Technical Working Party on Testing Methods and Techniques

TWM/3/18 Add.

Third Session Beijing, China, April 28 to May 1, 2025 Original: English Date: May 1, 2025

ADDENDUM TO: USE OF MOLECULAR MARKERS AS A TOOL TO ENFORCE PLANT BREEDERS' RIGHTS (PBR) IN SOYBEAN IN URUGUAY

Document prepared by an expert from Uruguay

Disclaimer: this document does not represent UPOV policies or guidance

The annex to this document contains a copy of a presentation "Use of molecular markers in Soybeans", made by an expert from Uruguay, at the third session of the Technical Working Party on Testing Methods and Techniques (TWM).

[Annex follows]

TWM/3/18 Add.

ANNEX



ABOUT THE ORGANIZATIONS

INASE:

Is a non-state public law institute created in February 1997 by Law N° 16.811

INASE promotes the development of a competitive seed sector by encouraging the production and planting of high quality seed.

URUPOV:

Is a non-profit association representing plant breeders, seed companies, and research institutions involved in the development, production, and commercialization of new plant varieties. Its core mission is to promote and safeguard plant breeders' rights, foster innovation, and contribute to a fair and sustainable seed sector.



COOPERATION AGREEMENT

INASE-URUPOV 2016



IRUPC

OBJECTIVE

To create a SNP panel that allows the identification of the soybean varieties registered in Uruguay with the following purposes:

- "Trade control" Enforcement of plant breeders' rights (Farm Saved Seeds & Brown bagging)
- External services to Seed Co. & Farmers

We aim to reduce the number of trials (which are time-consuming and expensive) and speed up the time to obtain results - 48 hs. vs 6 months.

VARIETAL IDENTIFICATION: Traditional vs new techniques



Traditional techniques: Morphological, physiological and biochemical traits - Require time and resources





Molecular markers allow the varieties available on the market to be identified quickly, accurately & efficiently - Reduce costs











BIOINFORMATICS	
GBS Sequences Tassel 5v2 HapMap	

RESULTS

The initial HapMap resulting from the Tassel Pipeline consisted of 14147 SNPs

It was filtered using R software to clean low quality SNPs by missing data and Minor Allele Frequency (MAF)



SELECTION OF THE MINIMAL MATRIX

The cleaned matrix was subsequently filtered by "**Polymorphism Information Content Iterative Filtering**" to obtain a minimal matrix:

With a panel of 34 SNPs, it was possible to identify the 200+ Uruguayan varieties of soybeans

	- 10 10 10 10 hours	1997 / Acres 1997 19	S DESERVED F		100 ISBN 54			Sec. 1
		SNP 1	SNP 2	SNP 3	SNP 4	SNP 5	SNP 34	
	Variety A	А	Т	С	т	G	С	
	Variety B	G	Т	С	А	Т	С	
	Variety C	G	С	А	Т	Т	A	
	Variety D	А	Т	A	А	G	A	and the second
and addied 1 185	Variety E	G	С	А	Т	G	С	
a man and	24	1	1200	1	-	4		Constant & and reasons a sure of a second

VALIDATION

Validation of 34 SNPs subset as molecular markers with **KASP** technology

Parameters:

- Repeatability
- Specificity
- Precision
- Robustness

KASP (Kompetitive Allele Specific PCR) – LGC Genomics







$N\,^\circ$ of FSS Samples analyzed by KASP 2022-24:

2022	
N° samples analyzed:	272
Concordance between reported and detected	the variety
Consistent	97
Doesn't match	175

272

2023	
N° samples analyzed:	243
N° samples analyzed: Concordance between the r	24 reported

and detected variety	
Consistent	120
Doesn 't match	11
Total	24

2024	
N° samples analyzed:	203
Concordance between	the
reported and detected	variety
Consistent	123
Doesn't match	80
Total	203

....

18

Total



Soybean area by seed origin (23/24):

Certified Seeds Farm Saved Seeds (SVT)	695.000 425.500	52%
Farm Saved Seeds (SVT)	425500	32%
		02/0
TOTALLEGAL	1120.500	84%
Illegal seed+No SVT	219500	16% 🛛 🕽
TOTAL	1340.000	100%

20



Area of action for exercising PBRs´ enforcement



SAMPLING



Each year, a comprehensive database is compiled listing all producers cultivating soybeans in the country.

Leaf Sampling:

- From the full universe of soybean growers, URUPOV selects a representative sample of farmers for leaf collection.
- Once the crop is established, the selected fields are visited. GPS coordinates are recorded to georeference each plot and where the sample was taken.
- Field walks are conducted, during which 10 to 20 leaf sections from individual plants are collected to create a composite sample.

Seed Sampling:

- URUPOV also collects samples from all seed lots reserved for FSS seed processing facilities nationwide.
- Samples are also taken from seed lots stored at producers' own facilities.
- Samples are processed at the URUPOV office, sub-sampled, and packaged in small jars for laboratory shipment.

>> Analysis and Comparison: Both leaf and seed samples are submitted to the laboratory for analysis. Results are received within one week after shipment. These results are then compared with the list of varieties that farmers have legally acquired and declared as FSS.

Code_ID: by farmer, variety, and volume

3 years of results: *leaves* + *seeds*

	ОК	Infringement ?	Total	%						
N° Samples	4.483	294	4.777	6%						
N° Growers	2.650	263	2.913	9%						
50% with infringements										



The cases in which differences are detected are reported to **INASE Uruguay** so to proceed and carry out the corresponding **official** verifications.

24

	(25 inase.uy/Infrac	ciones/			\$	<u>२ २ (</u>	Introducir frase
			E Y	,	ORMATIVA	REGISTR	OS GESTIO	NES EN LÍNEA &
							Inicio /	INFRACCIONES
Р	UBLICATION OF	REGISTRO DE EMPRE	SAS INFRACTORA	S (*)				
		Empresa	N° de expediente	Infracción	Calificación	Sanción	Fecha de resolución	Fecha de publicación
11	INFRINGEMENTS		2022-900-1- 0000130	Infracción a lo dispuesto por el articulo 46° literales a) y b) del Decreto 438/004 de fecha 16.12.04 en su redacción dada por el Decreto 219/010 de fecha 14.07.10; Lote de semilla de Soja ov. 5958 RSF IPRO de 10.000 kg, de reserva para uso propio, sin origen legal a su nombre en incorrectamente identificado. Se presentó factura de compra a nombre de familiar directo.	Grave	20 UR	17/06/2024	19/12/2024
			2022-900-1- 0000129	Infracción a lo dispuesto por el artículo 46° literales a) y b) del Decreto 438/004 de fecha 16.12.04 en su redacción dada por el Decreto 219/010 de fecha 14.07.10; Lote de semilla de Soja ox. 5958 RSF IPRO de 30.000 kg, de reserva para uso propio, sin origen legal a su nombre en incorrectamente identificado. Se presentó factura de compra a nombre de familiar directo.	Grave	20 UR	17/06/2024	19/12/2024
* It is sanction	important to highlight that few ns imposed by the PVP office have		2023-900-1- 0000008	Infracción a lo dispuesto por el artículo 46° literal a) del Decreto 438/004 de fecha 16.12.04 en su redacción dada por el Decreto 219/010 de fecha 14.07.10; Lote de semilla de soja or. DON MARIO 6.8i de 5.000 kg, de reserva para uso propio, sin origen legal.	Grave	45 UR	17/06/2024	19/12/2024
been le through	gally challenged by some producers		2023-900-1- 0000009	Infracción a lo dispuesto por el articulo 46° literal a) del Decreto 438/004 de fecha 16.12.04 en su redacción dada por el Decreto 219/010 de fecha 14.07.10; Lotes de semilla de soja del cv. DON MARIO 6.8 de 20.700 kg y del cv. 60162 RSF IPRO de 7.320 kg, de reserva para uso propio, sin origen legal. Presentó factura de compra a nombre de otre Empresa que explota el mismo establecimiento.	Grave	45 UR	17/06/2024	19/12/2024
the judg INASE,	ses have consistently ruled in favor of never questioning the validity of the		2024-900-1- 0000031	Infracción a lo dispuesto por los artículos 14*, 41*, 63*, 70* y 71* de la Ley 16.811 en su redacción dada por la Ley 18.467. Ofrecimiento ilegal de semilla de Soja cv. 60/62 RSF IPRO (cultivar protegido). Volumen de mercadería en infracción: 5 - 10 toneladas.	Grave	20 UR	15/07/2024	14/01/2025
tools us	ed to support those decisions.		2023-900-1- 0000006	Infracción a lo dispuesto por el articulo 46° literales a) y b) del Decreto 438/004 de fecha 16.12.04 en su redacción dada por el Decreto 219/010 de fecha 14.07.10. Lote de 20.910 kg de semilla de soja cv. 5958 RSF IPRO, para reserva de Uso Propio, sin orican lead e incorrectamente identificado.	Grave	290 UR	05/08/2024	14/01/2025

Final remarks

25

26

These tools enable faster and more cost-effective detection of infringements, while ensuring transparency and fairness for all parties involved (INASE, Farmers, and Breeders).

Additionally, they allow for:

- Timely application of sanctions (within 48 hours compared to 6–13 months)
- Greater effectiveness in implementing corrective measures
- Sanctions are effective, proportionate, and dissuasive
- Significant savings in resources typically allocated to field trials
- Improves collaboration & trust: Provides a neutral and science-based basis for disputes between

farmers, breeders, and enforcement authorities.



OPTICAL MARKERS w/AI & Machine learning











[End of Annex and of document]