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Exchange of germoplasm and genetic diversity to enrichment the *Desmanthus* genebank

Intercâmbio de germoplasma e diversidade genética para enriquecimento do bag *Desmanthus*

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ABSTRACT - The genus *Desmanthus* presents wide ecogeographical distribution and complex taxonomy. *Desmanthus pernambucanus* (L.) Thellung, popularly known in Brazil as Jureminha, is a leguminous species native to the Northeast region of Brazil, which stands out by its high protein content, resistance to droughts, and by presenting no toxicity to animals. The objective of this work was to evaluate the genetic diversity of 15 progenies from the Commonwealth Scientific and Industrial Research Organization (CSIRO; Australia), through germplasm exchange for enrichment of the *Desmanthus* genebank of Embrapa Tabuleiros Costeiros, in Nossa Senhora das Dores, SE, Brazil. Fifteen ISSR markers were used, from which 8 were selected. The progenies identified as 242, 245, 246, 255, and 268 were the most genetically distant, and the most recommended to be introduced to the genebank.

Keywords: Jureminha. *Ex situ* conservation. Forage. Native legume. Genetic diversity.

RESUMO - O gênero *Desmanthus* apresenta ampla distribuição ecogeográfica e complexa taxonomia. O *Desmanthus pernambucanus* (L.) Thellung, popularmente conhecido como Jureminha, é uma leguminosa nativa do Nordeste brasileiro, que se destaca pelo elevado teor de proteína, resistência à seca e por não apresentar toxicidade aos animais. O trabalho foi desenvolvido com o objetivo de avaliar a diversidade genética de 15 progênies oriundas do CSIRO (*Commonwealth Scientific and Industrial Research Organization*, Austrália), através de intercâmbio de germoplasma, para o enriquecimento do Banco Ativo de Germoplasma de *Desmanthus* da Embrapa Tabuleiros Costeiros (BAG), em Nossa Senhora das Dores, SE, Brasil. Foram utilizados 15 marcadores ISSR, dos quais oito foram selecionados. As progênies identificadas como 242, 245, 246, 255 e 268 foram as mais distantes geneticamente, e as mais indicadas para serem introduzidas no BAG.

Palavras-chave: Jureminha. Conservação *ex situ*. Forragem. Leguminosa nativa. Diversidade genética.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

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INTRODUCTION

The genus *Desmanthus* (Mimoseae tribe; Mimosaceae family) is native to the Americas and Caribbean Islands (LUCKOW, 1993), presenting a wide ecogeographical distribution, with eight species found in the United States and 14 in Mexico, where it presents high diversity. It is also found in South America countries, such as Argentina, Brazil, and Peru (RANGEL et al, 2015). In Brazil, five native species of this genus have developed in the South, Southwest, and Northeast regions, namely: *Desmanthus leptophyllus* Kunt, *Desmanthus paspalacus* (Lindm), *Desmanthus tatuhyensis, Desmanthus pernambucanus* (L.) Thelhung, and *Desmanthus virgatus* (L.) Wild. (LIMA; MELO, 2015). Most of them are unexplored; thus, there is lack of information on their ecological, geographic, and taxonomic limits and on available genes of these species.

In the Northeast region of Brazil, the species *Desmanthus pernambucanus* (L.) Thellung is popularly known as Jureminha. It was described by Luckow (1993) as an invasive pantropical species, with origin centers outside the Americas (Pacific and Indian Ocean Islands, southwestern Africa, and southeaster Asia). Luckow (1993) described the occurrence of *Desmanthus* species in the Northeast region of Brazil and reported *D. pernambucanus* as the single species found. Pengelly and Liu (2001) studied *Desmanthus* species using RAPD (Random Amplified Polymorphic DNA) markers and found that most species described as *D. virgatus* were actually *D. pernambucanus*, indicating that its origin is the Northeast region of Brazil.

D. pernambucanus plants present high seed production, yellow flowers, bipinnate leaves, and penetrating, resistant, hard roots. These plants are

autogamous and present sexual reproduction (SANTOS et al., 2012). They stand out by their high protein content, resistance to droughts, and regrowth and colonization capacity. They present no toxicity to animals and high nutritional value (COSTA et al., 2017; QUEIROZ, et al, 2021). These plants are palatable, present high yields (ARAGÃO et al., 2019), are tolerant to droughts, and present high seed production, which is a key-component for persistence of plants (QUEIROZ, 2016). They are also highly efficient in biological nitrogen fixation (FREITAS et al., 2011).

The first introductions of *Desmanthus* species to germplasm collections were carried out almost 70 years back. However, only after the 2000's they have been studied as species with economic potential. Researches on their genetic diversity are also recent (MELO et al., 2011). Molecular tools can be efficient for identification of superior genotypes, making it possible the concentration of efforts for combinations of promising individuals.

In 2016, Queiroz (2016) evaluated five accessions of the Active Germplasm Bank of the Federal Rural University of Pernambuco (UFRPE; Serra Talhada, PE, Brazil) through AFLP (Amplified Fragment Length Polymorphism) markers and suggested that the geographical distance does not affect the genetic distance, as geographically close accessions from the same species did not present genetic similarities, contrasting with geographically distant accessions from different species. Costa et al. (2017) evaluated 26 accessions of the UFRPE Active Germplasm Bank by using 8 ISSR (Inter Simple Sequence Repeats) markers and recommended 8 accessions to be used in breeding programs.

The objective of this work was to evaluate the genetic diversity of *Desmanthus pernambucanus* (L.) Thellung from Australia for enrichment of the *Desmanthus* genebank of Embrapa Tabuleiros Costeiros.

MATERIAL AND METHODS

The seeds used for multiplication and production of progenies were obtained from germplasm exchange with the Genebank of the Commonwealth Scientific and Industrial Research Organization (CSIRO: Tropical Plants and Pastures, Canberra, Australia).

The processing was carried out at the Laboratory of Seeds of Embrapa Tabuleiros Costeiros, in Aracaju, Sergipe, Brazil. The seeds were subjected to asepsis treatment using 12.5 mL of a sodium hypochlorite solution diluted into 500 mL of distilled water. The seeds were immersed in distilled water at temperature of 80 °C for 3 minutes to overcome dormancy and then withdrawn and dried at room temperature (±25 °C) (Figure 1).

Figure 1. Processing of *Desmanthus pernambucanus* (L.) Thellung seeds.

Seeds of each accession were separately placed inside germination boxes (Gerbox) on previously sterilized germination paper (Germitest). The paper was moistened with distilled water and the boxes were maintained in a BOD (Biochemical Oxygen Demand) germination chamber at 12-hour photoperiod and temperature of 28 °C (Figure 2).

After germination, 24 seedlings of each accession were transferred to 500-mL plastic cups containing a substrate composed of black soil + manure + coconut powder (1:1:1), and kept under a shade screen at approximately 28 °C in the headquarters of Embrapa Tabuleiros Costeiros, Aracaju, SE, Brazil. Irrigation was carried out twice a day for 15 minutes, using a micro sprinkler system.

The progenies were coded according to their numbers of origin, i.e., accession number in the CSIRO (243, 269, 249, 270, 242, 255, 246, 239, 245, 268, 261, 263, 251, 257, 247). After 60 days in a greenhouse, the seedlings were planted in pits of 25 cm depth. The soil of each pit was fertilized with 30 g of simple superphosphate and covered with a fine soil layer before placing the seedling. Each accession was properly identified in the field (Figure 3).

Figure 2. Process for germination of *Desmanthus pernambucanus* (L.) Thellung seeds in germination boxes (Gerbox) with germination paper (Germitest) maintained in a BOD chamber.

Figure 3. Planting of *Desmanthus pernambucanus* (L.) Thellung seedlings in the Jorge do Prado Sobral Experimental Field of Embrapa Tabuleiros Costeiros, Nossa Senhora das Dores, Sergipe, Brazil.

Young leaves were collected from the progenies for extraction of DNA (ROMANO; BRASILEIRO, 2003), which was quantified in a NanoDrop 2000c (Thermo Scientific®). The DNA solutions (10 ng mL^{-1}) were prepared by diluting the samples into a TE buffer solution (Tris-HCL 10 mM, pH 8.0, and EDTA 1 mM) and then stored at -20 °C. Fifteen primers were tested in PCR (Polymerase Chain Reaction) assays (Table 1). The PCR reactions were carried out using 1 µL of genomic DNA (10 ng μL^{-1}), 1.0 µL of each primer (5 mM), 14.8 µL of sterilized MilQ water, 2 µL of reaction buffer 10X, 0.6 µL of $MgCl_2$, 0.4 µL of dNTP (10 nM), and 0.2 µL of Taq DNA Polymerase (5 U μL^{-1}), totaling a final reaction volume of 20 µL. The material was amplified in a thermal cycler (Proflex®) and subjected to denaturation at 94 °C for 4 minutes, followed by 40 amplification cycles. Denaturation at 94 °C for 45 seconds, annealing for 1 minute, and extension at 72 °C for 2 minutes were carried out for each cycle. After the reaction cycles, the process was ended with a final extension at 72 °C for 7 minutes, followed by cooling at 10 °C.

Table 1. ISSR primers tested for *Desmanthus pernambucanus* (L.) Thellung and the respective sequences and annealing temperature.

Primers	Sequence	AT (°C)
ISSR 1	CAC ACA CAC ACA GG	51
ISSR 2	CTC TCT CTC TCT CTC TAC	51
ISSR 3	CTC TCT CTC TCT CTC TTG	51
ISSR 4	CAC ACA CAC ACA AC	51
ISSR 5	CTC TCT CTC TCT CTC TGC	44
ISSR 6	CAC ACA CAC ACA AG	44
ISSR 7	CAC ACA CAC ACA GT	44
ISSR 8	GAG AGA GAG AGA GG	39
ISSR 9	GTG TGT GTG TGT CC	39
ISSR 10	GAG AGA GAG AGA CC	39
ISSR 11	GTG TGT GTG TGT CC	39
ISSR 12	CAC CAC CAC GC	39
ISSR 13	GAG GAG GAG GC	39
ISSR 15	CTC CTC CTC GC	52

*Annealing temperature.

The reaction products were subjected to electrophorese (250 V, 145 mA, and 120 W) for 3 hours in 2% agarose gel. The banding standardization was carried out using 10 µL of the 100 bp molecular weight marker (Promega, Madison, South Dakota, USA). The visualization of fragments was obtained in a Gel doc L□pix image system (Loccus Biotechnology, Cotia, SP). Boostraps were carried out from simulations with resampling of different sizes (from 60 with increases of 10), each one repeated 5000 times per application in the software DBOOT, to assess whether the number of markers generated was enough for analyzing the sampling group.

Number of observed alleles (N_a), number of effective alleles (N_e), expected heterozygosity (H_e), and Shannon Index (SI) were calculated for dominant markers, using the program Genalex 6.5. Correlation and stress values were estimated in the program Genes (CRUZ, 2006). The genetic similarities between individuals were calculated using the Jaccard coefficient, and the development of a dendrogram was obtained with the aid of the program NTSYS-pc 2.0, based on the genetic similarity matrix using the method UPGMA (Unweighted Pair Group Method with Arithmetic Mean). The analysis of genetic structure was based on Bayesian statistics and estimated using the software Structure 2.3.4. The admixture ancestry model was used, and the results were based on 100,000 simulations with burn-in of 10,000. The software Structure Harvester (EARL; VONHOLDT, 2012) was used to determine the number of groups (K).

RESULTS AND DISCUSSION

Eight out of the fifteen primers tested were used for analyzing the genetic diversity in accessions of *Desmanthus* because of their high reproducibility. The selected primers amplified 38 fragments, with 71.05% polymorphism. The highest polymorphism percentage was shown by ISSR13 (100%), and the lowest by ISSR12 which presented only monomorphic fragments (0% polymorphism) (Table 2). The indication of a minimum number of bands in genetic diversity studies contributes to optimize the use of resources and time and decrease the number of representative markers needed for the characterization of genetic diversity (GONÇALVES et al., 2014).

Table 2. ISSR primers, total number of bands, number of polymorphic fragments and polimorphism percentage, and base width generated by PCR reactions for the study of genetic diversity of progenies *of Desmanthus pernambucanus* (L.) Thellung.

ISSR primers	Total number of fragments	Number of polymorphic fragments	Polimorphism percentage (%)
ISSR 2	7	5	71
ISSR 3	3	2	66
ISSR 4	4	2	50
ISSR 5	6	5	83
ISSR 7	7	6	85
ISSR 12	2	0	0
ISSR 13	6	6	100
ISSR 15	3	1	33
Total	38	27	

The reliability of the results was verified considering estimates of correlation, which presented a value of 0.998 and a stress value of 0.018, confirming the stability of the number of primers selected, as stress values equal to or less than 0.05 denote that the estimates are accurate (KRUSKAL, 1964). The number of fragments was lower than that reported by Costa et al. (2017), who used 8 primers and found 95 fragments, resulting in a stress value of approximately zero. Soares et al. (2020) used RAPD primers for evaluating the genetic diversity of 242 individuals from three ecogeographic regions of the state of Sergipe, Brazil (Zona da Mata, Agreste, and Semiarid regions), and found 96.33% polymorphism.

The mean number of alleles (N_a) was 1.76 and the number of observed alleles (N_e) was 1.49. Regarding genetic variability indexes, expected heterozygosity (H_e) and Shannon Index (SI) presented values of 0.28 and 0.42, respectively, which are moderate to low values (Table 3).

Table 3. Number of individuals, number of observed alleles (N_a), number of effective alleles (N_e), Shannon Index (SI), expected heterozygosity (H_e), and observed heterozygosity (H_o) for progenies of *Desmanthus pernambucanus* (L.) Thellung, obtained through ISSR markers.

Number of individuals	N_A	N_E	I	H_E	H_O
15	1.76	1.49	0.42	0.28	0.29

ISSR markers have shown efficiency for several other species, for example, a study on 5 *Trifolium* species (autogamous) found a mean genetic diversity of 0.175 (HWANG; HUH, 2016), and a study on *Vigna unguiculata* L. found a mean value of 0.6383 (IGWE et al., 2017). Similar results were found by Queiroz (2016) when using AFLP markers for accessions of *Desmanthus* sp., with H_e of 0.29, denoting low genetic diversity; and by Soares et al. (2020), who found H_e of 0.25 when using RAPD. These results are probably connected to the species reproduction system, which can affect the genetic diversity among individuals (SOARES et al., 2016)

The PCR-ISSR technique enabled the development of a Jaccard similarity matrix, which presented values between 0.50 and 0.97 (Table 4). The pairs formed by the progenies 269 × 243, 257 × 243, and 257 × 247 were the most genetically similar, presenting, respectively, indexes of 0.97, 0.91, and 0.91. Contrastingly, the pairs 246 × 243 and 251 × 246 presented lower values (0.50), indicating greater genetic differentiation between them.

Table 4. Jaccard similarity matrix using 8 primers by the ISSR technique for 15 progenies of *Desmanthus pernambucanus* (L.) Thellung.

	Progenies														
Progenies	243	269	249	270	242	255	246	239	245	268	261	263	251	257	247
243	1.00														
269	0.97	1.00													
249	0.80	0.82	1.00												
270	0.87	0.90	0.79	1.00											
242	0.65	0.67	0.58	0.74	1.00										
255	0.55	0.57	0.48	0.63	0.85	1.00									
246	0.50	0.52	0.53	0.57	0.61	0.48	1.00								
239	0.76	0.78	0.74	0.80	0.68	0.63	0.57	1.00							
245	0.61	0.63	0.55	0.64	0.70	0.80	0.57	0.64	1.00						
268	0.61	0.63	0.55	0.64	0.70	0.64	0.57	0.59	0.81	1.00					
261	0.88	0.90	0.79	0.87	0.69	0.59	0.53	0.87	0.66	0.66	1.00				
263	0.83	0.80	0.76	0.76	0.61	0.52	0.43	0.71	0.53	0.53	0.82	1.00			
251	0.77	0.74	0.70	0.71	0.65	0.55	0.50	0.66	0.61	0.61	0.76	0.88	1.00		
257	0.91	0.88	0.73	0.84	0.63	0.53	0.48	0.74	0.59	0.59	0.85	0.86	0.80	1.00	
247	0.88	0.85	0.81	0.82	0.61	0.52	0.52	0.82	0.58	0.58	0.88	0.89	0.83	0.91	1.00

Formation of three groups was observed, considering the similarity of 0.67 (Figure 4). The first group was formed by the progeny 246, considered the most isolated; the second group was formed by the progenies 242, 255, 245, and 268; and the third group was formed by the progenies 239, 249, 251, 263, 247, 257, 270, 261, 269, and 243. The groups were considered different and had no duplicates, which would make management difficult and raise the cost of maintaining the

genebank.

The genetic distances were subjected to Principal Component Analysis (PCA), which allowed for the identification of four clusters; the sum of the two first components explained 52.38% of the variability (Figure 5). The PCA formed the groups 1 (progeny 246), 2 (255, 245, 268, and 242), 3 (239, 270, 261, 269, 249, and 243), and 4 (247, 257, 263, and 251). The similarity between the individuals 245 (-0.9), 268 (-0.8), 255 (-1.0), and 242 (-0.7) in the first principal component was determinant for the formation of groups, different from the second principal component in which only the individual 251 (-0.8) presented higher correlation. These results reinforce the efficiency of the genetic diversity study using ISSR markers.

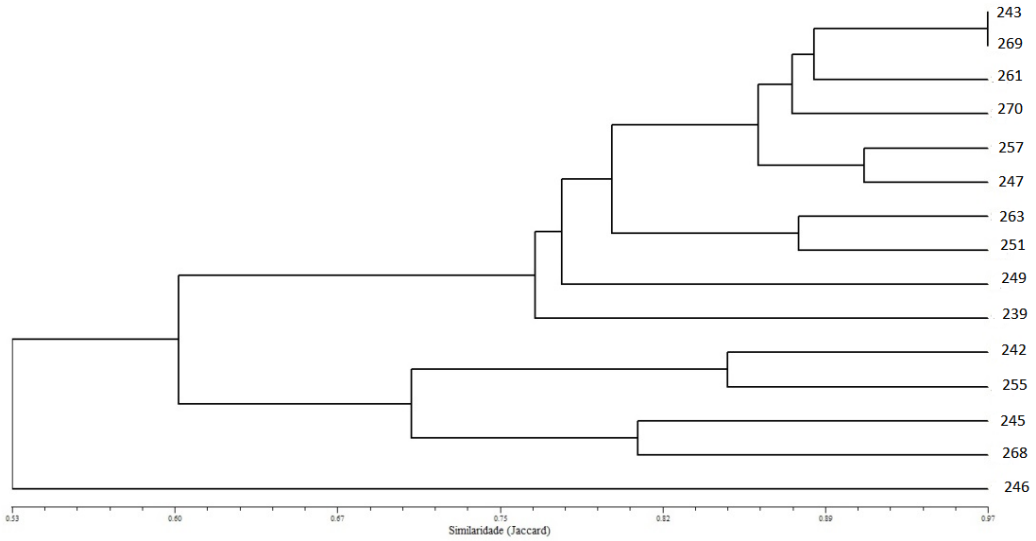

Figure 4. Dendrogram developed using the UPGMA method based on the similarity genetic index by the Jaccard coefficient for 15 progenies of *Desmanthus pernambucanus* (L.) Thellung.

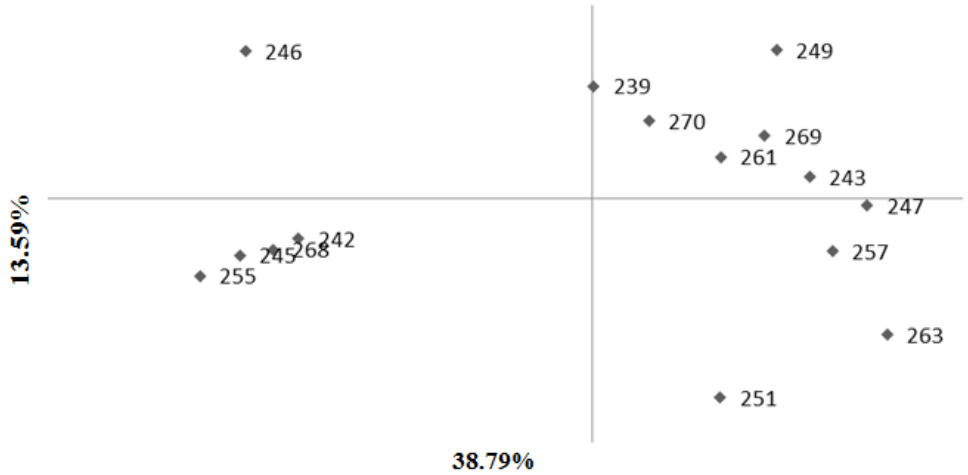

Figure 5. Principal Component Analysis (PCA) for progenies of *Desmanthus pernambucanus* (L.) Thellung.

Bayesian analysis was used to evaluate the genetic structure of the progenies (Figure 6). The software Structure was used to estimate the most probable number of clusters (K), by calculating the data log probability for each K value and by the ΔK statistics. The K that better represented the dataset was K=4. The first group was formed by the progenies 245, 255, 268, and 242; and the second by 269, 261, 270, 249, 239, and 243; the third group was formed only by the progeny 246, which was more isolated, forming a group by its own; and the fourth group was formed by 263, 251, 257, and 247. These results corroborate those of the PCA.

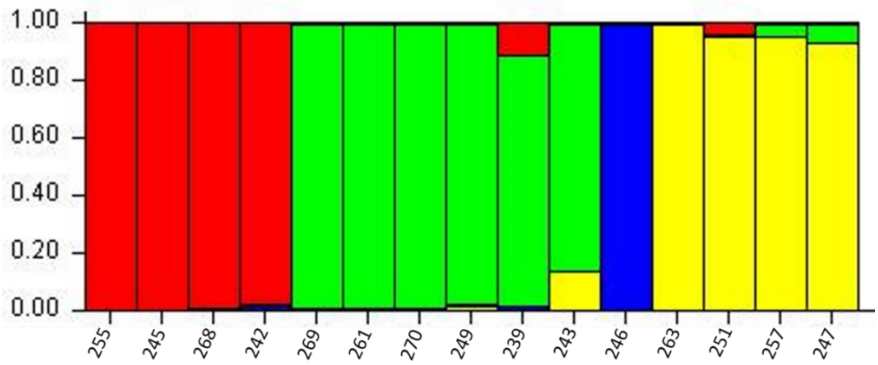

Figure 6. Representation of 15 progenies of *Desmanthus pernambucanus* (L.) Thellung in groups, according to molecular data of 8 ISSR primers, using the program Structure (ΔK=4).

CONCLUSIONS

The ISSR markers used were efficient for the study of genetic diversity of *Desmanthus pernambucanus* (L.) Thellung, showing a moderate diversity among the progenies. The progenies 242, 245, 246, 255, and 268 were the most divergent and are recommended to be introduced to the *Desmanthus* genebank of Embrapa Tabuleiros Costeiros.

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