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Genetic divergence of colored cotton based on inter-simple sequence repeat (ISSR) markers

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The management of colored cotton is an agricultural activity widely adopted by farmers located at Brazilian semiarid region. The fiber colors currently available are still limited to green and shades of brown, however, there is possibility to broaden the variability for this trait by using accessions from *Gossypium* Brazilian bank in breeding programs. Therefore, it is necessary to know the genetic diversity of available accessions in the collection. Here, the genetic divergence in colored fiber accessions was estimated in order to identify promising candidates for further use in hybridization procedures of cotton improvement. DNA of twelve accessions were extracted from leaves and used in inter simple sequence repeat-polymerase chain reaction (ISSR-PCR) assays, using commercial oligonucleotides. The genetic divergence was estimated by clustering-unweighted pair group method with arithmetic mean (UPGMA) method. Five groups were clustered among them, three were contributive results for further use in hybridization procedures, including Brazilian cultivars and Peruvian accessions. Based on level of divergence, we suggest that lines generated from these materials could generate news shades of fiber colors in further use for selection procedures in cotton breeding.

Key words: *Gossypium*, molecular marker, variability, genetic improvement.

INTRODUCTION

Plant genetic resources represent a valorous portion of the biological diversity and contribute towards achieving security and sustainable development from preservation of cultivars, landraces, and wild relatives of important

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plant species. Germplasm banks are reservoirs with an important hole to preserve these resources for further use in both applied and basic researches. The primary importance of these banks is that they carry undefined variation that proves to be a valuable resource for breeders to develop new crop cultivars (Sachs, 2009). Maintenance of germplasm banks have generally occurred in regions and by nations associated with crop production and commerce.

Cotton (*Gossypium hirsutum* L.) represents the most important natural fiber in the world. The genetic resources of *Gossypium* are extensive, dispersed globally across five continents, and consist of approximately 45 diploid (2n=2x=26) and five allotetraploid (2n=4x=52) species (Harlan and Wet, 1971; Fryxell, 1992; Stewart, 1994; Brubaker et al., 1999). Two allotetraploid species, *G. hirsutum* L. (upland cotton) and *Gossypium barbadense* L. (Pima, Sea Island or Egyptian cotton), account for the majority of cotton world production, although the former is widely grown worldwide (>90% of the total area) due to fiber yield and broad adaptation to several environments (Campbell et al., 2010; Percy et al., 2014). The quality of fibers from *G. barbadense* L. is better than *G. hirsutum* L., however the transference of fiber traits into upland genotypes provides limited success due to hybrid breakdown and segregation toward either parents (Gore et al., 2012; Gore et al., 2014).

The major cotton collections are located in United States, Russia, Uzbekistan, China, India, Brazil, Australia, and France (Campbell et al., 2010; Percy et al., 2014). The National Cotton Germplasm Collection (NCGC) has nearly 10,000 accessions of *Gossypium* accessible at the website www.ars-grin.gov. Each one has a single plant introduction number (PI) at the time the accession enters the collection. The first major breeding effort to incorporate the development and maintenance of a cotton germplasm collection was implemented in Trinidad in 1926 by the Empire Cotton Growing Corporation (Frelichowski and Percy, 2015).

The Brazilian collection is maintained by the Brazilian Agricultural Research Corporation (Embrapa) at the National Center for Genetic Resources and Biotechnology and currently has more than 3,000 accessions, several of them used for cotton breeding program to Savanna (Cerrado) and semiarid regions.

White fibers are desirable for most Brazilian textile industries, because they provide a uniform substrate for dyeing and finishing. Onto new market trends, other niches have emerged, such as the naturally colored fibers, that required no or less dying in the textile processing, reducing the pollution to the environment due to minor residual chemical toxicant (Xiao et al., 2007; Yuan et al., 2012; Feng et al., 2013). This technology is a differentiated product and therefore with higher value-added, representing an alternative model of innovation, to promote social and sustainable transformations (Cavalcanti, 2012).

Colored fibers appear as brown or green during the fiber development process. Generally, the resistance, length and fiber percent are lower in colored than in white accessions due to the pleiotropic effects of fiber color genes (Carvalho et al., 2011; Lacape et al., 2005), but Brazilian breeders have made efforts in order to improve this trait via genetic improvement. According to Kohel (1985), several mutants conditioning fiber color and quality traits were described and mapped. Among the many fiber color variants described, almost all have had a dominant expression over the white fiber color of commercial cottons.

The Brazilian Company of Agricultural Research (Embrapa Cotton) coordinates a robust program to colored cotton, involving improvement to yield, fiber quality and environmental adaptation. Currently, six cultivars are commercially available and others top lines are in progress (Carvalho et al., 2011). Periodically, new different accessions are introduced and evaluated in selection procedures, aiming to identify promising materials to assist the colored fiber breeding. In this work the genetic divergence of new lines of cultivated and wild *Gossypium* accessions were estimated based on polymerase chain reaction-inter simple sequence repeat (PCR-ISSR) molecular markers.

MATERIALS AND METHODS

Genetic resources and ISSR-PCR assays

Seeds of twelve cotton accessions, including wild and commercial cultivars, were used in this work. The genealogy and origin of materials are found in Table 1. DNA from seeds were extracted (Dellaporta et al., 1983) and further used in PCR assays. Twelve ISSR oligonucleotides, from University of British Columbia, were used in reactions (Table 2).

The PCR assays were performed in a 0.2-ml reaction tube with total volume of 25 µl containing 20 ng of template DNA, 1 µl each of ISSR oligonucleotide (10 µM), 0.5 µl dNTP mix (10 mM), 1.4 µl $MgCl_2$ (25 mM), 1× PCR assay buffer, and 1 U Taq DNA polymerase (Fermentas). PCR amplifications were performed in Amplitherm Thermal Cyclers, with initial denaturation at 96°C/5 min followed by 30 cycles of denaturation at 96°C/45 s, annealing at 40°C/45 s, and extension at 72°C/1 min. A final extension step was added at 72°C/5 min. Amplicons were separated by agarose gel (1.5%) and photodocumented. All reactions were carried out in triplicate.

Genetic analysis of cotton accessions

Amplification products were scored as presence (1) or absence (0) of the band, for each accession. A binary data matrix was

Table 1. Genealogy and origin of cotton accessions used in ISSR-PCR assays.

Accession	Species	Genealogy	GB/Origin	Fiber color
PI 608.352	*G. barbadense*	*Wild/comensal*	Peru/GRIN	Orange brown
BRS Topázio	*G. hirsutum*	Cultivar	Paraíba, Brazil	Tan
BRS 336	*G. hirsutum*	Cultivar	Goiás, Brazil	White
BRS 200	*G. hirsutum*	Cultivar	Paraíba, Brazil	Brown
PI 435.250	*G. barbadens*	*Wild/comensal*	Peru/GRIN	Dark brown
PI 435.259	*G. barbadense*	*Wild/comensal*	Peru/GRIN	Purple brown
PI 528.086	*G. barbadense*	*Wild/comensal*	Peru/GRIN	Yellowish brown
BRS Verde	*G. hirsutum*	Cultivar	Paraíba, Brazil	Green
BRS Rubi	*G. hirsutum*	Cultivar	Paraíba, Brazil	Reddish brown
BRS 286	*G. hirsutum*	Cultivar	Goiás, Brazil	White
MO	*G. barbadense*	*Wild/commensal*	Peru/GRIN	Dark brown
V3	*G. hirsutum*	*Land race*	Paraíba, Brazil	White

GB: Germplasm bank; CENARGEN: Embrapa Genetic Resources and Biotechnology; GRIN: Germplasm Resources Information Network, EUA.

Table 2. Sequence of ISSR oligonucleotides used in genetic analysis of colored cotton fiber.

Oligonucleotide	Sequence (5'→ 3')	TNB	NBP	Polymorphism rate (%)
UBC 812	GAGAGAGAGAGAGAGAA	5	3	60
UBC 813	CTCTCTCTCTCTCTCTT	10	4	40
UBC 820	GTGTGTGTGTGTGTGTC	8	1	12
UBC 824	TCTCTCTCTCTCTCTCG	9	6	67
UBC 827	ACACACACACACACACG	13	7	54
UBC 834	AGAGAGAGAGAGAGAGYT	9	4	44
UBC 853	TCTCTCTCTCTCTCTCRT	7	5	71
UBC 866	CTCCTCCTCCTCCTCCTC	11	8	73
UBC 868	GAAGAAGAAGAAGAAGAA	9	4	44
UBC 872	GATAGATAGATAGATA	9	6	67
UBC 884	HBHAGAGAGAGAGAGAG	14	2	14
UBC 892	TAGATCTGATATCTGAATTCCC	9	5	56
Total	-	106	50	-

TNB: Total number of bands; NBP: number of polymorphic bands.

generated, from which it was calculated genetic similarity index between all individuals compared two by two, using the index agreement Jaccard (Sneath and Sokal, 1973).
The similarities (*Sji*) were calculated, according to the expression:

$$S_{ji} = \frac{a}{(a+b+c)}$$

where *a* means the presence of bands on both accessions; *b*, presence of band in first accession and absence in second and *c* is the presence in second and absence in the former.

Clustering was done using symmetric matrix of similarity coefficient. A dendrogram based on *Sij* values was constructed using clustering technique of unweighted pair group method with arithmetic mean (UPGMA). In order to eliminate the non-hierarchical effects, the cophenetic correlation coefficient was estimated (Sneath and Sokal, 1973). Analysis was performed using the software GENES, version 2013.5.1 (Cruz, 2013).

RESULTS AND DISCUSSION

The ISSR oligonucleotides used for genetic analysis were contributive to identify divergent groups in cotton accessions. An average of 9 bands/oligo was obtained, with polymorphism rate varying from 75 to 12% (Table 2).

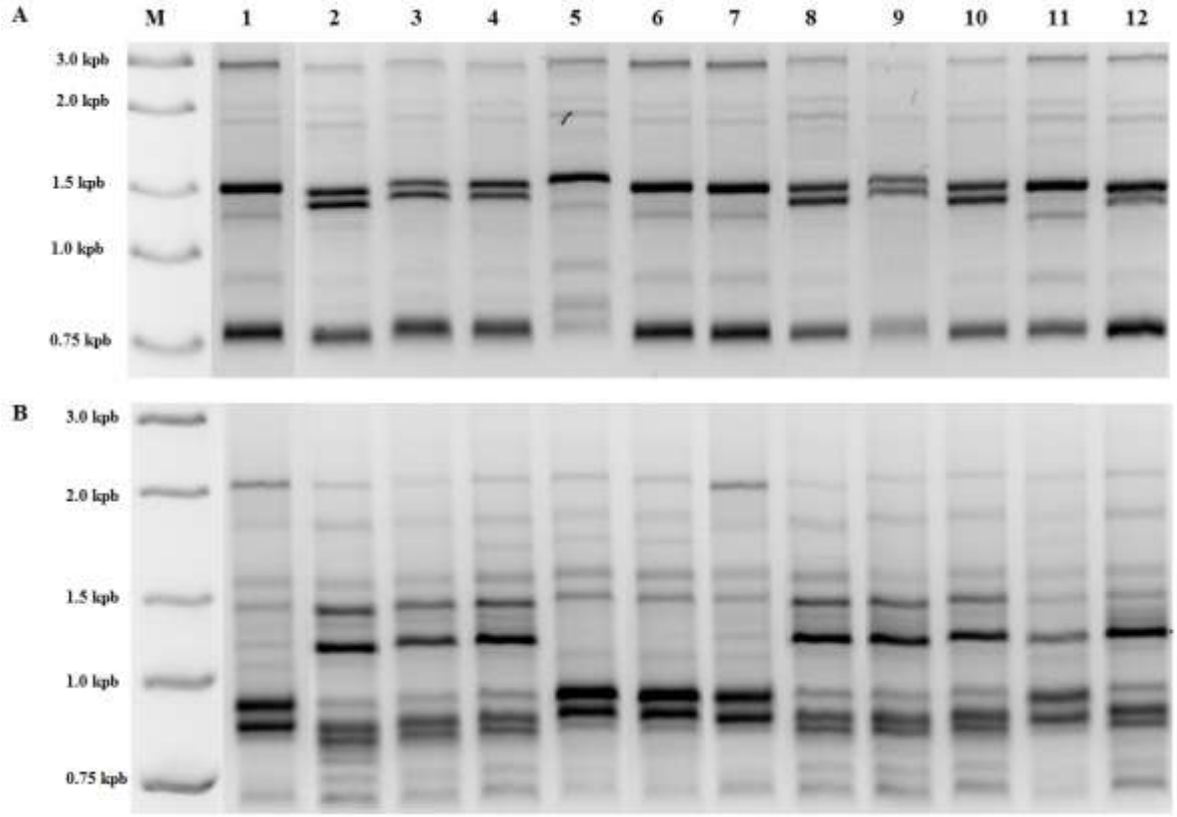

Figure 1. Band pattern obtained with oligonucleotides UBC 866 (A) and UBC 853 (B). M - marker 1 Kb (Ludwig Biotec); Accessions: 1. PI 608.352, 2. BRS Topázio, 3. BRS 336, 4. BRS 200, 5. PI 435.250, 6. PI 435.259, 7. PI 528.086, 8. BRS Verde, 9. BRS Rubi, 10. BRS 286, 11. MO, 12. V3.

UBC 866 and UBC 853, both rich in CT repetitions, were highly polymorphics, with rate of 73 and 71%, respectively. The pattern of bands obtained with these oligos is found in Figure 1.

Amplicons generated by ISSR-PCR assays were used to estimate the genetic divergence of cotton accessions by UPGMA method. A detail of fiber colors is found in Figure 2. Five groups were clustered (Figure 3), showing the following composition: Group A- compounded by five *G. hirsutum* L. accessions: BRS Topázio, BRS Verde and BRS Rubi, all colored fibers, and BRS 286 and V3, both white fiber. The peculiarity of this group is that all accessions are mid-cycle (140 to 160 days) and widely adapted to Brazilian Northeast region. V3 is a land race in pre-breeding proceeding and BRS 286 is full-sib of BRS Rubi. Both have the same parent, the drought tolerant CNPA 7H, developed by Embrapa to semiarid environments (Pedrosa et al., 2009; Carvalho et al., 2011).

About the other groups, the most relevant results were seen in B and D, both clustered wild *G. barbadense* accessions, from Peru, with fiber shades varying from cream to brown (Table 1 and Figure 3). Group C contained cvs. BRS 336 (white) and BRS 200 (brown), both *G. hirsutum,* with excellent fiber length. The last group contained only one *G. barbadense* accession, which remained distant from other Peruvians genotypes, because it is a land race with wild phenotype.

In overall, the use of accessions from groups A, B and D could be contributive to broaden the genetic basis of new lines of colored fibers. The white fiber accessions could contribute to improve the fiber qualities, providing genetic gains in selection procedures, while BRS Topázio and BRS Rubi, two Brazilian colored fibers of high yield and satisfactory fiber traits, could contribute to minimizing the deleterious effects often resulting from interspecific *Gossypium* crossings (Carvalho et al., 2011).

Based on results, there is a possibility to obtain new shades by using Peruvians accessions PI 608.352 (1), PI 435.259 (6) and PI 435.250 (5), with BRS 286 (10). For green shades, crossings between BRS Verde (8) and BRS 336 (3) is recommended. According to Morello et al.

Figure 2. Detail of fiber color of accessions: 1. PI 608.352, 2. BRS Topázio, 3. BRS 336, 4. BRS 200, 5. PI 435.250, 6. PI 435.259, 7. PI 528.086, 8. BRS Verde, 9. BRS Rubi, 10. BRS 286, 11. MO, 12. V3.

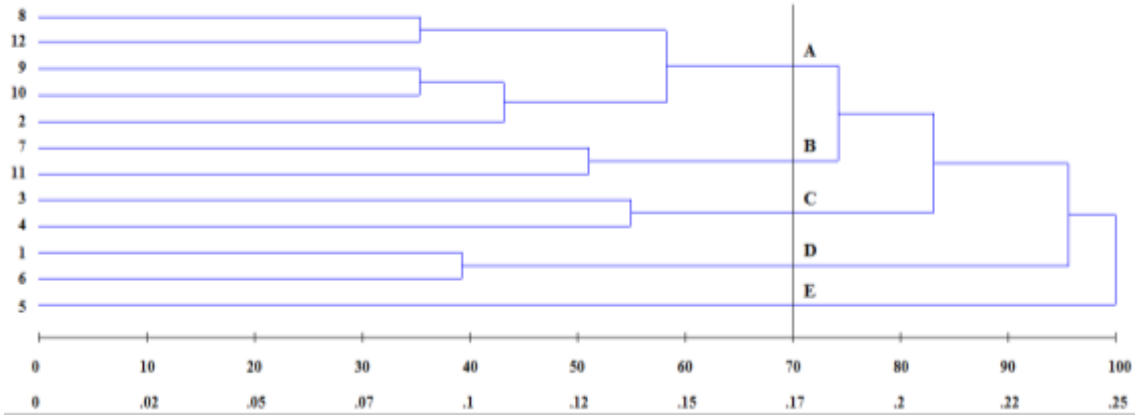

Figure 3. Dendrogram obtained by hierarchical clustering method UPGMA, from the dissimilarity matrix of 12 cotton genotypes. Cophenetic correlation coefficient: 0.80. Dotted line represents adopted selection screen based on the 70% similarity index. Access: 1. PI 608.352, 2. BRS Topázio, 3. BRS 336, 4. BRS 200, 5. PI 435.250, 6. PI 435.259, 7. PI 528.086, 8. BRS Verde, 9. BRS Rubi, 10. BRS 286, 11. MO, 12. V3.

(2012), this last cultivar has broad adaptability, high yield and excellent fiber quality.

Conclusion

Groups formed with cotton accessions offer opportunity to generate new colored lines, by using crossing works, with high yield and fiber quality for further use in selection procedures in breeding program.

Conflict of interests

The authors have not declared any conflict of interests.

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