GMR
The Original by FUNPEC-RP

Genetic diversity and structure of pigeonpea germplasm assessed by microsatellite markers

A.E. da S. Costa[1] and C.A.F. Santos[2]

[1] Programa de Pós-Graduação em Agronomia, Melhoramento Genético de Plantas, Universidade Federal Rural de Pernambuco (UFRPE), Recife, PE, Brasil
[2] Embrapa Semiárido, Petrolina, PE, Brasil

Corresponding author: C.A.F. Santos
E-mail: carlos-fernandes.santos@embrapa.br

Genet. Mol. Res. 20 (1): gmr18807
Received January 18, 2021
Accepted March 10, 2021
Published March 31, 2021
DOI http://dx.doi.org/10.4238/gmr18807

ABSTRACT. The pigeonpea is an important grain legume in various regions of the world. It is a pulse that is cultivated mainly by small farmers in semi-arid tropical regions for various purposes, including food security, livestock feed, and agroforestry systems. We examined the genetic diversity and structure of 73 pigeonpea accessions from 10 origins, including regions of Brazil, Central America, and India. Allelic diversity and polymorphism, molecular analysis of variance (AMOVA), Bayesian analysis (Structure) and a dendrogram were estimated based on data concerning 11 microsatellite loci. AMOVA revealed wide genetic differentiation among the pigeonpea populations, with F_{ST} ranging from 0.22 to 0.25. Expected heterozygosity in the accessions was greater than the observed heterozygosity in eight of the 11 loci, with the observed heterozygosity ranging from 0.06 to 0.55. Polymorphic information content ranged from 0.13 to 0.67 among the 11 loci. Genetic similarity among pigeonpea accessions ranged from 0.26 to 0.93, indicating considerable variability among them, with the formation of three groups, one of which included all Brazilian accessions. Two groups were formed in the Structure analysis, indicating reduced allele sharing between the populations of Northeast Brazil and other populations. This information highlights the importance of incorporating germplasm from the Brazilian

Northeast into pigeonpea breeding programs in Brazil, to increase the variability in germplasm collections.

Key words: Bayesian analysis; Brazil Northeast; *Cajanus cajan*; Dendrogram; Pulses; Redgram

INTRODUCTION

Pigeonpeas (*Cajanus cajan*), like other pulses in the Fabaceae family, are important sources of protein, especially for the poorest populations with restricted access to animal protein. In addition to the archaeological evidence (Fuller and Harvey, 2006), molecular studies, such as Saxena et al. (2014), confirm that pigeonpea is a species of Indian origin. This crop is the sixth pulse in terms of worldwide importance, with a production of 6.81 Mt in 2017 (FAOSTAT, 2020).

Pigeonpea is a crop that is resilient to environmental limitations and climate change, being grown mainly in semiarid regions (Varshney et al., 2017). It has the ability to absorb phosphorus from soils poor in this nutrient (Ae et al., 1990). In Brazil, the pigeonpea is used for grain production, especially in areas of transition from the semiarid to more humid areas (the northeastern agreste). It has been an alternative to help meet the growing demand for forage production. (Santos et al., 1999).

Despite the agronomic potential of this crop, the pigeonpea is underexploited in plant breeding in Brazil, and the available cultivars are result of line selection within populations (Godoy et al., 2013) or the introduction of germplasm from other countries (Santos et al., 1999). Germplasm collections of this legume are rare; the main one in Brazil includes almost 400 accessions available at Embrapa Semiárido in Petrolina, Pernambuco.

Estimates of genetic diversity parameters in germplasm collections are important for breeding programs. Microsatellites are essential tools for genetic studies due to multiallelism, abundance and codominance (Sharma et al., 2018). Polymorphic pigeonpea microsatellites have been widely reported (Odeny et al., 2009; Saxena et al., 2010; Bohra et al., 2011; Sarika et al., 2013). Zavinon et al. (2020) studied genetic diversity and population structure in 77 Benin landraces, using 30 loci. Sharma et al. (2018) used 33 loci to assess genetic diversity among a set of Indian accessions of male sterile, maintainer and germplasm lines. In Brazil, Sousa et al. (2011) estimated the diversity in 77 accessions of pigeonpea adapted to the south-southeast region, using 43 loci. However, a compressive study of pigeonpea Brazilian germplasm is still lacking.

We examined genetic diversity and structure in 73 accessions of pigeonpea from 10 origins/regions, including eight from Brazil, using microsatellite loci, to help guide programs of genetic resources and improvement of this pulse.

MATERIAL AND METHODS

Plant materials and DNA extraction

Seventy-three pigeonpea accessions from 10 different regions were evaluated; these included seven regions of northeast Brazil and one from the southeast region of Brazil, one from India and the other from the FAO office in Central America (Caribbean), these being Abaíra, BA (six accessions), Anagé, BA (seven accessions), India (nine accessions), Jacobina e Saúde, BA (nine accessions), Mairi, BA (six accessions), Moreilândia and Exu, PE (eight accessions),

Seabra, BA (five accessions), Triunfo, PE (seven accessions), São Carlos, SP (nine accessions) and Central America (seven accessions).

Northeast Brazilian accessions are the result of germplasm collections carried out in the northeast, while the southeast accessions are from Embrapa Pecuária Sudeste, São Carlos, SP. Indian accessions are from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). All accessions are preserved in the pigeonpea Embrapa Semiárido Germplasm collection.

Leaf samples from young plants were collected from all accessions and stored at -80°C. DNA extraction was performed according to the Doyle and Doyle (1990) protocol, with some minor modifications: centrifugations at 10,000 rpm, 2% beta mercaptoethanol concentration, and sample incubation time for 30 minutes at 60°C. After re-suspending the final pellet in TE buffer (10 mM Tris pH 8.0, 1 mM EDTA), the DNA solution was treated with RNAse to remove co-isolated RNAs. DNA quantification and integrity were verified on 0.8% agarose gel, followed by genomic DNA dilution to 40 ng mL^{-1}.

PCR and microsatellite amplification

Microsatellite loci developed by Bohra et al. (2011) were used for accession genotyping. Initial screening was carried out with a set of 42 loci on five *C. cajan* accessions. According to the quality of the amplification, a polymorphic locus was chosen for each pigeonpea linkage group (n = 11) (Table 1), according to a consensus genetic map (Bohra et al., 2012). Polymerase chain reactions (PCRs) for loci amplifications were performed for the final reaction volume of 12 µL, containing 1x of the PCR buffer (Ludwig Biotec, Brazil), 2 mM $MgCl_2$ (Ludwig Biotec, Brazil), 0.2 mM dNTPs, 5 ng μL^{-1} DNA, 0.2 µM of each primer, and 0.1 U of Taq DNA polymerase.

DNA fragments were amplified with a touchdown PCR program (Biometra, model-T1 Thermoblok): initial denaturation for 5 minutes at 95°C, followed by five cycles of denaturation for 20 seconds at 94°C, annealing for 20 seconds at a temperature according to locus (Table 1) (annealing temperature for each cycle was reduced by 1°C per cycle), and an extension for 30 seconds at 72°C. Subsequently, 30 cycles of denaturation at 94°C for 20 seconds followed by annealing for 20 seconds at a temperature according to Table 1 and an extension for 30 seconds at 72°C, and 15 minutes of final extension at 72°C. PCR amplification products were resolved in 6% denatured polyacrylamide gel electrophoresis on a 60-well sandwich-type glass plate. Gels were stained with a silver nitrate protocol, according to Creste et al. (2001).

Molecular data analysis

Only microsatellite loci that presented consistent amplification and polymorphism, with bands easy to view and interpret, were selected. Genotypic data for each accession were subjected to analysis to estimate parameters of genetic diversity: major allele frequency (MAF), number of genotypes (NG), total number of alleles (NA), number of private alleles (NPA), expected heterozygosity (H_E), observed heterozygosity (H_O) and polymorphism information content (PIC), using the software PowerMarker (Liu and Muse, 2005) and the Excel macro GenAlEx (Peakall and Smouse, 2006).

Microsatellite loci data were coded for presence (1) or absence (0) to estimate genetic relationships between pigeonpea accessions, with a dendrogram constructed by the UPGMA method (unweighted pair group method with arithmetic mean), based on the similarity coefficient Jaccard. The adjustment of the dendrogram was evaluated by the cophenetic

correlation, that is, the correlation between the real distance and the distances represented graphically, in the NTSYSpc software (Rohlf, 2000).

Analysis of molecular variance (AMOVA), without regional data structure, and with the decomposition of the total genetic variation between populations, between individuals and within individuals, using the mean squares, was performed using the Excel macro GenAlEx (Peakall and Smouse, 2006), with the 'Codom-Allelic' option to estimate F_{ST}. The significance of the F_{ST} and number of migrants (Nm) estimates were obtained by the randomization method, with 9999 permutations.

Allelic structure of each accession of a population was evaluated using the Bayesian clustering method with the Structure software (Pritchard et al., 2009). Bayesian analysis was performed with a burn-in of 50,000 iterations and 500,000 simulations of the Markov Chain Monte Carlo (MCMC) method. A continuous series of K was tested, from 1 to 10, in 10 runs. Prior knowledge data on the origin of the population was introduced, subdividing accesses into ten initial groups, according to the origin sites. The determination of the most likely value of K was performed using the *ad hoc* ΔK statistics, which are based on the rate of change of the logarithmic probability of the data about the number of groups inferred by Structure (Evanno et al., 2005). After defining the K, the genetic structure was inferred through a new execution in the same software, this time using a burn-in period of 100,000 and an execution length of 1,000,000 repetitions of MCMC with the same model mentioned above. For the graphical presentation of Structure results, Clumpak server was used (Kopelman et al., 2015).

RESULTS

Genetic diversity and UPGMA analysis

At least one microsatellite locus was identified in the 11 pigeonpea linkage groups (Table 1), among the 42 loci initially evaluated. Forty alleles (NA) were revealed by the 11 loci in the 73 accessions of *C. cajan* (Table 1), with an average of 3.64 alleles per locus.

Table 1. Linkage group (LG), annealing temperature (Ta) and genetic parameter estimates* in 73 pigeonpea accessions, based on 11 microsatellite loci.

Loci	LG	Ta (°C)	MFA	NG	NA	NPA	NE	H_O	H_E	PIC
CcM2485	1	60	0.76	9	4	0	1.49	0.16	0.21	0.37
CcM492	2	59	0.40	12	6	0	2.29	0.45	0.52	0.65
CcM1791	3	64	0.60	3	2	0	1.72	0.41	0.41	0.36
CcM1962	4	59	0.39	12	6	6	2.24	0.25	0.50	0.67
CcM810	5	59	0.44	6	3	0	1.95	0.25	0.45	0.55
CcM2542	6	59	0.45	8	4	4	1.99	0.55	0.46	0.53
CcM402	7	64	0.92	3	2	0	1.14	0.06	0.08	0.13
CcM2911	8	60	0.69	3	2	0	1.34	0.19	0.24	0.33
CcM126	9	61	0.54	11	5	0	1.92	0.28	0.40	0.58
CcM1573	10	64	0.49	5	3	1	1.71	0.50	0.40	0.40
CcM2735	11	59	0.48	6	3	0	1.65	0.45	0.36	0.48
Total				78	40	11				
Mean			0.56	7.09	3.64	1	1.77	0.32	0.37	0.46

*MAF = major allele frequency; NG = number of genotypes; NA = total number of alleles; NE = Number of Effective Alleles; NAP = number of private alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; PIC = polymorphism information content.

The major allele frequency (MAF) ranged from 0.39 (CcM1962) to 0.92 (CcM402), with a locus average of 0.56. The number of genotypes (NG) was 78 with an average of 7.09 per

locus, with the smallest NG in the CcM402, CcM1791 and CcM2911 loci and 12 the highest number in the CcM492 and CcM1962 loci. The content of polymorphic information (PIC) ranged from 0.13 (CcM402) to 0.67 (CcM1962) among the 11 loci, with 0.46 average (Table 1).

Expected accession heterozygosity (H_E) was greater than the observed heterozygosity (H_O) in eight of the 11 loci. H_O values ranged from 0.06 (CcM402) to 0.55 (CcM2542), with an average of 0.32 between the loci. For H_E values, the variation was from 0.08 (CcM402) to 0.52 (CcM492), with a 0.37 average (Table 1). Three loci (CcM1573, CcM1962 and CcM2542) contained one or more of the private alleles (NPA) (Table 1). All private alleles were identified in accessions of Indian origin. Six accessions harbored one private alleles (ICPL 88018, ICPL 90044, ICPL 90050, ICPL 90052) or two private alleles (ICPL 90046 and ICP 2582).

Genetic similarity among the accessions of the 10 pigeonpea populations ranged from 0.26 to 0.93, indicating high variability among them (Figure 1).

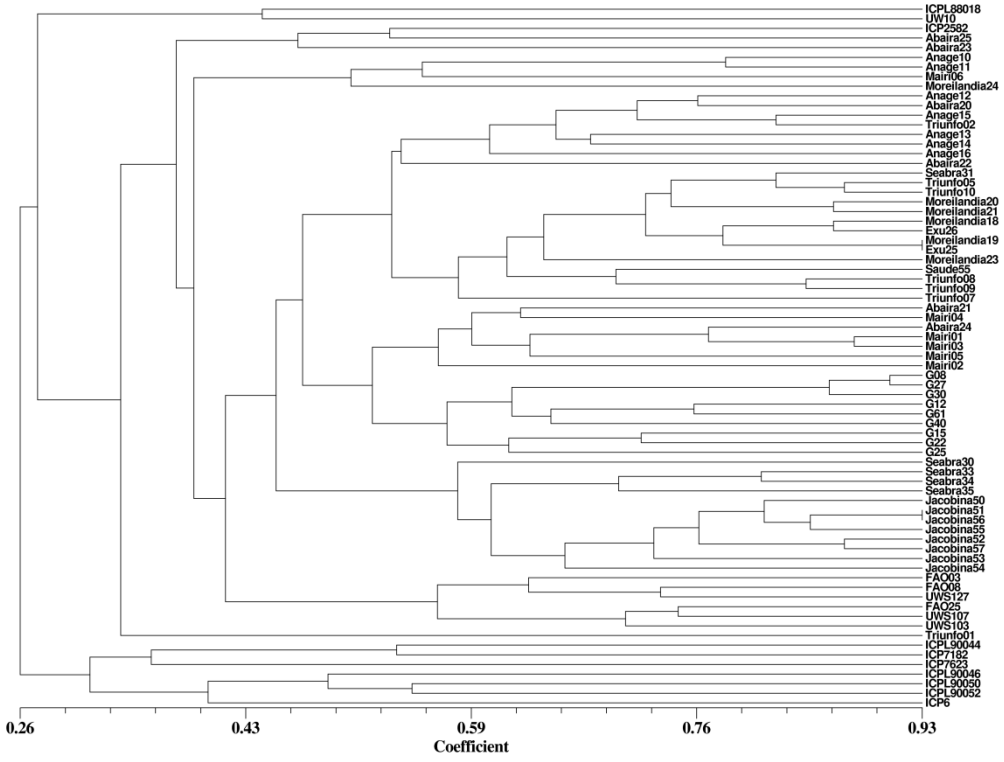

Figure 1. UPGMA dendrogram of the Jaccard similarity coefficient among 73 pigeonpea accessions, from 10 regions/origins, assessed with 11 microsatellite loci. Cophenetic correlation=0.82. India (ICPL 88018, ICPL 90044, ICPL 90046, ICPL 90050, ICPL 90052, ICP 6, ICP 2582, ICP 7182 and ICP 7623); Anagé, BA (Anage10, Anage11, Anage12, Anage13, Anage14, Anage15, Anage16); Abaira, BA (Abaira20, Abaira21, Abaira22, Abaira23, Abaira24, Abaira25), Seabra, BA (Seabra30, Seabra31, Seabra33, Seabra34 and Seabra35); Jacobina, BA (Jacobina50, Jacobina51, Jacobina52, Jacobina53, Jacobina54, Jacobina55, Jacobina56, Jacobina57 and Saude55); Mairi, BA (Mairi01, Mairi02, Mairi03, Mairi04, Mairi05 and Mairi06); Triunfo, PE (Triunfo01, Triunfo02, Triunfo05, Triunfo07, Triunfo08, Triunfo09 and Triunfo10); Moreilândia, PE (Moreilandia18, Moreilandia19, Moreilandia20, Moreilandia21, Moreilandia23, Moreilandia24, Exu25 and Exu26); São Carlos, SP (G08, G12, G15, G22, G25, G27, G30, G40 and G61) and; Central America (FAO03, FAO08, UW10, FAO25, UWS103, UWS107 and UWS127).

At the cut-off point of 0.30, it is possible to observe the formation of three groups: one group with all Brazilian accessions, the second group with accessions UW10 (Central America) and ICPL 88018 (India) and the third group with accessions of Indian origin (Figure 1). The greatest similarities (0.93) were observed between the Jacobina51 and Jacobina56 accessions and between the Morelândia19 and Exu25 accessions (Figure 1). The lowest similarity coefficients were observed with seven accessions of Indian origin (Figure 1). The cophenetic correlation of 0.82 indicates a moderate adjustment between the actual distance and the graphical distances.

Genetic structure by AMOVA and Bayesian inferences

Analysis of molecular variance (AMOVA) revealed wide genetic differentiation among the 10 pigeonpea populations, F_{ST} = 0.25 (Table 2). In the eight Brazilian populations, F_{ST} was 0.23 and after reducing to the seven populations sampled in northeastern Brazil, F_{ST} was 0.22 (Table 2), also indicating moderate to high genetic differentiation (Wrigth, 1978). The number of migrants (Nm) was close to 0.80 in the three situations, being slightly lower among accessions in Northeast of Brazil (Table 2). All population F_{ST} values were significant, indicating moderate to high differentiation between pigeonpea population pairs (Table 3).

Table 2. Analysis of molecular variance (AMOVA) in 73 pigeonpea accessions assessed with 11 microsatellite loci.

Source of variation	DF	MS	Estimated Variance	Total variance (%)	F_{ST} Statistic	P-value	Nm
Among Pops	9	14.11	0.78	25	0.25	<0.001	0.73
Among Indiv	63	2.82	0.54	18			
Within Indiv	73	1.74	1.74	57			
Total	145		3.06	100			
Among Pops BR	7	11.36	0.63	23	0.23	<0.001	0.84
Among Indiv BR	49	2.40	0.28	10			
Within Indiv BR	57	1.84	1.84	67			
Total	113		2.75	100			
Among Pops NE	6	10.15	0.57	22	0.22	<0.001	0.88
Among Indiv NE	41	2.30	0.28	11			
Within Indiv NE	48	1.75	1.75	67			
Total	95		2.60	100			

p-value based on 9999 permutations. DF=Degrees of freedom. MS=Mean square. Nm=Number of migrants. BR=Brazil. NE= Northeast Brazil.

Table 3. Genetic differentiation (F_{ST}) in pairs between 10 pigeonpea populations assessed with 11 microsatellite loci.

Origin	Anagé	Abaira	Seabra	Jacobina	Mairi	Triunfo	Moreilândia	São Carlos	Central America
India	0.20**	0.13**	0.28**	0.34**	0.22**	0.31**	0.34**	0.22**	0.20**
Anagé, BA		0.08*	0.30**	0.32**	0.22**	0.23**	0.25**	0.18**	0.29**
Abaira, BA			0.17**	0.18**	0.07*	0.06*	0.17**	0.12**	0.25**
Seabra, BA				0.12*	0.29**	0.29**	0.26**	0.32**	0.40**
Jacobina, BA					0.27**	0.27**	0.30**	0.29**	0.35**
Mairi, BA						0.22**	0.32**	0.12**	0.33**
Triunfo, PE							0.09*	0.26**	0.41**
Moreilândia, PE								0.35**	0.41**
São Carlos, SP									0.15**

Significance of F_{ST} based on 999 permutations, **P<0.01 and *P<0.05

Bayesian clustering method identified the highest value for K=2 (Figure 2), indicating that pigeonpea germplasm were divided into a group with accessions with long adaptation in Brazil and another group with accessions from Central America and Indian accessions. The genetic structure indicates the sharing of alleles among accessions in São Carlos, SP, Central America and India, and the absence of allele sharing in accessions collected in northeastern Brazil, with some exceptions in Mairi, BA, Anagé, BA and Abaíra, BA (Figure 3).

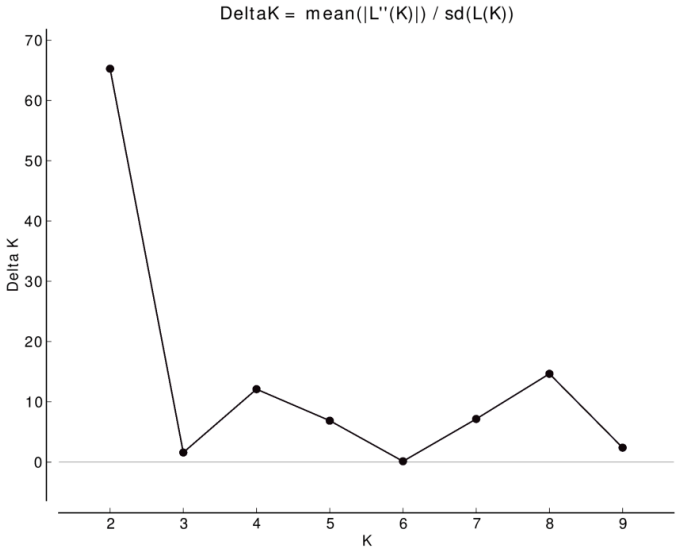

Figure 2. Estimation of the optimum number of clusters (K) of pigeonpea accessions in the STRUCTURE according to ΔK criterion of Evanno et al. (2005).

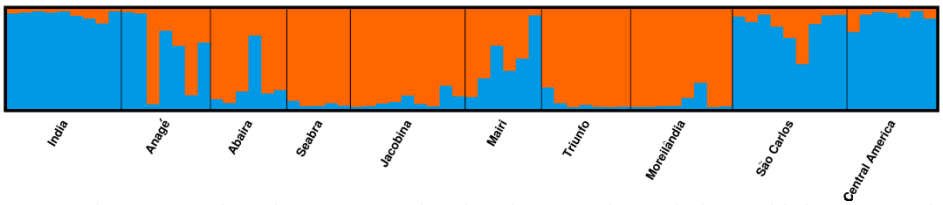

Figure 3. Genetic structure of 73 pigeonpea accessions based on Bayesian analysis, considering K=2. Each line represents a pigeonpea accession. Each color represents the probable lineage of the set from which the accession or partial accession was derived. Analysis performed with 11 microsatellite loci, Structure 2.3.4.

DISCUSSION

Pigeonpea is grown in some Brazilian regions, which reflects the reduced number of accessions preserved in germplasm collection, available almost exclusively from Embrapa Semiárido, Petrolina, PE, and Embrapa Pecuária Sudeste, São Carlos, SP. This pioneering study considers the broadest evaluation of accessions from Northeast Brazil, sampled in regions where f this pulse is widely cultivatedwidespread, comparing its genetic structure with germplasm from two important regions of the world of pigeonpea cultivation: India and Central America.

UPGMA analysis showed high genetic similarity, ranging from 0.26 to 0.93, corroborating the estimates by Manju et al. (2017), which ranged from 0.45 to 0.93 in 40 Indian

accessions. Minor genetic similarity was reported by Sharma et al. (2018), ranging from 0.2 to 0.32 in 96 Indian accessions and by Songok et al. (2010), ranging from 0.1 to 0.45 in 88 accessions of Indian and East African origin. The UPGMA analysis of the present study suggests the crossing of accessions of the three different identified groups, with emphasis on the crossings of accessions of Indian origin with Brazilian accessions. The main characteristics of accessions of Indian origin are precocity and small plant size, while Brazilian accessions present larger grains and pods, and adaptation to Brazilian environmental conditions.

Eleven loci identified in our study can be used for genetic structure estimates, as they presented a PIC greater than 0.33, except CcM402 in linkage group seven, which should be replaced by another locus of higher PIC. As discussed by Hamilton (2009), with the application of unlinked loci or on different chromosomes associated with the allelic frequency, it is possible to identify unique homozygous plants or cultivar among billions, making these estimates extremely valuable in plant breeding and even in commercial disputes. Sharma et al. (2018) analyzed pigeonpea accessions with 44 microsatellite loci distributed in the 11 linkage groups, of which only CcM402 is common to the loci evaluated in the present study, indicating the need for standardization of loci for population studies in the species.

The average number of alleles/locus in the present study, 3.64, was higher than the 1.7 average reported by Petchiammal et al. (2015), and below the average of 5.4 reported by Sousa et al. (2011). Observed heterozygosity (0.32) was lower than expected (0.37), typical of species that have a high frequency of self-fertilization. Sousa et al. (2011) also reported heterozygote deficiency in studies with pigeonpea germplasm. Loci PIC values ranged from 0.13 to 0.67, most loci used, except CcM402, were classified, according to Botstein et al. (1980), as medium to highly informative (Table 2), and are recommended for studies of population genetic diversity in pigeonpea. According to Serrote et al. (2020) the higher the PIC, the greater the polymorphism detection capacity of a locus.

Proportion of rare alleles observed in the present study was lower than in other studies. Songok et al. (2010) report the existence of 39% of alleles classified as rare in pigeonpea. According to Dwivedi et al. (2017) this class of alleles, with a frequency of less than 5%, despite being less likely to control agronomic traits of interest, persist even with natural or human selection and are potential alleles in natural populations for some less common characteristics in domesticated plants.

F_{ST} ranged from 0.22 to 0.25 in the pigeonpea populations, considering all populations indicates high genetic differentiation between the populations analyzed. It is suggested additional sampling in other Brazilian regions, especially in others Brazilian Northeast regions to increase the variability of pigeonpea collections. Low to moderate differentiation among pigeonpea populations has been reported by Kumari et al. (2014) (F_{ST}=0.17), by Zavinon et al. (2020) (F_{ST}=0.12), while Saxena et al. (2014) reported high differentiation among populations from different regions (F_{ST}=0.36), corroborating with the present study.

Number of migrants (Nm) estimated in the present study was low, around 0.8 in all three situations, 1) among all 10 populations, 2) among eight Brazilian populations and 3) among seven the northeastern Brazil populations, indicating limited gene flow among them. Zavinon et al. (2020) estimated an Nm of 17.0 with microsatellite loci and 1.6 with single nucleotide polymorphisms (SNP) loci. According to Wang (2004), a general rule, widely accepted by the conservation scientists, is that one migrant per generation in a population is the appropriate level of gene flow.

Formation of only two groups in the Bayesian analysis indicates the sharing of alleles among accessions of São Carlos, SP (Southeast Brazil), Central America and India, (blue color, Figure 3), while the accessions sampled in the Brazilian Northeast presented limited alleles

sharing (orange color, Figure 3), with some exceptions in the region of Mairi, Abaíra and Anagé. Regions of Jacobina, Seabra, Triunfo and Moreilândia have a unique profile, with little sharing with Indian accessions. India is the center of origin of the pigeonpea (Fuller and Harvey, 2006) and the results of the present study are unexpected, suggesting further investigations are required to assess the origin and evolution of germplasm in these regions of Northeast Brazil. Pigeonpea germplasm of northeastern Brazil has a long-term adaptation to tropical and semi-arid conditions, with selections made by growers mainly for semi-arboreal plant size, greater 100 seed weight, greater pod length and forage production, making it important to use this germplasm in pigeonpea improvement, at least in Brazilian conditions (Santos et al., 1999).

Pigeonpea genetic population structures showed moderate to high differentiation, limited gene flow and restricted allele sharing among populations from Northeast Brazil with populations from India, São Paulo, SP, and Central America. Variability of the germplasm collection can be increased with new samples and collections of pigeonpea populations in other regions of Northeast Brazil.

ACKNOWLEDGMENTS

The authors would like to thank MSc. Deisy Aiane Lima Aquino and MSc. Viviane Nunes dos Santos for their support in laboratory activities. To Capes for the Antonio E. S. Costa scholarship. Carlos A. F. is also a CNPq researcher.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ae N, Arihara J, Okada K, Yoshihara T, et al. (1990). Phosphorus uptake by pigeonpea and its role in cropping systems of the Indian subcontinent. *Science*. 248: 477-480. https://doi.org/10.1126/science.248.4954.477.

Bohra A, Dubey A, Saxena RK, Penmetsa RV, et al. (2011). Analysis of BAC-end sequences (BESs) and development of BES-SSR markers for genetic mapping and hybrid purity assessment in pigeonpea (*Cajanus* spp.). *BMC Plant Biol.* 11(1): 56. https://doi.org/10.1186/1471-2229-11-56.

Bohra A, Saxena RK, Gnanesh BN, Saxena K, et al. (2012). An intra-specific consensus genetic map of pigeonpea [*Cajanus cajan* (L.) Millspaugh] derived from six mapping populations. *Theor. Appl. Genet.* 125(6): 1325-1338. https://dx.doi.org/10.1007%2Fs00122-012-1916-5.

Botstein D, White RL, Skolnick M and Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32(3): 314.

Creste S, Neto AT and Figueira A (2001). Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant Mol. Bio. Rep.* 19(4): 299-306. https://doi.org/10.1007/BF02772828.

Doyle JJ and Doyle JD (1990). Isolation of plant DNA from fresh tissue. *Focus.* 12: 13-15.

Dwivedi SL, Scheben A, Edwards D, Spillane C, et al. (2017). Assessing and exploiting functional diversity in germplasm pools to enhance abiotic stress adaptation and yield in cereals and food legumes. *Front Plant Sci*. 8: 1461. https://doi.org/10.3389/fpls.2017.01461.

Evanno G, Regnaut S and Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14: 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

FAOSTAT (2020). Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data/QC. Accessed 15 december 2020.

Fuller DQ and Harvey EL (2006). The archaeobotany of Indian pulses: identification processing and evidence for cultivation. *Environ archaeol.* 11(2): 219-246. https://doi.org/10.1179/174963106x123232.

Godoy R, Souza FHD and Santos PM (2013). Pigeonpea selection and breeding. In: Forage breeding and biotechnology (Jank L, Chiari L, Valle CB, Simeão RM, eds.), Embrapa, Brasília.

Hamilton MB (2009). Population genetics. Wiley-Blackwell, Chichester.

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, et al. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Mol. Ecol. Resour*. 15(5): 1179-1191.

Kumari R, Agrawal K and Bhat KV (2014). Analysis of genetic diversity and its stratification in pigeonpea germplasm using BES-derived microsatellite markers. *Indian J. Biotechnol*. 13: 473-485.

Liu K and Muse SV (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*. 21: 2128-2129.

Manju Y, Kumar YY, Pushpendra K, Kumar SR, et al. (2017). Molecular diversity analysis as an improvement tool for pigeonpea [*Cajanus cajan* (L.)]. *Res. J. Biotechnol.* 12: 75-86.

Odeny DA, Jayashree B, Gebhardt C and Crouch J (2009). New microsatellite markers for pigeonpea (*Cajanus cajan* (L.) Millsp.). *BMC Res. Notes*. 2(1): 35. https://doi.org/10.1186/1756-0500-2-35.

Peakall R and Smouse PE (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*. 6: 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x.

Petchiammal KI, Muthiah AR and Jayamani P (2015). Molecular characterization of cultivated and wild Cajanus species using Simple Sequence Repeat markers. *Legume Res.* 38(6): 742-747. https://doi.org/10.18805/LR.V38I6.6717.

Pritchard JK, Stephens M and Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genetics*. 155: 945-959.

Rohlf FJ (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system, Version 2.10. Exeter Publishing, Setauket.

Santos CAF, Menezes EA and Araújo FP (1999). Introdução coleta e caracterização de recursos genéticos de guandu para produção de grãos e forragem. In: Recursos genéticos e melhoramento de plantas para o Nordeste (Queiroz MA, Goedert CO, Ramos SRR, orgs). Embrapa, Petrolina. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/104415/1/Carlos-Antonio.pdf.

Sarika AV, Iquebal MA, Rai A and Kumar D (2013). PIPEMicroDB: microsatellite database and primer generation tool for pigeonpea genome. *Database. 2013*: bas054. https://doi.org/10.1093/database/bas054.

Saxena RK, Prathima C, Saxena KB, Hoisington DA, et al. (2010). Novel SSR markers for polymorphism detection in pigeonpea (*Cajanus* spp.). *Plant Breed.* 129(2): 142-148. https://doi.org/10.1111/j.1439-0523.2009.01680.x.

Saxena RK, Von Wettberg E, Upadhyaya HD, Sanchez V, et al. (2014). Genetic diversity and demographic history of *Cajanus* spp. illustrated from genome-wide SNPs. *PLoS One.* 9(9): 1-9. http://dx.doi.org/10.1371/journal.pone.0088568.

Serrote CML, Reiniger LRS, Silva KB, Rabaiolli SMS, et al. (2020). Determining the Polymorphism Information Content of a Molecular Marker. *Gene.* 726: 144175. https://doi.org/10.1016/j.gene.2019.144175.

Sharma P, Singh I, Sirari A, Singh S, et al. (2018). Genetic divergence studies through microsatellite markers in pigeonpea [*Cajanus cajan* (L) Millsp.]. *Legume Res*. 43: 312-319. https://doi.org/10.18805/LR-4022.

Songok S, Ferguson M, Muigai AW and Silim S (2010). Genetic diversity in pigeonpea [*Cajanus cajan* (L.) Millsp.] landraces as revealed by simple sequence repeat markers. *Afr. J. Biotechnol*. 9(22): 3231-3241.

Sousa ACBD, Godoy R, Sforça DA, Campos TD, et al. (2011). Genetic diversity analysis among pigeonpea genotypes adapted to South American regions based on microsatellite markers. *Sci. Agric.* 68(4): 431-439. https://doi.org/10.1590/S0103-90162011000400007.

Varshney RK, Saxena RK and Jackson SA (2017). The Pigeonpea Genome: An Overview. In: The Pigeonpea Genome. Compendium of Plant Genomes A book series (CPG) (Varshney RK, Saxena RK, Jackson S eds). Springer, Cham.

Wang J (2004). Application of the One-Migrant-per-Generation Rule to Conservation and Management. *Conserv. Biol.* 18(2): 332-343. https://doi.org/10.1111/j.1523-1739.2004.00440.x.

Wrigth S (1978). Evolution and the Genetics of Population, Variability within and among Natural Populations. University of Chicago Press, Chicago.

Zavinon F, Adoukonou-Sagbadja H, KeilWagen J, Lehnert H, et al. (2020). Genetic diversity and population structure in Beninese pigeonpea [*Cajanus cajan* (L.) Huth] landraces collection revealed by SSR and genome wide SNP markers. *Genet. Resour. Crop. Evol*. 67: 191-208. https://doi.org/10.1007/s10722-019-00864-9.