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Research article

Moringa oleifera Genebank in Brazil: current status and future approaches

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ABSTRACT:*Moringa oleifera* Lam. is a tropical tree that belongs to the Moringaceae family and is popularly known worldwide for its multiple applications. This study aimed to evaluate the genetic variability of individuals from the Moringa Genebank of Embrapa Tabuleiros Costeiros, Sergipe state, Brazil. The Moringa Genebank comprises 25 accessions, represented by 177 genotypes, of which 18 were transferred in from an exchanged germplasm of the University of Florida, USA, and the others were from different states in Brazil. Leaves of each genotype were collected for DNA extraction and polymerase chain reaction (PCR) analysis using 20 Inter simple sequence repeats (ISSR) primers. A total of 141 bands were amplified, and 100 % were polymorphic. The average expected heterozygosity (He) and Shannon's index were 0.11 and 0.16, respectively. The highest genetic divergence was found between the M4 and M18 accessions, both from Florida, USA, whereas the closest pair of accessions was M23 and M24, both from Brazil. The cluster analysis obtained through the Structure software divided moringa genotypes into two groups. These results suggest low genetic diversity between the accessions in the Moringa Genebank. Therefore, the introduction of new accessions in the Moringa GeneBank is essential to increasing the genetic variability of the species to ensure its conservation and improvement.

Keywords: ISSR markers, Moringaceae, genetic resources

Introduction

Moringa oleifera Lam. belonging to the monogeneric Moringaceae family composed of 13 species, is the most popular and only cultivated species (Farooq and Koul, 2020). Native to India and spread over tropical areas, the species grows well in Africa, China, and Mexico. It is a fast-growing tree, which can reach up to 12 m in height and be propagated through both sexual and asexual reproduction. This plant has low demands for soil nutrients and water and is considered resistant to drought and diseases. *Moringa oleifera* is composed of a slender stem with drooping branches, white flowers, tripinnate leaves, and white-brown seeds with papery wings. It is a deciduous perennial tree, recognized for its multipurpose quality since every part of this plant has a specific application, including human and animal nutrition, medicine, soil fertilizer, foliar nutrients, green manure, biogas and biodiesel (Bancessi et al., 2020; Pandey et al., 2011).

The leaves of this plant have a variety of nutrients, such as minerals, proteins, vitamins A, B1, B2, B3, C, and E, carotenoids, and flavonoids, among other bioactive compounds. Due to these properties, moringa has been used as a food fortificant. Moreover, it has been recently recognized as a superfood, a valuable product for human and animal nutrition (Boopathi et al., 2021). This species has also been reported to have potential pharmacological effects, including antioxidants, anti-inflammatory, and anti-diabetic activity (Singh et al., 2020). The seeds are used for water purification, in which the powder extracted from the seeds has natural coagulant properties for water treatment (Milla et al., 2021). Additionally, the seeds have a high content of oil, known as "Ben", and can be used as a lubricant in both the culinary and cosmetic industries (Nair et al., 2021). In Brazil, it was introduced as an ornamental tree around 1950 and is now well distributed throughout the country, mainly in the northeastern region (Rivas et al., 2013).

Given the relevance of this species, it is essential to ensure that genetic variability is preserved to improve this resource for future breeding programs. Collections and genebanks are considered the key to this purpose (Leone et al., 2015), and in order to understand and conserve the genetic variability of *M. oleifera*, the Empresa Brasileira de Pesquisa Agropecuária (Embrapa) established the Moringa Genebank in 2009, located in Nossa Senhora das Dores, Sergipe state, Brazil. The initial objective was to identify superior individuals as regards nutritional characteristics for use in animal production, mainly in the production and quality of the leaves. Currently, the genebank consists of 25 accessions represented by 177 genotypes.

The evaluation of a germplasm collection represents an important step in promoting conservation actions and selecting crucial traits in a crop improvement program (Nair et al., 2021). Therefore, this study aimed to evaluate the genetic diversity of moringa genotypes from a Genebank of the Embrapa using Inter simple sequence repeats (ISSR) markers.

Materials and Methods

Collection of plant material

The Moringa Genebank was established in the experimental field in the city of Nossa Senhora das Dores, Sergipe state, Brazil (10°46′33″ S, 37°19′16″ W, altitude 204 m) (Figure 1A and B). It comprises 177 genotypes from 25 accessions, of which 18 are from exchanged germplasm of the University of Florida, USA, and the others from different states in Brazil, such as Sergipe, Pernambuco, Paraíba, Rio Grande do Norte, and Ceará (Table 1). Leaves of each genotype were collected, stored in ice to be transferred to the laboratory, and kept at –80 °C until DNA extraction.

DNA extraction

DNA was extracted using the protocol described by Doyle and Doyle (1990). The leaves (300 mg) were manually macerated using a mortar and pestle with the aid of liquid nitrogen using 1 mL of Cetyltrimethylammonium bromide (CTAB) buffer. A Nanodrop 2000c spectrophotometer quantified the DNA. Samples were diluted in Tris EDTA (Ethylenediaminetetraacetic acid) at a concentration of 10 ng μL^{-1}, and then kept at –20 °C to be used in polymerase chain reaction (PCR) reactions.

PCR

The PCR mix (20 µL for each sample) consisted of 1 µL of genomic DNA solution, 2 µL 10X PCR Buffer, 0.4 µL dNTP (deoxynucleoside triphosphate) (10 mM), 0.6 µL $MgCl_2$ (50 mM), 1 µL of each primer, 0.2 µL Taq DNA polymerase Ludwig and 14.8 µL of ultrapure water.

The samples were placed in an Axygen Maxygene thermocycler programmed to amplify the DNA. Each reaction consisted of a denaturation stage at 95 °C for 4 min, 45 cycles of amplification (denaturation at 94 °C for 45 s, annealing at each primer temperature for 45 s, and first extension at 72 °C for 2 min), followed by a final extension at 72 °C for 7 min. Twenty ISSR primers were tested (Table 2).

Electrophoresis and ISSR marker analysis

Samples, after amplification by PCR, were submitted to electrophoresis analysis. For this, a 2 % agarose gel was

Table 1 – Provenance and number of genotypes in the Moringa Genebank. Experimental field of Embrapa Tabuleiros Costeiros in Nossa Senhora das Dores, Sergipe state, Brazil.

Provenance	Accession	Number of genotypes
Florida, USA	M1	8
	M2	8
	M3	8
	M4	8
	M5	8
	M6	10
	M7	8
	M8	5
	M9	7
	M10	9
	M11	6
	M12	7
	M13	4
	M14	7
	M15	5
	M16	9
	M17	9
	M18	2
Fortaleza, Ceará state, Brazil	M19	2
Mossoró, Rio Grande do Norte state, Brazil	M20	3
Petrolina, Pernambuco state, Brazil	M21	7
Aracaju, Sergipe state, Brazil	M22	7
Frei Paulo, Sergipe state, Brazil	M23	10
Itabaiana, Sergipe state, Brazil	M24	10
Queimadas, Paraíba state, Brazil	M25	10
Total		177

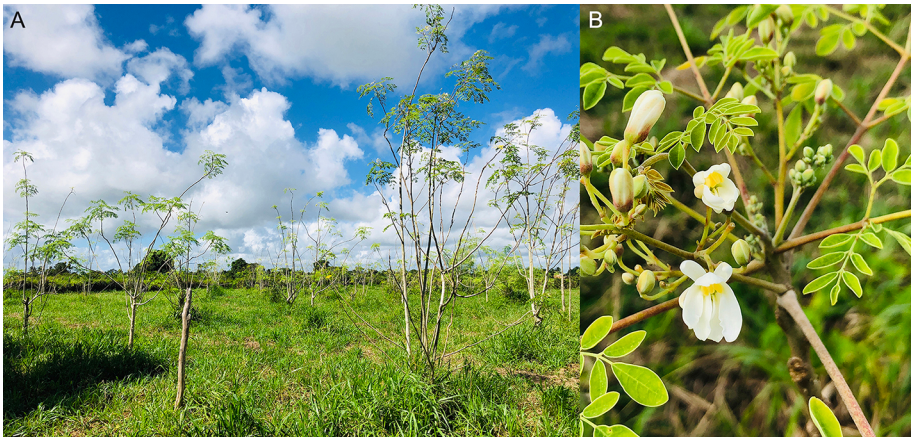

Figure 1 – A) Moringa Genebank and (B) Inflorescence. Experimental field of Embrapa Tabuleiros Costeiros in Nossa Senhora das Dores, Sergipe state, Brazil.

Table 2 – Sequence, annealing temperature (AT) and guanine-cytosine content (GC content) inter simple sequence repeats (ISSR) markers used to characterize the diversity and genetic structure of Moringa Genebank of Embrapa Tabuleiros Costeiros in Nossa Senhora das Dores, Sergipe state, Brazil.

Primer	Sequence	AT °C	% GC content
UBC 807	5' AGA GAG AGA GAG AGA GT 3'	47.0	47.1
UBC 809	5' AGA GAG AGA GAG AGA GG 3'	57.2	52.9
UBC 813	5' CTC TCT CTC TCT CTC TT 3'	44.6	47.1
UBC 811	5' GAG AGA GAG AGA GAG AC 3'	46.8	52.9
UBC 816	5' CAC ACA CAC ACA CAC AT 3'	54.8	47.1
UBC 818	5' CAC ACA CAC ACA CAC AG 3'	57.2	52.9
UBC 823	5' TCT CTC TCT CTC TCT CC 3'	57.2	52.9
UBC 825	5' ACA CAC ACA CAC ACA CT 3'	54.8	47.1
UBC 826	5' ACA CAC ACA CAC ACA CC 3'	57.2	52.9
UBC 827	5' ACA CAC ACA CAC ACA CG 3'	57.2	52.9
UBC 845	5' CTC TCT CTC TCT CTC TG 3'	58.8	52.9
UBC 848	5' CAC ACA CAC ACA CAC AG 3'	58.8	50.0
UBC 855	5' ACA CAC ACA CAC ACA CT 3'	53.1	47.1
UBC 856	5' ACA CAC ACA CAC ACA CA 3'	56.5	47.1
UBC 860	5' TGT GTG TGT GTG TGT GA 3'	46.9	47.1
UBC 864	5' ATG ATG ATG ATG ATG ATG 3'	50.8	33.3
ISSR 1	CAC ACA CAC ACA GG	52.6	57.1
ISSR 2	CTC TCT CTC TCT CTC TAC	57.6	50
ISSR 4	CAC ACA CAC ACA AC	49.7	50
ISSR 6	CAC ACA CAC ACA AG	49.7	50

placed under a constant voltage of 182V, 91 mA, and 17W for 115 min, followed by staining with ethidium bromide solution (0.5 µL mL^{-1} of water) for 30 min. Next, the agarose gels were submitted to visualization under ultraviolet light using Gel Doc L-pix photo documentation equipment (Loccus Biotecnologia).

Genetic diversity and statistical analysis

The bands' presence (1) and absence (0) were transformed into a binary matrix for each gel. The genetic variability for each accession was assessed using the Shannon index, which allows for the distinguishing of the genetic variation between populations with the same number of alleles, and expected heterozygosity (He), that represents the diversity of a locus describing the proportion of heterozygous genotypes expected under the Hardy-Weinberg equilibrium (Nei, 1973), using the Genalex 6.5 software program (Peakall and Smouse, 2012).

The genetic variance between and within accessions was estimated using the Analysis of Molecular Variance (AMOVA) and the level of significance was determined by 9.999 permutations. The genetic distance between accessions (GST) was estimated (Nei, 1973), corresponding to the total genetic variation, and its significance was tested by 10.000 bootstraps. Additionally, the genetic distance of Nei between the accessions was estimated. The analyses used the Genalex 6.5 software (Peakall and Smouse, 2012).

The model of Rogers (1972) was used to evaluate the genetic distance between the accesses and was visualized by constructing a dendrogram using the unweighted pair group method with arithmetic means (UPGMA) algorithm. The analysis was carried out using the poppr package (Kamvar et al., 2014) in the software R program. The FigTree 1.4.1 software program was used to format the dendrogram. At the individual level, the principal coordinate analysis (PCoA) was carried out using the Genalex 6.5 software program.

The genetic structure of 25 accessions was estimated by the Bayesian analysis using the Structure software package v.2.3.4. The values of genetic groupings (k) varying from 1 to 25 (number of accesses) were tested, and ten independent repetitions were performed for each k. Each replicate had a burn-in period of 50,000 iterations, followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations, assuming the mixture ancestry model and uncorrelated allele frequencies. The number of genetic groups (k) was identified by the ΔK method described by Evanno et al. (2005), implemented by the Structure Harvester software program. Accessions that showed membership values higher than 0.2 and lower than 0.8 were considered to have mixed ancestry.

Results

Genetic diversity indexes

The 20 ISSR primers used in our study generated 141 bands, of which 100 % showed signs of polymorphism (Table 3). The minimum and maximum number of amplified bands ranged from three (UBC 818) to 10 (UBC 825 and 848), with an average of seven bands per primer. The He showed variations between 0.03 (ISSR2) and 0.17 (UBC 825), with an average value of 0.11. Similarly, the Shannon index ranged between 0.04 (ISSR2) and 0.25 (UBC 825), with an average value of 0.16 (Table 3; Figure 2). These results are corroborated by the AMOVA analysis, with 56 % of variance revealed within accessions and 44 % variance among accessions (Table 4).

In our study, the highest coefficient of Nei's Genetic Distance was found between two accessions from the Florida exchange, M4 and M18 (0.310). On the other hand, the closest pair of accession is M23 and M24 (0.048), both from Brazil. Nei's genetic distance matrix obtained with pairwise accessions is exhibited in Table 5.

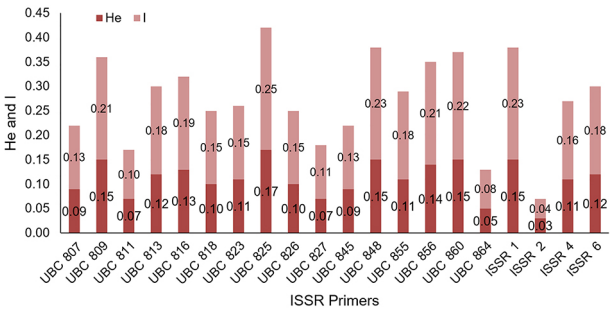

Figure 2 – Comparison for Shannon index (I) and expected heterozygosity (He) to 25 accessions represented by 177 genotypes from of Moringa Genebank of Embrapa Tabuleiros Costeiros in Nossa Senhora das Dores, Sergipe state, Brazil. ISSR = inter simple sequence repeats.

Table 3 – Inter simple sequence repeat (ISSR) primers used in moringa with their respective total fragments, polymorphic fragments, polymorphism percentages, Shannon index (I) and expected heterozygosity (He).

Primer	Total of bands	Polymorphic bands	Polymorphism (%)	He	I
UBC 807	5	5	100	0.09	0.13
UBC 809	4	4	100	0.15	0.21
UBC 813	4	4	100	0.12	0.18
UBC 811	6	6	100	0.07	0.10
UBC 816	8	8	100	0.13	0.19
UBC 818	3	3	100	0.10	0.15
UBC 823	8	8	100	0.11	0.15
UBC 825	10	10	100	0.17	0.25
UBC 826	9	9	100	0.10	0.15
UBC 827	9	9	100	0.07	0.11
UBC 845	8	8	100	0.09	0.13
UBC 848	10	10	100	0.15	0.23
UBC 855	9	9	100	0.11	0.18
UBC 856	7	7	100	0.14	0.21
UBC 860	9	9	100	0.15	0.22
UBC 864	7	7	100	0.05	0.08
ISSR 1	8	8	100	0.15	0.23
ISSR 2	4	4	100	0.03	0.04
ISSR 4	6	6	100	0.11	0.16
ISSR 6	7	7	100	0.12	0.18
Average	7	7	100	0.11	0.16

Table 4 – Analysis of molecular variance (AMOVA) showing the genetic variation within and among accessions from Moringa Genebank of Embrapa Tabuleiros Costeiros based on 20 inter simple sequence repeats (ISSR) primers.

Source of variation	df	SS	MS	Est. var.	%	GST
Among Accessions	24	1597.69	66.57	7.99	44	0.440***
Within Accessions	152	1549.39	10.19	10.19	56	
Total	176	3146.98	-	18.92	100	

***p < 0.001. df = degree of freedom; SS = sum of squares; MS = mean of squares; Est. var. = estimate of variance; % = percentage of total variation; GST = genetic distance between accessions.

Table 5 – Matrix using the coefficient of Nei's Genetic Distance from Moringa Genebank of Embrapa Tabuleiros Costeiros.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	0.000																								
2	0.102	0.000																							
3	0.085	0.081	0.000																						
4	0.102	0.124	0.066	0.000																					
5	0.117	0.116	0.099	0.071	0.000																				
6	0.111	0.103	0.144	0.142	0.134	0.000																			
7	0.097	0.108	0.121	0.103	0.089	0.052	0.000																		
8	0.115	0.089	0.098	0.107	0.093	0.097	0.068	0.000																	
9	0.131	0.105	0.140	0.129	0.091	0.099	0.080	0.088	0.000																
10	0.117	0.103	0.108	0.111	0.098	0.097	0.076	0.095	0.053	0.000															
11	0.142	0.145	0.133	0.125	0.111	0.158	0.139	0.125	0.130	0.114	0.000														
12	0.144	0.179	0.145	0.149	0.173	0.178	0.159	0.133	0.167	0.125	0.103	0.000													
13	0.130	0.157	0.117	0.129	0.154	0.180	0.157	0.151	0.148	0.116	0.081	0.083	0.000												
14	0.209	0.212	0.192	0.211	0.225	0.218	0.234	0.251	0.222	0.201	0.169	0.191	0.131	0.000											
15	0.223	0.240	0.235	0.258	0.243	0.236	0.269	0.294	0.242	0.213	0.191	0.203	0.166	0.089	0.000										
16	0.192	0.182	0.204	0.211	0.232	0.183	0.225	0.237	0.224	0.202	0.186	0.194	0.174	0.131	0.118	0.000									
17	0.235	0.235	0.261	0.266	0.254	0.237	0.249	0.255	0.225	0.220	0.192	0.218	0.178	0.131	0.150	0.081	0.000								
18	0.246	0.264	0.267	0.310*	0.262	0.265	0.269	0.285	0.277	0.268	0.241	0.265	0.205	0.161	0.164	0.171	0.097	0.000							
19	0.264	0.227	0.240	0.263	0.232	0.259	0.267	0.271	0.259	0.241	0.194	0.232	0.188	0.141	0.175	0.157	0.128	0.100	0.000						
20	0.206	0.192	0.199	0.240	0.237	0.209	0.224	0.260	0.224	0.207	0.197	0.250	0.226	0.178	0.171	0.163	0.132	0.136	0.120	0.000					
21	0.228	0.231	0.222	0.240	0.279	0.265	0.254	0.293	0.241	0.228	0.207	0.232	0.137	0.126	0.143	0.129	0.102	0.159	0.178	0.153	0.000				
22	0.219	0.211	0.251	0.264	0.257	0.232	0.258	0.264	0.237	0.221	0.208	0.239	0.176	0.114	0.116	0.117	0.087	0.088	0.111	0.130	0.093	0.000			
23	0.196	0.189	0.207	0.233	0.251	0.222	0.206	0.228	0.213	0.209	0.167	0.218	0.156	0.127	0.147	0.134	0.110	0.128	0.134	0.106	0.076	0.070	0.000		
24	0.218	0.206	0.207	0.228	0.232	0.236	0.222	0.246	0.214	0.210	0.163	0.206	0.164	0.127	0.146	0.114	0.105	0.134	0.116	0.122	0.077	0.077	0.048*	0.000	
25	0.238	0.225	0.225	0.217	0.254	0.250	0.228	0.268	0.221	0.211	0.189	0.223	0.167	0.128	0.175	0.156	0.152	0.179	0.155	0.161	0.073	0.089	0.059	0.050	0.000

*M4 and M18 (0.310) = highest coefficient of Nei's Genetic Distance; M23 and M24 (0.048) = lowest coefficient of Nei's Genetic Distance.

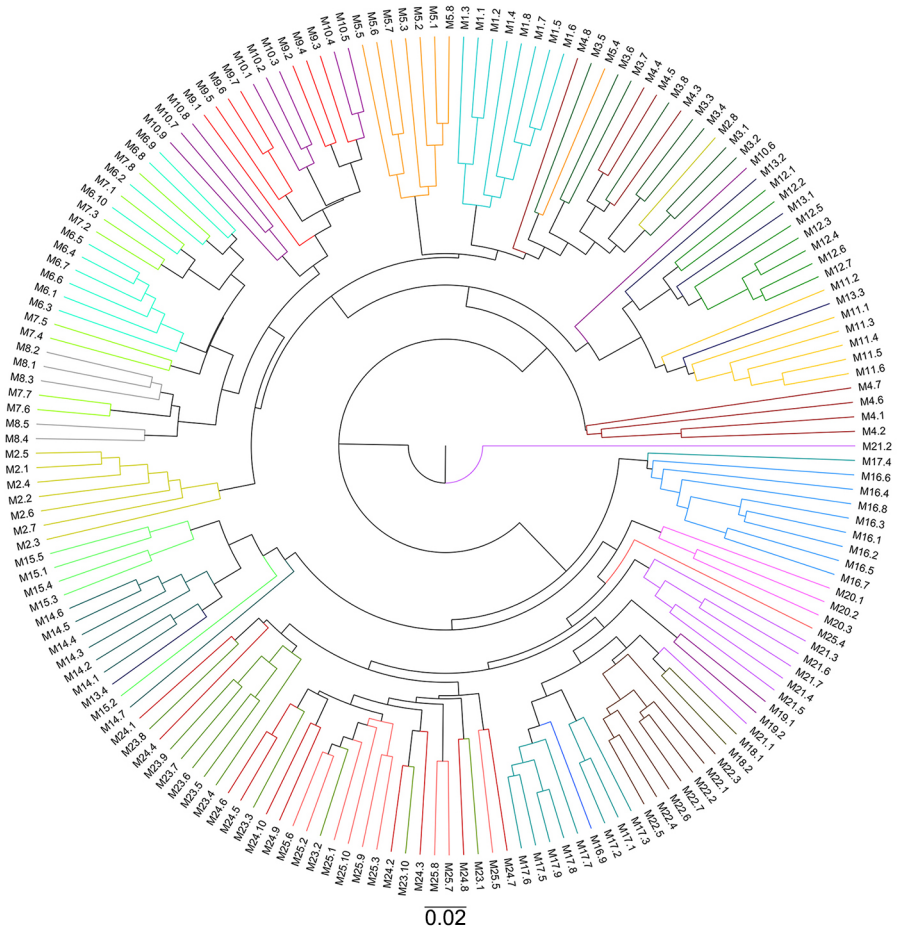

Figure 3 – Matrix using the coefficient of Rogers from the 25 accessions represented by 177 genotypes from of Moringa Genebank of Embrapa Tabuleiros Costeiros in Nossa Senhora das Dores, Sergipe state, Brazil. Different color represents different accessions.

Genetic distance

The relationship of all 177 genotypes was revealed by Roger's genetic distance (Figure 3). Moringa genotypes were categorized into two groups. One cluster was composed of M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, and M13, in which genotypes M4.1, M4.2, M4.6, and M4.7 are highlighted for being in a different subgroup from the others. The second cluster was composed of M14, M15, M16, M17, M18, M19, M20, M21, M22, M23, M24, and M25. Within these clusters, three sub-clusters were identified, which included most of the genotypes from M16 in one sub-cluster; M14 and M15 in the second sub-cluster; and the others in the third sub-cluster. In general, the cluster analysis did not correlate with the geographic origin of the accessions.

Genetic structure

Similarly, the Bayesian analysis provided by the Structure software program grouped moringa accesses into two broad clusters (K = 2) (Figure 4). Group I

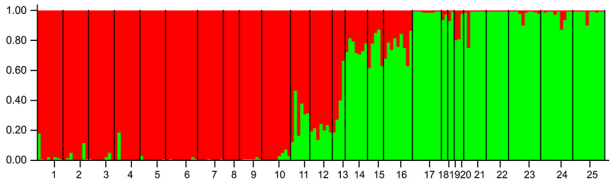

Figure 4 – Genetic structure analysis of 25 accessions represented by 177 genotypes from Moringa Genebank of Embrapa Tabuleiros Costeiros in Nossa Senhora das Dores, Sergipe state, Brazil, according to the Bayesian analysis groupings using the Structure program for K = 2 with separated by accession. Colors represented the two groups identified and the length of each color represents the genomic composition of each individual. 1 to 28 = accessions studied. Group I = red (M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13) and Group II = green (M19, M20, M21, M22, M23, M25).

(red) included all genotypes from the Florida exchange (M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12, M13), whereas accession M11, M12, and M13 were categorized as admixture (values of membership

higher than 0.2). On the contrary, all accessions from Brazil (M19, M20, M21, M22, M23, M25) were ranked in Group II (green), except for M17 and M18, which were from the Florida exchange. The accessions M14, M15, and M16 were also categorized as an admixture for having membership values lower than 0.8.

Principal coordinate analysis (PCoA) with ISSR markers showed the distribution of genetic diversity among the accessions across the two axes. The percentage of variation explained was 26.01 % (PCoA 1: 20.12 % and PCoA 2: 5.89 %) (Figure 5), which agreed with both groups formed in the aforementioned cluster analysis.

Discussion

Despite the wide cultivation of moringa in America, there are only a few studies that have attempted to assess the genetic variation of the species, including studies from Mexico (Avila-Treviño et al., 2017) and Colombia (Chaves-Bedoya et al., 2017). Previously, the first molecular characterization of the Moringa Genebank was conducted with only 16 accessions from the exchanged germplasm of the University of Florida, using Random Amplified Polymorphic DNA (RAPD) markers (Silva et al., 2012). Low genetic diversity between the accessions evaluated was found. Since then, new accessions of moringa collected in Brazil have been introduced, and our study represents the first molecular evaluation of genotypes from Brazil using ISSR primers for this species.

Previous studies have reported ISSR primers as an effective molecular marker for characterizing genetic diversity in moringa (Hassan et al., 2020; Hassanein, 2018; Rajalakshmi et al., 2019; Saini et al., 2013). In our study, all observed bands were polymorphic. Similar

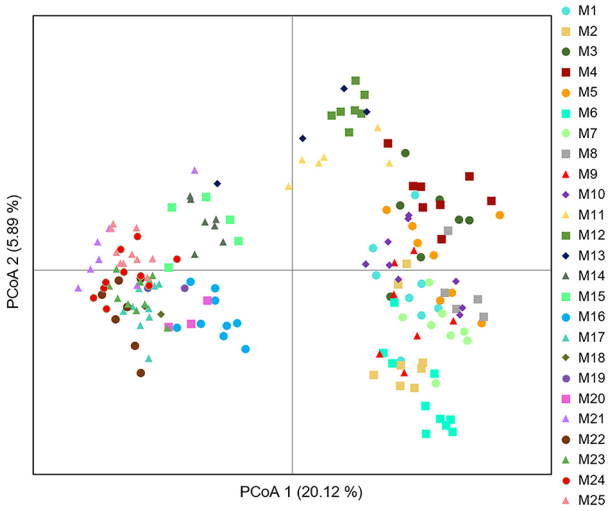

Figure 5 – Principal coordinate analysis (PCoA) in 25 accessions represented by 177 genotypes from Moringa Genebank of Embrapa Tabuleiros Costeiros in Nossa Senhora das Dores, Sergipe state, Brazil.

findings were reported by Hassanein (2018), in which 10 ISSR primers amplified 65 bands and 90.8 % were polymorphic for both species *Moringa oleifera* and *Moringa peregrine* (Forssk.) Fiori. Six ISSR primers were used to detect genetic variability among eight Indian cultivars of *M. oleifera*, and it was found to be the most suitable marker as compared to RAPD and cytochrome P450 based markers, and the results showed a 48.57 % polymorphism rate (Saini et al., 2013). Similarly, the genetic variability of 97 accessions of *M. oleifera* from India was assessed using 15 ISSR markers, which amplified 100 bands, showing 59.6 % polymorphism (Rajalakshmi et al., 2019).

Within the population, greater genetic variation than that observed in the AMOVA analysis in our study was found –86 % and 95 % of the variation (Ganesan et al., 2014; Rajalakshmi et al., 2019). Since moringa is a cross-pollinated plant, it was expected that most of the variation would be found within the population (Leone et al., 2015). Moreover, different factors may affect the genetic variability in moringa populations, such as aleatory mating patterns, genetic drifts caused by changes in allelic frequency, spontaneous mutation, and migration events of alleles within the population (Lakshmidevamma et al., 2021).

The Shannon index infers genetic diversity, ranging from zero to one, in which more diversity is found in values close to 1 (Perry and McIntosh, 1991). Our study's average Shannon index was 0.16, indicating low genetic diversity. Another indicator of genetic diversity was the He which varied from 0.03 to 0.17 (mean of 0.11), indicating low variability in the 177 moringa genotypes. Natural populations typically have He values exceeding zero due to the incorporation of new alleles by crossing (Silva et al., 2014). Similarly, low genetic diversity in 45 accessions of moringa from Colombia was found, in which values of He ranged from 0.13 to 0.29 (Chaves-Bedoya et al., 2017). The authors reported that the genotypes from Colombia were probably from a single or a small number of populations.

The opposite was observed when evaluating genetic materials from India where more genetic diversity is found since it is the center of origin (Muluvi et al., 1999). A high level of genetic diversity was found when evaluating seven advanced breeding lines from different locations in India (Kumar et al., 2017). Higher genetic diversity was observed for genotypes from India and Myanmar (He 0.36 to 0.76) using microsatellite markers (Wu et al., 2010). Nineteen SSR primers were used to assess genetic diversity among 300 genotypes from 12 populations from northern (Himachal Pradesh) and southern (Tamil Nadu) India (Ganesan et al., 2014).

They also found high genetic diversity in the Indian collection, reinforcing the idea that moringa originated in northern India and progressively established itself in the south, where it became more diverse. Another study using genotypes of natural

populations from India, Malawi and Kenya showed that Nei's average values ranged from 0.040 (Kenya) and 0.122 (Indian population), and the highest level of genetic diversity was found in Indian populations (Muluvi et al., 1999).

The Bayesian analysis provided by the Structure software program is a well-established tool used to obtain information about the population structure using bands of molecular markers (Pritchard et al., 2000). In our study, Bayesian analysis was also a valuable grouping method for categorizing moringa genotypes, making it possible to identify which genotypes are considered an admixture by establishing a threshold score (Figure 2). Even though Moringa Genebank accessions were divided into two groups and most of the accession from the University of Florida were allocated in Group I, some of them (M11, M12, M13, M14, M15, M16, M17, and M18) share similarities with accessions collected in Brazil, which belongs to Group II. Similar observations were obtained from the PCoA plot that together with structure data and cluster dendrogram can provide a more reliable outlook from the results (Figure 5).

A possible explanation for the lack of correlation between genetic variability and geographic origin is the center of origin of moringa. The majority of genetic studies on moringa are carried out in Asia, more specifically in India, its center of origin (Muluvi et al., 1999; Saini et al., 2013; Ganesan et al., 2014; Kumar et al., 2017; Ravi et al., 2020). In addition, studies with cultivated and natural accessions that quantify the genetic diversity of moringa across the world are considered meagre, although the conservation of genetic resources for this species by using germplasm banks is increasing (Boopathi et al., 2021).

The literature reports that moringa was introduced as an ornamental tree in the USA in 1915, more specifically in southern Florida, with seeds from Cuba and Nicaragua. The Director of the Educational Concerns of Hunger Organization (ECHO) has shown interest in moringa due to its adaptability. He has distributed seeds to other countries, such as Haiti and Brazil. In Brazil, the author reported a seed shipment to the Maranhão state, which resulted in the planting of 25,000 trees (Morton, 1991).

In Pakistan, they evaluated genetic diversity in 131 accessions from a wild population and 30 accessions obtained from ECHO (Florida), which were from nine different countries: Haiti, Mexico, Belize, USA (Florida), Zimbabwe, Mozambique, Tanzania, Senegal, and India. Interestingly, although there was high genetic diversity in the Pakistani wild collection, low genetic diversity was found in the accessions obtained from ECHO (Shahzad et al., 2013). Consequently, the authors suggest that those accessions are probably from the same population or a few populations since moringa was introduced from India to different countries by traders or immigrants and ECHO received seeds from these farmers. It has been reported that the low genetic diversity in introduced populations may be due to the use of related accessions when they were introduced into a new location. In addition, high selection factors can be operating to reduce the genetic diversity in these populations (Muluvi et al., 1999). Our findings also agree with this same pattern; that is to say, the collection of accessions was probably collected from the same or a few populations, which would explain the low genetic diversity observed.

Our study observed a discrepancy in genetic diversity between the Moringa Genebank in Brazil and the species' center of origin, indicating a low representation of moringa natural diversity. Therefore, actions to expand the bank are required to cover the species' wide diversity and provide genetic material for crop improvement in future plant breeding programs to develop local varieties, which include exchanges of genetic material between other germplasm banks, especially in regions of high diversity such as India.

The ISSR markers were effective in assessing genetic variability in *M. oleifera*. Overall, the 25 accessions of Moringa Genebank were distributed over two large groups. However, it was not possible to group them geographically. In addition, low genetic diversity was found between the accessions. The information obtained from this study can be used in management actions of the Moringa Genebank to improve genetic resources, such as the introduction of new genotypes. The goal is to ensure conservation strategies for this species and encourage future breeding programs. We suggest future studies using high throughput genotyping molecular markers to discriminate the germplasm and the morphological characterization of this collection.

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Authors' Contributions

Conceptualization: Soares TFSN, Silva AVC. **Data curation:** Silva AVC, Muniz EN. **Formal analysis:** Soares TFSN, Gois IB. **Funding acquisition:** Muniz EN, Soares TFSN. **Investigation:** Muniz EN, Silva AVC, Soares TFSN, Gois IB. **Methodology:** Silva AVC, Gois IB. **Project administration:** Muniz EN, Silva AVC, Ledo AS. **Resources:** Muniz EN, Silva AVC, Ledo AS. **Supervision:** Silva AVC. **Writing-original draft:** Soares TFSN, Gois IB, Silva AVC. **Writing-review & editing:** Silva AVC, Souza JL, Soares TFSN, Muniz EN, Gois IB, Ledo AS.

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Declaration of use of AI technologies

The authors have no use of AI technologies.

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