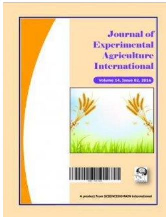

Journal of Experimental Agriculture International

33(5): 1-10, 2019; Article no.JEAI.41780
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Evaluation of Genetic Diversity by Molecular Markers ISSR of Algodoeiro (*Gossypium mustelinum*) in Native Populations of Pernambuco, Brazil

Luiz Sergio Costa Duarte Filho[1*], Edson Ferreira da Silva[1], Danielson Ramos Ribeiro[1], Allison Vieira da Silva[1] and Iêda ferreira de Oliveira[1]

[1]*Department of Biology, Universidade Federal Rural de Pernambuco, Brazil.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2019/v33i530154
Editor(s):
(1) Dr. Lanzhuang Chen, Professor, Laboratory of Plant Biotechnology, Faculty of Environment and Horticulture, Minami Kyushu University, Miyazaki, Japan.
Reviewers:
(1) Marcos Vinicius Bohrer Monteiro Siqueira, Universidade do Sagrado Coração, Brazil.
(2) Ajay BC, ICAR - Directorate of Groundnut Research, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/41780

Original Research Article

Received 19 April 2018
Accepted 06 June 2018
Published 03 April 2019

ABSTRACT

In order to assure and evaluate the genetic diversity, wild populations of Cotton (*Gossypium mustelinum*) were collected and evaluated from the coastal plain north of Pernambuco, Brazil. Such populations occur in urban areas in a state of real expansion and with imminent risks of extinction. As a result of these risks and the state of real expansion, aiming at the *ex situ* conservation of these genetic resources, branches of 66 plants were collected in three populations of *G. mustelinum* that are located in restinga vegetation in the localities of Ponta de Pedras and Bara of Catuama, both in the municipality of Goiana and in the locality Sossego Beach in the municipality Island of Itamaracá. The collected genotypes were inserted in a new Germplasm Bank (BAG) at the Federal Rural University of Pernambuco, after which a sample composed of 24 genotypes contained in the BAG was collected to perform genetic diversity studies using molecular markers of ISSR type. For the molecular analysis, 24 accesses with 4 ISSR primers were analyzed, which produced a total of 36

**Corresponding author: E-mail: luizsergiocdfilho@hotmail.com;*

bands, with a mean of 1,52 alleles per amplified locus. The genetic dissimilarity values, calculated according to the complement of the Jaccard index, ranged from 0.000 to 0.080. The UPGMA method grouped the accesses into three groups. The UFRPE30, UFRPE42 and UFRPE45 accessions were more dissimilar and UFRPE-48, UFRPE-50, UFRPE-52, UFRPE-55, UFRPE60, UFRPE06, UFRPE28, UFRPE29, UFRPE1, UFRPE2, UFRPE17 the least dissimilar. The ISSR markers used in this study demonstrated efficiency in the detection of molecular polymorphisms, revealing genetic variability among the 24 accessions. Considering the results obtained in this work, it is possible to infer that there is considerable genetic variability among the accessions of cotton, demonstrating the importance of the markers in the analysis of variability of species not studied, such as (*G. mustelinum*).

Keywords: Genetic resources; genetic variability; wild species.

1. INTRODUCTION

The Brazilian flora is composed of numerous wild species that constitute important reservoirs of genes, which can be introgressed in the cultivated species, aiming the availability of a greater genetic variability for the conservation and the improvement of plants. In this general work, five native species from Brazil will be studied with focus on diversity and existing genetic conservation, the first species to be studied is *G. mustelinum* Miers ex Watt.

Cotton is the common name given to several species of the botanical genus *Gossypium* L., from the family Malvaceae. There are about 40 species, shrubs, native to the subtropical and tropical regions, some of which are used for the production of the textile fiber known as cotton. The genus *Gossypium* constitutes important reservoirs of genes, which can be introgressed into the cultivated species of cotton, aiming the genetic improvement of plants [1]. The wild species *G. mustelinum* Miers ex Watt is endemic only in northeastern Brazil and has been described in the states of Rio Grande do Norte (RN), Ceará (CE), Bahia (BA) and Pernambuco (PE). Recently, three populations of *G. mustelinum* were reported on the northern coast of the State of Pernambuco (PE), located in the restinga area of Goiana and Ilha de Itamaracá. The three populations are extremely vulnerable because they occur in urban areas and are subject to genetic erosion. Therefore, it is necessary to characterize them morphologically and molecularly in order to provide strategies for the conservation of the genetic diversity of this species. This work was proposed to collect the accessions for the implantation of an *ex situ* collection to assure the conservation of the germplasm.

2. MATERIALS AND METHODS

The plant material was collected in the coastal plain north of the state of Pernambuco, Northeast Brazil, in restinga vegetation in the areas of Ponta de Pedras (S 07º37,255 '' W 34º48,728 '') and Barra de Catuama (S 07º40.493 'W 34º49.900'), both in the municipality of Goiana and in Praia do Sossego (between coordinates S 07º40,584 "W 34º49,158" and S 07º43, 208 "W 34º50,165") in the municipality Ilha de Itamaracá (Fig. 1), a material belonging to UFRPE study groups from Paraíba was also used in this study.

After the collection on October 15, 2017, the genotypes were planted in plastic bags of 5 liters containing a mixture of soil and substrate of coconut powder and kept in a greenhouse located in the Department of Agronomy of the Federal Rural University of Pernambuco - Recife PE. After 90 days, a germplasm bank (BAG) containing the 66 genotypes distributed in four populations was implemented at UFRPE (Fig. 2). Then, a sub-sample with 24 genotypes was stipulated to perform genetic diversity assessments by ISSR, divided among the three populations collected in Pernambuco and a contrasting population from Paraíba (Table 1).

After five weeks of development, leaves of the 24 genotypes of the sub-sample were collected for DNA extraction (CTAB protocol) [2]. The DNA samples were quantified on 0.8% agarose gel, 0.5X TBE buffer (0.045 mM Tris-Borate, 0.001 M EDTA, pH 8.0), stained with Blue Grenn Loading Dye I and visualized in transliner (Hing Performance Ultraviolet Transilluminator). For the electrophoretic run, 10 μl of a solution containing 1 μl of 10X diluted DNA plus 2 μl of loading buffer (4 g of sucrose + 0.025 g blue of

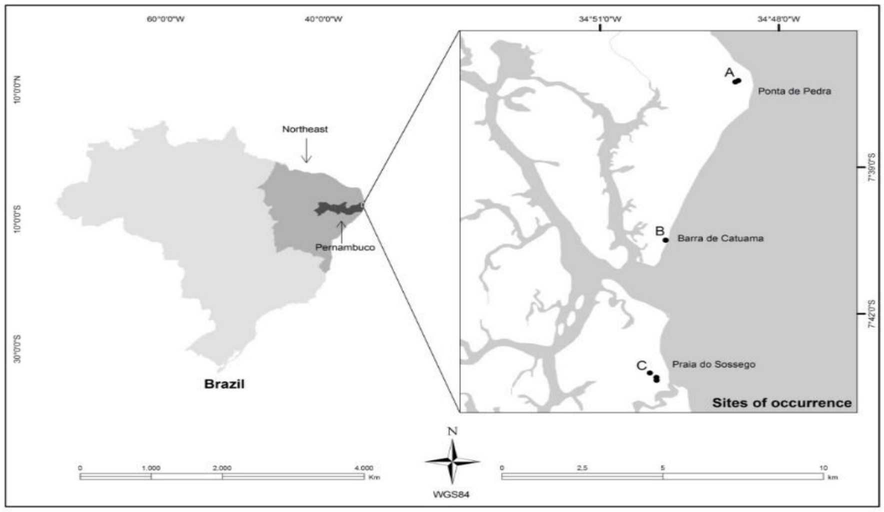

Fig. 1. Location of the populations: Ponta de Pedras (A), Barra de Catuama (B) and Sossego Beach (C), Pernambuco, Brazil

Fig. 2. Genotypes under development in the plant house, and implementation of the Active Germplasm Bank (BAG)

bromophenol) and 7 µl of MilliQ water were applied.

The DNA quantification of the samples was performed by comparing them to bacteriophage lambda DNA whose concentration was 25 ng/µL. The 24 subsamples selected in the Germplasm Bank (Table 1) were submitted to evaluations with ISSR-type markers (Table 2), using four oligonucleotides flanking semi-specific regions of DNA, ISSR markers were previously developed and available from the Laboratory of Genetic Resources of the Federal University of Alagoas.

Cyclination was obtained in thermocyclers (Eppendorf-Mastercycher Gradient), where initially the DNA was denatured at 95°C for 12 minutes. 47 cycles of denaturation - annealing - extension followed. In the first 11 cycles of amplification, denaturation was done at 94°C for 15 seconds; the annealing temperature in the first cycle was 65°C for 30 seconds, being decreased one degree at each cycle (touch down), reaching 55° C in the eleventh cycle. For the extension, a temperature of 72°C for 1 minute was used. The remaining thirty-six cycles occurred at 94°C for 15 seconds; 55°C for 30 seconds and 72°C for 1 minute. Final extension

Table 1. Identification of the 24 cotton subsamples of the UFRPE BAG

Identification	Source	Identification	Source
UFRPE-01	Ponta de Pedras	UFRPE-21	Ilha de Itamaracá
UFRPE-02	Ponta de Pedras	UFRPE-27	Ilha de Itamaracá
UFRPE-04	Ponta de Pedras	UFRPE-28	Ilha de Itamaracá
UFRPE-05	Ponta de Pedras	UFRPE-29	Ilha de Itamaracá
UFRPE-06	Ponta de Pedras	UFRPE-30	Ilha de Itamaracá
UFRPE-07	Ponta de Pedras	UFRPE-35	Ilha de Itamaracá
UFRPE-08	Barra de Catuama	UFRPE-46	Paraíba
UFRPE-09	Barra de Catuama	UFRPE-48	Paraíba
UFRPE-10	Barra de Catuama	UFRPE-50	Paraíba
UFRPE-12	Barra de Catuama	UFRPE-52	Paraíba
UFRPE-15	Barra de Catuama	UFRPE-55	Paraíba
UFRPE-17	Barra de Catuama	UFRPE-60	Paraíba

Table 2. Identification of the four ISSR oligonucleotides used for the 24 genotypes of the UFRPE BAG

Locus Identification of the ISSR	Composition of the ISSR
UFAL-09	(ACA) Tri-nucleotídeo
UFAL-10	(AAC) Tri-nucleotídeo
UFAL-11	(AAG) Tri-nucleotídeo
UFAL-12	(GA) Di-nucleotídeo

was followed at 72°C for 6 minutes. The amplified fragments were separated by 2.5% agarose gel, 0.5X TBE buffer (0.045 mM Tris-Borate, 0.001 M EDTA, pH 8.0), stained with Blue Grenn Loading Dye I and visualized in transliner (Hing Performance Ultraviolet Transilluminator.

To analyze the ISSR loci we used the GenAlEx version 6.5 program [3]. The mean number of alleles per locus was obtained by the ratio between the total number of alleles and the total number of loci. The diversity among the subsamples was calculated from the allelic frequency (pi), of the expected heterozygosity between the subsamples and the genetic distance.

From the distances obtained by the dissimilarity index, cluster analyzes were performed using the agglomerative hierarchical arithmetic mean method between unweighted pairs (UPGMA) and the Tocher hierarchical method. From the distance matrix obtained by the UPGMA grouping method, the simplified representation of the distances was done by means of a dendrogram, using the Program for the determination of genetic diversity.

3. RESULTS AND DISCUSSION

The DNA extraction using the methodology of [2] was successfully obtained, and the genomic DNA samples were suitable for the PCR reactions performed in the study as shown in Fig. 3.

Fig. 4 shows the existence of genetic variability for *G. mustelinum* genotypes evaluated by the ISSR markers UFAL-11 and UFAL-12, evidenced by the segregation of the populations. The genotypes that make up each phenotype class are presented in Table 1. The 24 subsamples belonging to the Cotton Germplasm Bank of UFRPE, when evaluated through 4 ISSR oligonucleotides, presented 20% polymorphism.

The four ISSR loci amplified a total of 36 alleles, with an average of 1,521 alleles per amplified locus, distributed among the 24 cotton subsamples. Oligonucleotides UFAL-09 and UFAL-10 were monomorphic for all subsamples, amplifying only one allele per locus. Of the 4 evaluated oligonucleotides 1 amplified five alleles (UFAL-11), and the UFAL-12 oligonucleotide amplified a total of seven alleles. The allele that showed the highest frequency of 0.500 is in the UFAL-11 oligonucleotide and the one that

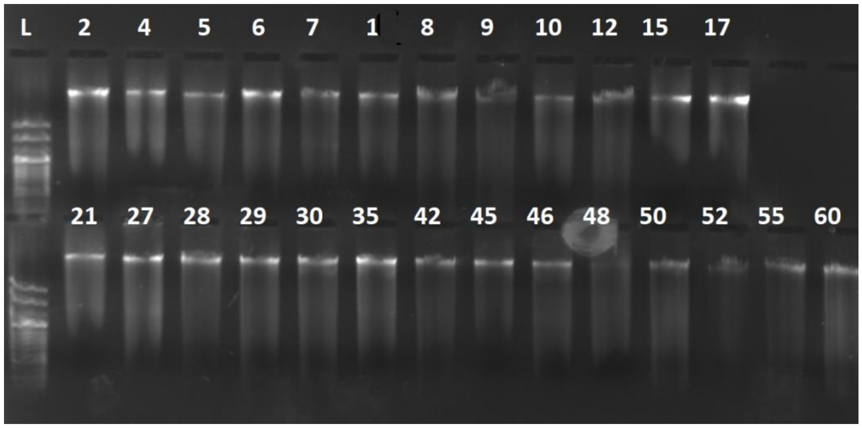

Fig. 3. Shows 0.8% agarose gel plated genomic DNA samples which are suitable for identification of collected genotypes

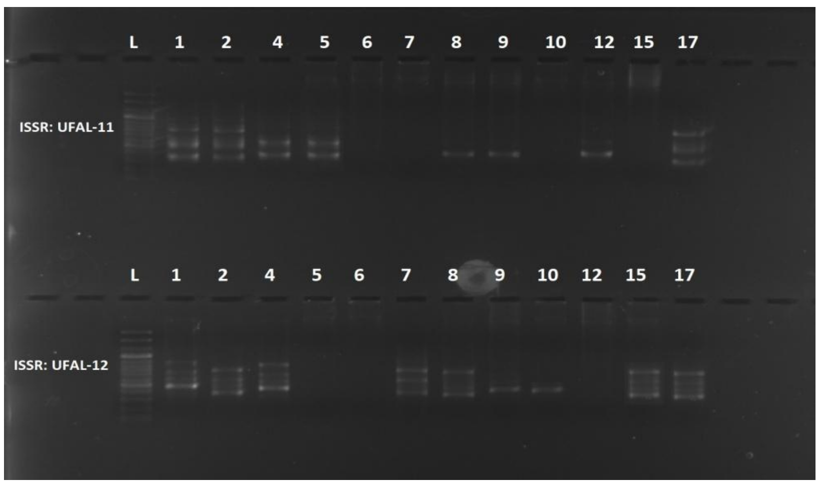

Fig. 4. 2.5% agarose gel with amplified PCR products on UFAL-11 and UFAL-12 primers which depicts the pattern of segregation of the population

showed the lowest frequency (0.041) is in the UFAL-12 oligonucleotide.

The evaluation of the genetic diversity among the subsamples of the Cotton Germplasm Bank of UFRPE, using molecular markers ISSR, showed the formation of close groups with 19% variability between the groups, which should have been caused mainly by the large number of subsamples that originated from those collected.

The results obtained in this work are compatible with those presented by [4], which evaluated the genetic diversity of cotton subsamples from the use of 56 pairs of BNL oligonucleotides, verified the amplification of 62 polymorphic loci, of which a total of 325 alleles were amplified, with a mean of five per marker. Likewise, [5], seeking to identify the genetic diversity and the population structure of 43 cotton cultivars and strains, observed that of the 33 ISSR markers used, 15 presented to the amplification of 104 polymorphic alleles. However, [6], studying 65 strains and four cotton genotypes, verified that only 9 of the 19 ISSR primers used showed polymorphism, with each primer amplifying only one locus, with the number of alleles produced at the polymorphic locus equal to 2.

The [7] affirm that ISSR markers are useful in the identification of cultivars and evaluation of genetic diversity, due to the high reproducibility, thus presenting advantages over other methods such as those based on PCR. In the same sense [8] states that the simple internal repetitive sequence (ISSR) technique can be used for rapid differentiation between related individuals due to the high degree of polymorphism, reproducibility and also because it contains a low cost.

The highest heterozygosity rate (0.098) was obtained for Population 3 (Ilha de Itamaracá), followed by Population 1 (0.088) native of Ponta de Pedras. The lowest rates of (0.018 and 0.067) were observed for Populations 2 (Barra de Catuama) and Population 4 (Paraíba) respectively, the mean heterozygosity was 0.068, corresponding to a low level of genetic diversity.

The low rate of determined heterozygosity among the cotton populations of the UFRPE BAG can be determined by the high kinship index between the subsamples. According to [9] inbreeding results from the mating of related individuals, being able to alter the genetic makeup of the population. This is done by increasing homozygosity and, consequently, by decreasing heterozygosity, thus altering the genotype frequency, but not the gene frequencies. In plants such as cotton that have a mixed breeding system, different inbreeding coefficients are found due to variations in the rates of natural self-fertilization [10].

The frequency values observed in Fig. 5 may infer that the size of a given population is a determining factor for the conservation of an allele. That is, in small groups of individuals the existence of reduced generations already makes possible the occurrence of allelic fixation, from the gene drift. In larger groups, the lead should take longer. The presence of private bands in POP3 (Itamaracá Island) and POP4 (Paraíba) populations can also be observed in Fig. 5, indicating a favorable relationship between these populations, thus favoring the introduction of a possible characteristic of interest and / or increased genetic variability.

It can be observed in Fig. 6 that some genotypes have high genetic similarity and can be considered as siblings or clones, such as genotypes (UFRPE-48, UFRPE-50, UFRPE-52, UFRPE-55, UFRPE-60 and UFRPE -06) and the genotypes (UFRPE-28 and UFRPE-29) presented in the upper left quadrant. The genotypes (UFRPE-1, UFRPE-2 and UFRPE-17) and genotypes (UFRPE-42 and UFRPE-45) were also presented with high genetic similarity. Considering the result of this distribution, it is correct to inform that the accomplishment of crosses between these related individuals is not advisable, because it favors inbreeding and diminishes the genetic diversity among the accesses.

The genetic dissimilarity values presented in Table 3 ranged from 0.000 to 0.080, with the highest dissimilarities (0.080) among the accessions (UFRPE-30 x UFRPE-01 - UFRPE-30 x UFRPE-02 - UFRPE-30 x UFRPE-17), (UFRPE-35x UFRPE-01-UFRPE-35 x UFRPE-02-UFRPE-35 x UFRPE-10 and

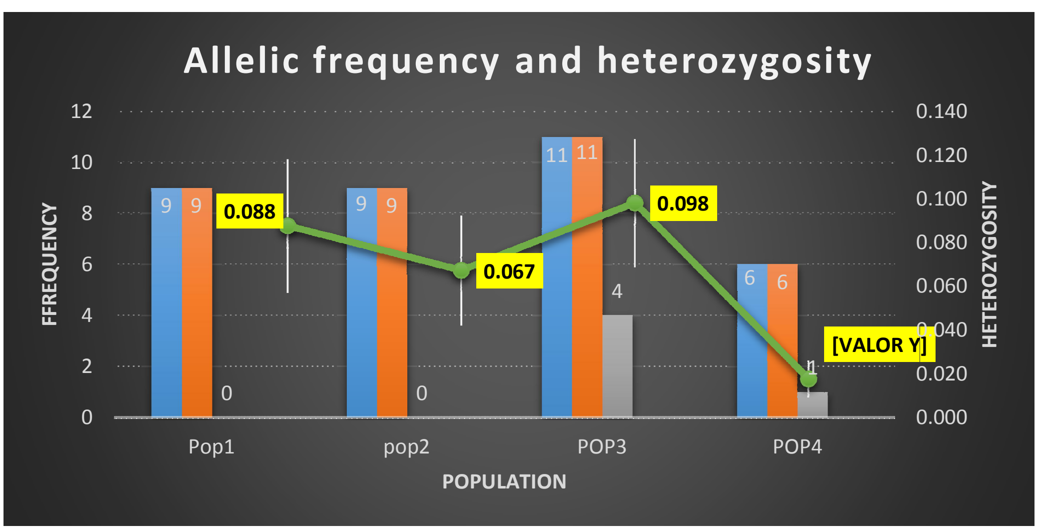

Fig. 5. Allelic frequency and heterozygosity of 24 Gossypium accessions estimated by 4 ISSR primers, which predicts *size of obtained population which is a determining factor for the conservation of an alleles*

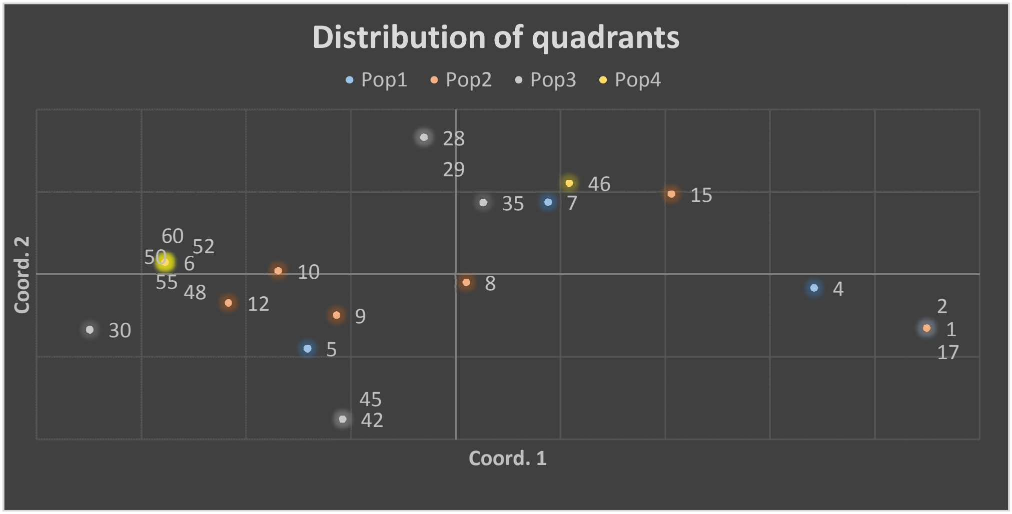

Fig. 6. Distribution of the similar genotypes of siblings within 24 accesses within Distribution quadrants

UFRPE-35 x UFRPE-17) 28 and UFRPE-42 x UFRPE-29), (UFRPE-45 x UFRPE-15-UFRPE-45 x UFRPE-28 and UFRPE-45 x UFRPE-29) and (UFRPE-46 x UFRPE-42 and UFRPE- -45), with a general average of 0.037 between the accessions, indicating that they are promising crosses for the introgression of desired characteristics.

Studying plant genetic diversity through ISSR, [11], found values of dissimilarity varying from 0.06 to 0.67, showing high genetic diversity among the accessions. Considering the above, it is possible to infer that the results of genetic dissimilarity, in this work, reflect a genetic variability from considerable to low among cotton accessions. The dissimilarity found is justified by the fact that the number of ISSRs evaluated is still low and that the populations collected and evaluated may belong to groups with a high inbreeding rate, a fact already discussed in this study.

In the agglomerative dendrogram UPGMA (Fig. 7), the formation of three large groups can be observed, considering the mean distance of 0.19 between groups. The G1 group consists of 14 accessions (UFRPE-55, UFRPE-60, UFRPE-52, UFRPE-50, UFRPE-48, UFRPE-06, UFRPE-10, UFRPE-09, UFRPE-12, UFRPE-30, UFRPE UFRPE-05, UFRPE-42 and UFRPE-45); The group G2 is composed of 7 accesses (UFRPE-07, UFRPE-15, UFRPE-04, UFRPE-04, UFRPE-01, UFRPE-02 and UFRPE-17), UFRPE-28 and UFRPE-29.

According to [12], molecular markers have been an important tool in the genetic improvement of plants, since they detect polymorphism directly at the DNA level, allowing observations in the genome on the relations between genotype and phenotype. Thus, the incorporation of molecular markers in cotton breeding helps, from the choice of the best breeders for a cross, to the identification of superior genotypes.

For [13], many deleterious recessive traits are hidden by dominant alleles in heterozygous forms, and appear after inbreeding. In this case, the author recommends the use of unrelated individuals in crosses, avoiding these undesirable effects.

Based on the observations made by [12] on the efficiency of the use of molecular markers, and in the observations made by [13] on the use of unrelated individuals in genetic crosses, and in the magnitudes of the characteristics evaluated in the current work, it is suggested crosses between individuals of groups that are allocated by the dendrogram of Fig. 7, since they indicate a greater degree of diversity, less possibility of inbreeding effects and greater capacity to explore the genetic variability of the groups evaluated.

The results generated in this work are in accordance with the literature on the importance of molecular markers, when compared to other works, such as the one performed by [14], demonstrating the importance of the markers in the analysis of variability of species little studied.

Table 3. Gene dissimilarity matrix among 24 cotton accessions calculated based on the complement of the Jaccard coefficient, using 4 ISSR primers. Recife, PE, 2017 to 2018

	1	2	4	5	6	7	8	9	10	12	15	17	28	29	30	35	42	45	46	48	50	52	55	60	
1	0,0																								**1**
2	0,0	0,0																							**2**
4	1,0	1,0	0,0																						**4**
5	5,0	5,0	4,0	0,0																					**5**
6	7,0	7,0	6,0	2,0	0,0																				**6**
7	4,0	4,0	3,0	5,0	3,0	0,0																			**7**
8	4,0	4,0	3,0	3,0	3,0	2,0	0,0																		**8**
9	5,0	5,0	4,0	2,0	2,0	5,0	3,0	0,0																	**9**
10	6,0	6,0	5,0	3,0	1,0	4,0	4,0	1,0	0,0																**10**
12	6,0	6,0	5,0	1,0	1,0	4,0	2,0	1,0	2,0	0,0															**12**
15	3,0	3,0	2,0	6,0	4,0	1,0	3,0	4,0	3,0	5,0	0,0														**15**
17	0,0	0,0	1,0	5,0	7,0	4,0	4,0	5,0	6,0	6,0	3,0	0,0													**17**
28	7,0	7,0	6,0	6,0	4,0	3,0	5,0	6,0	5,0	5,0	4,0	7,0	0,0												**28**
29	7,0	7,0	6,0	6,0	4,0	3,0	5,0	6,0	5,0	5,0	4,0	7,0	0,0	0,0											**29**
30	8,0	8,0	7,0	3,0	3,0	6,0	4,0	3,0	4,0	2,0	7,0	8,0	7,0	7,0	0,0										**30**
35	8,0	8,0	7,0	7,0	7,0	6,0	6,0	7,0	8,0	6,0	7,0	8,0	3,0	3,0	4,0	0,0									**35**
42	5,0	5,0	6,0	2,0	4,0	7,0	5,0	4,0	5,0	3,0	8,0	5,0	8,0	8,0	3,0	7,0	0,0								**42**
45	5,0	5,0	6,0	2,0	4,0	7,0	5,0	4,0	5,0	3,0	8,0	5,0	8,0	8,0	3,0	7,0	0,0	0,0							**45**
46	5,0	5,0	4,0	6,0	4,0	3,0	5,0	4,0	3,0	5,0	2,0	5,0	4,0	4,0	7,0	7,0	8,0	8,0	0,0						**46**
48	7,0	7,0	6,0	2,0	0,0	3,0	3,0	2,0	1,0	1,0	4,0	7,0	4,0	4,0	3,0	7,0	4,0	4,0	4,0	0,0					**48**
50	7,0	7,0	6,0	2,0	0,0	3,0	3,0	2,0	1,0	1,0	4,0	7,0	4,0	4,0	3,0	7,0	4,0	4,0	4,0	0,0	0,0				**50**
52	7,0	7,0	6,0	2,0	0,0	3,0	3,0	2,0	1,0	1,0	4,0	7,0	4,0	4,0	3,0	7,0	4,0	4,0	4,0	0,0	0,0	0,0			**52**
55	7,0	7,0	6,0	2,0	0,0	3,0	3,0	2,0	1,0	1,0	4,0	7,0	4,0	4,0	3,0	7,0	4,0	4,0	4,0	0,0	0,0	0,0	0,0		**55**
60	7,0	7,0	6,0	2,0	0,0	3,0	3,0	2,0	1,0	1,0	4,0	7,0	4,0	4,0	3,0	7,0	4,0	4,0	4,0	0,0	0,0	0,0	0,0	0,0	**60**
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**15**	**17**	**28**	**29**	**30**	**35**	**42**	**45**	**46**	**48**	**50**	**52**	**55**	**60**	

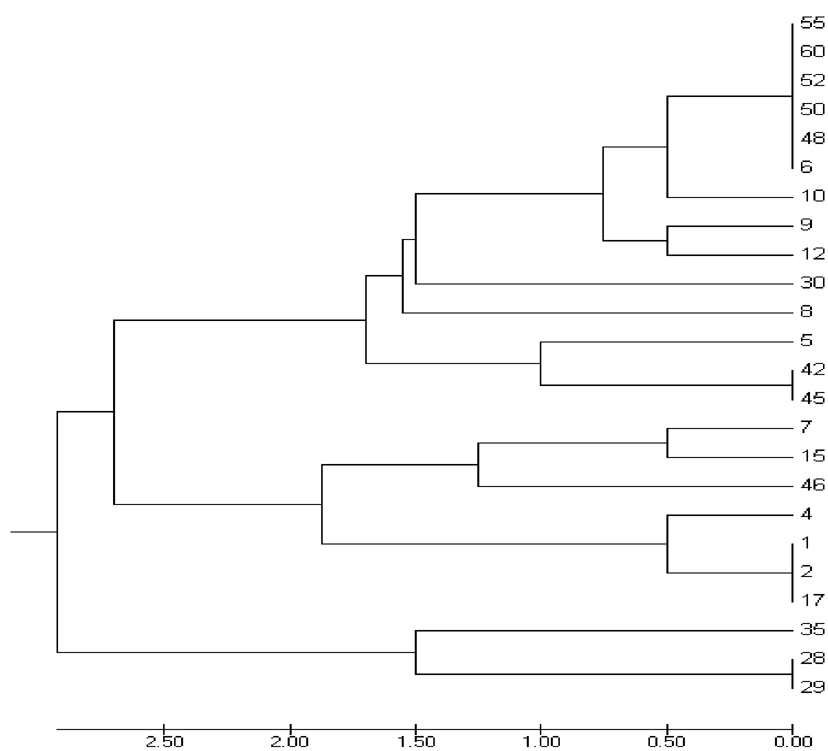

Fig. 7. Dendrogram obtained by the agglomerative method UPGMA, relating the 24 accessions of cotton based on the information of 4 loci of ISSR

4. CONCLUSION

The results obtained with the analyzes of the present study allow to conclude that:

The collection of genotypes and implantation of the BAG in UFRPE to ensure genetic variability was performed successfully;

ISSR markers were efficient in the detection of molecular polymorphisms and, therefore, of genetic variability among accessions of *G. mustelinum.*

The UFRPE-30, UFRPE-35, UFRPE-42 and UFRPE-45 accessions were the most divergent in relation to the others;

Based on the similarity evaluated by the agglomerative dendrogram UPGMA, the accessions formed three large genetic groups.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gotmare V, Singh P, Mayee C D, Deshpande V, Bhagat C. Genetic variability for seed oil content and seed index in some wild species and perennial races of cotton. Plant Breeding. 2000;123(2):207-208.
2. Ferreira ME, Grattapaglia D. Introdução ao uso de marcadores moleculares em análise genética. 3rd ed. Brasília: Embrapa – Cenargem; 1998. Portuguese.
3. Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics. 2012;28(19):2537-2539.
4. Liu S, Saha S, Stelly D, Burr B, Cantrell RG. Chromosomal assignment of microsatellite loci in cotton. Journal Hereditaridade. 2000;91(4):326-332.
5. Moiana LD, Vidigal Filho PS, Gonçalves Vidigal MC, Lacanallo GF, Galván MZ, Carvalho LP, Maleia MP, Pacheco CM, Ribeiro T, Zeni Neto H, Coimbra GK. Genetic diversity and populations structure of cotton (*Gossypium hirsutum* L. race *latifolium* H.) using microsatellite markers. African Journal of Biotechnology. 2012;54(11):11640-11647.
6. Menezes IPP, Hoffmann LV, Alves MF, Morello CL, Barroso PAV. Distância genética entre linhagens avançadas de germoplasma de algodão com uso de marcadores de RAPD e microssatélites. Pesquisa Agropecuária Brasileira, Brasília. 2008;43(10):1339-1347. Portuguese.
7. Goulão L, Oliveira CM. Molecular characterisation of cultivars of apple (Malus x domestica Borkh.) using microsatellite (SSR and ISSR) markers. Euphytica. 2001;122(1):81-89.
8. Borba RS, Garcia MS, Kovalleski A, Oliveira AC, Zimmer PD, Branco JSC, Malone G. Dissimilaridade genética de linhagens de Trichogramma Westwood (Hymenoptera: Trichogrammatidae) através de marcadores moleculares ISSR. Neotropical Entomology. 2005;34(4):565--569. Portuguese.
9. Queiroz SA. Efeito da endogamia sobre características de crescimento de bovinos da raça gir no Brasil. Revista Brasileira de Zootecnia, Viçosa. 2000;29(4):1014-1019. Portuguese.
10. Pedrosa MB. Potencial genético para seleção de uma população de algodoeiro de fibra colorida. 2005. 75 f. Tese (Doutorado em Fitotecnia) – Curso de Pós-graduação em Fitotecnia, Universidade Federal do Ceará, Fortaleza, 2005.
11. Pinto LR, et al. Avaliação de marcadores do tipo microssatélites para determinação do perfil molecular dos materiais do programa cana-IAC. In: Congresso Nacional da Sociedade dos Técnicos Açucareiros e Alcooleiros do Brasil - STAB, 9, 2008, Maceió. Anais… Maceió: STAB Regional Leste, p. 419-423, 2008.
12. Oliveira MSP, Amorim EP, Santos JB. Dos, Ferreira DF. Diversidade genética entre acessos de açaizeiro baseada em marcadores RAPD. Ciência e Agrotecnologia, Lavras. 2007;31(6):1645-1653. Portuguese.
13. Ronzelli Junior P. Melhoramento genético de plantas. Curitiba: Graffice: Editora Gráfica; 1996. Portuguese.

14. Junqueira KP, Faleiro FG, Bellon G, Junqueira NTV, Fonseca KGF, Lima CA, Santos EC. Variabilidade genética de acessos de pitaya com diferentes níveis de produção por meio de marcadores RAPD. Revista Brasileira de Fruticultura, Jaboticabal. 2010;32(3):840-846. Portuguese.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/41780