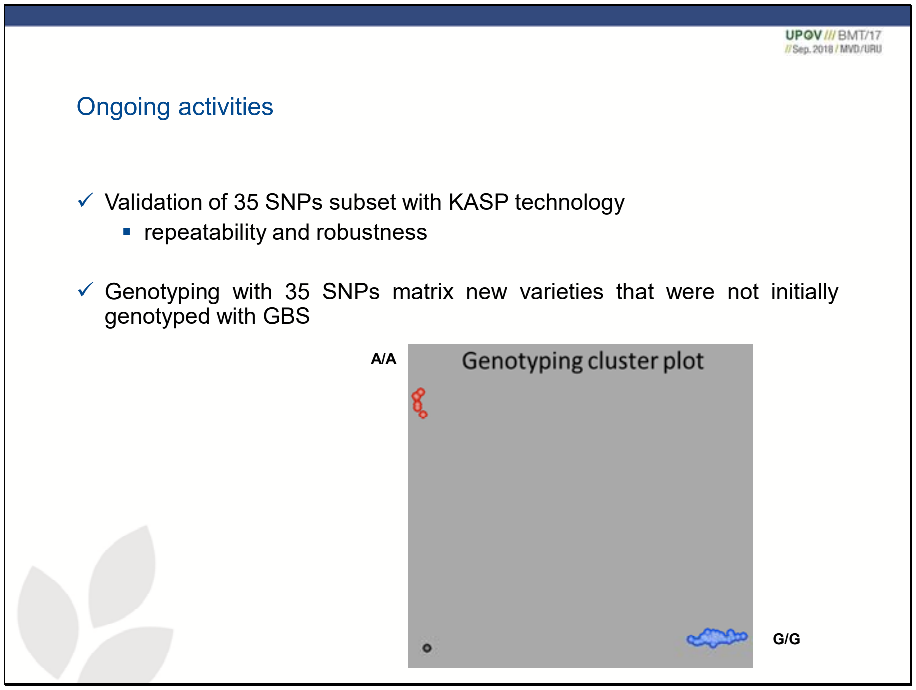
Distance matrix of 190 varieties with 35 SNPs set



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Ongoing activities

- ✓ Validation of 35 SNPs subset with KASP technology
  - repeatability and robustness
- ✓ Genotyping with 35 SNPs matrix new varieties that were not initially genotyped with GBS

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THANK YOU AND WELCOME TO URUGUAY!!!!

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[End of Annex and of document]