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# GENETIC DIVERSITY STUDIES AMONG COLOURED COTTON GENOTYPES BY USING RAPD MARKERS

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**Abstract**

Estimation of genetic diversity and the relationship between varieties are valuable sources of cotton breeding. Present study employed 11 colored cottons (10 genotypes belonging to *Gossypium hirsutum* and one to *Gossypium arboreum*) and five white-linted genotypes (4 of these belonging to *Gossypium hirsutum* and one to *Gossypium arboreum*) collected from different research stations of Pakistan for genetic diversity assessment *via* RAPDs. Out of 45 decamer randomly sequenced primers applied for RAPD analysis, 25 showed polymorphism. All of the 25 primers produced 205 fragments, out of which 144 were polymorphic accounting for 70.24% of the total number of fragments. Cluster analysis showed clear-cut separation of the *Gossypium hirsutum* and *Gossypium arboreum* genotypes while, narrow genetic base was observed among colored cottons. Four clusters were formed (I, II, III and IV). All the cotton genotypes except 'CIM-446' and 'NIAB-801' clustered together. Cluster IV contained *Gossypium arboreum*. The study demonstrated the efficient and reliable use of RAPDs for analyzing genetic variation in colored and white-linted genotypes of cotton.

## Introduction

Cotton of commerce are almost all white creamy yellow to bright white. In recent years, there has been renewed interest in naturally colored cottons, which have existed for over 5000 years (Hua *et al*., 2007). These cotton varieties are mutants of plants that normally produce white fiber.

Coloured cotton is a naturally pigmented fiber that grows in shades of green and brown. Its cultivation dates back to around 2700 B.C., in Indo-Pakistan, Egypt and Peru (Vreeland, 1999). Certain disadvantages associated with the color cotton like lower yield than white varieties, limited color range, inability of the fiber to be machine spun and uncertain market demands lead to their reduced cultivation and finally the industrial revolution and its newly invented looms, displaced short-fibered colored cotton by longer white cotton.

After disappearing for about a century, naturally colored cotton suddenly reappeared as environmentally friendly cotton in the early 1990's due to the efforts of Sally Fox, an inventor from California. She cultivated long fiber colored cotton, and created her own patented cotton called "Fox Fibre". Most Fox Fibre is grown organically (called Fox Fibre Colorganic) and requires minimal processing (Vreeland & James, 1987; 1993). It is used in a wide variety of products, including clothing, bedding and furniture. To date, studies involving colored cotton revealed a long list of benefits associated with colored cotton that greatly outweigh afore-mentioned disadvantages. Naturally colored cottons are unique, because they reduce the use of synthetic dyes. They are popular with environmentally conscious consumers and those who are allergic to the dyes in regular cotton. Naturally, colored cottons have a noticeably soft texture and the color does not

fade but deepens with washing (Narayanan *et al*., 1996). Cotton farmers use approximately 23% of the world's insecticides and 10% of the world's pesticides to combat pests such as the boll weevil. These compounds not only harm the workers who use them but also leach into the soil, reaching groundwater, rivers and streams, killing fish and contaminating livestock. Naturally, colored cottons on the other hand, resist pests, salt and drought better, so they reduce toxic pesticide application thereby causing less environmental pollution and are very adaptable to dry land and organic farming (Fox, 1987; Lee, 1996). These cotton varieties also eliminate the bleaching and dyeing costs and an excessive energy usage. Besides being low yielder, colored lint brings premium price for its growers; it sells for $1.30 to $1.40 per pound, compared with 65 cents to 70 cents for white cotton.

Today China is the leading producer of colored cotton in the world (Chen *et al*., 2007). On commercial scale, various shades of only brown and green colors are available. Yellow is expected to be released in the next few years, while reports of blue color cotton also appeared in Uzbekistan and other central Asian countries. A colored cotton-breeding programme in the USA is said to have been undertaken under organic conditions (Backe, 1994). However, as most of the colored cotton work is being done in the private sector so seeds of improved colored cotton varieties are inaccessible.

Due to recent increasing demands of colored cotton, scientists are putting concerted efforts to breed new colored cotton varieties with longer, stronger fibers and higher yields (Basbag & Temiz, 2004). Although it is widely assumed that genetically diverse parents can generate superior progeny, only a few studies have reported the development of successful cultivars from distant crosses. Recently, new coloured cotton varieties with all the desirable attributes, such as Kc 94-2, have been developed which overcome many of the problems of the parents.

These breeding strategies for colored cotton improvement are supplemented with the biotechnological approaches including tissue culture and genetic transformation. An improved green variety should be available in about two years and a genetically engineered blue version is on the way (Qui, 2000; Sheng-Wei *et al*., 2006).

The DNA-based markers have been exploited extensively for molecular characterization and DNA finger printing of cotton. Several types of PCR-based DNA markers have been utilized in cotton genome research, including RAPD, AFLP, RGA, and SRAP (Zhang *et al*., 2008). Among these, RAPDs being relatively simple, less expensive and reliable and are the most widely used molecular technique well suited for estimating similarity and differences among different cotton cultivars (Preetha & Raveendran, 2008; Sheidai *et al*., 2007; Rana & Bhat, 2005; Abdel Ghani & Zaki, 2003; Lu & Myers, 2002).

However, very few reports employed the molecular marker approaches for colored cotton studies (Punish & Raveendran, 2004). In this context, present investigation was carried out to the genetic divergence in coloured and selected white-linted cottons using RAPDs and to estimate the genetic base of these germplasm to be used in future cotton breeding program in Pakistan.

## Plant materials and DNA extraction

**Plant materials:** Sixteen genotypes including eleven colored and five white-linted cotton genotypes collected from different breeding stations/ research institutes of Pakistan were evaluated in the investigation (Table 1).

**Table 1. List of cotton genotypes used in the study.**

| Sr. No. | Name | Research station | Parentage | Type | Ploidy Level |
|---|---|---|---|---|---|
| 1. | Reddish Semi Okra | CRS[1], Bahawalpur | Unknown | Reddish | Tetraploid |
| 2. | Brown Okra | CRS[1], Bahawalpur | Unknown | Brown | Tetraploid |
| 3. | Off White | CRS[1], Bahawalpur | Unknown | Off White | Tetraploid |
| 4. | Brown | CRS[1], Bahawalpur | Unknown | Brown | Tetraploid |
| 5. | Brown cotton | AARI[2], Faisalabad | Unknown | Brown | Tetraploid |
| 6. | Brown cotton | Uni. of Agri. Faisalabad | Unknown | Brown | Tetraploid |
| 7. | American Light Khaki | AARI[2], Faisalabad | Unknown | Light Khaki | Tetraploid |
| 8. | American Light Green | AARI[2], Faisalabad | Unknown | Light Green | Tetraploid |
| 9. | American Khaki | AARI[2], Faisalabad | Unknown | Khaki | Tetraploid |
| 10. | Desi Khaki | AARI[2], Faisalabad | Unknown | Khaki | Diploid |
| 11. | Desi White | Uni. of Agri. Faisalabad | Unknown | White | Diploid |
| 12. | American Dark | AARI[2], Faisalabad | Unknown | Dark Brown | Tetraploid |
| 13. | CIM-707 | CCRI[3], Multan | CIM-243 x 738-6/93 | White | Tetraploid |
| 14. | BH-118 | CRS[1], Bahawalpur | BS-48 x 829-4/90 | White | Tetraploid |
| 15. | CIM-446 | CCRI[3], Multan | CP 15/2 x 512 | White | Tetraploid |
| 16. | NIAB-801 | NIAB[4], Faisalabad | (NIAB-78 x RIBAP 288) 25 kr radiation | White | Tetraploid |

[1]CRS, Bahawalpur: Cotton Research Station, Bahawalpur, [2]AARI, Faisalabad: Ayub Agri. Research Institute, Faisalabad, [3]CCRI, Multan: Central Cotton Research Institute, Multan, [4]NIAB: Nuclear Institute of Agriculture and Biology

**DNA isolation:** For each cultivar, genomic DNA was isolated as described by Khan *et al*., (2004). DNA was quantified by a spectrophotometer (CECIL CE 2021 2000 Series, Cambridge, UK) at 260 nm. For quality assessment, DNA was electrophoresed on 0.8% agarose gel (Khan *et al*., 2004). The dilutions of extracted DNA (15 ng $L^{-1}$ in $d_3H_2O$) were verified again by spectrophotometery.

**RAPD analysis:** Polymerase chain reaction was performed in a thermal cycler (Eppendorf AG No. 533300839, Germany) with 15 ng DNA as template. Amplification reactions contained 1.0 U of Taq DNA polymerase (MBI, Fermentas, Vilnius, Lithuania), 50 mM KCl, 3 mM MgCl2, 100 lM of each dNTP, 0.2 lM decamer primer (Gene Link Company, Hawthorne, NY, USA). The DNA amplification protocol was 94°C for 5 min., followed by 40 cycles of 94°C for 1 min., 36°C for 1 min., 72°C for 2 min., and finally 72°C for 10 min. All amplification products were electrophoresed on 1.2% (w/v) agarose gels at 80 V for 2 h, stained with Ethidium bromide, visualized in a UV transilluminator at 300 nm and photographed in a gel doc. System (SynGen, Synoptics Ltd, UK).

**Data analysis:** The DNA fragments amplified by a given primer were scored as present (1) or absent (0) for all the genotypes studied. Both monomorphic and polymorphic primers were included in the analysis and only reproducible fragments were considered. Coefficient of similarity among cultivars was calculated according to Nei & Li (1979). A dendrogram based on these similarity coefficients was constructed by using Unweighted Pair Group Method of Arithmetic means (UPGMA). This similarity matrix was analyzed using NTSYS-pc 2.01 (Rohlf 1997) and clustered with UPGMA (Unweighted Pair Group Method of Arithmetic Average) algorithm to determine the genetic relationships among the 16 cotton genotypes.

## Results and Discussion

Forty-five random decamer primers were used for analyzing the RAPD patterns of 16 (including 11 coloured cotton and 5 white-linted) genotypes. For the assessment of variation among-cultivars, 45 RAPD primers were applied but only 25 primers proved to be polymorphic. The selected 25 polymorphic primers generated 205 bands with an average of about 8.2 bands per primer, where 144 (70.24%) of them were polymorphic over the 16 cultivars assayed. The total number of bands resolved per primer ranged from a minimum of 4 (GLC-5) to a maximum of 18 (GLD-18). These results are in agreement with the report of Anolles (1994) who observed that up to 20 amplified fragments could be produced by RAPD analysis. The number of polymorphic bands scored for individual primers ranged from 2 (GLD-11 and GLD-16) to 14 (GLC-8) with an average of 5.76 bands. The number of polymorphic bands was low as compared to the previous studies on colored cotton where Punitha & Raveendran (2004) observed 76.31% polymorphic bands.

Correlation refers to the method used for the measurement of mutual relationship between two or more characters. In the present study, it was found that the total number of bands generated had no correlation with the proportion of polymorphic bands. For example, primer GLD-18 showed 18 bands but the level of polymorphism observed was 72.22%, as against primer GLA-4 giving 11 bands with 90% polymorphism. According to the similarity index the maximum similarity was observed between colored cotton genotypes, Reddish Semi Okra and Brown Okra (94.12%), while minimum similarity was observed between Desi Khaki (diploid colored cotton) and CIM-446 (tetraploid white cotton) (50.49%) followed by Off White (tetraploid colored cotton) and Desi Khaki (diploid colored cotton) (51.96%). The observed similarity was higher than previously observed by Punitha & Raveendran (2004) while working on colored cotton (87.3%).

Nei's similarity was used to carry out the cluster analysis and to generate a dendrogram showing the relationships among the selected genotypes. All 16 genotypes were grouped into four clusters (Fig. 1). The first cluster comprised of six (tetraploid) colored cottons and one white-linted (tetraploid) genotype (BH-118), collected from CRS, Bahawalpur and AARI, Faisalabad. The second cluster on the other hand consisted of only 2 tetraploid genotypes viz; American Dark (colored cotton) and CIM-707 (white-linted cotton). This genetic differentiation of cotton genotypes is in agreement with earlier reports (Iqbal *et al*., 1997). Both of the diploid genotypes used in the study were clustered together, although one was colored while the other was white-linted cotton. The diploid status of both the genotypes led to a clear-cut differentiation from tetraploid ones. Recently developed Chinese coloured germplasm also has low genetic base as compared to the basic coloured cotton lines (Sun et al, 2009). Present results depicted efficient use of RAPD technique to determine genetic distance among genotypes.

It is therefore concluded that in spite of the relatively low level of genetic variation present in the colored cotton genotypes, RAPDs can be used as a reliable technique for genotype identification as well as inter specific variation. Thus by judicious augmentation of these systems of evaluation, rapid progress can be achieved in developing colored cotton genotypes with important quality parameters.

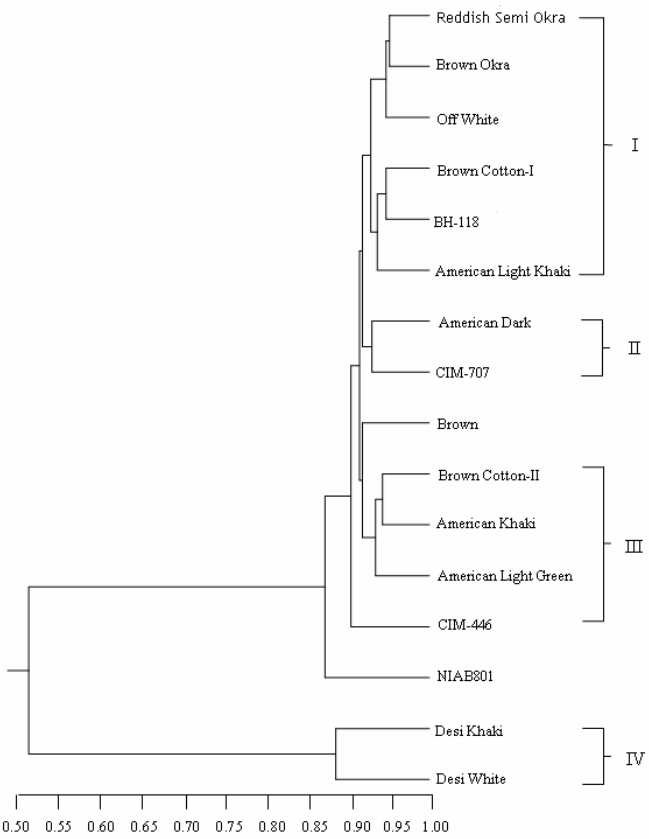


Fig. 1. UPGMA dendrogram illustrating the genetic relationships of the 16 Cotton genotypes (11 cloured and 5 white).

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