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Full Length Research Paper

Genetic divergence among African and American cotton (*Gossypium hirsutum* L. race *latifolium* H.) cultivars and inbred lines through random amplification of polymorphic DNA (RAPD) markers.

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Cotton (*Gossypium* spp.) is an important cash crop and the second largest source of textile fiber and edible oil throughout the world. This study was conducted to investigate the genetic divergence through random amplified polymorphism (RAPD) molecular markers among the introduced African and American cultivars and inbred lines of cotton (*Gossypium hirsutum* L. raça *latifolium* H.) in Mozambique. We used 24 RAPD primers that amplified a total of 166 bands, identifying 90.96% of polymorphism. The intra and inter group genetic variability quantification evidenced significant variability of 16.30% between the African and American groups. The highest genetic similarity was observed among the African commercial cotton cultivars, whereas American cultivars and inbred lines were considered the most dissimilar ones. The arithmetic complement of Jaccard, obtained with 151 RAPD molecular markers showed that African cultivars Albar BC853 and STAM 42 were the most similar, while the most dissimilar combinations were TAMCOT Sphinx and ISA 205 followed by TAMCOT Sphinx vs ALBAR BC853 and TAMCOT Sphinx vs REMU 40 combinations.

Key words: Molecular variance analysis**,** decamer primers, dissimilarity, *Gossypium hirsutum*, molecular markers.

INTRODUCTION

Cotton (*Gossypium spp.*) belongs to Malvaceae family and *Gossypium* genus, including 45 diploid species (2n = 2x = 26) and six allopolyploids (2n = 4x = 52), with some being cultivated and others wild (Fryxell, 1992; Brubaker et al., 1999). There are four cultivated species from *Gossypium* genus, two of them are diploids originally from Old World (*Gossypium herbaceum* and *Gossypium arboreum*) and the others are allopolyploids from the New World (*Gossypium hirsutum* and *Gossypium barbadense*). The diploid species involve A, B, C, D, E, F, G and K genomes and allopolyploids correspond to two sub genomic groups similar to A and D genomes (Endrizzi et al., 1985; Stewart, 1995).

Nowadays, *G. hirsutum* is one of the outstanding species due to its important world's cotton yield, which is estimated between 90 and 95% (Iqbal et al., 2001; Altaf-Khan et al., 2002; Penna, 2005). Additionally, seven distinguished geographic races has been described for this species (*latifolium, marie-galante, morrilli, palmeri, punctatum, richmondi* and *yucatanense*), according to variations that happened during the spreading of cotton crop. The *latifolium* race formerly from Mexico and

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Abbreviations: RAPD, Random amplified polymorphic DNA; **AMOVA,** analysis of molecular variance; **PCR,** polymerase chain reaction.

Guatemala originated the commercial upland cotton cultivars, which are widely cultivated in the world (Neves, 1965).

Cotton (*G. hirsutum* L. race *latifolium*) is cultivated in tropical and subtropical regions in different types of soil and it is grown as an annual crop, even though it is basically a tropical perennial plant (Prentice, 1972; Fryxell, 1984). Cotton is responsible for 40% of all world's fiber production (Ozyigit, 2009) and two thirds of that from regions of latitudes superior to 30°N, where the main producers are located, such as United States (US) and China (FAO, 2009).

In Mozambique, cotton is mainly cultivated by small holders in small areas equivalent to 1.0 ha or even less, contributing approximately 90% of total Mozambique cotton production (Mahalambe, 2003). However, the crops have a mean yield of 541 kg ha^{-1} which is considered very low when compared to world's mean yield, which is about 1.251 kg ha^{-1} of cottonseed (FAO, 2009). The practice to introduce commercial cotton cultivars from other African countries, known by their good productivity characteristic was adopted by the Mozambique Cotton Breeding Program. In 2006, in order to expand the germplasm bank and use it in breeding programs, another four cotton cultivars and eight lineages of *G. hirsutum* originally from US were introduced. On the other hand, the genetic diversity of this germplasm was not being evaluated; thus, this procedure is important to allow the proper use of these genetic sources, enabling the breeder to adequately identify and select superior parents for breeding programs (Falconer, 1989).

Molecular markers are important tools in genetic diversity analyses because they allow the obtaining of more precise and detailed information regarding to polymorphism, independence from effects caused by environmental conditions and the physiological stage of the plant (Agarwal et al., 2008). Among the current available molecular markers, random amplified polymorphic DNA (RAPD) have been used to identify the genetic diversity in germplasm banks because of its simplicity, the need of low DNA concentration and also by their ability to produce polymorphism (Williams et al., 1990). In several studies, RAPD technique was efficient in identifying genetic diversity in cotton (Zuo et al., 2000; Lu and Myers, 2002; Linus et al*.,* 2002; Mehetre et al*.,* 2004; Vafaie-Tabar et al*.,* 2004; Rana and Bhat, 2005; Sheidai et al*.,* 2007; Patil et al*.,* 2007; Esmail et al., 2008; Mahmood et al., 2009).

The present study was conducted to estimate the genetic divergence through RAPD molecular markers among the introduced African and American cultivars and inbred lines of cotton (*G. hirsutum* L. raça *latifolium* H.) in Mozambique.

MATERIALS AND METHODS

Plant materials

The study was conducted at the Molecular Biology Laboratory from Núcleo de Pesquisa Aplicada a Agricultura (Nupagri), Universidade Estadual de Maringá (UEM), Maringá, Paraná, Brazil. A total of 13 cultivars (nine African and four American) and eight American cotton inbred lines (*G. hirsutum* L.) were analyzed. The American cultivars and lineages were recently introduced in Mozambique by the Cotton Research and Seed Multiplication Center of Namialo - CIMSAN (Table 1). The eight lineages were developed by Texas Agricultural Experimental Station as part of an ongoing effort to develop germplasm with improved fiber quality (Smith, 2001; Smith, 2003; Thaxton et al*.,* 2005a; Thaxton et al*.,* 2005b; Thaxton et al*.,* 2005c)

Genomic DNA extraction and quantification

DNA extraction was carried out according to protocol described by Zhang and Stewart (2000). Seeds of each cultivar and lines were grown in trails containing washed sand in the greenhouse, where they were kept until the harvesting of young leaves. A total of four plants of each cultivar and lines were obtained.

At 20th day after the emergence of seedling, fresh young leaves were collected from four plants of each cultivar and inbred line, which were immediately placed in eppendorf tubes and maintained in a foam box containing liquid nitrogen to preserve the DNA. DNA extraction was carried out according to method previously described (Zhang and Stewart, 2000). DNA concentration was measured spectro-photometrically (Femto 700S, Femto Indústria e Comércio de Instrumentos Ltd., São Paulo, Brazil) using 260/280 nm absorbance ratio method. Finally, DNA samples were dilluted in sterile Milli-Q H_20 with a concentration approximately of 10 ng DNA μl^{-1}. The stock DNA samples were stored at -20°C.

Oligonucleotides (primers)

For the genetic divergence assay, 46 decamer arbritary primers were obtained from *Operon Technologies*, but only 24 were selected since they were considered the most polymorphic ones and also because they presented well defined bands (Table 2).

Polymerase chain reaction (PCR) amplification and genomic DNA analysis

Amplification reaction was carried out in a total volume of 25 µL containing 0.2 mM of each dNTP, 5 mM $MgCl_2$, 0.2 mM de primer, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1U Taq polymerase (Invitrogen®), template DNA (50ng μL^{-1}) and ultra pure water to complete the volume.

Amplification was performed using an initial denaturation of 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 60 s, annealing at 35°C for 60 s, extension at 72°C for 2 min. The last cycle was followed by a final extension at 72°C for 5 min and a 4°C hold.

Amplicons were visualized in a 1.2% agarose gel, containing ethidium bromide (0.02 µl mL^{-1}), in buffer solution TAE 1X (TAE 50X: Tris base - 242 g; glacial acetic acid – 57.1 ml; EDTA 0.5 M pH 8.0 - 100 ml). Amplification products were visualized by UV light on Spectroline and images captured with digital camera Canon Power Shot A620. A DNA ladder ranging from 100 to 2,072 bp (Invitrogen®) and GeneLine (version 2.0) software were used to determine the size of amplicons. Only clear and well defined amplified fragments were selected for statistical analysis.

Statistical analysis

Band-profiles for each cultivar and inbred line studied were

Table 1. List of cultivars and inbred line used for genetic divergence analysis.

Id.	Cultivar/ inbred line	Geographic group	Origin
1	Albar SZ9314	African	Zimbabwe
2	Albar FQ902	African	Zimbabwe
3	Albar BC853	African	Zimbabwe
4	STAM 42	African	Senegal
5	CA 222	African	Ivory Cost
6	CA 324	African	Ivory Cost
7	IRMA 12-43	African	Cameroom
8	ISA 205	African	Ivory Cost
9	REMU 40	African	Mozambique
10	TAMCOT 22	American	US -Texas
11	TAM 96WD-69s [(L)]	American	US -Texas
12	TAMCOT PYRAMID	American	US -Texas
13	TAM 98D -102 [(L)]	American	US -Texas
14	TAM 96WD-18 [(L)]	American	US -Texas
15	TAM 94J-3 [(L)]	American	US -Texas
16	TAM 88G-104 [(L)]	American	US -Texas
17	TAMCOT Sphinx	American	US -Texas
18	TAM 98D-99ne [(L)]	American	US -Texas
19	TAM 94WE-37s [(L)]	American	US -Texas
20	TAM 94L-25 [(L)]	American	US -Texas
21	TAMCOT Luxor	American	US -Texas

US: United States. [(L)] Inbred line. Numbers from 10 to 21 refer to the four cultivars and eight inbred lines from US introduced in Mozambique in 2006.

Table 2. Decamer arbritary primers used for DNA amplification.

Primer	Nucleotide sequence (5' – 3')	Primer	Nucleotide sequence (5' – 3')
OPA-11	CAA TCG CCG T	OPF-16	GGA GTA CTG G
OPA-13	CAG CAC CCA C	OPG-14	GGA TGA GAC C
OPA-18	AGG TGA CCG T	OPG-19	GTC AGG GCA A
OPB-04	GGA CTG GAG T	OPH-14	ACC AGG TTG G
OPB-07	GGT GAC GCA G	OPI-03	CAG AAG CCC A
OPB-12	CCT TGA CGC A	OPJ-04	CCG AAC ACG G
OPC-06	GAA CGG ACT C	OPK-08	GAA CAC TGG G
OPC-20	ACT TCG CCA C	OPK-09	CCC TAC CGA C
OPE-04	GTG ACA TGC C	OPK-20	GTG TCG CGA G
OPF-05	CCG AAT TCC C	OPO-19	GGT GCA CGT T
OPF-06	GGG AAT TCG G	OPW-07	CTG GAC GTC A
OPF-07	CCG ATA TCC C	OPY-20	AGC CGT GGA A

designed as 1 for presence or 0 for absence of a given band, considered for analysing only the polymorphic bands. Clustering of cotton cultivars and inbred lines was carried out by the hierarchical UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method (Sneath and Sokal, 1973), using a dissimilarity matrix corresponding to arithmetic complement of Jaccard (1908). The Genetic variability quantification of inter and intra-geographic groups (nine African commercial cultivars and a total of twelve American cultivar and inbred lines) through molecular variance analysis (AMOVA), was conducted by Excoffier et al. (1992) method based on 151 polymorphic bands. Statistical significance of variances was tested by using 1,000 random permutations. To evaluate the intra geographic group divergence, a graphic projection was done, using bidimensional space obtained by Jaccard's

Table 3. The number of amplified RAPDs bands among 21 cotton cultivars and inbred lines.

Primer	Total of band	Polymorphic band	Monomorphic band	Polymorphism (%)
OPA-11	8	7	1	87.50
OPA-13	4	4	0	100.00
OPA-18	2	2	0	100.00
OPB-04	10	10	0	100.00
OPB-07	4	2	2	50.00
OPB-12	8	8	0	100.00
OPC-06	6	6	0	100.00
OPC-20	4	4	0	100.00
OPE-18	9	8	1	88.89
OPF-05	7	7	0	100.00
OPF-06	8	8	0	100.00
OPF-07	5	4	1	80.00
OPF-16	8	7	1	87.50
OPG-14	5	3	2	60.00
OPG-19	7	7	0	100.00
OPH-14	10	10	0	100.00
OPI-03	7	7	0	100.00
OPJ-04	9	8	1	88.89
OPK-08	8	8	0	100.00
OPK-09	8	6	2	75.00
OPK-20	9	8	1	88.89
OPO-19	7	6	1	85.71
OPW-07	8	6	2	75.00
OPY-20	5	5	0	100.00
Total	166	151	15	90.96
Mean	6.92	6.29	0.63	-

dissimilarity matrix. Statistical analyses were done utilizing GENES software (Cruz, 2008).

RESULTS AND DISCUSSION

Genetic divergence analysis

The 24 RAPD primers amplified a total of 166 products with an average of 6.92 and amplitude from 2 to 10 bands per primer. From the band profile obtained, 151 were polymorphic (90.96% polymorphism), corresponding to 6.29 polymorphic bands per primer (Table 3).

Esmail et al. (2008) evaluated the genetic divergence among cotton elite inbred lines from US germplasm bank using 23 RAPD primers. A total of 113 bands were obtained, and 96 (84.96%) were polymorphic. On the other hand, Menezes et al. (2008) observed the presence of 280 amplified fragments when estimating genetic divergence among advanced inbred lines from cotton germplasm. From the profiles obtained, only 70 (25%) were polymorphic. In the present work, a higher polymorphism (90.96%) from those obtained in the studies previously was detected (Table 3). According to Colombo et al. (2000), the inter and intra group genetic relationships of vegetal species could be determined by the presence of 50 to 100 polymorphic bands.

In Table 3, it can be observed that primers OPB-04 and OPH-14 have amplified a higher number of fragments (10), whereas primer OPA-18 have obtained the lowest number of bands (2). The highest polymorphism percentage was noticed with the following primers: OPA-13, OPA-18, OPB-04, OPB-12, OPC-06, OPC-20, OPF-05, OPF-06, OPG-19, OPH-14, OPI-03, OPK-08 and OPY-20, showing 100% of polymorphism while the lowest polymorphism was exhibited by primer OPB-07 (50%).

The analysis of DNA band-profile (Figure 1) corresponding to amplification with primers OPA-11, OPA-13 and OPB-07, shows the polymorphism percentage observed among cultivars and inbred lines. Primer OPA-13 revealed 100% of polymorphism with amplification of two molecular markers with weight ranging from 500 to 700 bp, which were specific for cultivar TAMCOT Luxor. On the other hand, primer OPA-11 has amplified marker with 550 bp, which was specific for cultivar TAM 94L-25.

Genetic divergence estimative among 13 cultivars and eight inbred lines of cotton (*G. hirsutum*) was determined by 151 polymorphic bands through Jaccard's arithmetric

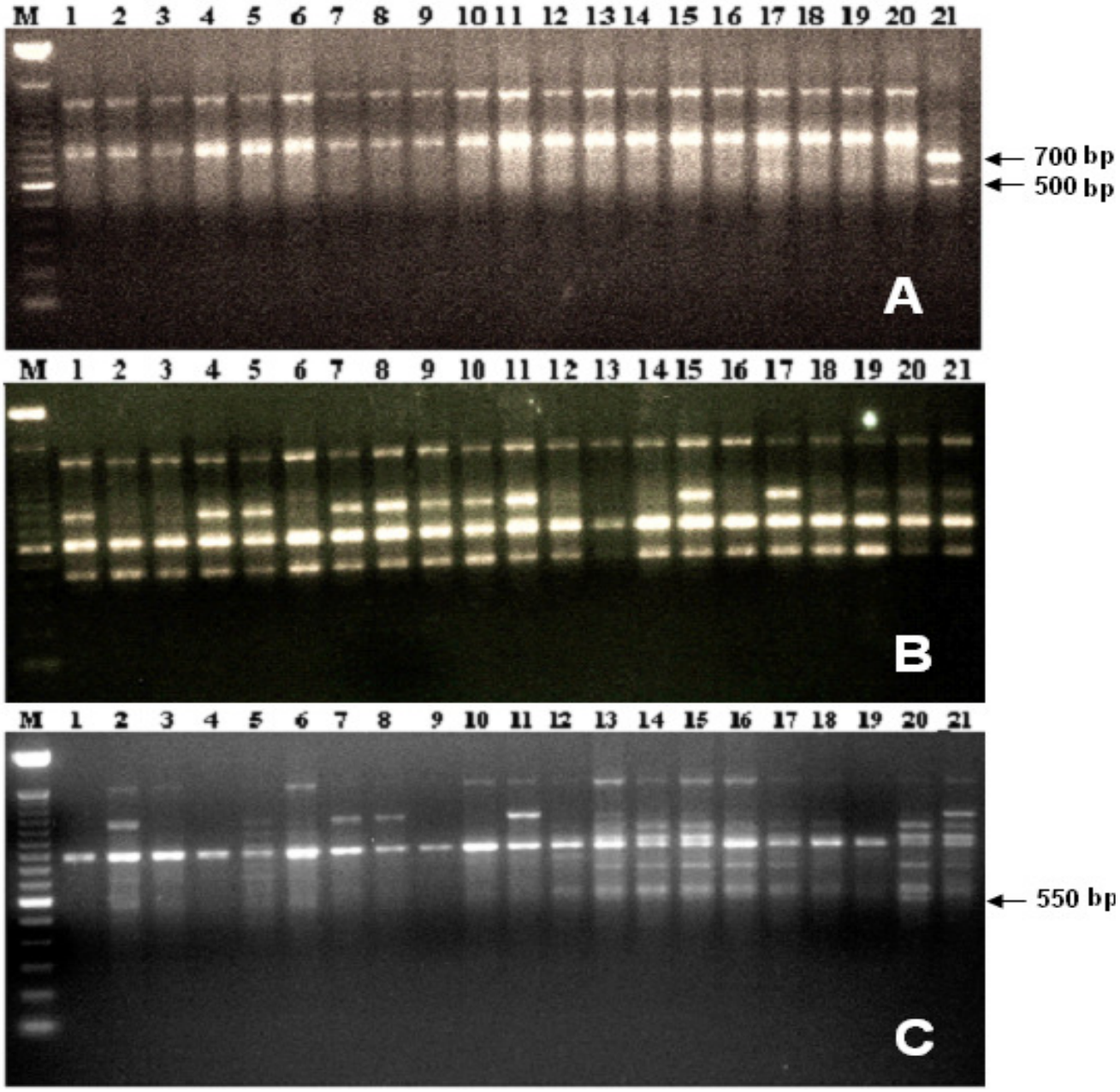

Figure 1. Electrophoretic profile of cotton amplified products of 13 cultivars and eight inbred lines (*G. hirsutum*) with primers OPA-13 (A), OPB-07 (B) and OPA-11 (C). M- 100 pb DNA ladder; 1-Albar SZ9314; 2- Albar FQ902; 3-Albar BC853; 4-STAM 42; 5- CA 222; 6-CA 324; 7-IRMA 12-43; 8- ISA 205; 9-REMU 40; 10- TAMCOT 22; 11-TAM 96WD-69s; 12-TAMCOT PYRAMID; 13-TAM 98D-102; 14-TAM 96WD-18; 15-TAM 94J-3; 16-TAM 88G-104; 17-TAMCOT Sphinx; 18-TAM 98D-99ne; 19-TAM 94WE-37s; 20-TAM 94L-25; 21-TAMCOT Luxor.

complement (Table 4). It is evidenced that dissimilarity between cultivars and inbred lines varied from 0.08 to 0.66 with an average of 0.29. The most divergent pairs composed by cotton cultivars or inbred lines were ISA 205 x TAMCOT Sphinx, followed by Albar BC853 x TAMCOT Sphinx. The American cultivar TAMCOT Sphinx formed the most divergent pairs with most African commercial cultivars ($d_{ii'} > 0.50$). The lowest genetic divergence was observed between cultivars Albar BC853 x STAM 42, with similarity lower than $d_{ii'} = 0.1$. In Table 4, in most cases, it is possible to note that the highest similarity was detected in African commercial cotton cultivars ($s_{ii'} > 0.80$).

In relation to genetic analysis of inter- and intra-geographic groups (African and American), it was verified that the dissimilarity of African group was lower (0.08 to

Table 4. Dissimilarity values obtained by arithmetic complement of Jaccard's index among 13 cultivars and eight inbred lines of cotton (*G. hirsutum*).

Cultivar/ Lineage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	0.00	0.27	0.26	0.24	0.31	0.25	0.26	0.27	0.27	0.29	0.29	0.31	0.32	0.26	0.32	0.30	0.61	0.29	0.39	0.33	0.37
2	-	0.00	0.13	0.15	0.22	0.15	0.13	0.22	0.19	0.32	0.27	0.25	0.22	0.24	0.25	0.24	0.61	0.22	0.38	0.25	0.28
3	-	-	0.00	0.08	0.19	0.12	0.14	0.26	0.16	0.28	0.23	0.21	0.22	0.22	0.28	0.26	0.65	0.21	0.40	0.24	0.30
4	-	-	-	0.00	0.15	0.14	0.11	0.23	0.10	0.27	0.22	0.22	0.24	0.23	0.25	0.26	0.62	0.16	0.38	0.26	0.28
5	-	-	-	-	0.00	0.17	0.18	0.26	0.20	0.31	0.26	0.28	0.28	0.27	0.29	0.28	0.61	0.24	0.44	0.30	0.31
6	-	-	-	-	-	0.00	0.15	0.24	0.16	0.25	0.23	0.22	0.18	0.16	0.25	0.19	0.61	0.19	0.37	0.24	0.16
7	-	-	-	-	-	-	0.00	0.16	0.11	0.28	0.21	0.22	0.24	0.23	0.26	0.22	0.59	0.15	0.36	0.25	0.28
8	-	-	-	-	-	-	-	0.00	0.21	0.38	0.28	0.33	0.30	0.32	0.30	0.26	0.66	0.26	0.44	0.38	0.39
9	-	-	-	-	-	-	-	-	0.00	0.27	0.23	0.25	0.23	0.25	0.23	0.25	0.63	0.14	0.38	0.25	0.29
10	-	-	-	-	-	-	-	-	-	0.00	0.17	0.26	0.20	0.23	0.28	0.28	0.61	0.30	0.40	0.33	0.33
11	-	-	-	-	-	-	-	-	-	-	0.00	0.19	0.21	0.23	0.26	0.27	0.60	0.24	0.38	0.28	0.28
12	-	-	-	-	-	-	-	-	-	-	-	0.00	0.19	0.18	0.30	0.26	0.60	0.23	0.36	0.24	0.29
13	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.16	0.23	0.22	0.62	0.25	0.42	0.26	0.27
14	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.20	0.18	0.58	0.20	0.32	0.21	0.23
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.19	0.62	0.25	0.37	0.29	0.27
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.56	0.25	0.38	0.28	0.29
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.61	0.54	0.57	0.60
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.37	0.24	0.25
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.44	0.39
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00	0.23
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.00

Average dissimilarity coefficient = 0.29; amplitude of dissimilarity coefficient (0.08 – 0.66). 1 = Albar SZ9324; 2 = Albar FQ902; 3 = Albar BC853, 4 = STAM 42; 5 = CA 222; 6 = CA 324; 7 = IRMA 12 - 43; 8 = ISA 205; 9 = REMU 40; 10 = TAMCOT 22; 11 = TAM 96WD-69s; 12= TAMCOT PYRAMID; 13 = TAM 98D - 102; 14 = TAM 96WD - 18; 15 = TAM 94J-3; 16 = TAM 88G-104; 17 = TAMCOT Sphinx; 18 = TAM 98D - 99ne; 19 = TAM 94WE - 37s; 20 = TAM 94L - 25; 21 = TAMCOT Luxor.

0.31) with an average of 0.19, which is considered very low to general average (0.29). Results of low similarity were also detected by other authors in other regions. Multani and Lyon, (1995) showed genetic divergence of nine Australian cotton cultivars whereas Iqbal et al. (1997) investigated 17 Asian cotton cultivars from *G. hirsutum* species through RAPD markers. The dissimilarity values observed in these studies varied, respectively, 0.01 to 0.08 and 0.07 to 0.18. Lukonge (2005) have also reported high similarity values ranging from 0.89 to 0.98, when evaluating 26 commercial cotton cultivars cultivated in Tanzania.

The low genetic divergence conferred by African commercial cultivars suggests that they might possess a narrow genetic base. This fact explains the limited use of parental genotypes with low divergence among themselves for breeding programs (Van-Esbroeck and Bownam, 1998; Iqbal et al., 2001).

The American group, which is mainly composed by inbred lines (Table 1), showed a relatively higher dissimilarity, ranging from 0.16 to 0.62 with an average of 0.32 above general average. Even though Lu and Myers (2002) have reported high similarity (92.7 to 97.7%) among 10 influential upland cotton varieties (*G. hirsutum* L.), these

Table 5. Molecular variance analysis of two geographic groups of cotton (*G. hirsutum*) cultivars and inbred lines obtained by RAPD molecular markers (151 bands).

VS	DF	SS	Variance Component	Variance percentage	F
Inter geographic groups	1	51.37	3.33	16.30**	0.163**
Intra geographic groups	19	325.06	17.11	83.70	
Total	20	376.43	20.44	100.00	

VS = Variation source; DF = Degrees of freedom; SS= Sum of squares. ** Significant at 1% of probability.

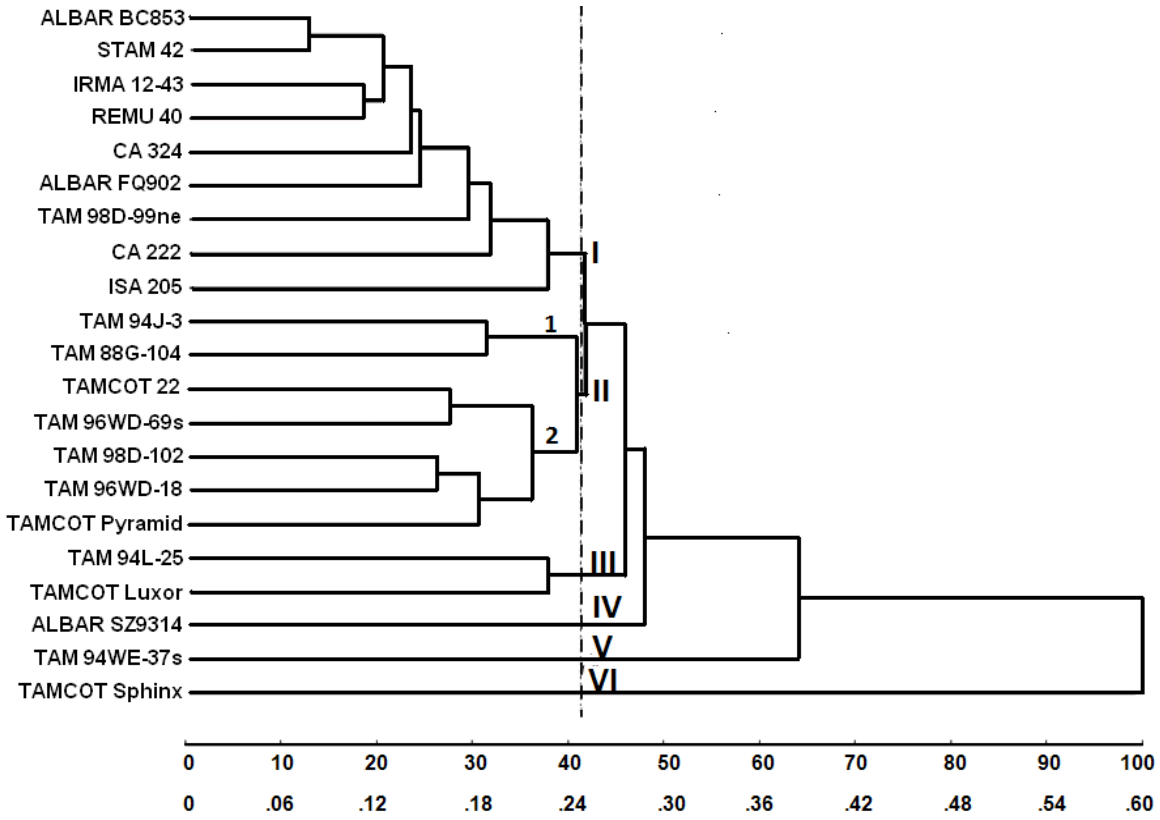

Figure 2. Dendrogram based on dissimilarity matrix corresponding to arithmetic complement of Jaccard's index among 21 (*G. hirsutum*) cotton cultivars and inbred lines by UPGMA criteria using 151 RAPD markers.

varieties genetically contributed for cotton.

AMOVA

The inter- and intra-groups variability quantification through AMOVA was estimated in 16.30 and 83.70%, respectively. According to Wright's (1978) methodology, F_{ST} value was high and significant ($P < 0.01$), evidencing a considerable inter-geographic groups genetic divergence (Table 5). From Table 5, it can also be inferred that no statistical evidences ($P > 0.05$) support the existence of intra-geographic group's variability among cultivars or inbred lines. On the other hand, the highest intra-geographic group's variance was noticed due to high divergence among American cultivars or inbred lines, since it was showed higher similarity among African commercial cultivars (Table 4). These results indicate that these two geographic groups studied could be considered genetically distinguished populations (Oliveira and Silva, 2008) and the American group the most divergent one.

Clustering analysis

Using RAPD polymorphisms, a dendrogram of studied African and American cotton cultivars and inbred lines was constructed with UPGMA clustering algorithm. The vertical dashed line in the dendrogram, at a distance of 41.30%, gave six groups (Figure 2). The first group was

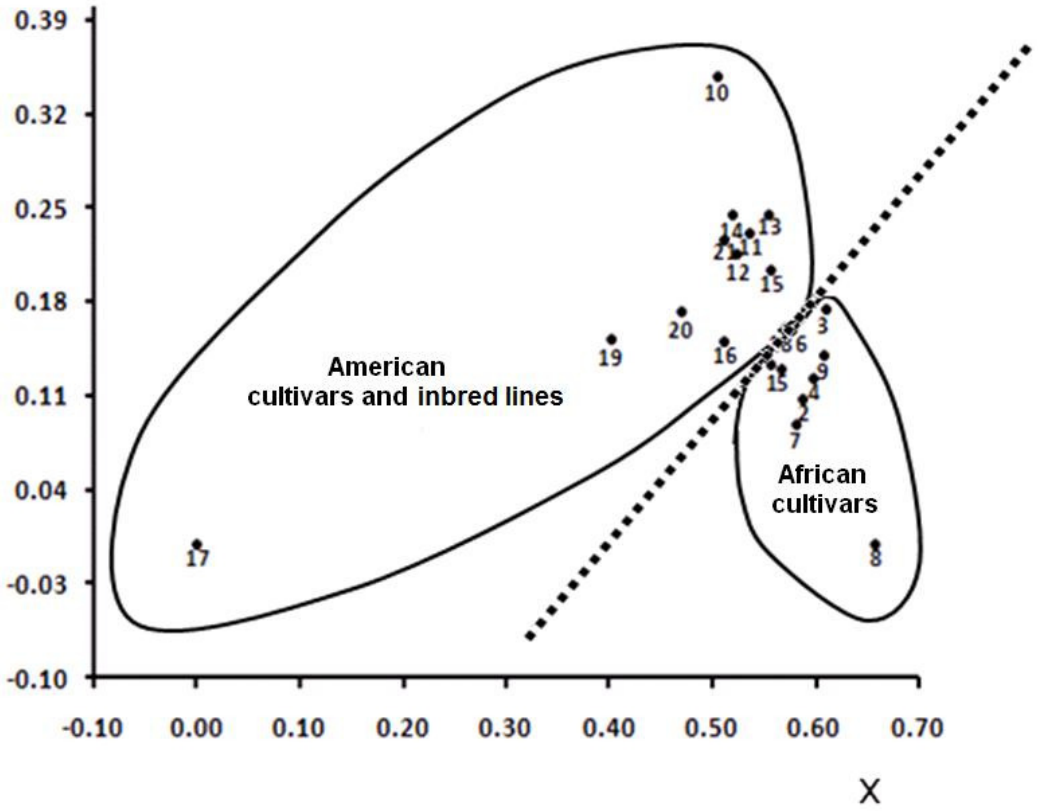

Figure 3. Graphic projection of genetic distance among cotton (*G. hirsutum*) cultivars and inbred lines based on arithmetic complement of Jaccard's index. Identification of cultivars and lineages are defined in Table 1.

composed of approximately 90% of commercial cultivars originally African, whereas ALBAR SZ9314 formed an isolate group (IV). American cultivars and inbred lines were arranged in four groups (II, III, V and VI); except for American inbred line TAM 98D-99ne which was allocated in the African group. When compared to the other American cultivars and inbred lines, TAM 98D-99ne does not have nectarines present in leaves and bracts (Thaxton et al*.,* 2005b). In group II the presence of two subgroups was noticed: The first one with similarity of 59.02% in relation to the second subgroup. On the other hand, group III was formed by inbred line TAMCOT 94L-25 and cultivar TAMCOT Luxor, revealing 61.04 % of similarity between themselves.

Groups IV, V and VI were only composed of either a cultivar or inbred line, as follow: ALBAR SZ9314, TAMCOT 94WE-37s and TAMCOT Sphinx, respectively. The cultivars and inbred lines mentioned formed singular and isolated groups, since they possess peculiar characteristics in relation to the others. Albar SZ9314 has most of its agronomic characteristics and fiber quality improved by Instituto de Investigação do Algodão de Zimbabwe, whereas TAMCOT Sphinx is evidenced by its high resistance to nematodes (*Rotylenchus reniformis*) (El-Zik and Thaxton, 1996). On the other hand, TAMCOT 94WE-37s is characterized by absence of trichomes in stalks and leaves (Smith, 2003).

Graphic projection of cultivars and inbred lines in bidimensional space obtained through Jaccard's dissimilarity matrix shows generalized genetic inter- and intra-geographic groups distance (Figure 3).

These results confirm the genetic inter- and intra-geographic groups (African and American) variability found in this study. The African group involves most similar commercial cultivars, while American group is composed by the most dissimilar cultivars and inbred lines. The results indicate that genetic diversity observed in cotton commercial cultivars in Mozambique was low. The recent introduction of American cultivars and lineages allowed the amplification of this low genetic variability among African commercial cultivars. In order to increase cotton yield in Mozambique, using the available germplasm, the following hybrid combinations are recommended: ISA 205 x TAMCOT Sphinx, Albar BC853 x TAMCOT Sphinx e REMU 40 x TAMCOT Sphinx. On the other hand, it is important to point out that cross between parents with great performance and also divergent among them simultaneously increase the possibility to obtaining superior segregants and consequently, better chances to succeed in breeding programs searching

great crop yield. For that reason, hybridization between ISA 205 and TAMCOT Sphinx would be the most indicated since they were the most divergent, and also because of the good adaptability and stability of cultivar ISA 205 (Maleia et al., 2010), which can be complemented by the ample resistance spectrum to pests and diseases found in cultivar TAMCOT Sphinx (El-Zik and Thaxton, 1996). Therefore, the results obtained in this study will be able to aim efficiency in researches, by providing consistent data for Cotton Breeding Program in Mozambique.

Conclusions

The use of RAPD molecular markers was efficient for genetic divergence in cotton cultivars and lineages, identifying a polymorphism of 90.96 %. Graphic projection of cultivars and inbred lines in bidimensional space obtained by arithmetic complement of Jaccard's index showed low distance among African commercial cultivars, whereas American cultivars and inbred lines were the most divergent among themselves. Arithmetic complement of Jaccard's, based on 151 RAPD molecular markers, indicated that the most divergent cultivars were ISA 205 and TAMCOT Sphinx, while the most similar were BC853 and STAM 42. The hybrid combinations recommended, using the germplasm studied, for cotton breeding programs in order to obtain better yield are as follow: ISA 205 x TAMCOT Sphinx, Albar BC853 x TAMCOT Sphinx e REMU 40 x TAMCOT Sphinx.

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REFERENCES

Agarwal MS, Hrivastava N, Padh H (2008). Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Rep. 27: 617-637.

Altaf-Khan M, Myers GO, Stewart JM (2002). Molecular markers, genomics and cotton improvement. In: Kang MS (ed.). Crop Improvement. New York: Food Products Press, pp. 253-284.

Brubaker CL, Bourland FM, Wendel JE (1999). The origin and domestication of cotton. John Wiley and Sons, New York, USA.

Colombo C, Second G, Charrier A (2000). Diversity within American cassava germplasm based on RAPD markers. Genet. Mol. Biol. 23:189-199.

Cruz CD (2008). Programa Genes: Diversidade genética. Editora UFV, Viçosa, Brazil.

El-Zik KM, Thaxton PM (1996). Registration of Tamcot Sphinx cotton. Crop Sci. 36: p. 1074.

Endrizzi JE, Turcotte L, Kohel RJ (1985). Genetics, cytology and evolution of Gossypium. Adv. Genet. 23: 271-375.

Esmail RM, Zhang JF, Abdel-Hamid AM (2008). Genetic diversity in elite cotton germplasm lines using field performance and RAPD markers. World J. Agric. Sci. 4: 369-375.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genet. 131: 479-491.

Falconer DS (1989). Introduction to quantitative genetics. Longman, New York, USA.

FAO [Food and Agricultural Organization] (2009). Faostat Fao Statistics Division. Available at: http://www.fao.org/es/ess/index_en.asp.

Fryxell PA (1984). Taxonomy and germplasm resources. In: Kohel RJ, Lewis CF (eds.). Cotton. Madison: American Society of Agronomy, Inc., Soil Sci. Society of America, Inc. pp. 27-57.

Fryxell PA (1992). A revised taxonomic interpretation of *Gossypium* L. (*Malvaceae*). Rheed. 2: 108-165.

Iqbal MJ, Aziz N, Saeed NA, Zafar Y, Malik KA (1997). Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theor. Appl. Genet. 94: 139-144.

Iqbal MJ, Reddy OUK, Ez-Zak KM, Pepper AE (2001). A genetic bottleneck in the evolution under domestication of upland cotton. (*Gossypium hirsutum* L.) examined using fingerprinting. Theor. Appl. Genet. 103: 547-554.

Jaccard P (1908). Novelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44: 223-270.

Linus AA, Bebeli PJ, Kaltsikes PJ (2002). Cultivar identification in upland cotton using RAPD markers. Aust. J. Agric. Res. 53: 637-642.

Lu HJ, Myers GO (2002). Genetic relationships and discrimination of ten influential upland cotton varieties using RAPD markers. Theor. Appl. Genet. 105: 325-331.

Lukonge EV (2005). Characterization and diallel analysis of commercially planted cotton (*Gossypium hirsutum* L.) germplasm in Tanzania. PhD thesis, University of the Free State, Bloemfontein, South Africa.

Maleia MP, Vidigal Filho, PS; Kvitschal, MV, Gonçalves-Vidigal, MGC (2010). Stability and adaptability of commercial cotton cultivars (*Gossypium hirsutum* L. race *latifolium* H.) in Mozambique. Afr. J. Agric. Res. 5: 539-550.

Mahalambe N (2003). Notas técnicas sobre a cultura do algodão. IAM, Maputo, Mozambique.

Mehetre SS, Gomes M, Eapen S (2004). RAPD analysis of hybrid nature of the offspring of *Gossypium hirsutum* × *G. raimondii.* Curr. Sci. 1: 25-28.

Menezes IPP, Hoffmann LV, Alves MF, Morello CL, Barroso PAV (2008). Distância genética entre linhagens avançadas de germoplasma de algodão com uso de marcadores de RAPD e microssatélites. Pesq. Agropec. Bras. 43: 1339-1347.

Mahmood Z, Raheel F, Dasti AA, Shahzadi S, Athar M, Qayyum M (2009). Genetic diversity analysis of the species of *Gossypium* by using RAPD markers. Afr. J. Biotechnol. 8: 3691-3697.

Multani DS, Lyon BR (1995). Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome, 38: 1005-1008.

Neves OS (1965). Cultura e adubação do algodoeiro. Instituto Brasileiro de Potassa, São Paulo, Brazil.

Oliveira MSP, Silva KJD (2008). Diferenciação genética entre procedências de açaizeiro por marcadores RAPD e SSR. Rev. Bras. Frutic. 30: 438-443.

Ozyigit II (2009). In vitro shoot development from three different nodes of cotton (Gossypium hirsutum L.). Not. Bot. Hort. Agrobot. Cluj. 37: 74-78.

Patil MD, Biradar DP, Patil BVC.; Janagoudar BS, Nadaf HL (2007). Analysis of genetic diversity of cotton genotypes using RAPD PCR technique. Karnataka J. Agric. Sci. 20: 215-217.

Penna JCV (2005). Melhoramento do algodão. In: Borém A (ed.). Melhoramento de espécies cultivadas. Viçosa: UFV, pp. 15-53.

Prentice AN (1972). Cotton: With special reference to Africa. Longman, London, UK.

Rana MK, Bhat KV (2005). RAPD markers for genetic diversity study among Indian cotton cultivars. Curr. Sci. 88: 1956-1961.

Sheidai M, Shahriari ZH, Rokneizadeh H, Noormohammadi Z (2007). RAPD and cytogenetic study of some tetraploid cotton (*Gossypium hirsutum* L.) cultivars and their hybrids. Cytol. 72: 77-82.

Smith CW (2001). Registration of TAM 88G-104 high-yielding upland cotton germplasm. Crop Sci. 41: 1369-1370.

Smith CW (2003). Registration of TAM 94WE-37s smooth-leaf germplasm line of upland cotton with improved fiber length. Crop Sci. 43: 743-744.

Sneath PHA, Sokal RR (1973). Numerical taxonomy. Freeman Publ., San Francisco, USA.

Stewart JMcD (1995). Potential for crop improvement with exotic germplasm and genetic engineering. In: Constable GA, Forrester NW (eds.). Challenging the future. Proceedings World Cotton Research Conference. Melbourne: CSIRO, pp. 313-327.

Thaxton PM, Smith CW, Cantrell R (2005a). Registration of TAM 96WD-69s glabrous upland cotton germplasm line. Crop Sci. 45: 1172 -1173.

Thaxton PM, Smith CW, Cantrell R (2005b). Registration of TAM 98D-102 and TAM 98D-99ne upland cotton germplasm lines with high fiber strength. Crop Sci. 45: 1668-1669.

Thaxton PM, Smith CW, Cantrell R (2005c). Registration of TAM 94L-25 and TAM 94J-3 germplasm lines of upland cotton with improved fiber length. Crop Sci. 45: p. 1172.

Vafaie-Tabar M, Chandrashekaran S, Rana MK, Bhat KV (2004). RAPD analysis of genetic diversity in Indian tetraploid and diploid cotton (*Gossypium* spp.). J. Plant Biochem. Biotech. 13: 81-84.

Van-Esbroeck G, Bownam DT (1998). Cotton germplasm diversity and its importance to cultivar development. J. Cotton Sci. 2: 121-129.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.

Zhang J, Stewart JM (2000). Economical and rapid method for extracting cotton genomic DNA. J. Cotton Sci. 4: 193-201.

Zuo KJ, Sun JZ, Zhang JF, Nie YC, Liu JL (2000). Genetic diversity evaluation of some Chinese elite cotton varieties with RAPD markers. Acta Genetica Sin. 27: 817-823.