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ESTIMATION OF GENETIC DIVERSITY AND HYBRID IDENTIFICATION IN AMERICAN COTTON (*GOSSYPIUM HIRSUTUM* L.) BY PCR BASED RAPD ANALYSIS

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ABSTRACT:- DNA of 15 cotton genotypes comprising five parents and their ten F_1 hybrids was extracted successfully from their immature leaves to estimate genetic diversity and hybrid identification. Out of the 26 RAPD primers used for DNA amplification, 14 primers produced polymorphic bands, while 12 produced monomorphic bands. These 14 primers yielded total 96 RAPD markers, out of which only 40 were found polymorphic which resulted in 41.66% polymorphism among the genotypes studied. The number of RAPD markers produced ranged from 4 to 12. Average number of 6.40 bands per primer was observed. On the whole, 87.50% genetic identity was found in material under investigation and it ranged from 68.07% to 96.88%. The polymorphism detected among the genotypes was employed as marker for hybrid identification. Comparing the banding patterns of RAPD of the parents with their particular hybrids, true hybrids were identified by six primers namely GLA-7, GLA-8, GLA-15, GLA-19, GLD-3 and GLD-5. Consequently, RAPD analysis can be used as a marker technique to ascertain the hybrid nature of offsprings and to evaluate parental lines in terms of genetic diversity.

Key Words: American Cotton; Genetic Diversity; Hybrid Identification; PCR; RAPD Analysis; Pakistan.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an important crop which provides the world's most important natural textile fibre and is the second most important oil seed crop in the world. It is commonly called as 'white gold' (Kumar et al., 2007). Pakistan is an agricultural country and cotton crop is the backbone for its foreign exchange earnings. It plays an important role in the economy of Pakistan by contributing 7.0% to the value added in agriculture and 1.5% to the GDP. About 50% of the total foreign exchange earnings obtained through exports of raw cotton and cotton products. Local cotton industry employs a huge labor force, and provides substantial amount of edible oil and cake meal (GoP, 2013).

Although, Pakistan is self sufficient in cotton production but still lacks in some fibre quality traits and per unit area production as compared to many other cotton growing countries of the world. Improvement in that direction is possible, if the existing scarce genetic sources are properly utilized in breeding programme. The first step being is the selection of parental lines with broad genetic base which are

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used in any hybridization programme to produce genetically modified and potentially rewarding new genotypes of cotton. Conventional breeding methods have contributed much in the evolution of new cotton varieties, however the use of molecular markers has been proposed as an alternative procedure for the identification of promising parental lines and identification of hybrids (Mehetre et al., 2004a). Molecular markers have equipped the plant breeders with a rapid and powerful tool to select parental lines with better potential and broad genetic diversity. In conventional breeding, true hybrid can be identified by comparing morphological traits of the parents with their hybrids by means of growing them in the field that is a lengthy and time consuming process. For this reason, an alternative method is needed for quick and reliable evaluation of true hybrids. Molecular markers analyses is more reliable and alternative way for identification of hybrids as these are independent of environmental variations because genetic interactions are anticipated on genotype basis. Several molecular techniques for instance SSR (microsatellite), ISSR, RFLP, AFLP, SNP and RAPD are being used for analysis of genetic diversity and evaluation of hybrids among different crop cultivars. The developments of DNA markers have greatly facilitated genetic studies in plant, animal and prokaryotic genomes (Mullis, 1990; Smith et al.,1990; Erlich et al., 1991; Khan et al., 2004). Various developments in DNA marker technology and marker-assisted selection have made cotton breeding more efficient and productive.

Among all these techniques, Random Amplified Polymorphic DNAs (RAPD) markers are more efficiently being used for genetic mapping and in plant breeding programmes for development of new cultivars (Williams et al., 1990). RAPD produced consistent results in various crops with optimized conditions and have the potential to be employed for phylogenetic relationships and taxonomic classification. This technique is technically the simplest, less expensive, fast, requires a small quantity of DNA and needs no prior information of target sequences to design primers. RAPD markers have been utilized by a number of scientist like Iqbal et al. (1997), Zuo et al. (2000), Rahman et al. (2002), Thangaraj et al. (2003), Mehetre et al. (2004a & b), Rana and Bhat (2005), Sheidail et al. (2007), Khan et al. (2010; 2011), Surgun et al. (2012) and Dongre et al. (2012) in cotton to quantify the genetic diversity and also for the identification of F_1 hybrids. RAPD analysis has also been utilized in other crops like maize (Iva et al., 2005), rice (Zhang et al., 1995; Mani et al. 2010; Skaria et al. 2011), wheat (Khan et al., 2005; Awan et al., 2008; Milad et al., 2011) for their genetic characterization and hybrid identification. Therefore, the present research work was conducted to study the genetic variability among selected cotton genotypes and the hybrid status of the offsprings at DNA level by using RAPD technique.

MATERIALS AND METHOD

Development of Experimental Material

The present studies were conducted at the Centre of Agricultural Bio-

chemistry & Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan. The experimental material was developed by crossing five parental genotypes (Coker-4601, MNH-552, S-14, Stoneville and Allepo-41) to generate 10 hybrids (Allepo-41 x Stoneville, Allepo-41 x S-14, Allepo-41 x MNH-552, Allepo-41 x Coker-4601, Stoneville x S-14, Stoneville x MNH-552, Stoneville x Coker-4601, S-14 x MNH-552, S-14 x Coker-4601 and MNH-552 x Coker-4601). The seed of parents was sown in earthen pots placed in greenhouse. During germination and growth, optimum growth conditions i.e., light and temperature were given and recommended agronomic practices were followed. At the time of flowering the parents were crossed to generate 10 F_1 crosses (direct). All desired preventive measures were taken to evade contamination of genetic material during crossing. At maturity, seeds obtained from the parents and crossed bolls were grown in plastic pots in the Ware House to obtain young fresh leaves as experimental material for DNA extraction.

DNA Extraction

This was done following CTAB method (Doyle and Doyle, 1990). The concentration of extracted genomic DNA of the 15 samples was measured on spectrophotometer (CECIL CE 2021-2000 Series, Cambridge, UK) at 260nm wavelength. DNA quality was assessed by running 5µL DNA mixed with 3µL bromophenol blue dye on 0.8 % agarose gel prepared in 0.5X TBE buffer. The DNA samples giving a smudge on the gel were rejected. Extracted DNA was diluted to make a PCR working solution of approximately 15ngμL^{-1} in d_3H_2O.

PCR Amplification and RAPD Analysis

In PCR amplification with RAPDs, the reaction condition is very important for obtaining reproducible and consistent results. For RAPD analysis, template DNA, Taq DNA polymerase and $MgCl_2$ with different concentrations were used to standardize the PCR conditions to find clear and reproducible RAPD banding pattern, as these factors may influence the reproducibility of RAPD banding pattern. Template DNA with five different concentrations of (7, 10, 15, 20, 25ng 25μL^{-1}) was tested but concentration of 15ng 25μL^{-1} produced more consistent and reproducible banding pattern. Similarly, $MgCl_2$ with eight different concentrations (1.7, 2.0, 2.3, 2.5, 2.8, 3.0, 3.3, 3.5 mM) was used but 3mM was found the best for constant results. Moreover, 1.0U of Taq DNA polymerase, provided best result for amplification of template DNA after studying different concentrations (0.2, 0.5, 0.7, 1.0, 1.5 unit 25μL^{-1}. Other conditions of PCR reaction were kept steady to achieve stable and reproducible RAPD banding pattern in each repeat.

The DNA amplification reactions were carried out with 26 primers (Gene Link Inc., UK) in a thermal cycler (Eppendorf AG No. 5333 00839) by using 15ng DNA as template. The polymerase chain reaction (PCR) was carried out with 25µL of reaction volume containing 8.3µL of d_3H_2O, 2.5µL of 10x Taq polymerase buffer (MBI, Fermentas, Vilnius 2028, Lithuania), 2.5µL of gelatin (0.025%), 3.0µL of 3mM $MgCl_2$ (MBI, Fermentas), 4.0µL of dNTPs

(MBI, Fermentas, Vilnius 2028, Lithuania), 2.0µL of oligonucleotide primer, 2.5µL of template DNA and 0.2 µL of Taq DNA polymerase (Fermentas).

The amplification of DNA include: initial extended step of denaturation at 94°C for 5 min followed by 40 PCR cycles of denaturation at 94°C for 1 min, primer annealing at 36°C for 1 min, extension at 72°C for 2 min and after 40 cycles final extension step for 10 min at 72°C.

All amplified products were electrophoresed in 1.2% agarose gel, stained with 1% ethidium bromide solution, at 80 V for approximately 2h using 0.5X TBE buffer along with a DNA marker of known molecular size. The fingerprints were examined under ultra violet transilluminator and photographed using the Syn Gene Gel Documentation System.

Data Analysis

All apparent RAPD fragments were calculated for each primer and counted as present (1) or absent (0) for each sample. Ambiguous bands which were not clearly distinguished were not counted. The bands were calculated from top to the bottom of each lane. Amplification profiles of each of 15 cotton samples were compared with each other. A Software Popgen Version 1.31 (Yeh et al., 1999) was used to calculate genetic identity matrix between genotypes studied following Nei (1978). This matrix was then used to produce a dendrogram by unweighted pair-group method of arithmetic averages analysis (UPGMA).

RESULTS AND DISCUSSION

Polymorphism

The genomic DNA of all the 15 cotton genotypes was of good quality for RAPD analysis as determined by gel electrophoresis. Out of the 26 RAPD primers used for DNA amplification, 14 primers produced

Table 1. Selected primers, their sequences and level of polymorphism.

Primers	Sequences (5'----3')	Total bands amplified	Polymorphic bands	%age polymorphism
GL DecamerA -4	AATCGGGCTG	12	4	33.33
GL DecamerA -7	GAAACGGGTG	8	4	50.00
GL DecamerA -8	GTGACGTAGG	4	1	25.00
GL DecamerA -9	GGGTAACGCC	7	2	28.50
GL DecamerA -11	CAATCGCCGT	10	7	70.00
GL DecamerA -12	TCGGCGATAG	8	3	37.50
GL DecamerA -13	CAGCACCCAC	8	3	37.50
GL DecamerA -15	TTCCGAACCC	6	1	16.60
GL DecamerA -17	GACCGCTTGT	6	2	33.33
GL DecamerA -19	CAAACGTCGG	5	4	80.00
GL DecamerA -3	GTCGCCGTCA	4	3	75.00
GL DecamerA -5	TGAGCGGACA	6	1	16.60
GL DecamerA -16	CACACTCCAG	6	2	33.33
GL DecamerA -8	GTCCACACGG	6	3	50.00
Total		96	40	41.66

polymorphic bands, while 12 produced monomorphic bands (Table 1). There are three types of bands i.e., monomorphic, polymorphic and unique bands. Bands produced in all the genotypes are called monomorphic while those produced in one or more but not in all genotypes are called polymorphic and bands produced in at least one genotype and not in any other are called unique bands. All the 14 selected random decamer primers generated 96 RAPD markers for the assessment of genetic variability between the genotypes studied. The number of RAPD markers ranged from 4 as produced by primers GLA-8 and GLD-3 (Figure 1 and 2) to 12 as yielded by primer GLA-4 (Figure 3). Among the 15 genotypes studied, hybrid H_1 produced maximum amplifications products (91) while minimum number (77) were yielded by hybrid H_6 with these 14 primers. Average number of bands yielded per primer was 6.40. Out of 96 RAPD markers amplified only 40 were polymorphic which resulted in 41.66% polymorphism. All the 14 primers yielded polymorphic products; however the level of polymorphism percentage assorted with each primer range from 16.6% to 80% (Table 1 Figures 1 - 4). All genotypes showed a varying degree of genetic diversity based on their amplification profile. Reactions were duplicated from time to time to check the consistency of amplified products.

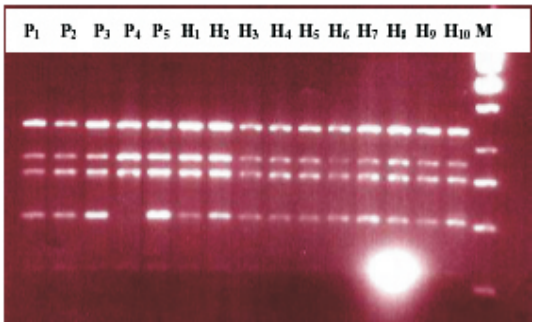

Figure 1. PCR amplification profile of cotton genotypes generated by primer GLA-8

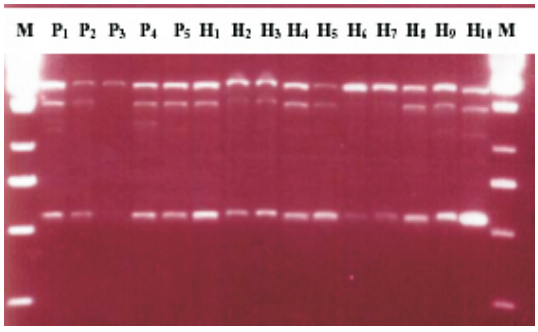

Figure 2. PCR amplification profile of cotton genotypes generated by primer GLD-3

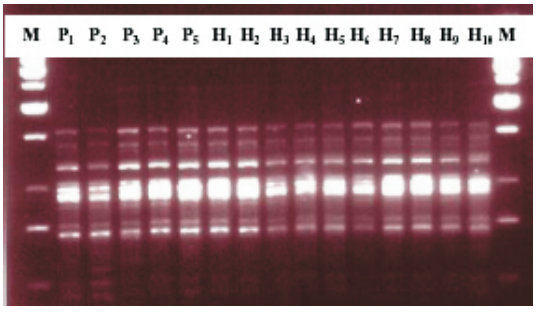

Figure 3. PCR amplification profile of cotton genotypes generated by primer GLA-4

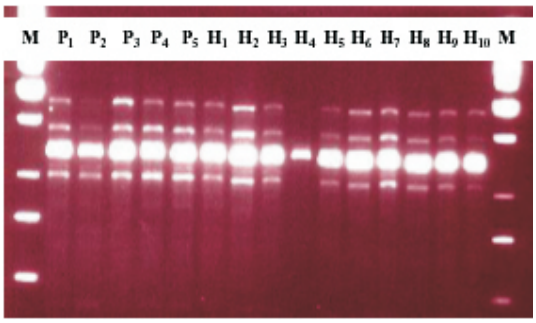

Figure 4. PCR amplification profile of cotton genotypes generated by primer GLA-19

Genetic Diversity

The percentage genetic identity among the 15 genotypes using RAPD primers ranged from 68.75% to 96.88%. These values were calculated after scoring the presence or absence of bands (Table 2). The highest genetic identity was found between MNH-552 and Allepo-41 x S-14 (96.88%) followed by S-14 x MNH-552 and Coker-4601 x MNH-552 (95.83%), Stoneville x S-14 and S-14 x MNH-552 (94.79%) and so on. The lowest genetic identity was observed between Allepo-41 x Coker-4601 and Stoneville x MNH-552 (68.75%). Overall, 87.50% average genetic identity was found in the material under investigation. As a result, all the genotypes showed a narrow genetic diversity.

Polymorphism other than RAPDs may also be due to change of base pairs in the sequences of priming sites, deletions or insertions in the priming sites that cause priming sites unable to carry out amplification. Deletions or insertions may also alter the size of a DNA fragment without disturbing its amplification (Williams et al., 1990). In this study, ambiguous polymorphism was also observed but not considered. It might have resulted from low discrimination by a primer between alternative priming site of faintly different nucleotide sequences. Bands of the same size amplified from different genotypes were considered as the same loci and bands of different sizes were considered as different loci. Amplification products of the same size resulted from different varieties might have homologous sequences. RAPD markers are inherited in Mendelian manner, but unlike other DNA level markers which are co-dominant, RAPD markers have complete dominant phenotypes (Rehman and Zafar, 2001).

In this study, polymorphic bands were generated by all primers. Iqbal et al. (1997) reported 98% of the primers yielded polymorphic bands and 89.1% polymorphism among all the 23 varieties of cotton in their study. A low level of polymorphism (41.66%) was examined among all the genotypes in the study. Lu and Myers (2002) also reported 13.5% polymorphism among all the ten varieties of cotton. Rahman et al. (2002) studied 66.2% polymorphism among 27 cultivars of cotton. This study showed narrow genetic base in the material under investigation, which might be due to almost similar genetic makeup of genotypes. The average genetic identity of 89.55% among 27 cotton genotypes was reported by Rahman et al. (2002). Vafai-Tabar et al. (2003) found 79% genetic identity in Indian cotton varieties. Linos et al. (2002) found a genetic identity range of 61.4%-92.2% in 28 upland varieties of cotton, representing a relatively narrow genetic base. Rana and Bhat (2005) reported 74% average genetic resemblance among 41 *G. hirsutum* cultivars. Khan et al. (2011) observed 88.89% - 42.48% genetic similarities among the 11 cotton accessions based on RAPD analysis. Surgun et al. (2012) calculated the rate of polymorphism between Turkish cotton genotypes which was 18.1%, and genetic similarity between any 2 genotypes ranged from 90.2% to 96.5% through RAPD-PCR analysis.

Contradictory results on the level of polymorphism in different studies could be accredited to the nature of

Table 2. Genetic identity matrix

Pop. ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 MNH-552	****	0.8854	0.9062	0.9167	0.9271	0.9167	0.9688	0.8646	0.8125	0.8854	0.8125	0.9062	0.9275	0.9167	0.8958
2 Allepo-41		****	0.8542	0.8438	0.8542	0.8854	0.8750	0.9167	0.8229	0.8958	0.8021	0.8750	0.8854	0.8438	0.8646
3 Stoneville			****	0.9062	0.8958	0.9062	0.9375	0.8333	0.7604	0.8542	0.8229	0.9167	0.9062	0.8854	0.8854
4 S-14				****	0.9062	0.9375	0.9271	0.8646	0.7917	0.8854	0.8542	0.8854	0.9167	0.8958	0.9167
5 Coker-4601					****	0.9271	0.9375	0.8542	0.8021	0.8750	0.8021	0.9167	0.9271	0.8438	0.9062
6 Allepo-41 x Stoneville						****	0.9271	0.8646	0.8333	0.9062	0.8333	0.9271	0.9375	0.8750	0.9167
7 Allepo-41 x S-14							****	0.8542	0.8021	0.8750	0.8438	0.9375	0.9271	0.8854	0.9062
8 Allepo-41x MNII-552								****	0.8438	0.8750	0.7812	0.8333	0.8438	0.8646	0.8229
9 Allepo-41 x Coker-4601									****	0.8854	0.6875	0.8229	0.8542	0.8125	0.8333
10 Stoneville x S-14										****	0.8021	0.8750	0.9479	0.8646	0.9062
11 Stoneville x MNH-552											****	0.8021	0.8125	0.8125	0.8125
12 Stoneville x Coker-4601												****	0.9271	0.8646	0.9271
13 S-14 x MNH-552													****	0.8958	0.9583
14 S-14 x Coker-4601														****	0.8750
15 Coker-4601 x MNH-552															****

the genetic makeup of all the cotton genotypes used in these studies. A low level of polymorphism was observed in the present study as compared to other studies might be due to the narrow based genetic material used in this study that belonged to same cotton species (*Gossypium hirsutum* L.).This indicates the fact that the more commonly cultivated genotypes have relatively narrow genetic base, which may be true due to the fact that universal parents are usually used for the development of new varieties of cotton. Therefore, the genetic base of recurrently cultivated varieties of cotton should be broadened by using uncommonly cultivated species of cotton in plant breeding programmes.

Cluster Analysis

Cluster Analysis of 15 genotypes of cotton by means of UPGMA revealed that Stoneville x MNH-552 and Allepo-41 x Coker-4601 were quite diverse from all others, tracked by a group of three genotypes (Allepo-41 x MNH-552, Allepo-41 and S-14 x Coker-4601). Excluding these five genotypes, the leftover was genetically more alike(Figure 5).

The most evident pairs observed were S-14 x MNH-552 vs. Coker-4601 x MNH-552, S-14 vs. Allepo-41 x Stoneville and MNH-552 vs. Allepo-41 x S-14. By comparison of these genetic interactions (Figure 5) to known parentage showed diminutive adequacy of grouping these genotypes. For example, Allepo-41 x S-14 grouped with MNH-552 but not with one of its parents (Allepo-41 or S-14). It might be due to involvement of common parents in the development of these genotypes. Same situation was observed in S-14 and Allepo-41 x Stoneville pair. However, Allepo-41 x MNH-552 paired with one of its parent (Allepo-41). Similarly S-14 x MNH-552 paired with Coker-4601 x MNH-552 because both have one parent (MNH-552) in common. A group of 3 genotypes Stoneville x Coker-4601, S-14 x MNH-552 and Coker-4601 x MNH-552 was closely linked with their two parents S-14 and Coker-4601 (Figure 5).

Hybrid Identification

The polymorphism examined between the genotypes was employed as marker for identification of hybrids. By comparing the banding pattern (bp) of RAPD markers of the parents with their pertinent hybrids, true hybrids from selfed progeny were identified. RAPD markers were categorized according to the presence or absence of bands into seven types as shown in Table 03. Similar RAPD banding patterns for hybrid identification were reported by Mehetre et al. (2004a) in their study. All the 10 F_1 hybrids were identified by different primers GLA-7, GLA-8, GLA-15, GLA-19, GLD-3 and GLD-5. Primer GLA-8 amplified a polymorphic marker of approximately 100bp in female parent Allepo-41 (P_1) and F_1 hybrid Allepo-41XMNH-552 (H3) but it was absent in male parent MNH-552 (P4) that helped to identify this hybrid. Similarly this polymorphic marker also helped to identify three other hybrids, H_6, H_8, and H_{10} Figure 01. The primer GLD-3 generated two polymorphic bands of 250bp and 125bp in male parent MNH-552 (P_4) and F_1 hybrid S-14XMNH-552 (H_1) but that band was not amplified in female parent S-14 (P_3), so hybrid

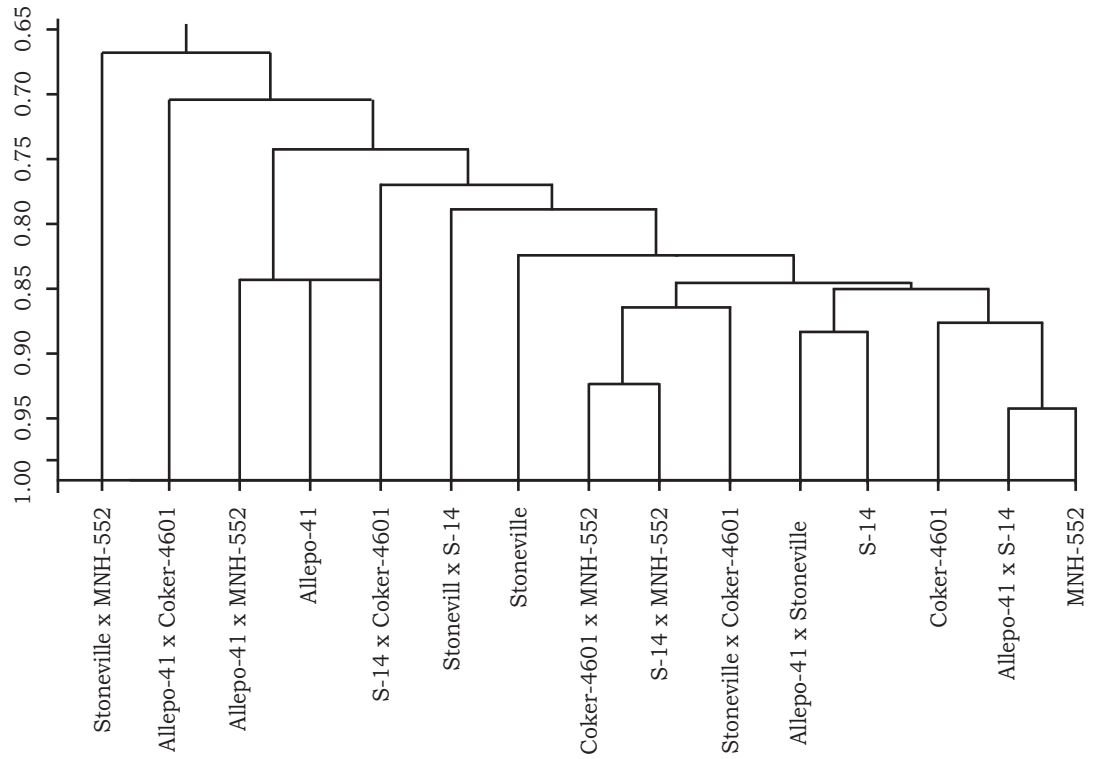

Figure 5. Dendrogram of 15 cotton genotypes based on UPGMA method

was identified (Figure 2). These two amplicons, amplified by GLD-3 also identified three other hybrids H_2, H_5, and H_9. Similarly, the hybrids H_1, H_3, H_4 and H_7 were identified by the primers GLA-7, GLA-15, GLA-19 and GLD-5. Of all the RAPD markers observed, Type IV are of particular importance because the occurrence of male-specific bands in the hybrid reveals that the hybrid is genuine, as it eliminates the prospect of self-pollination. Type I bands (Table 3) in the hybrids might have instigate due to reorganization of DNA sequences because of abolition or recombination between the chromosomes of parents during meiosis (Mehetre et al., 2004a). Banding pattern of type II and type IV markers were followed to identify the hybrids. Mehetre et al. (2004b) identified a genuine hybrid using RAPD markers. Dongre and Parkhi (2005) successfully used three DNA markers i.e. RAPD, ISSR and microsatellite for identification of a hybrid cotton H '6' and its parents G.Cot.10 (male) and G.Cot.100 (female). Ali et al. (2008),

Table 3. Classification of RAPD markers according to presence or absence of bands

Types	Property		
	Female	Offspring	Male
I	1	1	1
II	1	1	0
III	1	0	1
IV	0	1	1
V	1	0	0
VI	0	1	0
VII	0	0	1

Tafvizei et al. (2010) and Dongre et al. (2012) also identified genuine hybrids in cotton by comparing banding patterns of RAPD markers of hybrids with their pertinent parents. Consequently, RAPDs can be used as a marker to ascertain the hybrid nature of the offspring and for the evaluation of the parental lines and their hybrids.

LITERATURE CITED

Ali, M.A., M.T. Seyal, S.I. Awan, S. Niaz, S. Ali, and A. Abbas. 2008. Hybrid authentication in upland cotton through RAPD analysis Aust. J. Crop Sci. 2(3): 141-149.

Awan, S.I., S.D. A. Gardazi, S. Niaz, and M.A. Ali. 2008. Pattern of morphological and genomic diversity in wheat germplasm collection through a multivariate approach. Proc. Nat. Conf. Recent Advances in Agric. Biotech. March,18-19.148p.

Dongre, A., and V. Parkhi. 2005. Identification of cotton hybrid through the combination of PCR based RAPD, ISSR and micro-satellite markers. J. Pl. Biochem. Biotechnol. 14 (1): 53-55.

Dongre, A.B., M.P. Raut, V.M. Paikrao, and S.S. Pande. 2012 Genetic purity testing of cotton F_1 hybrid DHH-11 and parents revealed by molecular markers. Inter. Res. J. Biotech. 3(3): 32-36.

Doyle, J.J., and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus, 12:13 - 15.

Erlich, H.A., D. Gelfand, and J.J. Sninsky. 1991. Recent advances in the polymerase chain reaction, Science, 252:1643-1651.

GoP, 2013. Agriculture: Economic Survey of Pakistan 2012-13, Government of Pakistan, Islamabad. 19p.

Iqbal, M.J., N. Aziz, N.A. Saeed, Y. Zafar, and K.A. Malik. 1997. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. Theor. Appl. Genet. 94 (1): 139-144.

Iva, B., M. Snezana, F. Milomir, and K. Kosana. 2005. Genetic characterization of early maturing maize hybrids (*Zea mays* L.) obtained by protein and RAPD markers. Acta Biol. Jugosl. 37: 235-243.

Khan, I. A., F.S. Awan, A. Ahmad, and A.A. Khan. 2004. A modified mini-prep method for economical and rapid extraction of genetic DNA in plants. Plant Mol. Biol. Rep. 22: 89a-89e.

Khan, I. A., F.S. Awan, A. Ahmad, Y. B. Fu, and A. Iqbal. 2005. Genetic diversity of Pakistan wheat germplasm as revealed by RAPD markers. Genet. Resour. Crop Evol. 52: 239-244.

Khan, A. I., F.S. Awan, B. Sadia, R. M. Rana, and I. A. Khan. 2010. Genetic diversity studies among coloured cotton genotypes by using RAPD markers. Pakistan J. Bot. 42(1): 71-77.

Khan, A.I., I.A. Khan, F.S. Awan, H.A. Sadaqat, and S. Bahadur. 2011. Estimation of genetic distance based on RAPDs between 11 cotton accessions varying in heat tolerance. Genet. Mol. Res. 10 (1): 96-10.

Kumar, A., A.S. Halepyati, and M.G. Patil. 2007. Influence of integrated use of organic and inorganic sources of nutrients on yield and yield components of Bt and non-Bt cotton hybrids.

Karnataka J. Agric. Sci. 20(3): 596-597.

Linos, A.A., P.J. Bebeli, and P.J. Kaltsikes. 2002. Cultivar identification in upland cotton using RAPD markers. Aust. J. Agric. Res. 53(6): 637-642.

Lu, J., and O. Myers. 2002. Genetic relationships and discrimination of ten influential upland cotton varieties using RAPD markers. Theor. Appl. Genet. 105(2-3): 325-331.

Mani, P., T.M.J. Bastin, R.A. Kumar, and A.B.A. Ahmed. 2010. RAPD Analysis of genetic variation of four important rice varieties using two opr primers. J. Agri. Biol. Sci. 5(4): 12-15.

Mehetre, S.S., A.R. Aher, G.C. Shinde, M. Gomes, and S. Eapen, 2004a.RAPD analysis of interspecific hybrid between *Gossypium arboreum* and *Gossypium stocksii*. Caryologia, 57(2): 167-171.

Mehetre, S.S., M. Gomes, and S. Eapen. 2004b. RAPD analysis of hybrid nature of the offspring of *Gossypium hirsutum* x *G. aimondii*. Curr. Sci. 87 (1): 25-28.

Milad, S.I., L.E. Wahba, and M.N. Barakat. 2011. Identification of RAPD and ISSR markers associated with flag leaf senescence under water stressed conditions in wheat (*Triticum aestivum* L.). Aust. J. Crop Sci. 5(3): 337-343.

Mullis, K.B. 1990. The unusual origin of the polymerase chain reaction. Sci. Amer. 262: 56-65.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.

Rana, M.K., and K.V. Bhat. 2005. RAPD markers for genetic diversity study among Indian cotton cultivars. Curr. Sci. 88 (12): 1956-1961.

Rahman, M., and Y. Zafar. 2001. Genotyping of a new strain of lentil by DNA Fingerprinting. Pakistan J. Bot. 4: 423-428.

Rahman, M., M. Hussain, and Y. Zafar. 2002. Estimation of genetic divergence among elite cotton cultivars-genotypes by DNA fingerprinting technology. Crop Sci. 42(6): 2137-2144.

Sheidail, M., Z.H. Shahriari, H. Rokneizadeh, and Nour-mohammadi, Z. 2007. RAPD and cytogenetic study of some tetraploid cotton (*Gossypium hirsutum* L) cultivars and their hybrids. Cytologia, 72: 77-82.

Skaria, R., S. Sen, and P.M.A. Muneer. 2011. Analysis of genetic variability in rice varieties (*Oryza sativa* L.) of kerala using RAPD markers. Genet. Engg. Biotech. J. 24: 1-9.

Smith, O.S., J.S.C. Smith, S.L. Brown, R.A. Tenborg, and S.R. Wall. 1990. Similarities among a group of elite maize inbreds as measured by pedigree. F_1 grain yield, grain yield, heterosis and RFLPs. Theor. Appl. Genet. 80: 833-840.

Surgun, Y., B. Col, and B. Burun. 2012. Genetic diversity and identification of some Turkish cotton genotypes (*Gossypium hirsutum* L.) by RAPD-PCR analysis. Turk. J. Biol. 36: 143-150.

Thangaraj, K., T.S. Raveendram, R. Ravikesavan, and P. Azhaguvel. 2003. RAPD- A technique for genetic diversity and pedigree

studies in cotton. Plant & Animal Genomes XI Conference, January, 11-15, 2003.

Tafvizei, F., M. Sheidai, Z. Nourmohammadi, O. Alishah, and F. Farahani. 2010. Cyto-genetic and RAPD analysis of cotton cultivars and their F_1 progenies. Caryologia, 63(1): 73-81.

Vafaie-Tabar, M., S. Chandrashekaran, S.P. Singh, and M.K. Rana. 2003. Evaluation of genetic diversity in Indian tetraploid and diploid cotton (*Gossypium* spp.) by morphological characteristics and RAPDs. Indian J. Genet. 63: 230-234.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuc. Aci. Res. 18(22): 6531-6535.

Yeh, F.C., R. Yang, and T. Boyle, 1999. Popgene Version 1.31; Microsoft Window-based Freeware for Population Genetic Analysis. University of Alberta, Canada.

Zhang, Q., M.A. Saghai, J.P. Yang, K. D. Liu, Z.Q. Zhou, K.A. Gravois, C.J. Xu, and Y.J. Gao. 1995. Correlations between molecular marker polmorphism and hybrid performance in rice. Plant Genome IV Conference. San Diego, CA. 307p.

Zuo, K.J., J.Z. Sun, J.F. Zhang, and J.L. Liu. 2000. Genetic diversity evaluation of some Chinese elite cotton varieties with RAPD markers Yi Chuan Xue Bao. 27(9): 817-823.

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