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MOLECULAR APPROACHES FOR IDENTIFICATION OF THE APOMICTIC/SEXUAL REPRODUCTIVE MECHANISM AND GENETIC VARIABILITY IN BUFFEL GRASS (*Cenchrus* spp.) ACCESSIONS

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Cenchrus spp. encompasses forage grasses that are especially important for drylands. Thus, information on their reproductive mechanism and genetic diversity is needed. The Active Germplasm Bank (AGB) of the Embrapa Semiárido (Tropical semiarid research center) has 115 accessions of *Cenchrus* spp. that were not molecularly characterized. Therefore, the objective of this work was to evaluate the genetic diversity of *Cenchrus* spp. accessions in the AGB, and identify their reproductive mechanisms using DNA markers. Specific SCAR markers Q8H, UGT197, and PCAB10, in addition to the SCAR marker 4HS* were used to identify genotypes with apomictic and sexual reproduction modes, respectively; the genetic diversity was characterized using the ISSR D12 (GA) 6CG and HB14 (CTC)3GC markers. The reproductive mechanism was analyzed using images for the presence or absence of the band in the expected amplicon size. For the analysis of genetic diversity, the gel images were exported to the BioNumerics Software (Applied Maths), in which polymorphism data were obtained and the dissimilarity dendrogram constructed by the UPGMA clustering method using the Dice coefficient. The markers related to the apomictic region were positive for all accessions. However, only the accessions CPATSA 102 and CPATSA 134 had positive markers using the primers for both reproduction strategies, indicating that these individuals can be

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facultative sexual. The cluster analysis discriminated two large groups and the accessions clustered mainly by the place of origin. It was also possible to identify duplicates within AGB. The identification and exclusion of duplicate accessions provides financial and labor savings in maintaining and managing the AGB. The two sexual accessions were selected for the breeding programs of *Cenchrus* spp. in Brazil.

Key words: apomixis, selection assisted by markers, forage grasses, germplasm, breeding

INTRODUCTION

Human pressure on native vegetation areas has decreased animal feeding alternatives in the Brazilian semiarid region. However, new viable sources for animal feed have been considered. *Cenchrus ciliaris* (L.), known as buffel grass, is a perennial grass native to Africa that was introduced and used as pastures in semiarid regions worldwide, and is a good choice for the Semiarid region in the Northeast of Brazil. *C. ciliaris* (Poaceae, Panicoideae) is a polyploid grass (2n = 4x = 36) that is grown on large scales in Australia, South Africa, and India and is also found in Brazil, Mexico, and the United States (BHAT *et al.*, 2001; MARSHALL *et al.*, 2012; YADAV *et al.*, 2012).

This species was introduced to Brazil in 1952, and two cultivars (CPATSA 7754 and Áridus) were developed by the Brazilian Agricultural Research Corporation (Embrapa). The cultivar CPATSA 7754 was selected among accessions introduced and described by Oliveira (1999). However, little information about the genetic variability of these accessions is available. In addition, genetic improvement for this grass requires the identification of sexual sources and selection of genitors from hybrid crosses that produce viable seeds (VALLE *et al.*, 2009).

C. ciliaris has asexual reproduction as the main reproduction strategy, through apomixis, with dominance over sexual reproduction. Considering the low occurrence of plants with sexual reproduction in the natural populations and the low frequency of natural hybridizations between genotypes, the breeding of this species is challenging (YADAV *et al*., 2012; KUMAR *et al*., 2015). However, hybridization increases the existing genetic variability by artificial crosses between contrasting individuals and is an important tool for breeding programs (QUIROGA *et al.*, 2013) that can enable the development of new *C. ciliaris* cultivars with superior characteristics to the existing ones in the market.

DNA markers allow the analysis of genetic variability of accessions and the predominant mode of reproduction of species, identifying differences directly in the DNA of the genotypes, avoiding the effect of the environment and, consequently, identification errors in these analysis (BORBA *et al.*, 2005; SOUZA, 2015). Thus, they are an important tool for selecting accessions with characteristics of interest and can be used in Marker Assisted Selection (MAS), which reduces the time and resources for phenotyping in breeding programs.

Specific Sequence Characterized Amplified Regions (SCAR) markers were used by QUIROGA *et al.* (2013), JESSUP (2005), and JESSUP *et al.* (2002) in accessions of *Cenchrus* spp. to identify their reproductive mode. These authors found markers linked to the Apomixis Sequence Genomic Region (ASGR) in *C. ciliaris*. Kumar and Saxena (2016) developed a SCAR marker linked to the sexual reproductive mode in *Cenchrus.* This information is relevant for genetic breeding programs because it controls the reproduction mode in *Cenchrus* spp., making the breeding of these species less problematic regarding unpredictable variations in the progeny.

The Tropical Semiarid Researcher Center of Embrapa (henceforth Embrapa Semiárido) has an active germplasm bank (ABG) with 115 *Cenchrus* spp. accessions collected worldwide since 1970s. Some genotypes were evaluated regarding their morphological and agronomic traits (BRUNO *et al.*, 2017), however, molecular tools were not used to screening the accessions of this collection. In this context, the objective of this work was to evaluate the genetic diversity of accessions of *Cenchrus spp.* from the Active Germplasm Bank of Embrapa Semiárido and identify their reproductive mechanism by using DNA markers.

MATERIAL AND METHODS

Plant material

Young and fully expanded leaves were collected from 115 accessions of *Cenchrus spp.* of the AGB of the Embrapa Semiárido (Table 1) grown in the Caatinga Experimental Field in Petrolina, PE, Brazil. The material was identified and placed in autoclaved centrifuge tubes, macerated with liquid nitrogen, and stored at -20 °C. The sampling, storage, and maceration were performed according to FERREIRA AND GRATTAPAGLIA (1998), with adaptations.

Molecular Characterization

Approximately 40 mg of the macerated plant tissue was transferred to new tubes and the DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega). The extracted DNA was diluted to 50 ng μL^{-1} and stored in a freezer at –20°C. Specific SCAR markers (Q8H, UGT197 and PCAB10) (Table 2) associated with the apomixis sequence of the ASGR (OZIAS-AKINS *et al.*, 1998; JESSUP, 2005) were used to identify apomictic genotypes.

PCR reactions were performed according to Griffa (2010), with adaptations, with a final volume of 10 µL, containing 1X buffer, 25 mM MgCl2, 1 mM dNTP, 5 mM of each marker (front and back), 5 U Taq DNA polymerase, and the DNA sample of 50 ng μL^{-1}. The PCR program consisted of 94°C for 3 minutes and 10 cycles of 94°C for 30 seconds, 64°C for 30 seconds (-1°C per cycle) and 72°C for 45 seconds, followed by 36 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 45 seconds, and finally 72°C for 5 minutes and at 4°C ∞. In addition, the samples were stained with GelRed and applied to 2% agarose gel in the presence of TAE (Tris, Acetic acid, Edta) buffer in electrophoresis with 80 Volts for 3 hours.

The sexual sources were identified using the SCAR 4HS* marker to amplify the specific genomic region associated with sexual reproduction of the accessions of *C. ciliaris*. The PCR reaction using the 4HS* marker was performed using 1X buffer, 2 mM MgCl2, 200 µM dNTP, 0.5 µM of each primer (front and back) and 2U Taq DNA polymerase and the DNA sample of 50 ng μL^{-1}, with the final volume of 10 µL, according to Yadav *et al.* (2012), with adaptations.

PCR reactions for this primer were performed using 94°C for 3 minutes, 34 cycles (94°C for 1 minute, 63°C for 1 minute, 72°C for 1,5 minutes), 72°C for 10 minutes, and 4°C ∞. In addition, the samples were stained with GelRed (Biotium) and applied to 1.2% (w/v) agarose gel in the presence of TBE (Tris/Borate/EDTA) in electrophoresis with 80 Volts for 3 hours.

Table 1. Identification and origin of accessions of Cenchrus *spp. of the Germplasm Active Bank of the Embrapa Semiárido*.

127	*C. ciliaris*	USA	312	NI	NI	592	*C. ciliaris*	USA
128	*C. ciliaris*	USA	432	*C. ciliaris*	Australia	593	*C. ciliaris*	USA
129	*C. ciliaris*	USA	433	*C. ciliaris*	Australia	595	*C. ciliaris*	USA
130	*C. ciliaris*	USA	434	*C. ciliaris*	Australia	598	*C. ciliaris*	USA
131	*C. ciliaris*	USA	435	*C. ciliaris*	Australia	599	*C. ciliaris*	USA
132	*C. ciliaris*	USA	436	*C. ciliaris*	Australia	601	*C. ciliaris*	USA
134	*C. ciliaris* X *C. setigerus*	Brazil	437	*C. ciliaris*	Australia	602	*C. ciliaris*	USA
136	*C. ciliaris*	Brazil	438	*C. ciliaris*	Australia	603	*C. ciliaris*	USA
138	*C. ciliaris*	Brazil	439	*C. ciliaris*	Australia	608	*C. ciliaris*	USA
140	*C. ciliaris*	Brazil	616	*C. ciliaris*	Brazil	609	*C. ciliaris*	USA
141	*C. ciliaris*	Brazil	126	*C. ciliaris*	USA	611	*C. ciliaris*	USA
144	*C. ciliaris*	Brazil	541	*C. ciliaris*	Kenya	613	*C. ciliaris*	USA
145	*C. ciliaris*	Brazil	555	*C. ciliaris*	USA	614	*C. ciliaris*	USA
146	*C. ciliaris*	Brazil	556	*C. ciliaris*	USA	615	*C. ciliaris*	USA
147	*C. ciliaris*	Brazil	557	*C. ciliaris*	USA	617	*C. ciliaris*	USA
148	*C. ciliaris*	Brazil	558	*C. ciliaris*	USA	754	NI	NI
149	*C. ciliaris*	Brazil	559	*C. ciliaris*	USA	7754	*C. ciliaris*	Brazil
150	*C. ciliaris*	Brazil	156	*C. ciliaris*	Brazil	572	*C. ciliaris*	USA
Numbank	*C. ciliaris*	Australia	158	*C. ciliaris*	Brazil	573	*C. ciliaris*	USA
3	*C. ciliaris*	Brazil	164	NI	NI	574	*C. ciliaris*	USA
4	NI	NI	176	*C. ciliaris*	Brazil	575	*C. ciliaris*	USA
5	*C. ciliaris*	Brazil	177	*C. ciliaris*	Brazil	576	*C. ciliaris*	USA
6	*C. ciliaris*	Brazil	189	*C. ciliaris*	Brazil	577	*C. ciliaris*	USA
52	*C. ciliaris*	Brazil	192	*C. ciliaris*	Tanzania	578	*C. ciliaris*	USA
53	*C. ciliaris*	Brazil	193	*C. ciliaris*	Tanzania	579	*C. ciliaris*	USA
57	*C. ciliaris*	Brazil	194	*C. ciliaris*	Tanzania	580	*C. ciliaris*	USA
102	*C. ciliaris X Birdwood*	Brazil	195	*C. ciliaris*	Tanzania	581	*C. ciliaris*	USA
119	*C. ciliaris*	India	196	*C. ciliaris*	Tanzania	582	*C. ciliaris*	USA
120	*C. ciliaris*	Australia	198	*C. ciliaris*	Tanzania	584	*C. ciliaris*	USA
121	*C. ciliaris*	Australia	199	*C. ciliaris*	Tanzania	585	*C. ciliaris*	USA
122	*C. ciliaris*	Australia	200	*C. ciliaris*	Tanzania	588	*C. ciliaris*	USA
123	*C. ciliaris*	Australia	201	*C. ciliaris*	Tanzania	589	*C. ciliaris*	USA
124	*C. ciliaris*	USA	237	*C. ciliaris*	Brazil	590	*C. ciliaris*	USA
125	*C. ciliaris*	USA	302	*C. ciliaris*	Iran	591	*C. ciliaris*	USA

NI = No information

Table 2. Primers, targets and expected amplicon sizes used in this study

Target	Primer	Primer Sequence (5′ — > 3′)	Base Pairs	References
Apomixis Region	Q8H	GAGCTTGNCCAATCGGGAAA ATGGTGATGGATCTTTTGGAC	800	Ozias-Akins *et al.*, (1998)
	UGT197	GGATGAATAAAACGGTGTTGGGAG GAACAACCGCACAAGTGAGAGAA	850	
	PCAB10	TTCGAAATCGCATAGGTGAG GAGCCTTTCTTTATTTACCCAGTG	200	
Sexual Region	4HS*	AAGAGCAGGGGTTAGAGGTAA CACATTCAGCCTACGGAGTG	250	Yadav *et al.*, (2012)
Genetic Diversity	D12	(GA)6CG		Al-Soqeer (2011)
	HB14	(CTC)3GC		

Genetic diversity was evaluated using the ISSR D12 (GA)6CG and HB14 (CTC)3GC markers (Table 2). Four phenotypically contrasting accessions were used for the polymorphism test. PCR reactions were performed using the reagents and sequence: 1X buffer; 3.0 mM MgCl 2; 0.2 μM dNTP; 0.5 μM primer; 0.9 U of Taq DNA polymerase and the DNA sample 50 ng μL^{-1}. The thermocycler sequence was: 95 °C for 3 minutes; 35 cycles (94°C for 30s, 51°C for 45s, 72°C for 75s), 72°C for 5 minutes, and 4°C ∞. In addition, the samples were stained with GelRed and electrophoresed on a 2% agarose gel at a constant voltage of 80 Volts for 3 hours.

The gel images were captured under UV light by a LPix Image photo-documenter (Loccus Biotechnologia), and a 100 bp DNA Ladder (Ludwig Biotechnologia) was used as molecular weight marker to determine the size of the amplified fragments.

Data analysis

The analysis for identification of the reproductive mode was performed using images interpreted qualitatively regarding the presence or absence of the band. The analysis of genetic diversity was performed using images of gels exported to the BioNumerics software v. 7.6 (Applied Maths), in which polymorphism data were obtained, and the dendrogram of dissimilarity was developed using the UPGMA clustering method and the Dice coefficient.

RESULTS

Identification of apomictic and sexual genotypes

The three markers used (UGT197, Q8H, and PCAB10) amplified the respective fragment associated with the apomictic sequence of the ASGR (OZIAS-AKINS *et al.*, 1998; JESSUP, 2005), in the 115 accessions of *C. ciliaris* of the Embrapa Semiárido, a sample of the population is shown in Figure 1.

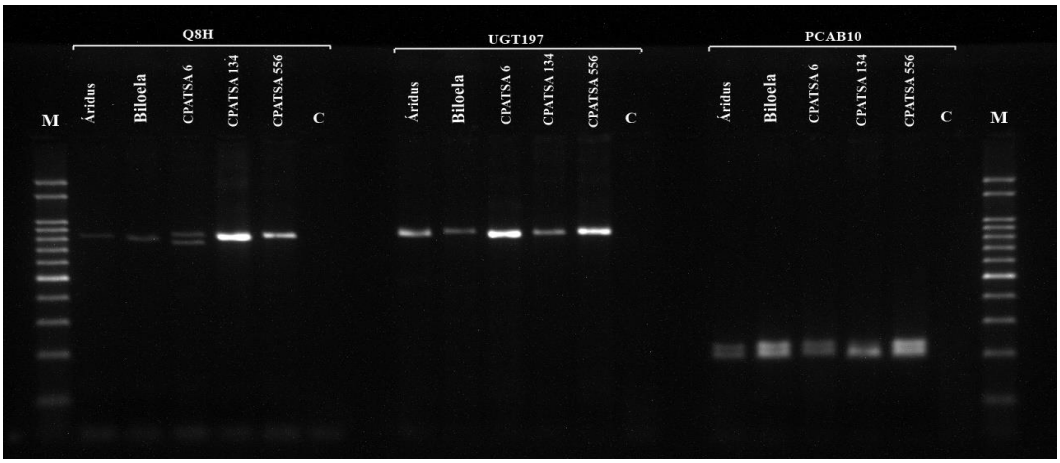

Figure 1. Gel electrophoresis of amplification with specific markers Q8H (800bp), UGGT197 (850bp) and PCAB10 (200bp) associated with the apomixis sequence of the genomic region in accessions of *Cenchrus spp.* of the Active Germplasm Bank of the Embrapa Semiárido. M: Marker 100 base pairs (Ludwig Biotechnology); C: Control (blank).

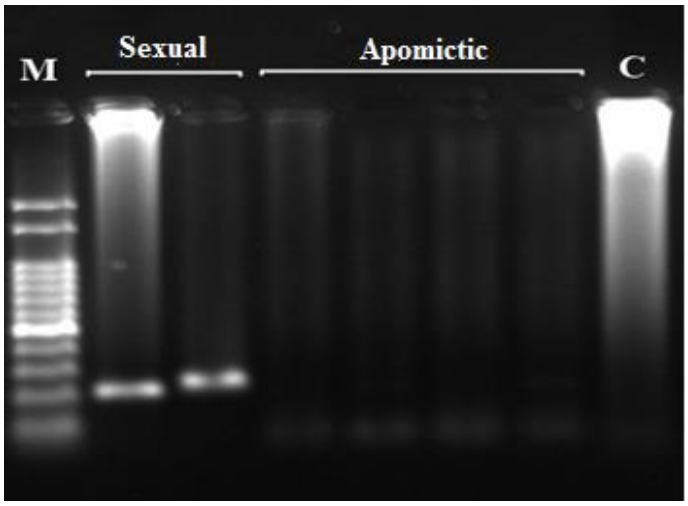

Figure 2. Gel electrophoresis with a specific marker associated with the reproductive sexual region 4HS* (250bp) revealing accessions of *Cenchrus spp.* of the Active Germplasm Bank of the Embrapa Semiárido with sexual reproduction mode. M: Marker 100 base pairs (Ludwig Biotechnologia); C: Control (Blank)

The efficiency of the markers used to identify apomictic genotypes was also verified by QUIROGA *et al.* (2013), who test them in sexual individuals and found no amplification.

The 4HS* marker developed by YADAV *et al.* (2012) was efficient in identifying sexual genotypes in accessions of *C. ciliaris* of the AGB of the Embrapa Semiárido (Fig. 2). Two out of 115 accessions (CPATSA 102, CPATSA 134) showed the DNA fragment linked to the sexual reproduction system. Furthermore, these two accessions also presented the gene fragment for apomixis; thus, they can be facultatively apomictic.

Genetic diversity of accessions of C. ciliaris of the AGB of the Embrapa Semiárido

The cluster analysis using the UPGMA method, and the Dice coefficient formed two groups. Group I was formed by accessions CPATSA 432 and CPATSA 590. Group II, was subdivided into seven subgroups - Subgroup IIa consisted of 31 accessions (CPATSA 573, CPATSA 574, CPATSA 579, CPATSA 575, CPATSA 609, CPATSA 194, CPATSA 615, CPATSA 436, CPATSA 616, CPATSA 576, CPATSA 613, CPATSA 588, CPATSA 611, CPATSA 3, CPATSA 131, CPATSA 195, CPATSA 603, CPATSA 581, CPATSA 582, CPATSA 598, CPATSA 558, CPATSA 557, CPATSA 580, CPATSA 599, CPATSA 138, CPATSA 585, CPATSA 556, CPATSA 577, CPATSA 141, CPATSA 140, and CPATSA 568); Subgroup IIb consisted of 43 accessions (CPATSA 120, CPATSA 124, Biloela, Numbank, CPATSA 4, CPATSA 127, Áridus, CPATSA 123, CPATSA 52, CPATSA 122, CPATSA 128, CPATSA 53, CPATSA 121, Molopo, CPATSA 130, CPATSA 126, CPATSA 199, CPATSA200, CPATSA 201, CPATSA 237, CPATSA 5, CPATSA 152, CPATSA 136, CPATSA 155, CPATSA 584, CPATSA 150, CPATSA192, CPATSA 193, CPATSA 6, CPATSA 158, CPATSA 129, CPATSA 144, CPATSA 148, CPATSA 57, CPATSA 591, CPATSA 601, CPATSA 563, CPATSA 608, CPATSA 312, CPATSA 102, CPATSA 119, CPATSA 125, and CPATSA 578); Subgroup IIc consisted of 7 accessions (CPATSA 541, CPATSA 614, CPATSA 302, CPATSA 570, CPATSA 595, CPATSA 602, and CPATSA 592); Subgroup IId consisted of 8 accessions (CPATSA 134, CPATSA 151, CPATSA 145, CPATSA 146, CPATSA 164, CPATSA 434, CPATSA 196, and CPATSA 198); Subgroup IIe consisted of four accessions (Gayndah, Gray, CPATSA 571 and CPATSA 572); Subgroup IIf consisted of 12 accessions (CPATSA 132, CPATSA 154, CPATSA 176, CPATSA 177, CPATSA 189, CPATSA 754, CPATSA 7754, CPATSA 433, CPATSA 147, CPATSA 438, CPATSA 149, and CPATSA 156); and Subgroup IIg consisted of 8 accessions (CPATSA 435, CPATSA 589, CPATSA 437, CPATSA 439, CPATSA 559, CPATSA 617, CPATSA 593, and CPATSA 555).

Three of these subgroups held genetically identical accessions: accessions CPATSA 581 and CPATSA 582 from the United States in subgroup IIa; accessions CPATSA 199, CPATSA 200, and CPATSA 201 from Tanzania, in addition to CPATSA 237 from Brazil in Subgroup IIb; accessions CPATSA 176 and CPATSA 177 from Brazil in the Subgroup IIf; and the cultivar CPATSA 7754 from Brazil and the accession CPATSA 754 without origin information, also in the Subgroup IIf (Fig. 3).

DISCUSSION

Cattle farming is an important activity in the semiarid region of Brazil as a source of animal protein for human consumption, and income for small farmers.

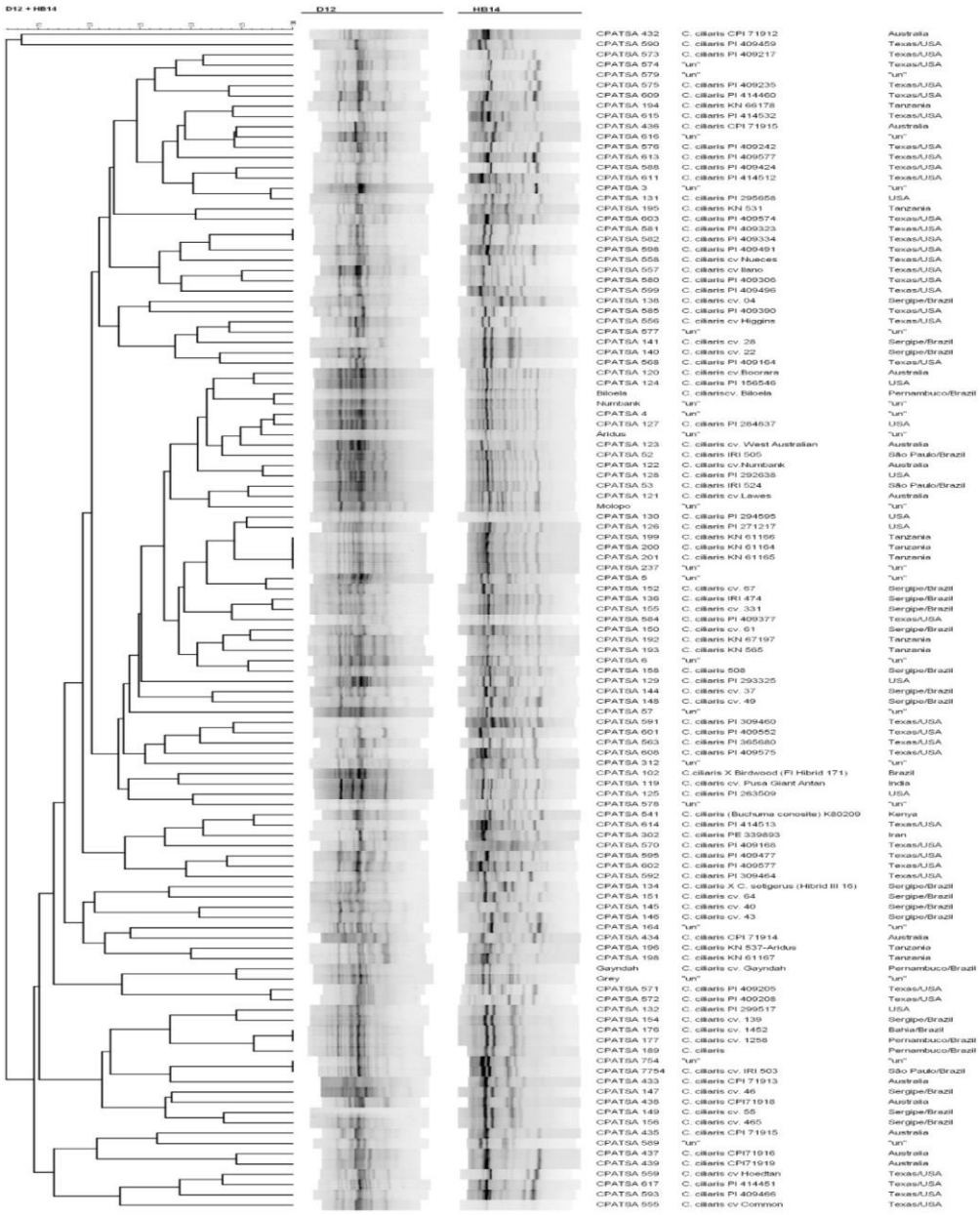

Figure 3. Dendrogram of dissimilarity build using the UPGMA method showing the clustering of accessions and cultivars of *Cencrhus spp.* of the Active Germplasm Bank of the Embrapa Semiárido. Right column: CPATSA registry; Species and cultivars; and Origin of the accessions.

These animals are raised mainly in extensive system on native pastures (Caatinga) in this region due to the low production costs since the forage is not harvested and does not need barns to store and supply it (GIULIETTI *et al.,* 2003).

However, the profitability of this system is not sufficient for small family farmers (VOLTOLINI *et al.,* 2010). Thus, the introduction of exotic forage species that can adapt to the Brazilian semiarid conditions can assist in the development of more sustainable agricultural production systems. *C. ciliaris* is promising for this purpose since it has a deep root system and high resistance to water deficit. However, the introduction of this grass species in these productive systems usually does not consider obtaining specific cultivars, which are still little used in the Brazilian semiarid region (RANGEL *et al.,* 2009).

Apomixis is an important tool for plant cloning but causes difficulties in obtaining cultivars for grass breeding programs. Apomixis may be facultative (some flowers occasionally exhibit meiotic sacs that can be fertilized and originate hybrids) or mandatory (when sexual reproduction is wholly excluded and all seeds will be clones of the mother plant) (HOJSGAARD *et al.,* 2014). This reproduction mechanism is controlled by one or more genes in the *C. ciliaris* species (JESSUP *et al.,* 2002; DWIVEDI *et al.,* 2007). Apomixis is predominant in almost all species that present this mode of reproduction (DWIVEDI *et al.,* 2007). Consequently, the occurrence of plants with sexual reproduction is rare in *Cenchrus* species. (KUMAR *et al.,* 2017). However, the same species may present variations in expressing facultative apomixis, indicating that several genes control apomixis. Two accessions in the presented study had DNA fragment linked to the sexual reproduction system (CPATSA 102, CPATSA 134).

The band fragment associated with the apomixis sequence of the ASGR (OZIAS-AKINS *et al.,* 1998; JESSUP, 2005) of all accessions of the AGB of the Embrapa Semiárido were amplified, using three markers UGT197, Q8H and PCAB10, and two of these accessions (CPATSA 102, CPATSA 134) also displayed DNA fragment linked to the sexual reproduction system. The use of DNA markers assists in selection and is efficient in discriminating accessions. According to KUMAR *et al.* (2017) apomixis-specific SCAR markers confirmed the presence of apomixes, but the use of only markers linked to the sexual system would be sufficient since the absence of the marker would indicate the apomictic mode of reproduction.

CPATSA 102 and CPATSA 134 displayed both fragments (sexual and apomictic mode of reproduction). Thus, they can be facultative apomictic. An apomictic facultative plant generates either zygotic as apomictic seeds; consequently, the progenies of these plants will segregate and form sexual hybrids and clones. KUMAR *et al.* (2017) report that facultative or apomictic sexual genotypes are heterozygous. However, it needs to be confirmed by analyzing their embryo sac, as performed by DWIVEDI *et al.,* (2007).

Cenchrus spp. with sexual reproduction in a population can be crossed with other plants, transferring characteristics of interest (protein content, palatability, and yield) to apomictic plants (QUIROGA *et al.,* 2013). First, however, breeding programs should access all available genetic variability, quantifying the genetic divergence between and within the accessions, through molecular markers, such as information about existing polymorphism at the DNA level.

It was possible to generate 100% of polymorphic bands with only two markers, with a mean amplification of 9.2 and 9.8 bands per accession. AZEVEDO *et al.* (2011), and LIMA *et al.* (2011) reported high percentages (>75%) of polymorphic bands characterizing genotypes of

Brachiaria spp. and *Pennisetum purpureum*, which may indicate high intraspecific genetic variability in *C. ciliaris* (GUTIERREZ-OZUNA *et al.*, 2009). AL-SOQEER (2011), reported that the use of these markers for diversity analysis could be efficient in identifying duplicates and assessing intra and interspecific genetic diversity in *Cenchrus spp.*; they also found, through cluster analysis, no identical genotypes in their evaluations, with the maximum value of similarity of 97% between genotypes.

Duplicate accessions might have been deposited to the AGB because of collection failures or because these individuals can be genetically identical even collected at different locations. Identifying and eliminating duplicates in the AGB provides financial and labor savings in maintaining and managing the bank.

The accessions tended to be grouped by country of origin (Fig. 3), forming large groups by genotypes, such as Group IIa with about 81% (CPATSA 573, CPATSA 574, CPATSA 579, CPATSA 575, CPATSA 609, CPATSA 194, CPATSA 615, CPATSA 576, CPATSA 613, CPATSA 588, CPATSA 611, CPATSA 3, CPATSA 131, CPATSA 195, CPATSA 603, CPATSA 581, CPATSA 582, CPATSA 598, CPATSA 558, CPATSA 557, CPATSA 580, CPATSA 599, CPATSA 585, CPATSA 556, CPATSA 577, and CPATSA 568), Group IIc with 71% (CPATSA 614, CPATSA 570, CPATSA 595, CPATSA 602, and CPATSA 592), and Group IIg with 62% (CPATSA 589, CPATSA 559, CPATSA 617, CPATSA 593, and CPATSA 555) of the accessions from the United States; and Group IId with 62% (CPATSA 134, CPATSA 151, CPATSA 145, CPATSA 146, and CPATSA 164), and Group IIf with 67% (CPATSA 154, CPATSA 176, CPATSA 177, CPATSA 189, CPATSA 7754, CPATSA 147, CPATSA 149, and CPATSA 156) of accessions from Brazil. However, Bruno *et al.* (2017) found significant morphological and agronomic differences between *Cenchrus ciliaris* accessions from the same country when evaluating 30 accessions of the same AGB, indicating high morphological variability of intra and interspecific regions, regardless of the country of origin.

The accessions that amplified the fragments for both sexual reproduction and apomictic reproduction, i.e., facultative sexual genotypes (CPATSA 102 and CPATSA 134), did not cluster together, although both came from Brazil. These accessions can be used in cross-breeding to obtain genetic variability for breeding programs. The CPATSA 134 accession (apomictic facultative) was grouped with the CPATSA 151, CPATSA 145, and CPATSA 146 accessions from Quissamã, (Sergipe State), Brazil, and had only the apomictic amplicon. This remarkable genetic similarity between accessions from the same place may be due to a common ancestor that originated these accessions.

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MOLEKULARNI PRISTUPI ZA IDENTIFIKACIJU APOMIKTIČNOG / SEKSUALNOG REPRODUKTIVNOG MEHANIZMA I GENETIČKE VARIJABILNOSTI KOD *Cenchrus* spp. UZORAKA

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Izvod

Cenchrus spp. obuhvata krmne trave koje su posebno važne za suva područja. Stoga su potrebne informacije o njihovom reproduktivnom mehanizmu i genetskoj raznolikosti. Aktivna banka germplazme (AGB) Embrapa Semiarido (istraživački centar za tropska poluaridna područja) ima 115 uzoraka *Cenchrus spp*. koji nisu bili molekularno okarakterisani. Stoga je cilj ovog rada bio da se proceni genetska raznolikost *Cenchrus spp*. uzoraka u AGB i identifikuju njihovi reproduktivni mehanizmi pomoću DNK markera. Specifični SCAR markeri K8H, UGT197 i PCAB10, pored SCAR markera 4HS *, korišćeni su za identifikaciju genotipova sa apomiktičnim i seksualnim načinima reprodukcije; genetička raznolikost je okarakterisana pomoću ISSR D12 (GA) 6CG i HB14 (CTC) 3GC markera. Reproduktivni mehanizam je analiziran pomoću slika na prisustvo ili odsustvo trake u očekivanoj veličini amplikona. Za analizu genetske raznolikosti, gelske slike su izvezene u softver Bionumerics (*Applied Maths*), u kojem su dobijeni podaci o polimorfizmu i dendrogram nesličnosti konstruisan metodom UPGMA klasterisanja koristeći Dice koeficijent. Markeri vezani za apomiktički region bili su pozitivni za sve uzorke. Međutim, samo su uzorci CPATSA 102 i CPATSA 134 imali pozitivne markere koristeći prajmere za obe strategije reprodukcije. Klaster analizom su diskriminisane dve velike grupe, a uzorci su se grupisali uglavnom prema mestu porekla. Takođe je bilo moguće identifikovati duplikate unutar AGB. Identifikacija i izuzeće duplikatnih uzoraka omogućava uštedu novca i rada u održavanju i upravljanju AGB-om. Dva uzorka su odabrana za oplemenjivačke programe *Cenchrus spp*. u Brazilu.

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