International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 5 | Issue 4| OCT-DEC| 2015 | 201-207
Original Research Article – Biological Sciences

VARIETAL CHARACTERIZATION OF COTTON GENOTYPES THROUGH RAPD

S. Ezhil Kumar*

Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Tamilnadu

***Corresponding Author Email: sezhil_5000@yahoo.co.in**

ABSTRACT

Cotton is one of the most important commercial fiber and oil yielding crops in India. Genetic variability and relationship between varieties are of important for cotton breeding. RAPD markers were used for identification and genetic diversity analysis of cotton genotypes. 10 cotton genotypes were subjected to RAPD analysis using 10 RAPD primers. PCR products were subjected to 1.0% agarose gel electrophoresis and banding patterns were compared. 10 RAPD primers produced 757 bands with 268 polymorphic markers and 35% polymorphism. RAPD primer PGF-03 identified four genotypes of cotton viz., Anjali, G.Cot-15, Kanchana and G.Cot-20. The primer PGF-03 identified the genotypes Anjali and G.Cot-15 with the presence of an unique band at 250 bp and identified the genotype Kanchana with the presence of three specific bands at 1000 bp, 1100 bp and 2500 bp and identified G.Cot-20 with the presence of unique band at 850 bp. The results revealed that RAPD technique was thus found to be efficient method for detecting DNA polymorphism and useful for varietal identification in cotton genotypes.

KEY WORDS

cotton, RAPD, varietal identification

INTRODUCTION

Cotton (*Gossypium spp*.) is the most important fiber crop, belonging to the family Malvaceae. It is considered as “king of fiber crop” and “hub of textile industry”, which provides the world’s most important natural textile fibre and is the second most important oil seed crop in the world. Cotton is also called white gold because of economic importance and cotton alone contributes about 4% of national GDP. India is the second largest cotton producer in the world next to China with cultivable area of 115.53 lakh ha with production of 375 lakh bales of 170 kg with the productivity of 552 kg per ha (Anon, 2013). Crop improvement programs in India have generated large member of varieties in the last 30 years. With the proliferation of newly developed varieties, identification of these varieties and maintaining their seed lots has become major concern. The varieties and hybrids attain acceptance when the farmer gets genetically pure seeds of high standards. For this purpose, each cultivar should be properly defined with suitable descriptors, so as to maintain its identify during seed production through field inspection and certification. Characterization of varieties provides description of the material essential for their identification, conservation, management, utilization in crop improvement programmes, identification of suitable lines for breeding purpose and to avoid duplication of varieties and also required for their protection under PPV & FR Act 2001. Varieties can be identified by different methods like morphological, chemical, biochemical and molecular methods. Morphological markers *viz.,* leaf size, leaf colour, leaf hairiness, plant morphology, pollen colour, petal colour and fruit characters etc. has been used for varietal characterization. Though the morphological characterization is simple and easy, their expression is influenced by the environment leading to errors in scoring. Biochemical analysis of seed storage protein shows promising results in characterization of genotypes, but, it is biased since a small portion of genome is represented by these markers and exhibits

low polymorphism. Molecular marker is powerful PCR based technique, indicating the differences in the nucleic acid sequences at a particular location or locations in the genome. Molecular markers have high discrimination power, enabling detection of closely related genotypes and they are efficient tool for characterization of the plant species. Various PCR based molecular markers are Inter Simple Sequence Repeat (ISSR), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Sequence Tagged Micro Satellite (STMS) etc., are useful in various applications of plant breeding. Among these markers, Randomly Amplified Polymorphic DNA (RAPD) can be applied without prior knowledge of gene sequence (Henry, 1997). Furthermore, it is easy to perform and does not require expensive equipment and RAPD markers are dominant because they are either present or absent and it has been shown that RAPD markers can be efficient in the routine assessment of variety identification and of hybrid seed purity. Hence, a study was undertaken to use RAPD markers in varietal identification in cotton.

MATERIALS AND METHODS

The experimental material consisted of 10 genotypes of cotton (*Gossypium spp*.) (Table 1) were collected from Central Institute for Cotton Research (CICR), Nagpur, India. The cotton plants were grown in two rows of 6 m length with 45 cm space between the plants in a completely randomized design with three replications. Single plant having fresh and young leaves, was selected randomly from any of the three replicates of each genotype. Fresh leaves (4-5 g) were collected and powdered in the presence of liquid nitrogen in a pre-chilled pestle and morter. DNA extraction buffer containing 2% polyvinylpyrrolidone (PVP) was added to avoid co-isolation of phenolics and polysaccharides in the DNA. Leaf tissues were cut into small pieces, homogenized and digested with extraction buffer (pH = 8.0): [1 M Tris-HCl, 0.5 M EDTA, 5 M NaCl, 2X CTAB, 4% PVP and β-mercaptoethanol]. After incubation at 65°C in water bath for an hour, the mixture was emulsified with an equal volume of phenol, chloroform, Isoamyl alcohol (25:24:1). Equal volume of ice-cold iso-propanol was added to precipitate DNA and centrifuged for pelleting. The pellets were washed with 70% alcohol, air-dried and resuspended in 100 µl of TE buffer (1 M Tris-HCl, 0.5 M EDTA, pH 8.0) and finally treated with 1 µl of RNase. Quantity and quality of extracted DNA was estimated spectrophotometrically and by gel electrophoresis. A total of ten RAPD primers synthesized from Bangalore Genei Pvt. Ltd. were used. List of RAPD primers used in this study was given in Table 2.

Table 1. List of cotton genotypes used in the study

Sl.No	Genotype
1.	Kanchana
2.	Khandwa -2
3.	Khandwa – 3
4.	JK-4
5.	Anjali
6.	G.Cot-20
7.	G.Cot-15
8.	KC-3
9.	NH-545
10.	MCU -10

Table 2. List of RAPD primers used and their sequences

S.No	Oligo Name	Sequence (5` - 3`)
1.	OPA-09	GGGTAACGCC
2.	OPA-10	GTGATCGCAG
3.	OPA-11	CAATCGCCGT
4.	OPC-11	AAAGCTGCGG
5	OPA-04	AATCGGGCTG
6	OPI-07	CAGCGACAAG
7	PGF-01	GAAACAGCGG
8	PGF-02	GGAGCCCAC
9	PGF-03	GCCGTCTACG
10	PGF-04	GGCATCGGCC

Molecular analysis

RAPD amplification

RAPD amplification was performed in 25 µl reaction volume containing 40 ng genomic DNA, 2.5 µl reaction buffer (10X Taq polymerase buffer with 15 mM $MgCl_2$), 200 µM of each dNTPs, 15 ng of RAPD primers, 1.5 U Tag polymerase. An initial denaturing step of 5 min at 94°C followed by 40 PCR cycles (denaturing at 94°C for 1 min, primer annealing at 60°C for 1 min and primer extension at 72°C for 1 min). A final step of extension at 72°C for 8 min was carried out for polishing the ends of PCR products. The PCR amplification was carried out in a thermal cycler (Thermal cycler gradient, Eppendorf). Amplified PCR products of RAPD were separated on 1.0 per cent agarose gel containing 0.1 ig/il of ethidium bromide for about 5 hours at 60 volts. Gels were photographed under UV light with gel documentation system. Fragment size was estimated by using 1 kb molecular size ladder.

Comparison of genotypes was carried out based on the presence and absence of fragment produced by RAPD amplification. The presence of each band was scored as 'I' and it absence as 'O'. The data matrix was read by NTSYS-PC version 2.02 (Numerical Taxonomy and Multivariate Analysis System for Personal Computer) developed by Rohlf (2004) and analyzed by SIMQUAC (similarity for qualitative data) program with Jaccard's similarity coefficient. The SIMQUAC is a program for computing a variety of similarity and dissimilarity coefficients for qualitative data. The qualitative nature of the absence (O) or presence (1) state of RAPD marker was used as the basis for similarity analysis among various cotton genotypes. A matrix of O and 1 act as the input and the output matrix of similarity or dissimilarity coefficients. The resultant similarity matrix was entered into SHAN (Sequential, Agglomerative, Hierarchical and Nested clustering method) clustering program, a tree matrix was produced and dendrogram was constructed using UPGMA. Primer banding characteristics such as total number of bands, number of plymorphic bands, number of monomorphic bands and percentage of polymorphic bands were obtained.

RESULTS AND DISCUSSION

The results obtained in the present study are given below

Initially nineteen RAPD primers were screened and ten primers produced scorable bands. All the ten RAPD primers showed polymorphism. A total of 757 RAPD markers were amplified, out of which 268 were polymorphic markers with 35.0 per cent polymorphism. Average number of polymorphic loci amplified per primer was 26.8. The size of RAPD amplicons was in between 250 bp in OPA-11 to 3000 bp in OPC-11 and OPA-04.

The number of polymorphic markers generated by each primer was given in Table 3. RAPD allelic profiles are shown in Plates 1& 2. The RAPD primer OPA-10 generated the maximum number (110) of markers and the RAPD primer PGF-02 generated the least number (37) of markers. The RAPD primers PGF-01 and OPA-04 had generated highly polymorphic profile. The RAPD primer OPA-07 produced low polymorphic profile. None of the primers was individually so informative as to differentiate all the genotypes studied. The RAPD primer PGF-03 was polymorphic in all the genotypes and produced seven unique bands in the genotypes viz., Anjali, G.Cot-15, Kanchana and G.Cot-20. The primer PGF-03 produced three unique bands at 1000 bp, 1100 bp and 2500 bp for the genotype Kanchana and it produced one unique band at 250 bp for the genotypes Anjali and G.Cot-15. The primer PGF-03 produced one unique band at 850 bp for G.Cot-20. The RAPD primer OPA-10 produced one unique band at 850 bp for the genotype Kanchana and produced one unique band at 900 bp for G.Cot-15 and NH-545. The RAPD primer OPI-07 produced one unique marker at 650 bp for the genotypes JK-4 and Khandwa-2. The RAPD primer OPC-11 produced one unique band at 1500 bp for JK-4 and another unique band at 1000 bp for Kanchana. The RAPD primer OPA-04 produced one unique band at 3000 bp for Kanchana. The RAPD primer OPA-11 produced one unique band at 3200 bp for the genotype JK-4. Genetic relationships among the ten cotton genotypes were estimated based on Jaccards pair wise similarity coefficients. In RAPD analysis, the cotton genotypes KC-3 with NH-545 showed maximum similarity (90.6%) followed by the genotypes G.Cot-15 with KC-3 (82.3%) among the ten cotton genotypes, while the genotypes Kanchana with Anjali showed least pair wise similarity (30.7%) followed by Khandwa-2 with Anjali (33.3%) among the ten cotton genotypes (Table 4).

Table 3. RAPD bands and their characteristics generated by 10 RAPD primers

Sl.No	Primers	Total Number of bands	Number of Polymorphic bands	Percentage Polymorphism	Band size
1.	OPA-04	80	39	49.0	350-3000
2.	OPA-09	76	28	37.0	750-3000
3.	OPA-10	110	23	21.0	600-2800
4.	OPA-11	85	31	36.0	250-3200
5.	OPC-11	73	20	27.0	300-3000
6.	OPI-07	94	19	20.0	300-2800
7.	PGF-01	37	27	72.0	300-3000
8.	PGF-02	61	22	30.0	400-1500
9.	PGF-03	61	20	33.0	250-2000
10.	PGF-04	80	39	48.0	400-3000

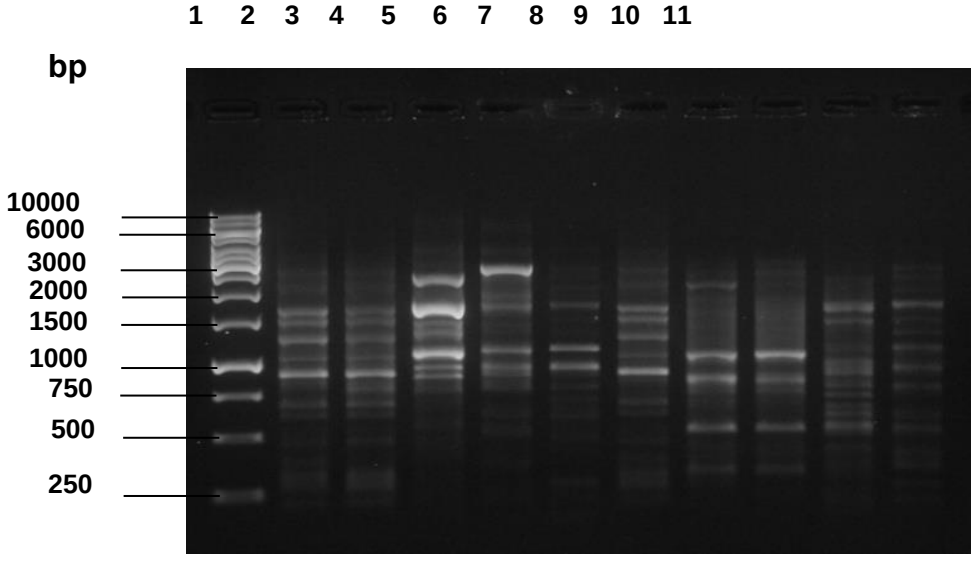
Table 3. Genetic similarity matrix for RAPD

	Kanchana	Khandwa-2	Khandwa-3	JK-4	Anjali	G.Cot-20	G.Cot-15	KC-3	NH5-45	MCU-10
kanchana	1.0000									
Khandwa2	0.6563	1.0000								
Khandwa3	0.4412	0.4735	1.0000							
JK4	0.5000	0.5882	0.4286	1.0000						
Anjali	0.3077	0.4333	0.4194	0.5758	1.0000					
GCot20	0.7500	0.7097	0.5313	0.5833	0.4167	1.0000				
GCot15	0.4750	0.6471	0.4444	0.6667	0.4595	0.5226	1.0000			
KC3	0.5526	0.6471	0.4444	0.7647	0.5000	0.6389	0.8235	1.0000		
NH545	0.5263	0.6176	0.3784	0.7353	0.4722	0.6111	0.7941	0.9063	1.0000	
MCU-10	0.4857	0.7500	0.3636	0.7097	0.4688	0.5758	0.6667	0.7188	0.7419	1.0000

Cluster analysis

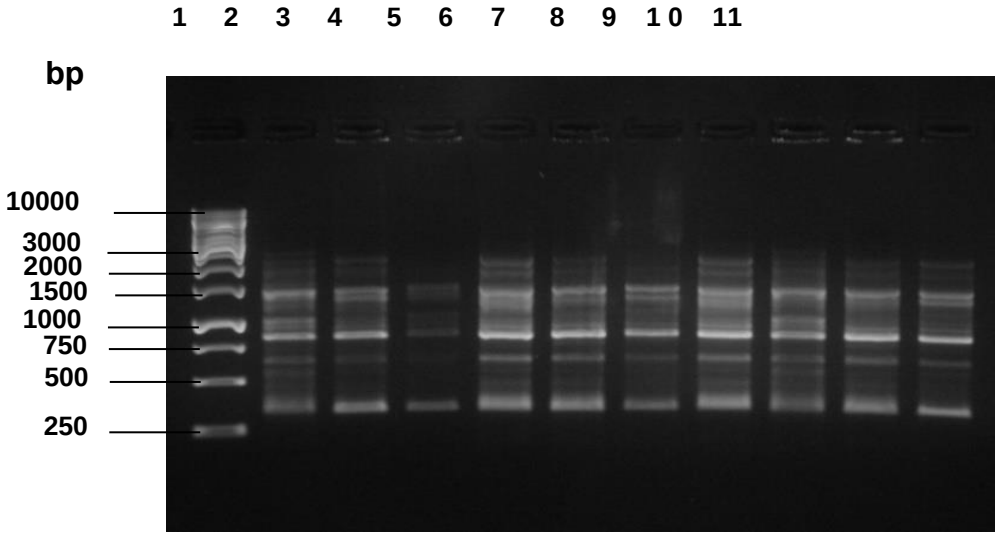
The dendrogram was constructed for ten cotton genotypes using UPGMA method (Nei and Lei *et al.* 1979). In RAPD analysis, the ten cotton genotypes were grouped into two main clusters (main clusters I and II) with an average of 43 per cent. The main cluster I consisted of the single genotype Khandwa-3, which was placed outside the major clusters at one end of dendrogram. The main cluster II consisted of nine genotypes and it was divided into two sub clusters (sub clusters II A and II B). The sub cluster II B consisted of a single genotype Anjali, which was different from the rest of the genotypes in mail cluster II. The sub cluster II A consisted of eight genotypes and it was again divided into two sub subclusters as II Ai and II Aii. The sub subcluster II Ai grouped into two genotypes *viz.,* Kanchana and G.Cot-20. The sub subcluster II Aii consisted of six genotypes viz., Khandwa-2, MCU-10, JK-4, G.Cot-15, KC-3 and NH-545. The sub cluster II B consisted of one genotype Anjali. The similarity between genotypes varied with a range of 47 to 91 per cent (Fig.3).

Fig.1. RAPD PROFILE ANALYSIS OF COTTON GENOTYPES WITH PRIMER - OPA-11



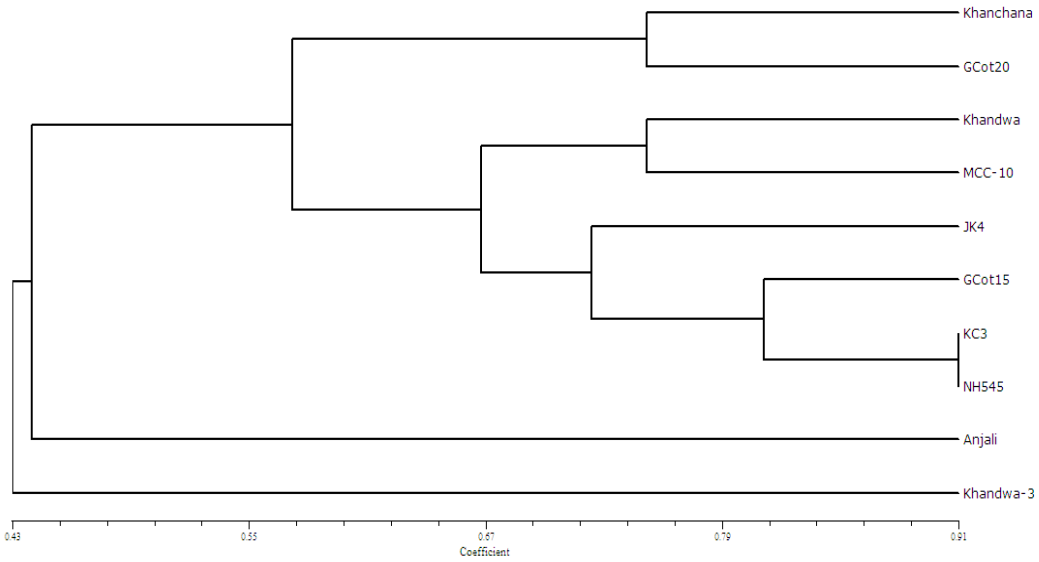
L1-DNA ladder, L2 kanchana, L3 Khandwa -2, L4 khandwa-3, L5 JK4, L6- Anjali, L7 G.cot - 20, L8 G.cot – 15, L9 –KC-3, L10 Nh-545, L11-Mcu-10

Fig 2. RAPD PROFILE ANALYSIS OF COTTON GENOTYPES WITH PRIMER OPA-04



L1-DNA ladder, L2 kanchana, L3 Khandwa -2, L4 khandwa-3, L5 JK4, L6- Anjali, L7 G.cot - 20, L8 G.cot – 15, L9 –KC-3, L10 Nh-545, L11-Mcu-10

Fig. 3. Dendrogram of 10 cotton genotypes developed from the RAPD data using UPGMA



DISCUSSION

A number of DNA markers have been used to study the extent of genetic variation in a number of crops and differ mainly in their principles and engender varying amounts of data. The present study was designed to explore genetic diversity among the ten cotton genotypes using RAPD markers.

Characterization of plant germplasm using molecular techniques has an important role in the management and utilization of plant genetic resources (Karp, 2002). It enhances on selection of diverse parents to widen the gene pool (Fu, 2006). These characterizations have provided useful information for understanding the genetic diversity and structure of cotton gene pools. This information could be incorporated into effective management of germplasm in cotton breeding program for control of genetic diversity

In the present study, ten RAPD primers were used to check polymorphism among the ten genotypes of cotton and all the ten primers were found to be polymorphic. The ten RAPD primers generated 757 markers for the assessment of genetic variability between the genotypes studied. The number of RAPD marker ranged from 37 as produced by the primer PGF-01 to 110 as produced by the primer OPA-10 among the ten cotton genotypes studied. Out of 757 RAPD markers

amplified, only 268 markers were polymorphic which had resulted 35.0 per cent polymorphism. All the ten primers produced polymorphic markers; however the level of polymorphic percentage assorted with each primer ranged from 20.0 to 49.0 per cent. All the genotypes showed a varying degree of genetic diversity based on their amplification profile. The average percentage of polymorphism was 26.8. A low level of polymorphism was observed among the ten cotton genotypes studied. These findings are in accordance with the findings of Lu and Myers (2002): Manikam *et al.* (2002) and Sheidai *et al*. (2007). A low level (35.0%) of polymorphism was observed among the ten genotypes in the present study. This study showed that narrow genetic base in the material under investigation, which might be due to almost similar genetic makeup of genotypes. Contradictory reports on the extent of observed polymorphism in cotton could be attributed to different types of genetic materials used in different studies.

Jaccards coefficient of similarity ranged from 30 to 90 per cent (Table 4). The highest similarity (90.63%) was found between the genotypes KC-3 with NH-545, followed by (82.35%) in G.Cot-15 with KC-3. On the other hand the lowest genetic similarity (30.7%) was found between the genotypes Kanchana with Anjali, followed by (33.0%) in Khandwa-2 with Anjali. The difference between the highest and lowest value of genetic similarity revealed the wide range of genetic variability present among the ten cotton genotypes. High similarity value between the genotype pairs were found due to difference in genetic constitution. The genotypes with least genetic similarity could be used as parental lines for breeding purpose. The RAPD diversity analysis suggested that, the genotypes Khandawa-3, Anjali and G.Cot-20 were to be most variable genotypes. The coefficient of the genetic similarity was found to be in the range of 30.0 to 90.0 per cent. This observation showed the existence of low genetic similarity in the studied ten genotypes of cotton. These findings are in accordance with the findings of Khan *et al*. (2011); Patil *et al*. (2007) and Sharaf *et al*. (2009).

The dendrogram from RAPD analysis formed two major clusters. The genotype Khandwa-3 remained isolated forming an out group from the rest of the genotype showing dissimilarity from the rest of the genotypes. The genotypes present in the same subclusters are more alike than others. Nei and Li's coefficient ranged from 0.43 to 0.91, with an average of 0.67. Sub cluster II A contained the genotype G. Cot-15 remained isolated forming an out group from the rest of the genotypes in this subcluster. The genotype KC-3 and NH-545 were most similar accounting for similarity coefficient of 0.99. The genotypes Kanchana, Khandwa-3 and Anjali are most dissimilar genotypes.

CONCLUSION

It is concluded that about seven genotypes of cotton were identified based on the presence or absence of unique markers. The RAPD primer PGF-03 could identify four genotypes of cotton *viz.,* Anjali, G.Cot-15, Kanchana and G.Cot-20. The primer PGF-03 could identiy the genotypes Anjali and G.Cot-15 with the presence of an unique band at 250 bp, could identify Kanchana with the presence of three specific bands at 1000 bp, 1100 bp and 2500 bp and could identify G.Cot-20 with the presence of unique band at 850 bp. The RAPD primer OPA-10 could identify the genotypes kanchana with the presence of unique band at 800 bp and could identify G.Cot-15 and NH-545 with the presence of unique band at 900 bp. The RAPD primer OPC-11 could identify the genotype JK-4 with the presence of a unique band at 1500 bp and identify Kanchana with the presence of a unique band at 1000 bp. The RAPD primer OPA-04 could identify the genotype Kanchana with the presence of a unique band at 3000 bp

REFERENCES

Anon. (2013). Annual report. All India Coordinated Crop Improvement Programme. 2013-2014.

Fu, Y. B., G. W.Peterson, J. K. Yu, L. Gao, J. Jia and K. W. Richards. (2006). Impact of plant breeding on genetic diversity of the Canadian hard red spring wheat germplasm as revealed by EST-derived SSR markers. *Theoretical and Applied Genetics,* 112 (7): 1239-1247.

Karp, A. 2002. The New Genetic Era: Will it Help us in Managing Genetic Diversity? *In: Managing Plant Genetic Diversity*. (Engels, J. M. M., V. Ramanatha Rao., A. H. D. Brown and M. T. Jackson eds.), 43-56.
IPGRI, Rome.

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. *Genetics and Molecular Research,* 10 (1): 96-101.

Lu, H. J. and G. O. Myers. 2002. Genetic relationships and discrimination of ten influential upland cotton varieties using RAPD markers. *Theoretical and Applied Genetics,* 105:325–331.

Manikam, A., C. Parameswari, M. Indumathi and A. H. Chandra. 2002. DNA fingerprinting in cotton (*Gossypium spp*.L) based on RAPD markers for varietal differentiation. *Research-on Crops*, 3(3): 635-642.

Nei, N. and W. Lei. 1979. Mathematiical model for studying genetic variation in terms of restriction endonuclease. *Proc. Natl. Acad. Sci.*76: 5269 – 5273.

Rohlf, F.J. (2004). NTSYS-pc Numerical Taxonomy and Multivariate Analysis System version 2.2, Exeter Software, Applied Biostatics, New York.

Sharaf, A. N., D. A. El-kadi, H. F. Alatwani, A. Y. Gamal El-Din and A. A. Abd El-Hadi. 2009. Genetic studies on some cotton genotypes using DNA molecular markers. In: 4th Conference on Recent Technologies in Agriculture.

Sharaf, A.N., D. A. El-kadi, H. F. Alatwani, A. Y. Gamal El-Din and A. A. Abd El-Hadi. 2009. Genetic studies on some cotton genotypes using DNA molecular markers. *4th Conference on Recent Technologies in Agriculture,* 167-177.

Sheidai, M., Z. H. Shahriari, H. Rokneizadeh and Z. Noormohammadi. 2007. RAPD and Cytogenetic study of some tetraploid cotton (*Gossypium hirsutum* L.) cultivars and their hybrids. *Cytologia,* 72(1): 77–82.

***Corresponding Author:**
S.Ezhil Kumar*
Email: **sezhil_5000@yahoo.co.in**