Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v45i1.64168

Genetic diversity among genotypes of *Parkia platycephala* (Benth.), a typical tree of northeastern Brazil

Raiane de Sousa Oliveira[1], Gérson do Nascimento Costa[2], Raimundo Nonato Oliveira Silva[3], Paulo Sarmanho da Costa Lima[4*], Raimundo Bezerra de Araújo Neto[4], Ângela Celis de Almeida Lopes[5] and Francielle Alline Martins[6]

[1]Departamento de Zootecnia, Universidade Federal do Piauí, Campus Universitário Ministro Petrônio Portella, 64049-550, Bairro Ininga, Teresina, Piauí, Brazil. [2]Departamento de Biologia, Universidade Estadual do Maranhão, Coelho Neto, Maranhão, Brazil. [3]Departamento de Biologia, Universidade Federal do Piauí, Campus Amilcar Ferreira Sobral, Floriano, Piauí, Brazil. [4]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Meio Norte, Teresina, Piauí, Brazil. [5]Departamento de Agronomia, Universidade Federal do Piauí, Campus Universitário Ministro Petrônio Portella, Teresina, Piauí, Brazil. [6]Departamento de Biologia, Universidade Estadual do Piauí, Teresina, Piauí, Brazil. *Author for correspondence. E-mail: paulo.costa-lima@embrapa.br

ABSTRACT. *Parkia platycephala* (Fabaceae) is a useful tree. The leaves are rich in protein, fiber and minerals and are good quality, low-cost fodder for animals, making it a great option for animal feed during the dry season in Northeast Brazil. In addition, wood is used in small buildings and as fuel. With the aim of exploring genetic variation within the species, we have assessed the molecular and morpho-agronomic characteristics of 10 accessions (F1 - F10) of the species originating from the same location and maintained in the Forage Collection at *Embrapa Meio Norte*. Clustering analyses based on the amplicons generated by 12 inter simple sequence repeat (ISSR) primers and on 36 qualitative/quantitative markers separated the accessions into two groups, the constituents of which were dependent on the characteristics considered. The most divergent genotypes according to ISSR analysis were F3 and F8. Morpho-agronomic analysis identified F2 and F7 as the most divergent, while the traits that contributed most (36.5%) to total diversity were, in order of importance, mature inflorescence length, stem length, immature inflorescence width, seed weight and pedicel length. The results revealed that there is sufficient genetic variability among the studied accessions. These accessions with greater diversity are candidates for actions that promote the conservation, domestication and genetic improvement of the species.

Keywords: faveira-de-bolota; forage legume tree; inter simple sequence repeat markers; morpho-agronomic traits; multivariate analysis.

Received on June 8, 2023.
Accepted on December 6, 2023.

Introduction

Parkia platycephala Benth. (Fabaceae), known locally as faveira-de-bolota, is a semi-deciduous tree that is distributed mainly in the Cerrado, Atlantic Forest and Caatinga transition zone in northeast Brazil (Santos, Farias, Silva, Dias, & Silva, 2019). This important forage species, which can grow to a height of some 20 to 30 m, has compound leaves and a capitular inflorescence with flowers that typically range in color from red to dark red but are sometimes yellow or even white. The dark leathery leguminous fruits hang from long peduncles and release a viscous resin when ripe. During the dry season, the ripe pods containing small and round or elongated seeds (Lorenzi, 2020) fall to the ground and serve as fodder for grazing animals and as a source of income for family farmers. The leaves are rich in protein, fiber and minerals and constitute good quality and low-cost forage for animals (Magalhães et al., 2014), while the wood is used in minor constructions and as a fuel.

Unfortunately, the indiscriminate exploitation of *P. platycephala* associated with the fragmentation of forest ecosystems has caused loss of genetic variability among native populations with economic and environmental potential (Melo Jr., Carvalho, Vieira, & Oliveira, 2012). Furthermore, the study and conservation of the species is limited by the lack of Morpho-agronomic descriptors and of information regarding the genetic diversity of accessions maintained in germplasm collections. In this context, inter simple sequence repeats (ISSR) markers are very helpful because they permit the assessment of the genetic diversity of natural populations in the absence of previous knowledge of the DNA and without the need for

phenotypic expression (Santana et al., 2016). In addition, the technique is straightforward, highly reproducible, inexpensive and particularly applicable to less studied species such as *P. platycephala*.

Considering that knowledge of the genetic diversity within populations of *P. platycephala* is important for the authentication of genotypes and their conservation in the germplasm banks, we aimed to perform the molecular and morpho-agronomic characterization of 10 accessions maintained in the Germplasm Collection of *Embrapa Meio Norte*, Teresina, Piauí State, Brazil.

Material and methods

Sampling leaf material

Ten accessions (F1 to F10) of *P. platycephala* from the Forage Collection at *Embrapa Meio Norte* (5°2'5.36" S; 42°48'8.33" W) were selected for analysis. The leaves of each of the accessions were collected, wrapped in paper towels, placed in plastic bags and stored at -20°C until required for analysis.

DNA extraction and analysis

Young leaf DNA was extracted according to the protocol described by Doyle and Doyle (1990) with some modifications. Briefly, leaf samples were macerated in a Precellys®24 tissue homogenizer (Bertin Instruments) by applying two cycles of 25 and 30 s at 6,600 rpm each followed by the immediate addition of extraction buffer [2% cetyltrimethylammonium bromide **(**CTAB) buffer, 1.4 M NaCl, 20 mM ethylenediaminetetraacetic acid (EDTA), 100 mM Tris-HCl pH 8.0, 1% polyvinylpyrrolidone (PVP), 3% polyvinylpolypyrrolidone (PVPP)] and 2-mercaptoethanol (20 µL). Samples were placed in a water bath at 37°C for 5 to 7 min and subsequently washed three times with chloroform: isoamyl alcohol (24:1) solution to remove polysaccharides. After the first and second washes, the samples were centrifuged at 17,000 x g for 10 and 5 min., respectively, while after the third wash centrifugation was performed at 4,383 x *g* for 5 min. The supernatants were removed and the DNA pellets resuspended in 50 µL of Tris-EDTA buffer pH 8.0. DNA integrity and purity were evaluated by 0.8% agarose gel electrophoresis together with a lambda-DNA marker. DNA concentration and purity were determined using a NanoDrop 20000 UV-Vis spectrophotometer (Thermo Fisher Scientific). Samples were diluted to 7 ng μL^{-1} and stored at -20ºC until required for polymerase chain reaction (PCR).

ISSR-PCR analysis

Twenty-five ISSR primers acquired from the University of British Columbia (Vancouver, BC, Canada) were subjected to preliminary screening with DNA from accessions F3, F7, and F10, and 12 were selected based on the level of polymorphism and band resolution (Table 1).

Table 1. ISSR primers used in the analysis of the genetic diversity of accessions of *Parkia platycephala*.

Primer	Annealing temperature (°C)	Primer sequence (5'-3')	GC (%)	Number of loci		Polymorphism (%)	Bands (pb)
				Total	Polymorphic		
UBC 807 [na]	48	AGAGAGAGAGAGAGAGT	47.06	10	8	80.0	200-1300
UBC 808 [na]	51	AGAGAGAGAGAGAGAGC	52.94	12	12	100.0	500-2000
UBC 810 [na]	47	GAGAGAGAGAGAGAGAT	47.06	6	5	83.3	400-1650
UBC 811 [na]	50	GAGAGAGAGAGAGAGAC	52.94	8	5	62.5	200-1000
UBC 812 [na]	51	GAGAGAGAGAGAGAGAA	47.06	7	6	85.7	500-1650
UBC 818 [na]	51	CACACACACACACACAG	52.94	11	7	63.6	500-2000
UBC 825 [na]	54	ACACACACACACACA	46.67	7	4	57.0	500-2000
UBC 827 [na]	54	ACACACACACACACACG	52.94	11	10	90.0	300-1500
UBC 834 [a]	54	AGAGAGAGAGAGAGAGYT	50.00	11	11	100.0	200-2000
UBC 856 [a]	54	ACACACACACACACACYA	50.00	9	8	88.8	400-2000
UBC 857 [a]	54	ACACACACACACACACYG	55.55	8	6	75.0	400-1500
UBC 890 [a]	52	VHVGTGTGTGTGTGTGT	58.82	13	12	92.3	300-1500
Total (mean)	-	-	-	113	94	83.1	200-2000

[a] = anchored primer; [na] = non-anchored primer; Y = C or T; V = A or C or G; H = A or C or T.

The PCR mixture contained 6.8 µL water, 1 µL 10 × Taq DNA polymerase buffer, 0.1 µL Invitrogen Taq DNA polymerase (1 U μL^{-1}; Thermo Fisher Scientific), 0.3 µL 50 mM $MgCl_2$, 1 µL 10 mM dNTPs, 0.3 µL primer (0.8 µM) and 0.5 µL genomic DNA (7 ng μL^{-1}). Amplifications were carried out in a thermal cycler model 2720 (Thermo Fisher Scientific) using the following parameters: initial denaturation at 94°C for 1.5 min. 40 cycles

of denaturation at 94°C for 40 s, annealing at 47-54°C according to primer (Table 1) for 45 s, extension at 72°C for 2 min. and final extension at 72°C for 7 min. Amplicons from all accessions, together with a 100 bp DNA ladder (Thermo Fisher Scientific), were submitted to electrophoresis for 4 hours at 80 V on 1.5% agarose gel pre-stained with 0.8 µL GelRed™ (Biotium). The gels were visualized on a transilluminator and images were captured and digitized using the MiniBis Pro gel documentation system (DNR Bio Imaging System).

Molecular analyses

In order to investigate genetic diversity among the 10 accessions, gels of the ISSR amplicons were scored for band presence (1) or absence (0) and a binary data matrix was constructed. A matrix of genetic similarity was generated according to the Jaccard coefficient of genetic distance and a dendrogram constructed using the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm. Bootstrap analysis with 1,000 replicates was performed to test the reliability of individual branching points of the phylogenetic tree and to calculate the cophenetic correlation coefficient (r). All analyses were performed with the aid of PAST version 3.08 software.

Morpho-agronomic analyses

The morpho-agronomic characterization of *P. platycephala* accessions took into account 18 quantitative and 18 qualitative traits that have been defined previously in the literature, and these included aspects relating to the anatomy of leaves and stem (n =10), flowers (n =10), fruits (n =10), and seeds (n = 6). The anatomical traits were evaluated by field observations performed in the flowering season during August 2017. However, F5 and F9 were excluded from the analysis because their morphological and agronomical traits could not be compiled at the time of study. Due to the lack of a catalog of descriptors for *P. platycephala*, it was necessary to elaborate morphoagronomic descriptors from the daily verification of the phenology of the plants, aiming to verify characters with variation within the germplasm collection, in addition, based on descriptors for the fava (*Phaseolus lunatus* L.) published by the International Plant Genetic Resources Institute (IPGRI, 2001). Leaf and stem traits included leaf length (LLE, cm; measured from the petiole to the terminal leaflets), leaf color (LCO), leaf hairiness (LHA; evaluated on the lower surface of leaflets), leaf stipules/thorns (LST), stem pigmentation (SPI), stem thorns (STH), stem texture (STE), stem length (SLE, m; measured from the ground to the first branches), stem diameter (SDI, cm; evaluated at the thickest point), and stem branching (SBR; influenced by the density of the branches). Flower traits were monitored from the emergence of the first inflorescences and included immature inflorescence length (IIL, cm), immature inflorescence width (IIW, cm), immature inflorescence color (IIC) and texture of the epidermis of immature inflorescence (IIT), all of which were evaluated before the onset of anthesis. The traits mature inflorescence color (MIC) and anther color (ACO) were evaluated immediately after flowering, while the average number of inflorescences per node (NIN), average pedicel length (PLE, cm; n = 10 random pedicels plant^{-1}), mature inflorescence length (MIL, cm), and mature inflorescence width (MIW, cm) were determined after maturation.

Fruit traits were assessed in 20 random pods per plant and comprised pod pubescence (PPU), pod curvature (PCU) and pod apex shape (PAS), all of which were evaluated in fully developed immature pods, along with number of pods per inflorescence (NPI), pod length (PLE, cm), pod width (PWI, cm) and pod color (PCO) assessed in mature pods, and pod dehiscence (PDE), mature pod weight (MPW) and number of loculi per pod (NLP) determined during maturation. Seed traits were evaluated in mature pods collected at random, and included number of seeds per pod (NSP), seed length (SLN, mm), seed width (SWI, mm), seed weight (SWE, mg; n = 100 seeds with moisture content of 12-14%), seed texture (STX), and seed color (SCO).

Data analyses

The diversity among accessions was evaluated taking into account the mean values of quantitative traits and the modal values of qualitative traits. Tocher`s optimization clustering method based on simple coincidence distance was used for qualitative descriptors, while the UPGMA method based on Euclidean distances was used for quantitative descriptors. A multivariate approach employing principal component analysis (PCA) was used to explain the relationship among quantitative traits and to determine those that best explained the total variability in the dataset (Singh, 1981). Analyses were carried out using GENES software version 2015.05.

Results

Molecular analyses

The 12 selected primers generated 113 amplified loci of which 94 (83%) were polymorphic (Table 1). The highest number of bands was obtained with primer UBC890 (n = 13) and the lowest with UBC810 (n = 6), whilst the average number of bands per primer was 9.4 and band sizes varied between 200 to 2000 bp. The non-anchored primer UBC808 (with AG repeats) and the anchored primer UBC834 (also with AG repeats) produced bands that were 100% polymorphic, while the best band resolution was obtained with primer UBC834 (Figure 1). Non-anchored primers UBC825, UBC811, and UBC818 produced fewer polymorphic bands than the others despite the presence of AG and AC repeats.

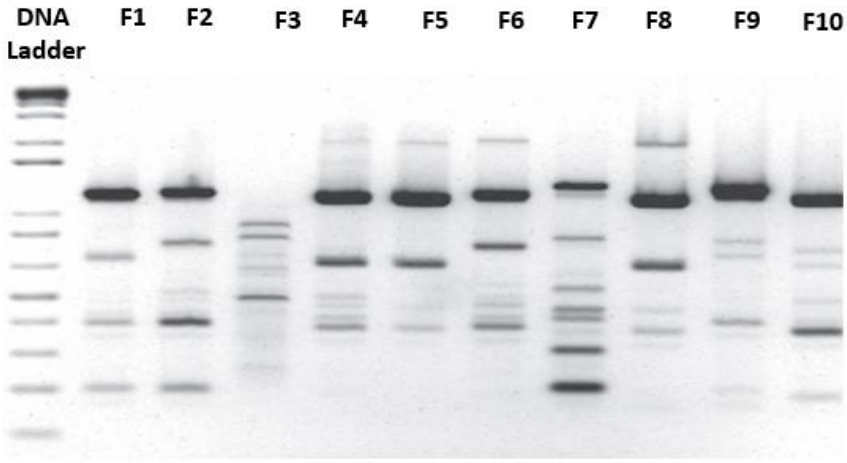

Figure 1. Electrophoretic profile of the PCR products obtained from the amplification of DNA from ten *Parkia platycephala* accessions using the ISSR primer UBC 834.

The UPGMA dendrogram constructed using Jaccard distances presented a high cophenetic correlation coefficient (r = 0.91) and revealed the existence of two genetically distinct groups, namely group I comprising all accession except the F3 accession that makes up the group II, only representative of the group (Figure 2).

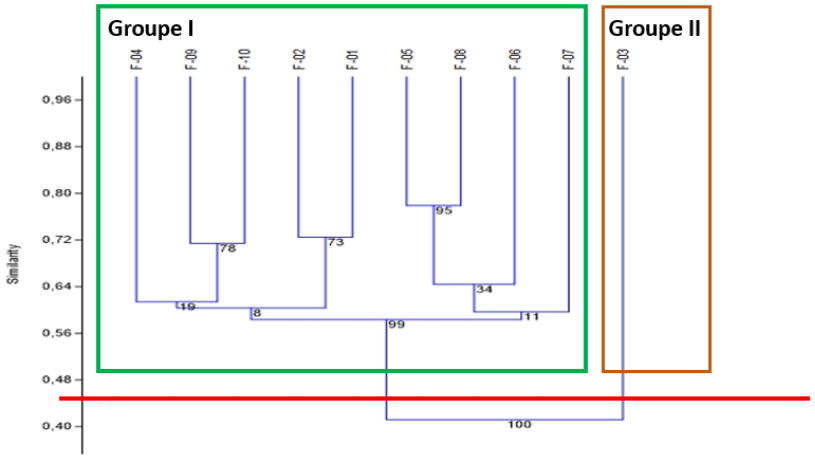

Figure 2. UPGMA dendrogram based on Jaccard distances of the ten *Parkia platycephala* accessions studied. The dashed red line shows the mean similarity coefficient.

Morph-agronomic analyses

According to the similarity matrix based on simple coincidence distances (Table 2), F6 and F7 were the most similar, whereas F2 and F7 were the most divergent. Analysis of the qualitative traits using the Tocher's clustering method separated eight of the ten accessions for which data were available into two groups, namely group I comprising F1, F3, F4, F6 - F8, and F10, and group II represented exclusively by F2, further indicating the low genetic diversity among *P. platycephala* accessions.

Table 2. Genetic similarity matrix based on simple coincidence distances between pairs of eight of the *Parkia platycephala* accessions studied.

Accessions	F1	F2	F3	F4	F6	F7	F8	F10
F1	1							
F2	0.3889	1						
F3	0.1667	0.3333	1					
F4	0.1667	0.3889	0.2222	1				
F6	0.2778	0.5000	0.2778	0.3333	1			
F7	0.2222	0.5556	0.2222	0.2778	0.0556	1		
F8	0.2222	0.3333	0.1667	0.3333	0.3333	0.2778	1	
F10	0.3333	0.4444	0.2222	0.2778	0.2778	0.2778	0.2778	1

Regarding the quantitative traits, the UPGMA dendrogram constructed on the basis of Euclidian distances presented a high cophenetic correlation coefficient (r = 0.84) and separated the accessions into two groups, namely group I comprising exclusively F6 and group II comprising F1 - F4, F7, F8, and F10 (Figure 3).

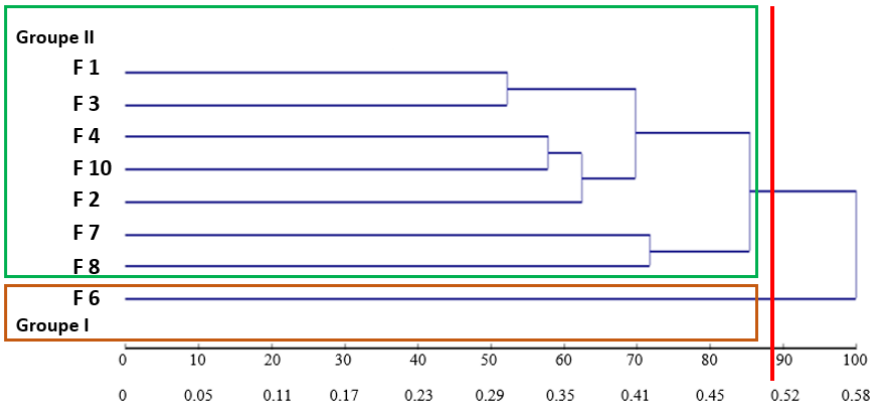

Figure 3. Dendrogram of eight Parkia accessions generated by UPGMA clustering using Euclidean distance.

The results of PCA showed that the first five components accounted for 96.54% of the variance (Table 3).

Table 3. Principal component (PC) analysis of 18 traits of eight of the *Parkia platycephala* accessions studied showing their relative contributions (eigenvalues) to the total variance.

PC	Variance	Eigenvalue (%)	Accumulated eigenvalue (%)
PC1	6.47	35.96	35.96
PC2	4.51	25.05	61.01
PC3	2.85	15.87	76.89
PC4	2.26	12.55	89.45
PC5	1.27	7.08	96.54
PC6	0.41	2.32	98.86
PC7	0.19	1.10	99.97
PC8	0.00	0.02	99.99
PC9	0.00	0.00	99.99
PC10	0.00	0.00	99.99
PC11	0.00	0.00	99.99
PC12	0.00	0.00	99.99
PC13	0.00	0.00	99.99
PC14	0.00	0.00	99.99
PC15	0.00	0.00	99.99
PC16	0.00	0.00	99.99
PC17	0.00	0.00	99.99
PC18	0.00	0.00	100.00

PC1 was responsible for 35.96% of the variance among eight of the accessions studied, while the traits with the highest weights were NIN (0.32), APL (0.30), MPW (0.30), SDI (0.29), and NPI (0.28) (Table 4).

Table 4. Principal component (PC) analysis of 18 traits of eight of the *Parkia platycephala* accessions studied showing their respective eigenvectors.

PC	Morphological and agronomic traits																	
	LLE	SLE	SDI	IIL	IIW	NIN	APL	MIL	MIW	NPI	PLE	PWI	MPW	NLP	NSP	SLW	SWI	SWE
PC1	0.03	0.24	0.29	0.26	0.20	0.32	0.30	0.25	0.20	0.28	0.05	0.01	0.30	0.24	0.09	0.29	0.23	0.19
PC2	0.11	0.26	0.19	0.12	0.16	0.03	0.14	0.34	0.30	0.08	0.39	0.26	0.16	0.20	0.34	0.02	0.34	0.25
PC3	0.47	0.13	0.09	0.06	0.15	0.19	0.01	0.10	0.04	0.32	0.17	0.42	0.07	0.19	0.27	0.30	0.03	0.36
PC4	0.08	0.07	0.15	0.44	0.47	0.00	0.21	0.07	0.31	0.21	0.04	0.08	0.32	0.32	0.27	0.12	0.07	0.12
PC5	0.44	0.12	0.29	0.05	0.10	0.37	0.28	0.08	0.07	0.16	0.35	0.30	0.03	0.16	0.05	0.30	0.25	0.13
PC6	0.09	0.65	0.32	0.20	0.01	0.18	0.28	0.00	0.35	0.22	0.07	0.06	0.19	0.14	0.04	0.01	0.02	0.24
PC7	0.10	0.27	0.26	0.01	0.21	0.03	0.47	0.27	0.24	0.11	0.30	0.27	0.19	0.35	0.26	0.07	0.02	0.11
PC8	0.09	0.06	0.14	0.01	0.01	0.00	0.07	0.06	0.05	0.05	0.14	0.12	0.07	0.09	0.04	0.45	0.81	0.18
PC9	0.33	0.25	0.21	0.01	0.09	0.20	0.20	0.09	0.11	0.22	0.38	0.33	0.27	0.26	0.19	0.37	0.17	0.05
PC10	0.36	0.01	0.42	0.30	0.24	0.10	0.19	0.00	0.20	0.06	0.05	0.10	0.62	0.01	0.09	0.00	0.00	0.16
PC11	0.08	0.01	0.01	0.22	0.47	0.03	0.41	0.22	0.04	0.24	0.01	0.05	0.13	0.59	0.21	0.01	0.00	0.02
PC12	0.18	0.22	0.05	0.10	0.08	0.76	0.26	0.09	0.04	0.07	0.38	0.00	0.00	0.12	0.11	-	0.00	0.21
PC13	0.14	0.31	0.32	0.10	0.04	0.01	0.13	0.01	0.02	0.06	0.48	0.00	0.00	0.05	0.05	-	0.00	0.70
PC14	0.16	0.11	0.04	0.17	0.27	0.08	0.18	0.73	0.30	0.04	0.03	0.00	0.00	0.19	0.36	-	0.00	0.04
PC15	0.30	0.18	0.34	0.26	0.33	0.05	0.09	0.21	0.55	0.05	0.09	0.00	0.00	0.06	0.44	0.00	0.00	0.00
PC16	0.00	0.08	0.06	0.62	0.33	0.00	0.11	0.20	0.32	0.04	0.05	0.03	0.34	0.02	0.42	0.00	0.00	0.10
PC17	0.19	0.20	0.02	0.03	0.08	0.19	0.17	0.01	0.08	0.64	0.08	0.47	0.26	0.28	0.12	0.02	0.02	0.11
CP18	0.23	0.05	0.30	0.05	0.11	0.00	0.11	0.11	0.03	0.34	0.07	0.44	0.12	0.11	0.06	0.59	0.25	0.17

Legend: LLE: Leaf Length; LCO: Leaf Color; LHA: Leaf Hairiness; LST: Leaf Stipules/Thorns; SPI: Stem Pigmentation; SLE: Stem Length; SDI: Stem Diameter; SBR: Stem Branching; IIL: Immature Inflorescence Length; IIW: Inflorescence Width; IIC: Immature Inflorescence Color; IIT: Immature Inflorescence Texture; MIC: Mature Inflorescence Color; ACO: Anther Color; NIN: Number of Inflorescences per node; PLE: Average Pedicel Length; MIL: Mature Inflorescence Length; and MIW: Mature Inflorescence Width.

The most important traits contained in PC2, which was responsible for 25.05% of the variance, were PLE (0.39), MIL (0.34), NSP (0.34), SWI (0.34), and MIW (0.30). PC3 included mainly the traits LLE (0.47), PWI (0.42), SWE (0.36), NPI (0.32), and SLN (0.30), and was responsible for 15.87% of the variance. PC4 was responsible for 12.55% of the variance and the traits with the highest weight were IIW (0.47), IIL (0.44), MPW (0.32), NLP (0.32), MIW (0.31), and NSP (0.27). PC5 included mainly the traits LLE (0.44), NIN (0.37), PLE (0.35), PWI (0.30), SLN (0.30), and SDI (0.29), and was responsible for 7.08% of the variance.

Overall, the most representative traits were MIL (8.7%), SLE (8.5%), IIW (6.8%), SWE (64%), and PLE (6.1%) which, when taken together, contributed 36.2% of the total diversity of the eight of the *P. platycephala* accessions studied (Figure 4).

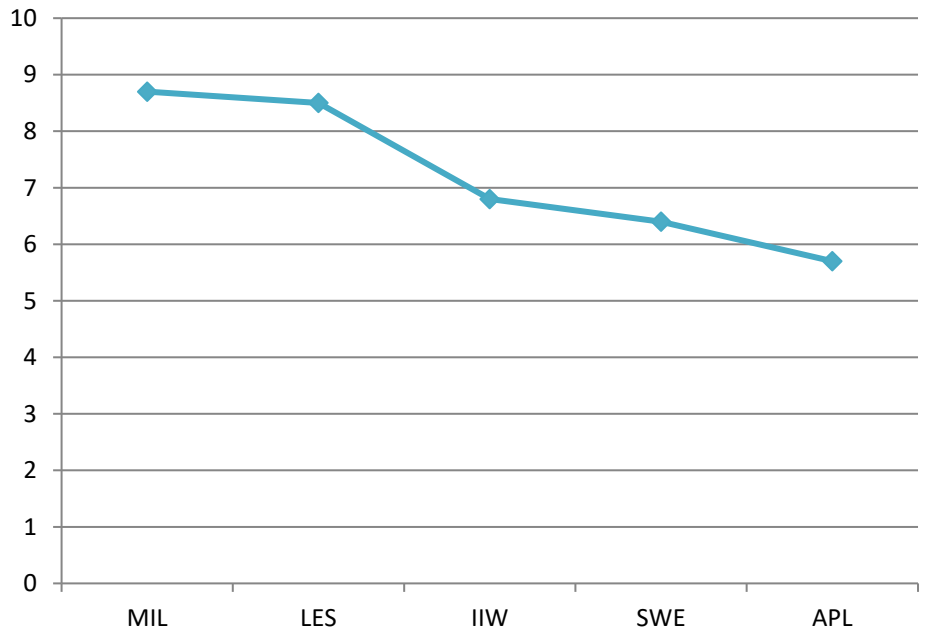

Figure 4. Relative contributions of 18 traits to genetic divergence among eight of the *Parkia platycephala* accessions studied. The most important traits are shown as: MIL, mature inflorescence length; SLE, stem length; IIW, immature inflorescence width; SWE, seed weight; and APL, average pedicel length.

Discussion

The selected ISSR primers were very efficient in determining the genetic variability of *P. platycephala* accessions since the mean percentage of polymorphic loci was high (83.1%). However, as noted previously by Souframanien and Gopalakrishna (2004), the anchored primers attained a higher level of polymorphism than those that were non-anchored. The level of polymorphism obtained for *P. platycephala* was similar to values reported for some other members of the Fabaceae, including *Galega officinalis* L. (Wang et al., 2011), *Caragana microphylla* Lam. (Huang et al., 2013), *Butea monosperma* (Lam.) Taub. (Vashishtha, Jehan, & Lakhanpaul, 2013), *Medicago ruthenica* (L). Ledeb. (Li et al., 2013), but greater than that reported for *Astragalus rhizanthus* Benth. (Anand, Srivastava, & Chaudhary, 2010).

Although all of the *P. platycephala* accessions tested originated from the same location and their genetic diversity was low, the classification of F3 in a separate group indicates that variability exists and could be explored. Furthermore, the genetic similarity coefficients obtained for *P. platycephala* are within the interval reported for *Medicago sp.* (Xavier, Kumar, & Srivastava, 2011). Despite their similar origin, the genetic diversity detected in *P. platycephala* could be explained by various factors including the non-domesticated condition of the species, its sexual reproduction mode (Lorenzi, 2020), its dependence on bats as principal pollinating agents (Luckow & Hopkins, 1995) and seed predation by coleoptera of the family Bruchidae that favors seed dispersion (Silva, Maimoni-Rodella, & Rossi, 2007).

In the present study, the assessment of morphological and agronomical traits was performed at the flowering stage in the month of August 2017, during which the air temperature varied from 18.9 to 37.5°C. The absence of flowering in accession F9 may have been induced by temperature stress caused by the wide thermal amplitude during the dry season. Indeed, flowering was also observed to be delayed in some other accessions. The phenology of trees of the Cerrado area and other seasonally dry forests remains somewhat obscure, although reasons for the observed periodicity have been attributed to abiotic/biotic factors or imposed by phylogenetic restriction. Vegetative growth and reproductive development are seasonal events and in some species, including *P. platycephala*, flowering along with the renewal of leaves occurs during the dry season (Bulhão & Figueiredo, 2002).

In the first two components, 61.01% of the variation was accumulated, however, Manly (2008) indicates that the principal components analysis does not always accumulate 80% of the variation in the first two or three components, as is the case of studies that use variables or original and/or poorly correlated characters.

The inflorescence and stem descriptors were the ones that most contributed to the verification of the variability between the studied genotypes, therefore, they should be considered in new studies of the genetic diversity of acorn bean.

The cophenetic correlation coefficients for the UPMGA trees constructed on the basis of Jaccard distances (molecular data; $r = 0.91$) as well as Euclidian distances (quantitative traits; $r = 0.84$) demonstrate the goodness of fit of the clustering performed in that the distance matrices were highly correlated with the cophenetic matrix.

Although diversity analyses based on molecular, quantitative and multi-categorical traits separated the accessions into two groups, there was little consensus between them. Such divergence may be explained by the intrinsic nature of the data, as for example the number of loci controlling the phenotypic traits. While ISSR markers amplify a fragment of the gene coding for a specific character, the morpho-agronomic descriptors are associated with the phenotype of the individual in which a specific character is conferred by the interaction between two or more alleles.

Conclusion

The ISSR and morpho-agronomic markers were very helpful in characterizing *P. platycephala*. Independent clustering analysis of the molecular, qualitative and quantitative traits revealed that sufficient genetic variability exists among the studied accessions of *P. platycephala*. These accessions with greater diversity are candidates for actions that promote the conservation, domestication and genetic improvement of the species. The most representative traits with potential for morphoagronomic descriptors to evaluate the diversity among faveira-de-bolota accessions were stem length and pedicel length, mature inflorescence length and immature inflorescence width, and seed weight.

Acknowledgements

The authors wish to thank the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) for a scholarship awarded to one of us (RSO) and to *Embrapa Meio-Norte* for providing the infrastructure.

References

Anand, K. K., Srivastava, R. K., & Chaudhary, L. B. (2010). Analysis of genetic diversity in *Astragalus rhizanthus* Benth. ssp. *rhizanthus* var. *rhizanthus* (Fabaceae) using molecular markers from India. *Journal of Botany*, *2010*, 1-9. DOI: https://doi.org/10.1155/2010/701975

Bulhão, C. F., & Figueiredo, P. (2002). Tree legume phenology in a marginal cerrado area in northeastern Maranhão. *Brazilian Journal of Botany*, *25*(3), 361-369. DOI: https://doi.org/10.1590/S0100-84042002000300012

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*(1), 13-15.

Huang, W. D., Zhao, X. Y., Zhao, X., Luo, Y. Q., Feng, J., & Su, N. (2013). Genetic variation within the sand-fixation species *Caragana microphylla* (Leguminosae) in Horqin Sandy land detected by inter-simple sequence repeats analysis. *Biochemical Systematics and Ecology*, *51*, 343-348. DOI: http://dx.doi.org/10.1016/j.bse.2013.10.006

International Plant Genetic Resources Institute [IPGRI]. (2001). *Descritores para Phaseolus lunatus*. Rome, IT: IPGRI.

Li, H.-Y., Li, Z.-Y., Cai, L.-Y., Shi, W.-G., Mi, F.-G., & Shi, F.-L. (2013). Analysis of genetic diversity of Ruthenia Medic (*Medicago ruthenica* (L.) Trautv.) in inner Mongolia using ISSR and SSR markers. *Genetic Resources and Crop Evolution*, *60*(5), 1687-1694. DOI: https://doi.org/10.1007/s10722-012-9950-3

Lorenzi, H. (2020). *Brazilian trees: identification and culture manual of tree species in Brazil* (8. ed.). Nova Odessa, SP: Plantarum.

Luckow, M., & Hopkins, H. C. F. (1995). A cladistic analysis of *Parkia* (Leguminosae: Mimosoideae). *American Journal of Botany*, *82*(10), 1300-1320. DOI: https://doi.org/10.2307/2446253

Magalhães, J. A., Fogaça, F. H. S., Costa, N. L., Rodrigues, B. H.N., Santos, F. J. S., Araújo Neto, R. B., ... Bitencourt, A. B. (2014). Efeito da adição da faveira (*Parkia platycephala*) sobre a composição químico-bromatológica da silagem de capim-elefante (*Pennisetum purpureum*) *PUBVET*, *8*(11), 1-17.

Manly, B. F. J. (2008). *Métodos estatísticos multivariados: uma introdução*. Porto Alegre, RS: Artmed/Bookman.

Melo Jr., F. A., Carvalho, D., Vieira, F. A., & Oliveira, D. A. (2012). Spatial genetic structure in natural populations of *Caryocar brasiliense* Camb. (Caryocareceae) in the North of Minas Gerais, Brazil. *Biochemical Systematics and Ecology*, 43, 205-209. DOI: https://doi.org/10.1016/j.bse.2012.02.005

Santana, J. G. S., Nascimento, A. L. S.,Costa, T. S., Almeida, T. M. B., Rabbani, A. R. C., & Muniz, A. V. C. S. (2016). Estimation of genetic diversity in a natural population of Cambuí tree (*Myrciaria tenella* O. Berg) using ISSR markers. *Genetics and Molecular Research*, 15(4), 1-11. DOI: http://doi.org/10.4238/gmr.15048819

Santos, L. M., Farias, S. G. G., Silva, R. B., Dias, B. A. S., & Silva, L. S. (2019). Ecophysiology of germination of *Parkia platycephala* Benth. seeds. *Floresta e Ambiente*, *26*(1), 1-7. DOI: https://doi.org/10.1590/2179-8087.028215

Silva, L. A., Maimoni-Rodella, R. C. S., & Rossi, M. N. (2007). A preliminary investigation of pre-dispersal seed predation by *Acanthoscelides schrankiae* Horn (Coleoptera: Bruchidae) in *Mimosa bimucronata* (DC.) Kuntze trees. *Neotropical Entomology*, *36*(2), 197-202. DOI: https://doi.org/10.1590/S1519-566X2007000200005

Singh, D. (1981). The relative importance of characters affecting genetic divergence. *Indian Journal of Genetics and Plant Breeding*, *41*, 237-245.

Souframanien, J., & Gopalakrishna, T. A. (2004). Comparative analysis of genetic diversity in black gram using RAPD and ISSR markers. *Theoretical and Applied Genetics*, *109*(8), 1687-1693. DOI: https://doi.org/10.1007/s00122-004-1797-3

Vashishtha, A., Jehan, T., & Lakhanpaul, S. (2013).Genetic diversity and population structure of *Butea monosperma* (Lam.) Taub. - a potential medicinal legume tree. *Physiology and Molecular Biology of Plants*, *19*(3), 389-397. DOI: https://doi.org/10.1007/s12298-013-0170-x

Wang, Z., Wang, J.-E., Wang, X.-M, Gao, H.-W., Dzyubenko, N. I., & Chapurin, V. F. (2011). Assessment of genetic diversity in *Galega officinalis* L. using ISSR and SRAP markers. *Genetic Resources and Crop Evolution*, *59*, 865-873. DOI: http://doi.org/10.1007/s10722-011-9727-0

Xavier, J. R., Kumar, J., & Srivastava, R. B. (2011). Characterization of genetic structure of alfalfa (*Medicago* sp.) from trans-Himalaya using RAPD and ISSR markers. *African Journal of Biotechnology*, *10*(42), 8176-8187. DOI: https://doi.org/10.5897/AJB10.2083