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Estudo molecular da variedade "Escoba Blanca" do *Sesamum indicum* L. usado no Paraguai

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Rev. Fac. Agron. (LUZ). 2024, 41(1): e244104
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron.v41.n1.04

Crop production
Associate editor: Dra. Lilia Urdaneta
University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela

Received: 27-10-2023
Accepted: 18-12-2023
Published: 09-02-2024

Keywords:
Genetic diversity
Genotype
Microsatellite markers
Sesamum indicum L.

Abstract

The high quality of sesame seeds originating in the country has led Paraguay to be among the main exporters. Of the varieties available in Paraguayan territory, the most widespread is "*Escoba Blanca*", which, possibly due to the multiplication process, could promote changes in its allele frequency, diversity, and genetic purity. This work was carried out, aiming to determine the genetic differentiation between 50 populations/seedbeds/banks from seven Paraguayan companies collecting "Escoba Blanca" sesame, using microsatellite markers. These seven banks/companies/cooperatives collect and represent samples from all the producers/seedbeds located in different departments of the Eastern and Western Region (Chaco) of the country, with whom they work, market, and collect sesame. Plant tissue was obtained to extract DNA, from seedlings planted especially for the purpose, using all the included samples/accessions. Six microsatellite markers were used: GBssrsa184, GBssrsa123, GBssrsa182, GBssrsa108, GBssrsa08, and GBssrsa72. The following were calculated: number and frequency of alleles, distance/groupings, differentiation between populations, and their genetic structure. The mean number of alleles per *locus* ranged from 1.33 to 3.00. In the markers, GBssrsa184 and GBssrsa108, three populations presented a higher frequency of alleles. The populations examined exhibited a wide degree of genetic differentiation between them, with the identification of four groups, with greater and less purity respectively.

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Resumen

La alta calidad en los granos de sésamo originados en el país ha conducido al Paraguay a situarse entre los principales exportadores. De variedades disponibles en territorio paraguayo, la más difundida es 'Escoba Blanca', que, posiblemente debido al proceso de multiplicación, pudo impulsar modificaciones en su frecuencia alélica, diversidad y pureza genética. Este trabajo fue realizado, objetivando determinar la diferenciación genética existente entre 50 poblaciones/semilleros/bancos provenientes de siete empresas paraguayas acopiadoras de sésamo 'Escoba Blanca', utilizando marcadores de microsatélites. Estos/as siete bancos/empresas/cooperativas, colectan y representan muestras de todos los productores/semilleros ubicados en diferentes departamentos de la Región Oriental y Occidental (Chaco) del país, con quienes trabajan, comercializan y acopian sésamo. Se obtuvo tejido vegetal para extraer ADN, desde plantines sembrados especialmente para el fin, usando todas las muestras/accesiones incluidas. Fueron utilizados seis marcadores microsatélites: GBssrsa184, GBssrsa123, GBssrsa182, GBssrsa108, GBssrsa08 y GBssrsa72. Fueron calculados: número y frecuencia de alelos, distancia/agrupamientos, diferenciación entre poblaciones y su estructura genética. El número medio de alelos por *locus* varió de 1,33 a 3,00. En los marcadores GBssrsa184 y GBssrsa108 tres poblaciones presentaron mayor frecuencia de alelos. Las poblaciones examinadas exhibieron amplio grado de diferenciación genética entre ellas, con identificación de cuatro grupos, con mayor y menos pureza respectivamente.

Palabras clave: diversidad genética, genotipo, marcadores microsatelites, *Sesamum indicum* L.

Resumo

A alta qualidade das sementes de gergelim originárias do país fez com que o Paraguai estivesse entre os principais exportadores. Das variedades disponíveis no território paraguaio, a mais difundida é a 'Escoba Blanca', que, possivelmente pelo processo de multiplicação, poderia promover alterações na sua frequência alélica, diversidade e pureza genética. Este trabalho foi realizado com o objetivo de determinar a diferenciação genética entre 50 populações/canteiros/bancos de sete empresas paraguaias que coletam gergelim 'Escoba Blanca', utilizando marcadores microssatélites. Estes sete bancos/empresas/cooperativas coletam e representam amostras de todos os produtores/sementeiros localizados em diferentes departamentos da Região Leste e Oeste (Chaco) do país, com os quais trabalham, comercializam e coletam gergelim. Foi obtido tecido vegetal para extração de DNA, de mudas plantadas especialmente para esse fim, utilizando todas as amostras/acessos incluídos. Foram utilizados seis marcadores microssatélites: GBssrsa184, GBssrsa123, GBssrsa182, GBssrsa108, GBssrsa08 e GBssrsa72. Foram calculados: número e frequência de alelos, distância/agrupamentos, diferenciação entre populações e sua estrutura genética. O número médio de alelos por locus variou de 1,33 a 3,00. Nos marcadores GBssrsa184 e GBssrsa108, três populações apresentaram maior frequência de alelos. As populações examinadas exibiram amplo grau de diferenciação genética entre si, com identificação de quatro grupos, com maior e menor pureza respectivamente.

Palavras-chave: diversidade genética, genótipo, marcadores microssatélites, *Sesamum indicum* L.

Introduction

In Paraguay, the cultivation of sesame (*Sesamun indicum* L.) has become one of the main income items for small and medium-scale family farming in the last decade (González and Causarano, 2014; Rabery *et al.*, 2020). The high quality of sesame seeds originating in the country has led Paraguay to become one of the world's leading exporters, gaining space in international markets (Melgarejo *et al*., 2020). At the same time, this significant boost in the production and marketing system has tripled the area planted per harvest, making it an item with a high socioeconomic impact.

Among the varieties available in Paraguay, the most widespread is the ''Escoba Blanca'' (Rabery *et al.*, 2020). Although this situation was positive, fields with "Foundation" category seeds were not maintained to guarantee varietal purity, in addition to the widespread practice among farmers of using their seeds. This is complemented by the dubious phylogenetic origin of this variety, which drives the concern to molecularly characterize the material. In that context, consideration of microsatellites, also known as simple sequence repeats (SSRs), is important. Due to their robustness, these SSRs have become an ideal molecular marker in population genetics research (Zhang *et al*., 2023).

So far, five stable and morphologically uniform genotypes have been identified, which means that they belong to the ''Escoba Blanca'' variety, but the level of genetic diversity between the selected and non-selected materials is still unknown, so performing a genetic analysis with microsatellite markers would provide more precise information than an analysis with morphological descriptors (Nyongesa *et al*., 2013). Thus, it was visualized to use microsatellite markers to develop the molecular study from samples from the most important national seedbeds in the country, in order to know distances, differentiations, and genetic structure between them, contributing to determining the purity and/or flow of this variety, widely disseminated.

Materials and methods

Sample collection

Existing samples were collected in the seven most important seedbeds in Paraguay, places where the reproductive material of the ''Escoba Blanca'' variety is periodically collected; directly from its production centers, located in areas of San Pedro, Concepción, Caaguazú, Cordillera, Central, Caazapá, Canindeyú, Alto Paraná and Misiones (Eastern Region), as well as from Boquerón and Presidente Hayes (Western Region or Chaco). These seedbeds correspond to companies, private cooperatives, and a university academic center, here denominated by discretion and ethics: *A*, *B*, *C*, *N*, *S*, *F*, and *L*. A total of 50 accessions/samples were obtained from the seven seedbeds. Each sample consisted of 200 g of seeds, randomly taken from the key batches, collected, and available in the seven seed banks, according to geographical origin. For sampling, metal drills were used, obtaining the reproductive material from the collection bag center (20 kg each). The samples were carefully stored and transported to a national biotechnology laboratory, where grain moisture (5 to 10 %) was quantified and kept in conservative chambers.

Obtaining plant tissue

Ten seeds from each population were sown in commercial substrates, composed of combinations of peat, nutrients, bark, vermiculites, and homogeneous black soil. They were placed in small plastic pots, taking care of the planting depth, watering, and

monitoring them permanently. After germination and emergence of the seedling, the leaf tissue of three seedlings per population was extracted, at the time that each one presented between four and six leaves/plant, becoming a biological sample. Then, DNA extraction was carried out with everyday material, a typical practice, which Alda *et al.* (2019) highlight as a simple procedure, but key for biotechnological services.

DNA Extraction with CTAB + DTT

DNA was extracted using the cetyltrimethylammonium bromide (CTAB) plus dithiothreitol (DTT) method, which is also used in other living beings (Angulo *et al*., 2020; Suazo *et al.*, 2020), here, modified and described below:

The material to be extracted was placed in a porcelain mortar and ground with the addition of liquid nitrogen until the total homogeneity of the product was achieved; it was then collected and placed in 2 mL tubes to which 750 µL of CTAB+DTT were added to each sample inside the fume hood. The samples were then placed in a thermal block and incubated at 65 °C for 30 min. After 15 minutes, they were taken out and reversed six to eight times, and then completed with the remaining 15 minutes. The samples were then given 750 µL of chloroform at -20 °C. They were again mixed by inversion and centrifuged at 1136 *g* for 8 min and at 4 °C. Labeled 1.5 mL eppendorf tubes were prepared and 450 µL of isopropanol or propanol-2 at -20 °C were added to each, and the supernatant was then added to the corresponding tube. It was mixed by inversion about six to eight times and incubated for 5 min at room temperature while resting, and then centrifuged at 441 *g* for 5 min. The isopropanol or propanol-2 was removed from a glass, taking care not to throw the *pellet* away. One milliliter of 75 % ethanol was added to wash it, passed for 30 seconds (s) through a vortex, and was subsequently centrifuged at 1153 *g* for 5 min. The supernatant was removed and the samples were dried in the hood. To dissolve the *pellet* well, ultrapure water was added. The samples were placed in the oven at 37 °C until the next day, and then the DNA content was quantified with a DS-11-DeNovix microvolume spectrophotometer. DNA integrity was determined by electrophoresis at 60 volts for 60 min in a 0.8 % agarose gel with tris-acetate-ethylenediaminetetraacetic (TAE) buffer that was stained with Promega's *Diamond Nucleic Acid Dye* intercalant and visualized using Promega's *Gel Doc* photodocumentation system. Samples of adequate quality and integrity were conveniently stored at -20 °C.

Molecular and statistical analysis

After a literature review of molecular studies with sesame, six SSR markers were chosen: GBssrsa184, GBssrsa123, GBssrsa182, GBssrsa108, GBssrsa08, and GBssrsa72 (Cho *et al.*, 2011). To perform the Polymerase Chain Reaction (PCR), each PCR reaction was carried out in a volume of 10 µL containing ultrapure water, 1.5 millimolar (mM) of $MgCl_2$, 1x 1.5 mM of $MgCl_2$ of 10x PCR buffer, 0.2 mM of deoxyribonucleotides (dNTPs), 250 nanomolar (nM) of each direct and reverse primer, 0.5 units of Taq DNA polymerase and 10 nanograms (ng) of DNA. The temperature profile used for PCR amplification comprised a denaturation step at 94 °C for 1 min, followed by the hybridization temperature of the primers at 45.2 - 53 °C for 1 min, and elongation at 72 °C for 1 min. After 34 cycles, the reaction was finished with 10 min at 72 °C for the final extension.

Subsequently, the PCR amplification products were run with a commercially available automatic sequence analyzer, typically used in paternity and genetic analysis in general, in a private laboratory of the national environment, considered a reference, which follows international protocols, validated in the academic and scientific community. Several alleles and allele frequencies were then tested using the Microsatellites Toolkit (Excel® MS Toolkit). Likewise, Nei's genetic distances were calculated considering the *Neighbor-Joining* clustering method, granting confidence intervals with 1,000 repeats (*bootstrap*) through Populations® version 1.2.28. Treeview® was used to visualize the genetic dendrogram. Correspondence factor analysis was performed in the process of genetic differentiation between populations, using Genetix®. Finally, the genetic structure between the sesame analysis groups and the correct allocation to the populations under study were analyzed (Structure® 2.1). These procedures were followed by other authors (Martínez-López, 2017; Martínez-López *et al*., 2019a, 2019b).

Results and discussion

Table 1 shows the allele frequencies for each of the six *loci* used, the average number of alleles, and the standard deviation of each group.

The highest average number of alleles was in the *L* population (3.00). This value would be due to the high number of samples obtained from it, considering that it is a seedbed that: a) harvests and distributes its germplasm in at least 12 communities distant from each other, b) It is one of the oldest to operate with this agricultural item in the Paraguayan Chaco. Concerning this parameter of genetic diversity, Surapaneni *et al.* (2014) in sesame cultivars (India); indicated an average of 2.5 alleles per *locus*. On the other hand, Pandey *et al.* (2015), verified an average of 3.37; while Dossa *et al.* (2016); confirmed 4.15 alleles per *locus*. On the other hand, Cho *et al.* (2011), showed that the number of alleles/*locus* depends on the diversity of the material, which in turn would be influenced by the geographical distribution and age of operation in the field.

In the present study, these concordances could not be verified.

On the other hand, for the allele frequencies (table 1) in the GBssrsa184 marker, populations *A*, *C*, and *L* exhibited greater extension in their distribution, occurring between the base pairs (bp) 169:177. In contrast, *L* showed an absence of fragments at the height of 169 bp. In GBssrsa08, reduced polymorphism was recorded, with the *F* population having a slightly larger extension of allele frequencies and relatively low variability with fragments of 138 and 148 bp. The other populations showed low variability. This dynamic was observed for the GBssrsa72 *locus,* where the only sample that revealed a slight amplitude in gene frequencies was *B*. The GBssrsa108 *locus* revealed that the *A, C*, and *L* populations were more polymorphic, while the frequencies for GBssrsa182 indicated that the sample with the highest polymorphism was "*L*". According to Abdellatef *et al.* (2008), employing RAPD (*Random amplified polymorphic DNA*) markers revealed high levels of genetic variability in sesame, even with the use of a limited set of primers. Previously, Laurentin and Karlovsky (2006) reported a high degree of variability in the sesame population studied with AFLP (*Amplified fragment length polymorphism*).

Molecular techniques as a basis of calculations for genetic distances are a very useful tool to delve into possible origins and formation of populations (Martínez-López *et al*., 2019a). Along these lines, figure 1 shows the dendrogram obtained based on the Nei distance.

Table 1. Allele frequencies per microsatellites and population, and average number of alleles of white sesame collected in Paraguay.

Locus/Marker	[1]pb	Population/Seedbeds						
		A	*B*	*C*	*N*	*S*	*F*	*L*
		[2]n=6	n=4	n=5	n=5	n=15	n=3	n=12
	161							4.17
	169	16.67		20.00				
GBssrsa184	171	50.00	75.00	40.00	100.00	86.67	33.33	66.67
	175	16.67		20.00			66.67	20.83
	177	16.67	25.00	20.00		13.33		8.33
GBssrsa08	138	100.00	100.00	100.00	100.00	100.00	83.33	100.00
	148						16.67	
	272	100.00	75.00	100.00	100.00	96.67	100.00	100.00
GBssrsa72	284		25.00					
	288					3.33		
	262		12.50			6.67		
	264	25.00	12.50	60.00		10.00	66.67	25.00
GBssrsa123	266	16.67	12.50		20.00			8.33
	267			20.00				
	268	58.33	62.50	20.00	80.00	76.67	33.33	66.67
	282					6.67		
	204	16.67		20.00				8.33
	206	50.00	50.00	60.00		16.67	66.67	25.00
GBssrsa182	260	33.33	25.00	20.00	100.00	83.33	33.33	58.33
	262		25.00					
	264							8.33
	173							4.17
	189	8.33	12.50	20.00	20.00	3.33		
	190					6.67		
GBssrsa108	191	58.33	75.00	30.00	80.00	76.67	66.67	75.00
	193	16.67		10.00				4.17
	199	8.33		10.00				
	203	8.33	12.50			13.33		8.33
	213			30.00			33.33	8.33
Average number of alleles		2.83	2.50	2.83	1.33	2.50	1.83	3.00
Standard Deviation		1.60	1.05	1.60	0.52	1.22	0.41	1.67

[1]bp: Base pairs, [2]n: number of samples

Three main lines/clusters have been found (figure 2), where the *L* population is the first, and most distant from the rest. This population (*L*) represents a productive cooperative in the center of the western region, which has been spreading and marketing this agricultural item for about three decades, perhaps being the pioneer in the area. The second group was made up of *A, C,* and *F*. Among these, *C* and *F* were genetically very close to each other. In terms of commercial and geographical dynamics, these three populations have had a lot of germplasm exchange in the last 15 years, both as collectors of "cross-producer", except for *F*, which represents an agrarian academic center and not an objective economic return. The cross-producer is the one who receives germplasm from a company, for its cultivation, but after the harvest, chooses to sell to another collector, looking for a better price, which would be the fundamental circuit for the mixture or deterioration of varietal purity.

The third group consisted of *B*, *N,* and *S*, where samples of *N* and *S* were genetically close to each other. However, the *B* samples were close to the *N* and *S* populations. All the proximities recorded between some of the accessions of the populations (*C* and *F*; *N* and *S),* would be due to the frequent exchange of germplasms given annually for the development of the crop in the country, taking into account that the company *S* and the cooperatives *F* and *N*, started the field in the 90s (Chaco). It is worth noting that the *bootstrap* indices of distance analysis give consistency to the results (Nei and Takezaki, 1996).

Next, the correspondence factor analysis of the seven Paraguayan white sesame populations.

The populations exhibited a high degree of differentiation between them, presenting an extensive distribution along the three axes of the graph that explain most of the genetic variation, except for the *N* and *S* populations, which did not present considerable differentiation between them. Likewise, a low degree of genetic differentiation between *S, B,* and *N* accessions was observed, showing congruence with results derived from the distance and differentiation analysis, discussed above (figure 1).

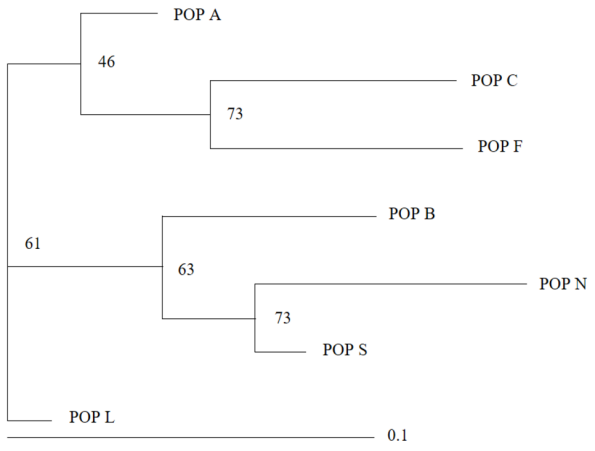


Figure 1. Dendrogram of genetic distances (Nei) among the seven Paraguayan populations (seedlings) of sesame var. "Escoba blanca".

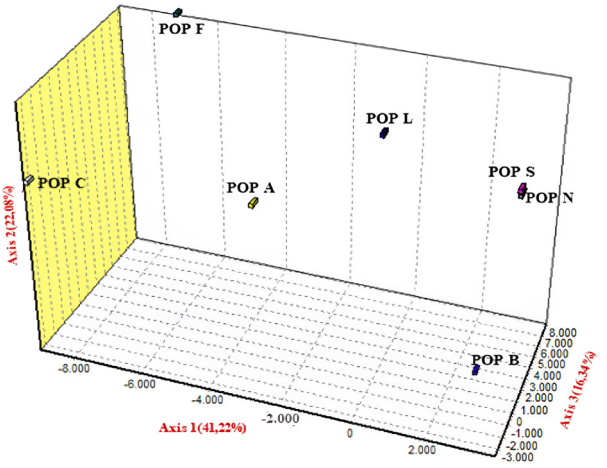
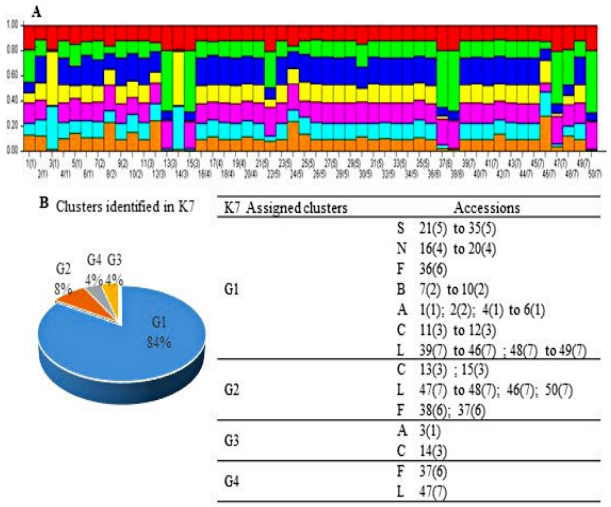


Figure 2: Genetic differentiation between white sesame populations, using correspondence factor analysis for the axes of greatest inertia.

In a study by Abdellatef *et al.* (2008) on Sudanese sesame, two main clusters were found, one of which was made up of the Elgadaref-1 and Elobaied-1 genotypes, while the other grouped the Abusitta and Ali Mahdi genotypes. Ali *et al.* (2007), showed that accessions from Japan, India, Myanmar, and Pakistan were closely related genetically, based on their origin. According to Arriel *et al.* (2006), the low diversity evidenced in sesame in Brazil can be attributed to the fact that sesame is an introduced crop. However, Pham (2011) found high genetic variation in sesame accessions evaluated, indicating that these were correlated with their geographical origin. However, the analysis also showed that some accessions were not grouped with others from the same geographical region, referring to the exchange of varieties between farmers from different regions as a possible cause. On the other hand, Surapaneni *et al.* (2014), did not find any relevant differentiation between sesame genotypes, according to their origin.

Molecular marker techniques, such as AFLP, RAPD, ISSR (*inter simple sequence repeats*), and SSR, have been widely used in studies of genetic diversity in sesame (Pham, 2011), agreeing with Teklu *et al.* (2021) and Teklu *et al*. (2022), who report that single-nucleotide polymorphisms (SNPs) are also widely used in plant species analyses. Although SSRs are considered in sesame, Pandey *et al.* (2015), argue that their application is limited in studies with this item, despite their relevance and usefulness. On the other hand, Dossa *et al.* (2016), reported limited studies considering large samples of sesame accessions from Africa and Asia. Also, Dar *et al.* (2017), indicated few studies on genetic diversity in sesame.

The result of the analysis of the genetic structure among all the accessions that constituted the germplasms analyzed is shown below (figure 3).



K7 Assigned clusters	Accessions
G1	S 21(5) to 35(5)
	N 16(4) to 20(4)
	F 36(6)
	B 7(2) to 10(2)
	A 1(1); 2(2); 4(1) to 6(1)
	C 11(3) to 12(3)
	L 39(7) to 46(7) ; 48(7) to 49(7)
G2	C 13(3) ; 15(3)
	L 47(7) to 48(7); 46(7); 50(7)
	F 38(6); 37(6)
G3	A 3(1)
	C 14(3)
G4	F 37(6)
	L 47(7)

Figure 3. Graphical representation of the results of the genetic structure analysis of the seven Paraguayan populations of sesame var. "Escoba blanca".

Genetic structure sharing between *C*, *F* populations and some *L* constituent samples was observed to be very consistent. This strong similarity supports the hypothesis that there is some kind of genotypic relationship of common origin between them; which leads to believe that there is still varietal purity of white sesame in these seed banks. The practical importance of genetic structure results is to identify samples or seedbeds that can be used for breeding programs, emphasizing the need for distant materials. Pham (2011) suggested that 40 % of the total, intra-varietal genetic variation can be found between accessions or different seed banks. On the other hand, Laurentin and Karlovsky (2006) postulated that the influencing factor in the current genetic structure of sesame is the human activity associated with the management of seed banks and their commercial distribution.

This work showed that the *S*, *N,* and *B* populations *are* genetically well related, as well as *C* and *F*; giving consistency to the hypothesis that these groups (*S-N-B* and C-*F*) share origin, a fact that can be attributed to the dynamic flow of germplasm exchange between them, or the unique origin in the distribution of white sesame seeds in the early years of cultivation in Paraguay. In the structure analysis (figure 3B), "K7" revealed four clusters (G1, G2, G3, and G4), where G1 had the highest number of individuals (84 %). These results conveniently contribute to the determination of selection criteria useful for making strategic decisions regarding the future of the crop, which is essential for effective breeding programs in the genetic management of this variety (Gediya *et al.*, 2019).

This scientific publication in digital format is a continuation of the Printed Review: Legal Deposit pp 196802ZU42, ISSN 0378-7818.

Conclusions

Sesame populations exhibit an interesting degree of genetic differentiation among them, with the identification of four groups of seedlings. The mean number of alleles per *locus* varies at low levels, showing reduced genetic variability in the white sesame used in Paraguay. Likewise, the GBssrsa184 and GBssrsa108 markers better determine the highest allele frequencies, identifying three populations with this characteristic in molecular diversity. Finally, it should be mentioned that the molecular study carried out is contributing and enriching, leaving to the discretion of the decision-makers of the sector, the type of use and program that they intend to establish in the future, from more or less genetically pure lineages, all useful in work of national impact.

Acknowledgment

To the Institute of Agricultural Biotechnology of Paraguay (INBIO).

Literature Cited

Abdellatef, E., Sirelkhatem, R., Mohamed, A. M. M., Radwan, K. H., & Khalafalla, M. M. (2008). Study of genetic diversity in sudanese sesame (*Sesamum indicum* L.) germplasm using random amplified polymorphic DNA (RAPD) markers. *African Journal of Biotechnology*, *7*(24), 4423–4427. https://www.ajol.info/index.php/ajb/article/view/59603

Alda, J., Marcos-Merino, J., & Gallego, R. (2019). Extracción de ADN con material cotidiano: desarrollo de una estrategia interdisciplinar a partir de sus fundamentos científicos. *Educación Química*, *30*(1), 58-69. https://doi.org/10.22201/fq.18708404e.2019.1.65732

Ali, G. M., Yasumoto, S., & Seki-Katsuta, M. (2007). Assessment of genetic diversity in sesame (*Sesamum indicum* L.) detected by amplified fragment length polymorphism markers. *Electronic Journal of Biotechnology*, *10*(1), 12-23. https://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0717-34582007000100002&lng=es&nrm=iso

Angulo, L., Perichi, G. A., Castro, L. D., & Figueroa, R. (2020). Comparación de diferentes métodos de extracción de ADN genómico en babosas plagas (mollusca: gasterópoda). *Revista Científica ECOCIENCIA*, *7*(4), 35-49. https://doi.org/10.21855/ecociencia.74.380

Arriel, N. H. C., Di Mauro, A., Di Mauro S., Bakke, O. A., Unêda-Trevisoli, S., Costa, M., Capeloto, A., & Corrado, A. R. (2006). Técnicas multivariadas na determinação da diversidade genética em gergelim usando marcadores RAPD. *Pesquisa Agropecuária Brasileira*, *41*(5), 801-809. https://doi.org/10.1590/S0100-204X2006000500012

Cho, Y.I., Park, J.H., Lee, C.W., Ra, W.H., Chung, J.W., Lee, J.R., Ma, K.H., Lee, S.Y., Lee, K.S., Lee, M.C., & Park, Y.J. (2011). Evaluation of the genetic diversity and population structure of sesame (*Sesamum indicum* L.) using microsatellite markers. *Genes & Genomics*, *33*(2), 187–195. https://doi.org/10.1007/s13258-010-0130-6

Dar, A. A., Mudigunda, S, Mittal, P. K., & Arumugam, N. (2017) Comparative assessment of genetic diversity in *Sesamum indicum* L. using RAPD and SSR markers. *3 Biotech*, *7*(10), 1-12. https://doi.org/10.1007/s13205-016-0578-4

Dossa, K., Wei, X., Zhang, Y., Fonceka, D., Yang, W., Diouf, D., Liao, B., Cissé, N., & Zhang, X. (2016). Analysis of genetic diversity and population structure of sesame accessions from Africa and Asia as major centers of its cultivation. *Genes*, *7*(4), 1-13. https://doi.org/10.3390/genes7040014

Gediya, L., Patel, D., Kumar, S., Kumar, P., Parmar, D., & Patel, S. (2019). Phenotypic variability, path analysis and molecular diversity analysis in chickpea (*Cicer arietinum* L.). *Vegetos*, *32*(2), 167-180. https://doi.org/10.1007/s42535-019-00020-9

González, A., & Causarano, H. (2014). Destino del nitrógeno aplicado en un cultivo de sésamo (*Sesamum indicum* L.) en un suelo degradado de Paraguay. *Acta Agronómica*, *63*(3), 253-261. https://doi.org/10.15446/acag.v63n3.42259

Laurentin, H., & Karlovsky, P. (2006). Genetic relationship and diversity in a sesame (*Sesamum indicum L.*) germplasm collection using amplified fragment length polymorphism (AFLP). *BMC Genetics*, *7*(1), 1–10. https://doi.org/10.1186/1471-2156-7-10

Martínez-López, O. R. (2017). El Pampa Chaqueño: raza bovina local del Paraguay. Argumentos técnicos y científicos que sustentan su reconocimiento como raza local. Artes Gráficas Visual. https://isbn.bibliotecanacional.gov.py/catalogo.php?mode=detalle&nt=11326

Martínez-López, O. R., Barbosa, S., Martínez, A. M, Delgado, J. V., & Landi, V. (2019a). Distancias genéticas entre dos poblaciones de bovinos criollos paraguayos. *AICA*, *14*,126-136. https://www.aicarevista.com/n %C3 %BAmeros/vol %C3 %BAmen-14-2019/

Martínez-López, O. R., Barbosa, S., Martínez, A. M., Delgado, J. V., & Landi, V. (2019b). Perfil genético de dos poblaciones bovinas criollas de Paraguay. *AICA*, *14*, 141-153. https://www.aicarevista.com/n%C3%BAmeros/vol %C3%BAmen-14-2019/

Melgarejo, M., Galeano, A., Amarilla, D., Maidana, E., Bogado, M., Franco, R., Mendoza, M., Colman, P., Silvero, O., Lugo, W., & Da Silva, M. (2020). Efecto de diferentes densidades de siembra sobre las características agronómicas del ajonjolí (*Sesamum indicum* L.) en el distrito de Curuguaty. *Idesia (Arica)*, *38*(3), 107-112. https://dx.doi.org/10.4067/S0718-34292020000300107

Nei, M., & Takezaki, N. (1996). The root of the phylogenetic tree of human populations. *Molecular Biology and Evolution*, *13*(1), 170-177. https://doi.org/10.1093/oxfordjournals.molbev.a025553

Nyongesa, B., Were, B., Gudu, S., Dangasuk, O., & Onkware, A. (2013). Genetic diversity in cultivated sesame (*Sesamum indicum L.*) and related wild species in East Africa. *Journal of Crop Science and Biotechnology*, *16*(1), 9-15. https://doi.org/10.1007/s12892-012-0114-y

Pandey, S., Das, A., Rai, P., & Dasgupta, T. (2015). Morphological and genetic diversity assessment of sesame (*Sesamum indicum* L.) accessions differing in origin. *Physiology and Molecular Biology of Plants*, *21*(4), 519-529. https://doi.org/10.1007/s12298-015-0322-2

Pham, T. (2011). *Analyses of Genetic Diversity and Desirable Traits in Sesame (Sesamum indicum L., Pedaliaceae): Implication for Breeding and Conservation* [Doctoral Thesis, Swedish University of Agricultural Sciences]. https://res.slu.se/id/publ/33897

Rabery, S., Enciso, V., Franco, R., & Lezcano, J. (2020). Incidencia del riego complementario en el rendimiento de granos de sésamo (*Sesamun indicum* L.). *Investigación Agraria*, *22*(2), 75-81. https://doi.org/10.18004/investig.agrar.2020.diciembre.2202642

Suazo, T., Miranda, S., Rivers, E., Lacayo, M., & Tenorio, D. (2020). Evaluación de metodologías de extracción de ADN de plantas recalcitrantes. *Revista Torreón Universitario*, *9*(24), 45-57. https://doi.org/10.5377/torreon.v9i24.9723

Surapaneni, M., Yepuri, V., Vemireddy, R., Ghanta, A., & Siddiq, E. (2014). Development and characterization of microsatellite markers in Indian sesame (*Sesamum indicum* L.). *Molecular Breeding*, *34*(3), 1185-1200. https://doi.org/10.1007/s11032-014-0109-0

Teklu, D., Shimelis, H., Tesfaye, A., Mashilo, J., Zhang, X., Zhang, Y., Dossa, K., & Shayanowako, A. (2021). Genetic variability and population structure of Ethiopian sesame (*Sesamum indicum* L.) germplasm assessed through phenotypic traits and simple sequence repeats markers. *Plants*, *10*(6), 1129. https://doi.org/10.3390/plants10061129

Teklu, D., Shimelis, H., Tesfaye, A., & Shayanowako, A. (2022). Analyses of genetic diversity and population structure of sesame (*Sesamum indicum* L.) germplasm collections through seed oil and fatty acid compositions and SSR markers. *Journal of Food Composition and Analysis*, *110*(104545),1-12. https://doi.org/10.1016/j.jfca.2022.104545

Zhang, J., Yang, J., Lv, Y., Zhang, X., Xia, C., Zhao, H., & Wen, C. (2023). Genetic diversity analysis and variety identification using SSR and SNP markers in melon. *BMC Plant Biology*, *23*(39), 2-10. https://doi.org/10.1186/s12870-023-04056-7