Genetic Diversity in Purslane (Portulaca Spp.) Using Simple Sequence Repeat Molecular Markers

Joseilson Moreira Araújo

joseilsondines@hotmail.com

Universidade Federal da Paraíba

Elizanilda Ramalho Rêgo

Universidade Federal da Paraíba

Mailson Monteiro Rêgo

Universidade Federal da Paraíba

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GENETIC DIVERSITY IN PURSLANE (*PORTULACA* SPP.) USING SIMPLE SEQUENCE REPEAT MOLECULAR MARKERS

Joseilson Moreira de Araújo [1], Ms.; Elizanilda Ramalho do Rêgo[2], Ph.D; Mailson Monteiro do Rêgo[2], Ph.D.

[1] Universidade Federal da Paraíba, Centre for Agricultural Sciences, Postgraduate Programme in Agronomy, Rodovia PB 079, Km 12, SN, University Campus II, 58.397-000, Areia-PB, Brasil.

[2] Universidade Federal da Paraíba, Centre for Agricultural Sciences, Department of Biosciences, Biotechnology and Plant Breeding Laboratory, Rodovia PB 079, Km 12, SN, University Campus II, 58.397-000, Areia –PB, Brasil.

Corresponding author: Joseilson Moreira de Araújo, Ms, Universidade Federal da Paraíba, Centre for Agricultural Sciences, Postgraduate Programme in Agronomy, Rodovia PB-079, Km 12, SN, University Campus II, 58.397-000, Areia-PB, Brasil, Email: joseilsondines@hotmail.com, Fone: 55 + 83 98150-5699.

Abstract

Purslane (*Portulaca* spp.) is a shrub-like plant that exhibits variation in the coloration of its leaves, stems, and flowers. Among its various attributes are its potential as an ornamental plant, its use in medicine, and its incorporation into human diets. Due to its importance, it has been the subject of studies in several breeding programs. For this purpose, molecular markers based on the Polymerase Chain Reaction are used, among which Simple Sequence Repeat markers stand out due to their codominant nature, consisting of repetitive, non-coding DNA regions made up of short tandem repeats (1–6 base pairs). Within this context, the objective of this study was to evaluate the genetic diversity of *Portulaca* spp. genotypes from the active germplasm bank of the Center for Agricultural Sciences at the Federal University of Paraíba, using Simple Sequence Repeat molecular markers. The experiment was conducted in the plant biotechnology laboratory of the Center for Agricultural Sciences/Campus II, located in the city of Areia. A total of 65 accessions from five purslane species were used.For DNA extraction, the method of Doyle & Doyle (1987), with modifications, was employed. Ten primer pairs were used, resulting in the amplification of 2,489 bands distributed across 187 loci, with 89.30% polymorphism. Based on the obtained data, the accessions PU16, PU28, and PU31 of *Portulaca umbraticola*; PO02 and PO08 of *Portulaca oleracea*; PA01 and PA02 of *Portulaca amilis*; and PG01 and PG03 of *Portulaca grandiflora* are suggested as potential parental lines.
Keywords**:** Diversity, Breeding, Genetics and *Portulaca*

INTRODUCTION

Purslane (Portulaca spp.) is a small herbaceous or subshrub plant characterized by high morphological variability, exhibiting differences in the size and coloration of its flowers, leaves, and stems. The family Portulacaceae comprises approximately 30 genera and 500 species, with a broad distribution across tropical and subtropical regions of the world, particularly in western North America, South America, and the African continent (ALBUQUERQUE et al., 2022). In Brazil, this family occurs across all geographic regions and is represented exclusively by the genus Portulaca L., which comprises 21 species (SANTOS & HASSEMER, 2022).

Among the species of this genus, P. oleracea, P. grandiflora, and P. umbraticola stand out due to their greater potential for agricultural use (OCAMPO & COLUMBUS, 2012). In addition to their agronomic relevance, Portulaca species have been widely used as vegetables in human diets and also for medicinal purposes in several countries, including the United States, Australia, China, and Pakistan (IRANSHAHY et al., 2017). In Brazil, P. umbraticola is commonly used for ornamental purposes, being frequently cultivated in pots and placed on window sills (SOUZA et al., 2024). Conversely, P. oleracea is recognized for its wide range of biological activities, including analgesic, antibacterial, muscle relaxant, wound-healing, anti-inflammatory, immunomodulatory, antioxidant, and antitumor properties (RAHIMI et al., 2019; XU et al., 2022).

Despite the growing number of studies worldwide addressing the nutritional value and medicinal properties of Portulaca species, research focused on their genetic diversity remains limited (ALAM et al., 2015). However, recent studies highlight that genetic diversity is essential for plant adaptation to abiotic stresses such as drought, salinity, and extreme temperatures (CORTÉS, 2024).

Genetic diversity in cultivated species can be assessed using morphological (phenotypic), biochemical, and molecular (DNA-based) markers (ALAM et al., 2015). Among molecular markers based on the Polymerase Chain Reaction (PCR), the most commonly used include Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), Simple Sequence Repeat (SSR), and Amplified Fragment Length Polymorphism (AFLP) (LABRA et al., 2003; LUNA-PÁEZ et al., 2007).

Microsatellite markers, also known as Simple Sequence Repeats (SSRs), are repetitive, non-coding regions of DNA consisting of short tandem repeat motifs (1–6 bp) (SAMAH et al., 2016). These markers are codominant, multiallelic, and highly polymorphic, allowing the assessment of genetic diversity, identification of accessions, and genetic mapping (JIANG et al., 2024).

The Portulaca Breeding Program at the Federal University of Paraíba, located in the municipality of Areia, Paraíba, Brazil, conducts research aimed at exploring the economic, ornamental, gastronomic, and medicinal potential of species within the genus Portulaca spp.

Within this context, the objective of this study was to evaluate the genetic diversity of Portulaca spp. genotypes from the Active Germplasm Bank of the Center for Agricultural Sciences of the Federal University of Paraíba (CCA–UFPB), using SSR molecular markers.

MATERIAL AND METHODS

The experiment was conducted at the Plant Biotechnology Laboratory of the Federal University of Paraíba (UFPB), Center for Agricultural Sciences, Campus II, located in the municipality of Areia, Paraíba, Brazil, within the Agreste mesoregion and the Brejo Paraibano microregion.

A total of 65 accessions belonging to five species and 22 hybrids of Portulaca spp. were used in this study, distributed as follows: P. grandiflora (3 accessions), P. umbraticola (33 accessions), P. oleracea (4 accessions), P. werdermannii (1 accession), and P. amilis (2 accessions); intraspecific hybrids of P. umbraticola (7 accessions), intraspecific hybrids of P. oleracea (2 accessions), and interspecific hybrids between P. umbraticola and P. oleracea (13 accessions). All materials are part of the Germplasm Bank (BAG) of the Plant Breeding Program of the aforementioned institution (Table 1).

86 Table 1. Active Germplasm Bank of purslane (*Portulaca* spp.) belonging to the Plant Breeding Program of the Federal University of Paraíba, Areia, Paraíba, Brazil.

Acesso	Nome do acesso	Cor da Flor	Cor da folha	Cor do Caule	Origem	Coordenadas geográficas	Espécie
1	PG01	White	Green	Green	Alagoa Grande, Paraíba, Brasil	7°05′20″S 35°38′06″W	*P. grandiflora*
2	PG02	Red	Green	Purple	Alagoa Grande, Paraíba, Brasil	7°05′20″S 35°38′06″W	*P. grandiflora*
3	PU01	Orange	Green	Purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
4	PU04	Pink	Green with purple margin	Light purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
5	PU05	Red	Green	Purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
6	PU02	Yellow	Green	Green	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
7	PU03	White	Green	Green	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
8	PU06	White	Green	Light green	João Pessoa, Paraíba, Brasil	7°7'7.92"S 34°52'53"W	*P. umbraticola*
9	PU07	Pink	Green with purple margin	Purple	João Pessoa, Paraíba, Brasil	7°7'7.92"S 34°52'53"W	*P. umbraticola*
10	PU08	Orange	Green with purple margin	Light purple	João Pessoa, Paraíba, Brasil	7°7'7.92"S 34°52'53"W	*P. umbraticola*
11	PU09	Orange	Green	Light purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
12	PU10	Fuchsia pink	Green with purple margin	Purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
13	PO04	Yellow	Dark green	Dark purple	Tifton, Georgia, Estados Unidos da América	*42º31'54"N 43º35'69"E*	*P. oleracea*
14	PO02	Yellow	Dark green	Dark purple	Tifton, Georgia, Estados Unidos da América	*42º31'54"N 43º35'69"E*	*P. oleracea*
15	PW01	Purple	Green	Green	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. werdemannii*
16	PO06	Yellow	Green	Dark purple	Tifton, Georgia, Estados Unidos da América	*42º31'54"N 43º35'69"E*	*P. oleracea*
17	PA01	Pink	Green	Purple	Athens, Georgia, Estados Unidos da América	31°27'2"N 83°30'31"W	*P. amilis*
18	PO08	Yellow	Green	Purple	Tifton, Georgia, Estados Unidos da América	*42º31'54"N 43º35'69"E*	*P. oleracea*
19	PO02 X PU10	Pink	Green	Dark purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
20	PO06 X PU05	Orange	Green	Dark purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
21	PO06 X PO09	Pink	Green with purple margin	Wine-colored	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
22	PO01 X PU10	Pink	Green	Dark purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
24	PO06 X PU09	Light pink	Green with purple margin	Purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
25	PO01 X PU06	Yellow	Green	Purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
26	PO01 X PU40	Yellow	Green	Dark purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
27	PO02 X PU01	Yellow	Green	Green with purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
29	PO9 X PU03	Light yellow	Green	Green with pink	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. oleracea*
30	PO02 X PO20	Red	Green	Dark purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
31	PO02 X PU40	Yellow	Green	Purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
32	PU04 X PU05	Yellow	Green	Green	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
33	PU04 X PU02	Yellow	Green	Pink	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
34	PU07 X PO05	Orange	Green	Purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
35	PU39 X PU07	Yellow	Green	Purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
36	PU02 X PU06	Pink	Green	Green	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
37	PU01 X PU02	Pink	Green	Green	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
38	PU39 X PU08	Orange	Green	Pink	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
39	PU05 X PU07	Dark pink	Green	Brown	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
40	PU11	Yellow	Green	Purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*

41	PU12	Dark pink	Dark green	Purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
42	PU13	Yellow	Green with purple margin	Purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
43	PU14	Dark pink	Dark green	Purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
45	PU15	Yellow	Green	Green	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
46	PU16	Yellow	Green	Purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
47	PU17	Yellow	Dark green	Purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
48	PU18	Dark pink	Green	Light purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
49	PU19	Pink	Green	Light purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
50	PU20	Red	Green	Purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
51	PU21	Dark pink	Green	Light purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
52	PU22	Lilac	Green	Green	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
53	PU23	White	Green	Green	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
54	PU24	Yellow	Dark green	Purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
55	PU25	Pink	Green	Green	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
57	PU26	Dark pink	Green	Light purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
58	PU27	Dark pink	Green	Green	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
61	PU28	White	Green	Green	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
62	PU29	Orange	Green with purple margin	Purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
63	PU30	White	Green	Green	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
64	PU31	Yellow	Green with purple margin	Light purple	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
66	PU32	White	Green	Green	Fortaleza, Ceará, Brasil	3°43′39″S 38°31′39″W	*P. umbraticola*
70	PU33	Pink	Green, white and pink margins	Purple	João Pessoa, Paraíba, Brasil	7°7'7.92"S 34°52'53"W	*P. umbraticola*
71	PA02	Pink	Dark green	Purple	Cabedelo, Paraíba, Brasil	6º58'52"S 34º50'02"W	*P. amilis*
73	PG3	White	Green	Purple	João Pessoa, Paraíba, Brasil	7°7'7.92"S 34°52'53"W	*P.grandiflora*
75	PO05 X PU06	Yellow	Green with purple margin	Dark purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
76	PO05 X PU01	Yellow	Green	Purple	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*
77	PU08 X PO02	Salmon	Green	Green	Universidade Federal da Paraíba, Areia, Paraíba, Brasil	6°57'42"S 35°41' 43"W	*P. umbraticola*

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Root Obtaining from Different Portulaca spp. Species

Five branches from each accession were collected and placed for rooting. The branches were kept in 200 mL glass containers containing 50 mL of water for a period of twenty days to induce root formation (Figure 1a). After this period, the roots were collected and subjected to disinfestation using a 1% sodium hypochlorite solution for 5 minutes. Subsequently, the material was rinsed three times with distilled water (Figure 1b). Excess water was removed using paper towels, and the samples were placed in Kraft paper bags and stored in 1-liter glass containers containing silica gel for 3 days to dehydrate the material.

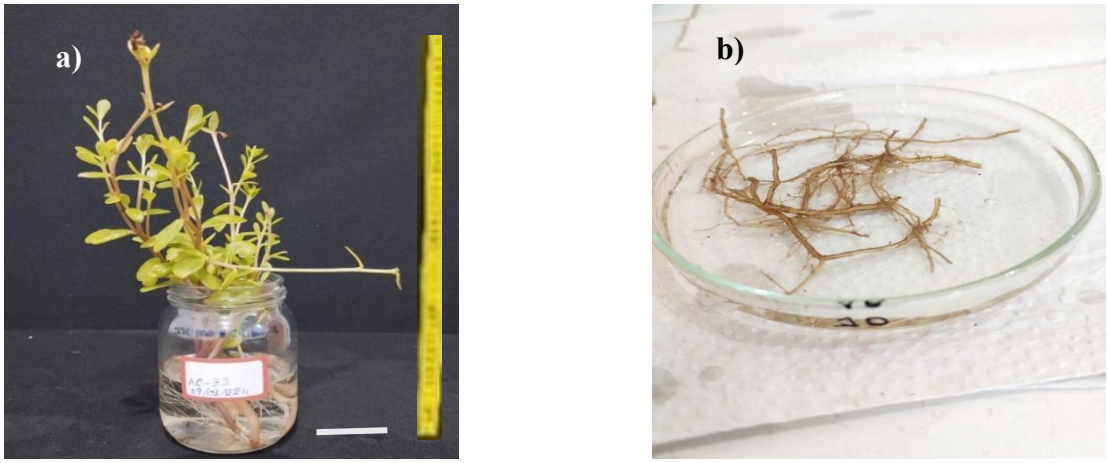

Figure 1. Conditioning and cleaning of plant material: (a) *Portulaca* spp. branches placed in 200 mL transparent glass containers containing 50 mL of water; (b) roots cleaned with distilled water and placed in a Petri dish. Scale bar = 1 cm.

DNA Extraction

DNA extraction was performed using the protocol proposed by Doyle & Doyle (1987), with modifications. Approximately 200 mg of roots were used, which were ground in liquid nitrogen using a mortar and pestle. The homogenized material was then transferred into 1.5 mL Eppendorf tubes.

To each tube containing the samples, 700 µL of 2% CTAB extraction buffer (supplemented with β-mercaptoethanol) was added. The samples were incubated at 65 °C for 30 minutes in a water bath and gently mixed every 10 minutes to ensure proper homogenization. After incubation, the samples were allowed to cool to room temperature. Subsequently, 600 µL of CIA (chloroform:isoamyl alcohol, 24:1 v/v) was added, and the tubes were manually inverted for 1 minute, followed by centrifugation at 13,400 rpm for 10 minutes.

The supernatant (600 µL) was carefully transferred to a new properly labeled tube, and an equal volume of phenol:chloroform:isoamyl alcohol was added for deproteinization. The mixture was again inverted for 1 minute and centrifuged at 13,400 rpm for 5 minutes.

The resulting supernatant was transferred to a new tube, adjusted to a final volume of 400 µL, and 40 µL of washing buffer (CTAB 10% + 1.4 M NaCl) was added. The samples were homogenized by gentle inversion for 5 minutes. Afterward, 500 µL of CIA was added, followed by centrifugation at 13,400 rpm for 5 minutes.

Then, 280 µL of the supernatant was transferred to a new tube, and nucleic acids were precipitated by adding 185 µL of cold isopropanol. The samples were centrifuged at 13,400 rpm for 5 minutes, resulting in visible DNA pellets. For samples in which the pellet was not immediately observed, incubation at −20 °C for 30 minutes was performed to promote precipitation.

At this stage, the supernatant was discarded, and the pellet was washed with 1,000 µL of 70% ethanol, followed by a wash with absolute ethanol to remove residual salts. For each washing step, samples were centrifuged at 13,400 rpm for 5 minutes. After removal of the final supernatant, the pellets were air-dried under natural conditions until complete evaporation of ethanol.

Finally, the DNA was resuspended in 50 µL of TE buffer (Tris-EDTA: 10 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} EDTA, pH 8.0).

DNA Quantification

To estimate the quantity and integrity of the extracted DNA, the material was subjected to electrophoresis on a 0.8% agarose gel. A volume of 10 µL of each DNA sample was mixed with 3 µL of bromophenol blue and 2 µL of Syber Green, and then loaded into the gel wells.

Electrophoresis was performed in 1× TBE buffer (0.04 M Tris-acetate and 1 mM EDTA) at 100 volts for 30 minutes. After electrophoresis, the gel was removed from the tank and photographed under UV light using a Gel Logic 112 photodocumentation system. DNA samples were compared to a 1 kb DNA ladder standard at a concentration of 100 ng.

PCR Reaction Using SSR Markers

For DNA amplification reactions, a PCR mix was prepared containing 2.5 µL of buffer, 1.5 µL of $MgCl_2$, 1.0 µL of dNTPs, 1.5 µL of SSR forward primer (F), 1.5 µL of SSR reverse primer (R), 0.4 µL of Taq DNA polymerase, and 14.6 µL of ultrapure water, resulting in a final reaction volume of 25 µL. Of this total, 23 µL corresponded to the reaction mix and 2 µL to the DNA template.

The genetic variation among accessions was analyzed using ten pairs of microsatellite (SSR) markers (Table 2).

Table 2. SSR (Simple Sequence Repeat) oligonucleotides and their respective sequences (Invitrogen™ – Thermo Fisher Scientific).

Nº	Primer	Sequence (5' – 3')	Tm
1	JMN-9(SSR)F	5'-CAATCATCATGCAGTGCTCT-3'	51º C
	JMN-9(SSR)R	5'-GTTGTAGGTCACTCCATTCC-3'	51º C
2	JMN-13(SSR)F	5'-GTTTGGCGGTGGATTGAGA-3'	53º C
	JMN-13(SSR)R	5'-ATGGTGAGGAGGATGTGGG -3'	53º C
3	JMN-15(SSR)F	5'-GTCAACAACCACAACCAAAT-3'	50º C
	JMN-15(SSR)R	5'-GTTGATGGCATTGTTGATCT-3'	50º C
4	JMN-19(SSR)F	5'-ATCATCATGCAGTGCTCTCT-3'	52º C
	JMN-19(SSR)R	5'-AACTGGTTGTTCACGTTGTT-3'	52º C
5	JMN-20(SSR)F	5'-TAAAGTAGGCCAACTGCAAA-3'	50º C
	JMN-20(SSR)R	5'-ACTTTGATTGGAGGAAGAGG-3'	50º C
6	JMN-22(SSR)F	5'-TCAACCTGCCAAAGTACAAT-3'	50º C
	JMN-22(SSR)R	5'-TTTTTCTTGGCTGTAGCTTG-3'	50º C
7	JMN-23(SSR)F	5'-ACTGACACAAATGGAAATGC-3'	50º C
	JMN-23(SSR)R	5'-GCTCGTCAATAGTGAGATCG-3'	50º C
8	JMN-26(SSR)F	5'-GAGAGACGACTGGCATACTG-3'	51º C
	JMN-26(SSR)R	5'-CCACCTGTTGACATCCTATG-3'	51º C
9	JMN-41(SSR)F	5'-TAACCCAGCTATGCCTTACA-3'	51º C
	JMN-41(SSR)R	5'-TCTCCTTCCTCTTTGACCTC-3'	51º C
10	JMN-46(SSR)F	5'-AGTATCTGGGATTCCTTTCG-3'	49º C
	JMN-46(SSR)R	5'-ACTGATGTTGGATGAGCAAC-3'	49º C

At the end of the PCR process, the amplified DNA samples were mixed with 3 µL of loading buffer and 2 µL of Sybr Green and homogenized by pipetting. From this mixture, 15 µL were loaded into the wells of a 3.0% agarose gel. The gel was placed in the electrophoresis chamber and submerged in 1× TBE buffer (Tris, boric acid, and EDTA) until completely covered, and then subjected to an electric current of 120 V for 55 minutes.

After electrophoresis, the gel was photographed using a Gel Logic 112 imaging system. The molecular weights of the PCR products were estimated using a 100 bp DNA standard (Invitrogen™ 1 kb Plus DNA Ladder).

Analysis of Genetic Diversity Within and Between *Portulaca* Species

Construction of the data matrix and genetic distance estimates

Genetic diversity was evaluated based on the banding patterns revealed in agarose gel electrophoresis. For each marker, the presence (1) or absence (0) of bands was recorded, resulting in the construction of a binary data matrix. Genetic distances among accessions were estimated using the binary distance proposed by Sokal and Rohlf (1962).

Cluster analyses

Based on the genetic distance matrix, hierarchical clustering was performed using Ward's method (1963). The optimal number of groups was determined by dendrogram cutting according to Mojena's criterion (1977). In addition, a bootstrap analysis was conducted to provide statistical support for the groupings.

Cluster validation was performed by estimating the cophenetic correlation 9pplied9nte (Sokal & Rohlf, 1962), whose significance was tested using Student's *t*-test at a 5% probability level. For non-hierarchical clustering, the Tocher optimization method was 9pplied as described by Rao (1952).

Molecular Variance Analysis (AMOVA)

Genetic variability within and among the analyzed species was estimated using Molecular Variance Analysis (AMOVA), applying a non-parametric approach implemented in the Genes software (Cruz, 2013). AMOVA allowed the assessment of the distribution of genetic variability within and between populations based on the SSR markers used. The F-statistics derived from this analysis were used to estimate genetic diversity between pairs of populations, as described by Excoffier et al. (1992).

RESULTS

The integrity and quantity of the extracted DNA were assessed using representative samples through electrophoresis on a 1% agarose gel (Figure 2). The electrophoretic profiles revealed clear and continuous bands in all analyzed samples, indicating the successful extraction of high-quality genomic DNA suitable for subsequent molecular analyses. A 100 bp molecular weight marker (Invitrogen™ 1 kb Plus DNA Ladder) was used as a reference, allowing the estimation of band sizes using GelAnalyzer software version 23.1.1.

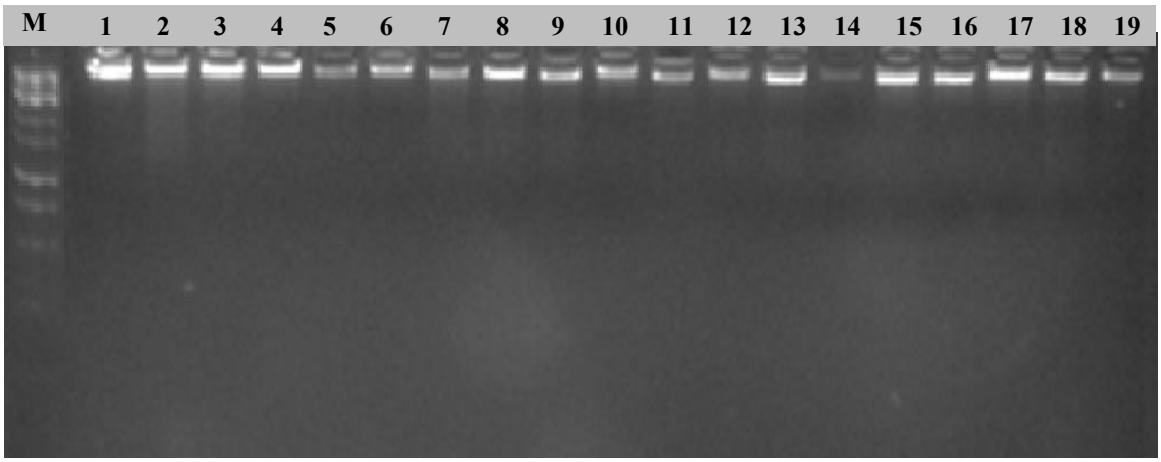

Figure 2. Electrophoretic profile on a 1% agarose gel of 19 *Portulaca* spp. accessions used to assess DNA quantity and quality. M: 100 bp molecular weight marker (Invitrogen™ 1 kb Plus DNA Ladder).

The use of a 3% agarose gel provided higher resolution of the amplified fragments, allowing clear visualization of the banding patterns in the electrophoretic profiles (Figure 3).

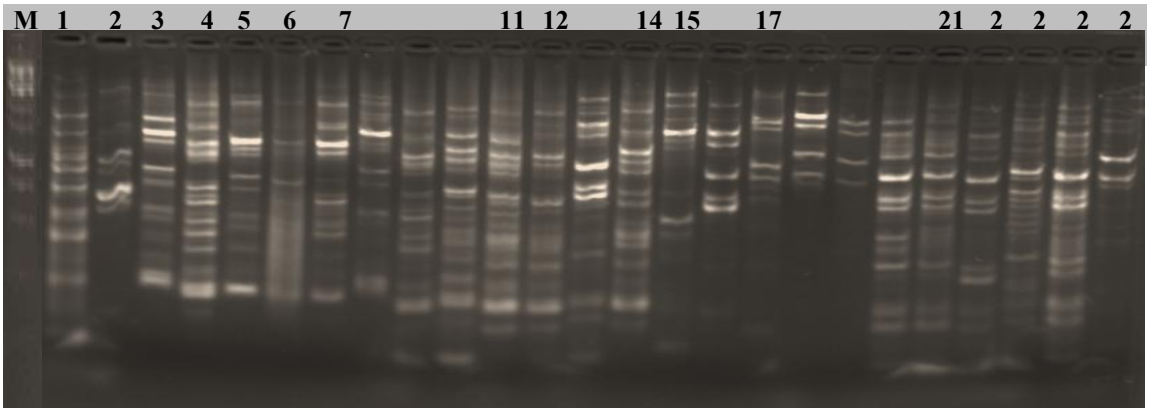

Figure 3**.** Electrophoretic profile on a 3% agarose gel of 25 purslane (*Portulaca* spp.) accessions generated with the SSR primer JMN-23 F–R. M: 100 bp molecular weight marker (ladder).

From the PCR reactions, a total of 2,489 bands were amplified, distributed across 187 loci, of which 167 were polymorphic and 20 monomorphic (Table 3). The detailed analysis of the amplified bands revealed that most loci exhibited polymorphism, indicating high genetic variability among the *Portulaca* spp. accessions. The high polymorphism rate observed (89.30%) (Table 3) demonstrates the efficiency of the SSR markers used.

Among the primers tested, the JMN-41 F–R SSR primer stood out, showing 100% polymorphic loci, highlighting its strong potential for diversity studies and accession identification (Table 3). These results confirm the applicability of microsatellite markers for the genetic characterization of *Portulaca* spp. populations.

Table 3. Markers and their sequences, number of amplified bands (NBA), polymorphic loci (NLP), monomorphic loci (NLM), percentage of polymorphic loci (PPL), polymorphic information content (PIC), resolving power (Rp), marker index (MI), and expected heterozygosity (He).

Marcador	Sequência (5'-3')	NBA	NLP	NLM	PLP (%)	PIC	R_p	MI	He
JMN-9(SSR)F	5'-CAATCATCATGCAGTGCTCT-3'	157	13	3	81,25	0,24	4,83	3,15	0,27
JMN-9(SSR)R	5'-GTTGTAGGTCACTCCATTCC-3'								
JMN-13(SSR)F	5'-GTTTGGCGGTGGATTGAGA-3'	155	13	5	72,22	0,21	4,76	2,76	0,22
JMN-13(SSR)R	5'-ATGGTGAGGAGGATGTGGG -3'								
JMN-15(SSR)F	5'-GTCAACAACCACAACCAAAT-3'	475	34	2	94,44	0,29	14,61	10,05	0,32
JMN-15(SSR)R	5'-GTTGATGGCATTGTTGATCT-3'								
JMN-19(SSR)F	5'-ATCATCATGCAGTGCTCTCT-3'	135	14	3	82,35	0,20	4,15	2,83	0,21
JMN-19(SSR)R	5'-AACTGGTTGTTCACGTTGTT-3'								
JMN-20(SSR)F	5'-TAAAGTAGGCCAACTGCAAA-3'	360	18	1	94,74	0,38	11,07	6,97	0,41
JMN-20(SSR)R	5'-ACTTTGATTGGAGGAAGAGG-3'								
JMN-22(SSR)F	5'-TCAACCTGCCAAAGTACAAT-3'	151	13	1	92,86	0,26	4,64	3,44	0,27
JMN-22(SSR)R	5'-TTTTTCTTGGCTGTAGCTTG-3'								
JMN-23(SSR)F	5'-ACTGACACAAATGGAAATGC-3'	389	21	1	95,45	0,38	11,96	7,99	0,41
JMN-23(SSR)R	5'-GCTCGTCAATAGTGAGATCG-3'								
JMN-26(SSR)F	5'-GAGAGACGACTGGCATACTG-3'	299	18	1	94,74	0,35	9,20	6,43	0,38
JMN-26(SSR)R	5'-CCACCTGTTGACATCCTATG-3'								
JMN-41(SSR)F	5'-TAACCCAGCTATGCCTTACA-3'	264	16	0	100	0,32	8,12	5,22	0,40
JMN-41(SSR)R	5'-TCTCCTTCCTCTTTGACCTC-3'								
JMN-46(SSR)F	5'-AGTATCTGGGATTCCTTTCG-3'	104	7	3	70,00	0,24	3,20	1,73	0,26
JMN-46(SSR)R	5'-ACTGATGTTGGATGAGCAAC-3'								
	TOTAL	2.489	167	20					
	MÉDIA	248,90	16,70	2,00	87,81	0,29	7,66	5,06	0,32

The polymorphic information content (PIC) values for the ten SSR primer pairs were relatively high, ranging from 0.20 to 0.38, with an average value of 0.29 (Table 3).

The resolving power (Rp) ranged from 3.20 to 14.61, with the highest value observed for the JMN-15 (SSR) F–R primer pair and the lowest for the JMN-46 (SSR) F–R pair, resulting in an average of 7.66.

The highest expected heterozygosity was observed for the primer pairs JMN-20 (SSR) F–R and JMN-23 (SSR) F–R, both presenting a value of 0.41, while the lowest value was recorded for the JMN-19 (SSR) F–R primer pair (0.21), with an overall mean of 0.32 (Table 3).

Genetic distances between pairs of accessions ranged from 0.40 to 1.00. The lowest genetic distance (0.40) was observed between the accessions (PO02 × PO20) and (PO02 × PU40), both hybrids sharing the same parental accession PO02.

In contrast, the lowest intraspecific distance was recorded between PU14 and PU15 (0.49), while the highest genetic distance was observed among the accessions PU16, PU28, and PU31, all belonging to *P. umbraticola*, showing maximum dissimilarity of 1.00. Regarding interspecific comparisons, the lowest dissimilarity was found between PU10 and PO02 (0.53).

Furthermore, the dissimilarity analysis revealed that accession PU16 was the most divergent among all accessions, presenting the highest intra- and interspecific genetic distances, ranging from 0.86 to 1.00.

Based on the collected data, a dendrogram (Figure 4) was constructed using Ward's method (1963) to analyze the genetic variability among the 65 *Portulaca* spp. accessions. The accessions were grouped into six distinct clusters, and a cut-off point was defined according to Mojena (1977).

Group I was formed by only two accessions, corresponding to the hybrid PU02 × PU06 and accession PU16. Group II comprised the hybrids (PO02 × PU01) and (PO09 × PU03).

Group III included the accessions PU21, PU25, PU28, PU33, PU29, PU31, PU27, PU24, PU26, PU30, and PU32, representing a more homogeneous group composed mainly of *P. umbraticola* accessions.

Group IV was composed of PA01, PA02, PG01, and PG03, whereas Group V included only two accessions, the hybrids (PU08 × PO02) and (PO05 × PU01).

Group VI comprised 44 accessions, corresponding to 67.69% of all genotypes analyzed. This group included the accessions PU01, PU02, PU03, PU04, PU05, PU06, PU07, PU08, PU09, PU10, PU11, PU12, PU13, PU14, PU15, PU17, PU18, PU19, PU20, PU22, PU23, PG02, PO02, PO04, PO06, PO08, and PW01, as well as several intra- and interspecific hybrids. Intraspecific hybrids included (PU04 × PU02), (PU04 × PU05), (PU39 × PU08), (PU39 × PU07), (PU01 × PU02), (PU05 × PU07), (PO02 × PO20), and (PO06 × PO09). Interspecific hybrids comprised (PU07 × PO05), (PO02 × PU40), (PO02 × PU10), (PO01 × PU40), (PO01 × PU06), (PO06 × PO09), (PO01 × PU10), (PO06 × PU05), and (PO05 × PU06) (Figure 4).

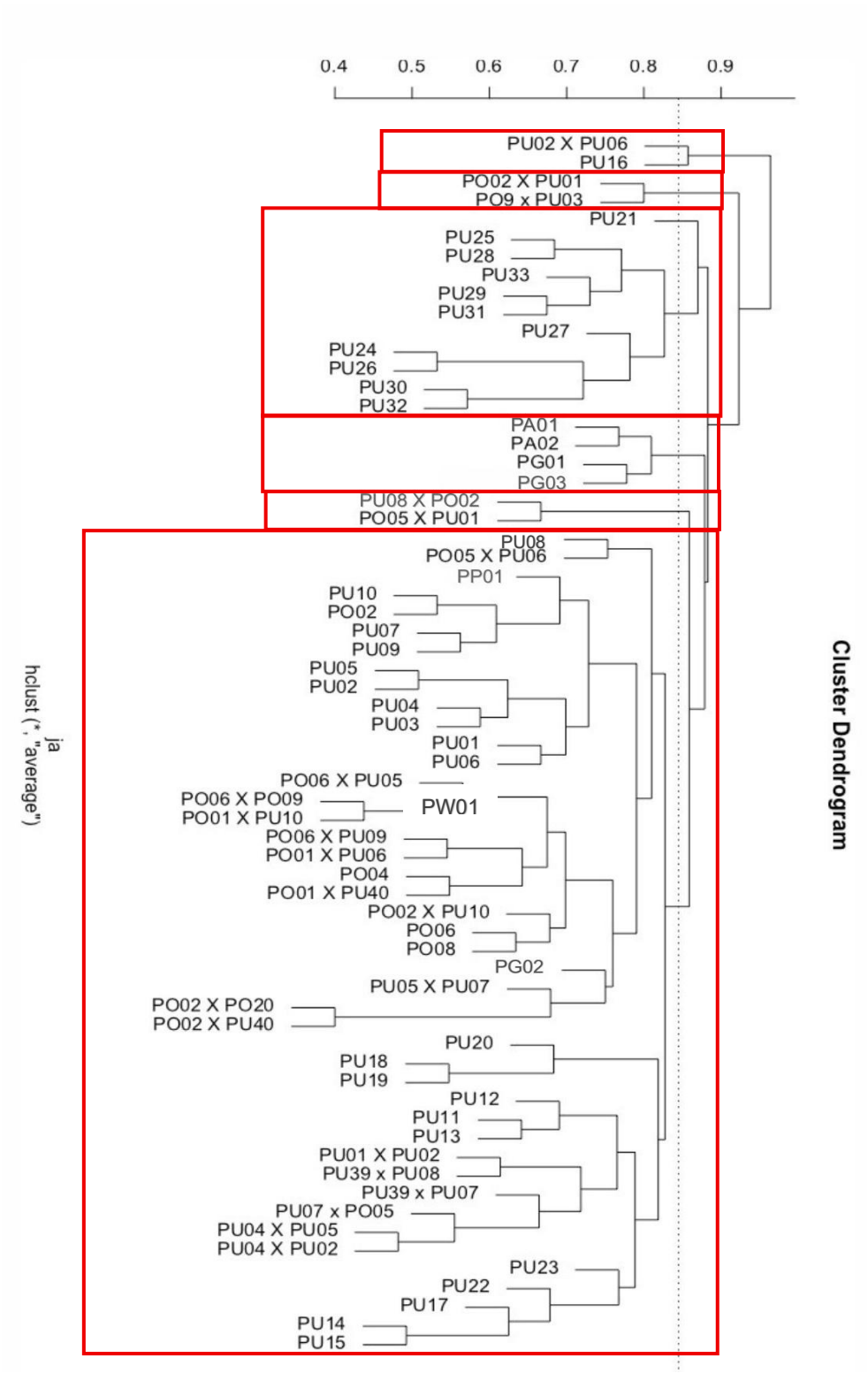

Figure 4. Dendrogram constructed using Ward's method, based on Jaccard's genetic similarity coefficient (Jaccard, 1912), in a population of 65 *Portulaca* spp. accessions generated from 10 SSR marker pairs.

Study of Genetic Diversity Based on AMOVA

The Molecular Analysis of Variance (AMOVA), based on microsatellite (SSR) markers, revealed statistically significant differences at the 5% probability level, indicating relevant genetic variation among *Portulaca* spp. accessions. The highest proportion of genetic diversity was detected within species

(90.93%), whereas only 9.07% was attributed to variation among species (Table 4).

Table 4. Molecular Analysis of Variance (AMOVA) in *Portulaca* spp. using SSR molecular markers, performed using Genes software (Cruz, 2006).

Variance components	Estimate	Percentage	P(f)
Among -Va	2.6334	9.07	<0,001
Within -Vd	26.3929	90.93	<0,001
Total	29.0263	100.0	<0,001
Statistic ØST	.0907		
Mean value of n	4.2442		

Discussion

The characterization of the genetic diversity of *Portulaca* spp. genotypes from the Active Germplasm Bank (AGB) of CCA-UFPB, performed using SSR markers, revealed significant genetic variability among the evaluated accessions. This diversity represents an essential resource to support breeding programs, conservation strategies, and the sustainable use of the genus germplasm.

Overall, the observed polymorphism patterns confirm the efficiency of SSR markers in genotype discrimination, corroborating their applicability in genetic diversity studies and reinforcing their value as a molecular tool in germplasm banks.

Information on the genetic status and species diversity of *Portulaca* contributes to understanding population structure and the occurrence of species adapted to specific environments (FELICIANO et al., 2022).

The distribution of genotypes into six distinct groups (Figure 4) indicates that the AGB of CCA-UFPB harbors genetically differentiated accessions, reinforcing the need for conservation and expansion of the germplasm collection.

Group VI stands out for comprising a combination of accessions of *P. umbraticola*, *P. oleracea*, *P. grandiflora*, and *P. werdemannii*, as well as intra- and interspecific hybrids. The formation of this heterogeneous group can be explained by the shared genetic similarities among *Portulaca* species.

The clustering of different species has also been reported by Jia et al. (2017), who, using SRAP markers to assess genetic diversity in *P. umbraticola* and *P. grandiflora* accessions, demonstrated that the clustering structure reflects complex patterns of variability within the genus. This structure is generally consistent with botanical classification, although some accessions exhibit signs of genetic admixture.

This phenomenon may be related to the reproductive system of the genus, since, according to Paniago & Valle (2016), in outcrossing plants there is the possibility of gene flow between generations, recombination among genomes, and maintenance of shared alleles.

According to Pinzón et al. (2021), the domestication process is closely related to gene flow, since the continuous exchange of genetic material among crops, wild plants, and weeds over time promotes the introduction of new genes, contributing to an increased degree of relatedness among genotypes.

Furthermore, it was observed that the hybrids present in this group share genetic relatedness with the other accessions, suggesting that the level of genetic relationship was a determining factor in the formation of this cluster. Supporting this interpretation, Purwantoro et al. (2023), when analyzing a subgroup in a study involving antelope orchid species and hybrids, reported high genetic similarity among the evaluated accessions and hybrids, with all hybrids clustering with their respective parental lineages. According to the authors, this result is plausible, since the hybrid parents were interspecific.

These findings highlight that the presence of parental genotypes and hybrids directly influences the clustering of different accessions in genetic similarity analyses.

The greatest genetic distances were observed among the accessions PU16, PU28, and PU31, with a value of 1.00, indicating a high level of intraspecific divergence. Accession PU16 stood out as particularly divergent, showing genetic distances ranging from 0.86 to 1.00 in relation to the other accessions.

From a breeding perspective, highly divergent accessions are important because they may generate progeny with a broader range of variability and greater heterotic potential. However, it is necessary to evaluate the reproductive compatibility among the individuals before their use in hybridization programs.

In *P. oleracea* accessions, the highest genetic distance (0.86) was recorded between PO02 and PO08. For *P. amilis*, represented by only two accessions (PA01 and PA02), the dissimilarity was 0.83. In *P. grandiflora*, the maximum observed value was 0.88 between PG01 and PG03.

These results reinforce the usefulness of genetic distance as a tool for parent selection, since more divergent accessions can broaden the genetic base of segregating populations and increase the probability

of favorable recombination events.

Aligned with these results, Wu et al. (2021) demonstrated that when species diversity is higher, populations are able to expand their geographic range more effectively and adapt better to new environments, indicating that more divergent parents present greater potential for plant breeding programs.

The primers used in this study proved to be effective and polymorphic, as evidenced by the Polymorphic Information Content (PIC), which ranged from 0.20 to 0.38, with a mean value of 0.29 (Table 3). According to Silveira et al. (2023), values below 0.25 are considered slightly informative, those between 0.25 and 0.50 reasonably informative, and values above 0.50 highly informative.

Most of the markers employed showed medium to high values, reinforcing the reliability of the data for genotype discrimination and genetic diversity assessment.

Finally, the molecular variance analysis (AMOVA) revealed that the highest proportion of genetic diversity is found within species (90.93%), while only 9.07% was attributed to variation among species (Table 4), results that are consistent with the dissimilarity matrix and the dendrogram. These findings indicate that, despite the existence of differences among species, intraspecific variability is predominant.

Consistent results were reported by Hu et al. (2024), who, using AMOVA, observed that 97.44% of the genetic variability occurred within populations, with only a small fraction explained by variation among populations. The authors attributed this pattern to the species' reproductive system, which is predominantly outcrossing, favoring high levels of intrapopulation variability.

CONCLUSION

The genetic analysis of Portulaca spp. accessions revealed high intraspecific diversity, as evidenced by Simple Sequence Repeat markers, genetic distance analyses, and Molecular Variance Analysis. The results confirm the efficiency of these markers in genotype discrimination and highlight the applicability of molecular data in assessing genetic diversity and in the planning of breeding programs.

Accessions PU16, PU28, PU31, PO02, PO08, PA01, PA02, PG01, and PG03 are recommended as potential parents in intraspecific crosses due to their high genetic divergence.

Statements and Representations

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Conflicts of interest
The authors declare that there is no conflict of interest.

Author contributions
The corresponding author, Ms. J.M.A., was directly responsible for the conception of the manuscript, as well as carrying out the entire research, writing, and publication process. Author E.R.R. contributed substantially to the preparation of the manuscript as an advisor and reviewer. Author M.M.R. served as a reviewer in the analysis and interpretation of two datasets.

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