Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 6 [8] July 2017: 51-57

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE **OPEN ACCESS**

Genetic diversity of parents using RAPD, ISSR and SSR molecular markers in upland cotton (*Gossypium hirsutum* L.)

Bilwal, B. B. 1* , Vadodariya, K.V. 1 , Rajkumar, B. K 1 , Upadhyay, A. K 2 and Lahane, G. R. 3
1 Main cotton Research Station (MCRS), Navsari Agricultural University, Navsari, Gujarat
2 School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, India.
3 Vasantrao Naik Marathwada Agricultural University, Parbhani Maharashtra
* Corresponding author email:bbilwal88@gmail.com

ABSTRACT
Present study aimed to analyze the genetic diversity among nine cotton genotypes using the molecular markers viz., RAPD, ISSR and SSR marker. A total of 25 RAPD, 22 ISSR and 16 SSR primers were used, which produced 171, 209 and 33 amplicons, respectively. The number of polymorphic amplicons was found to be 156 (RAPD), 142 (ISSR) and 24 (SSR) accounting for polymorphism to 91.22 % (RAPD), 67.94 % (ISSR) and 72.72 % (SSR), respectively. The genetic relationship was examined using the Numerical System Package (NTSYS-PC), UPGMA analysis was performed and dendrograms was constructed. Based on the RAPD, ISSR and SSR combined pooled data, the genetic similarities ranged from 0.74 to 0.93 per cent. Dendrograms clustered the nine genotypes into two major clusters (A and B) showing affinity at genus level; most of the genotypes (5 out of 9) falling into cluster A. Maximum similarity (93%) was found between the genotype DELTA-15 and KH-19; followed by 92 per cent similarity among GSHV-93/13 and DELTA-15; and GSHV-97/1016 and Kh-119. On the other hand least similarity of 0.72 was recorded among GJHV-337 and GSHV-97/1016; GJHV-337 and GISV8/1029; and GJHV-337 and DELTA-15; and GJHV-337 and KH-119. The results indicate that the genotypes can be separated from each other at the molecular level. Our results accord the suitability and reliability of molecular marker (RAPD, ISSR and SSR) to estimates genetic diversity and genetic relationships among the studied cotton genotypes.
***Key words**: Cotton (G. hirsutum L.), Cotton, genetic diversity, molecular markers, RAPD, ISSR, and SSR.*

Received 20.05.2017 Revised 15.06.2017 Accepted 26.06.2017

INTRODUCTION

Cotton, *Gossypium* spp., is an economically important crop that is grown throughout the world as a source of fibre, food and feed [1]. The cotton belonging to the genus *Gossypium* (Malvaceae) comprises of approximately 50 species, distributed in various continents except Europe. It has four cultivated species: *Gossypium hirsutum* L., *G. barbadense*, *G. arboreum* L. and *G. herbaceum* L. *G. hirsutum* is the predominant cultivated cotton with high yield and wide adaptation, while its closely related species, *G. barbadense* is grown for its extra-long, strong, and fine fibre. The diploid species, *G. arboreum* L. and *G. herbaceum* L. are cultivated only in very small acreage. Plant breeders select genotypes with desirable traits by looking at the phenotype. Most of these traits are polygenic with complex non-allelic and environmental interaction. Thus, DNA marker technology would provide a tool to the plant breeders to select desirable genotypes directly on the basis of genotype instead of phenotype. Conventional breeding methods generally aim to improve agronomically relevant or otherwise interesting traits by combining characters present in different parental lines of cultivated species or their wild relatives. Transfer of genes from wild species is time consuming, not always successful and it is difficult to assess the introgression of alien genes. Moreover conventional methods and mating designs to select the cultivars for development of superior hybrid. Which are time consuming and less precise processes as compared to direct selection of genotype based on molecular processes. Further conventional selection depends upon accurate screening methods and availability of lines with clear-cut phenotypic characters. With the use of molecular techniques it would be now possible to hasten the transfer of desirable genes among varieties and to introgress novel genes from related wild species into the local and popular genotypes which would accelerate the generation of new varieties [2]. Morphological and physiological features of plants have been used to understand the genetic variation.

Though morphological features are indicative of the phenotype, they are affected by environmental factors and growth practices. There are about 145 morphological markers identified in cultivated cotton but their utility in breeding programmes has remained limited because of their deleterious effect and the difficulties in accumulating multiple markers in a single genotype [3]. Due to the narrow genetic base of cotton germplasm that cotton breeders have been utilizing low efficiency of traditional selection methods, cultivar improvement in cotton has slowed down in the past 10–15 years. Analysis of genetic diversity and estimation of relatedness between species and genotypes identification [4] is useful in plant breeding programs because it provides a tool for accurate organization of germplasm and efficient parental selection. Genetic markers are important tools in the determination of genetic diversity, which is the first step for breeding projects and the protection of genetic resources. Researchers denote specific locations on chromosomes that constitute important clues for genome analysis. In general, morphological and molecular markers are used. During the past few decades, a number of molecular techniques have been recruited to complement traditional methods for the evaluation of biodiversity, estimation of relatedness and genotype identification. DNA markers are, by far, the most powerful and the most widely used tool to uncover informative polymorphism and genome variability [5] with the development of the polymerase chain reaction (PCR) technology, the number of useful DNA based marker types has been expanded considerably. Therefore, more reliable markers such as PCR based marker types are available now a days including Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR) and Simple Sequence Repeat (SSR) markers have come into use of the plant breeders. Keeping in view the above facts, the present investigation was conducted as an attempt to address the objectives to recon molecular diversity of nine parents of cotton genotypes.

MATERIALS AND METHODS

Plant material and DNA extraction: The cotton genotypes included in this study consisted of nine genotypes of *Gossypium hirsutum* that includes GSHV-97/13, GJHV-337, AET-5, GN.Cot-22, GSHV-97/1016, GISV-8/1029, DELTA-15, EC-10786 and KH119. Seed material was obtained from the collection of the Main Cotton Research Station, (MCRS), NAU, Surat. Total genomic DNA was isolated from young leaves of cotton genotype using a modified cetyltrimethylammonium bromide (CTAB) method [6]. After quantification, the DNA samples were diluted to 5 ng μL^{-1} with distilled H_2O. The DNA was then stored in a freezer (–20 °C) until it was used for PCR amplification.

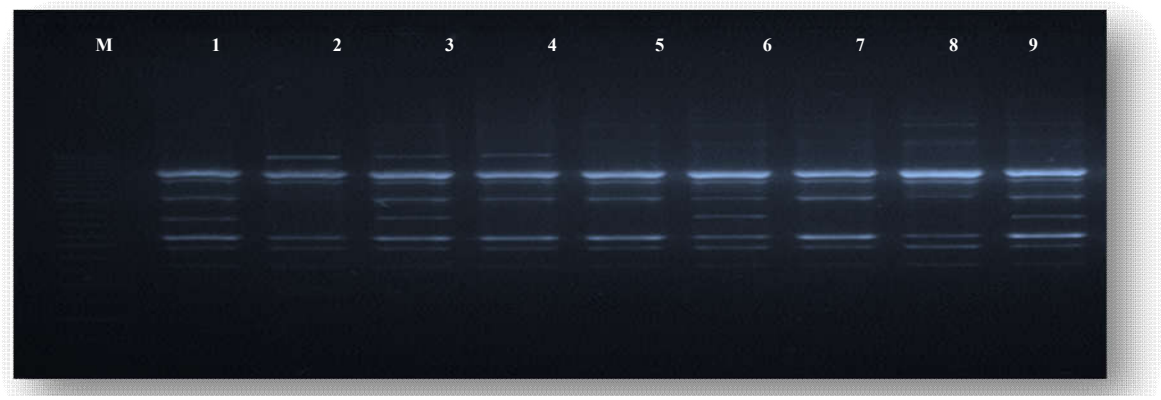
Molecular analysis: PCR amplification was performed using 25 random primers (RAPD), 22 (ISSR) primers and 16 (SSR) primer pairs (Table 1, 2 and 3). The amplified reaction for RAPD, ISSR and SSR were carried out in a reaction volume of 15 µL containing the following: 1.5 µL of 10× Taq buffer with KCl [100 mM Tris-HCl (pH 8.8), 500 mM KCl, 0.8% Nonidet 40], 1 µL of primer (10 µM), 2.4 µL of magnesium chloride (25 mM), 1 µL of dNTPs (10 mM), 0.25 µL of Taq DNA polymerase, 3.85 µL of ddH_2O, and 25 ng of genomic DNA as a template. PCR amplification conditions were as follows: first denaturation at 94 °C for 1 min, followed by 45 cycles of 94 °C for 1 min, 36 °C for 1 min, and 72 °C for 2 min. After the completion of 45 cycles, a final extension was carried out at 72 °C for 10 min. Subsequently, PCR products were analyzed using agarose gel electrophoresis (1.5 per cent agarose gels) and amplicon were detected by staining with ethidium bromide and visualized under UV light. A 1.0-kb DNA ladder was used as a molecular size standard (Fermentas).

Data analysis: The banding patterns generated by RAPD, ISSR and SSR were examined to determine the level of polymorphism and the genetic relatedness among the nine cotton genotypes. The amplified fragments were scored as present (1) or absent (0). Cluster analysis was based on similarity matrix obtained with unweighted pair group method using arithmetic average (UPGMA), and the relationships between genotypes were displayed as dendrogram based on NTSYs data analysis programme [7].

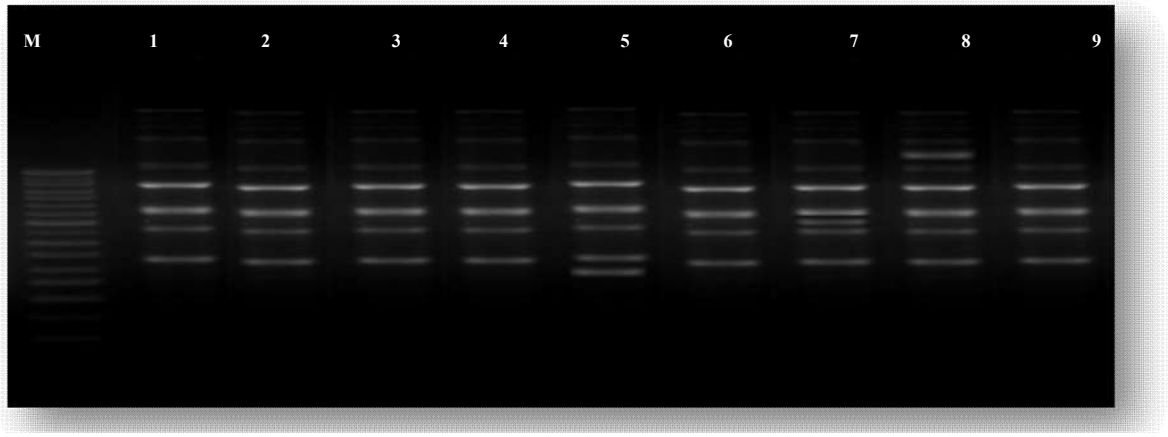
RESULTS AND DISCUSSION

Polymorphism as detected by RAPD analysis: A total of 25 random primers against nine cotton genotypes were selected for their scorable results. The amplification profiles of nine cotton genotypes by 25 RAPDs revealed a total of 156 polymorphic bands out of 171 reproducible products (Table 1), corresponding to 91.22 per cent polymorphism. The number of amplicons/ primers ranged from 3 (RPI 11) to 14 (RPI 23 and RPI 25), whereas the number of polymorphic bands/primer ranged from 0 (RPI 8 and RPI 15) to 11 (RPI 23). [8] Assayed 21 cotton accessions using 28 RAPD primers. The total number of amplicons detected was 323, while, the number of polymorphic amplicons was 191. Thus, the level of polymorphism among the 21 accessions was 59.1 per cent. Genetic diversity of 31 available *Gossypium* species, three sub-species and one interspecific hybrid was studied by [9] screened upland cotton genotypes by using 45 RAPD primers to distinguish the genotypes. The result indicated interspecific

genetic relationship of several species as related to their center of origin. The study further revealed a broader genetic base of most of species besides indicating the genetic relationship of *G. hirsutum* to standard cultivated *G. barbadense, G. herbaceum* and *G. arboretum.* They found RAPD markers are more efficient than morphological markers, isozymes and RFLP as RAPD detected the variation in closely related genotypes too. Genetic diversity of 13 cotton line was investigated at DNA level with the random amplified polymorphic DNA (RAPD) procedure by [10]. Out of eighty random decamer primer screened, 19 were found to be polymorphic and they generated 70 amplicons and 66 of them were polymorphic (94.20 %). Maximum genetic distance of 85 % and minimum genetic distance of 49 % was observed (Fig.1A).



(Fig.1A)



(Fig.1B).

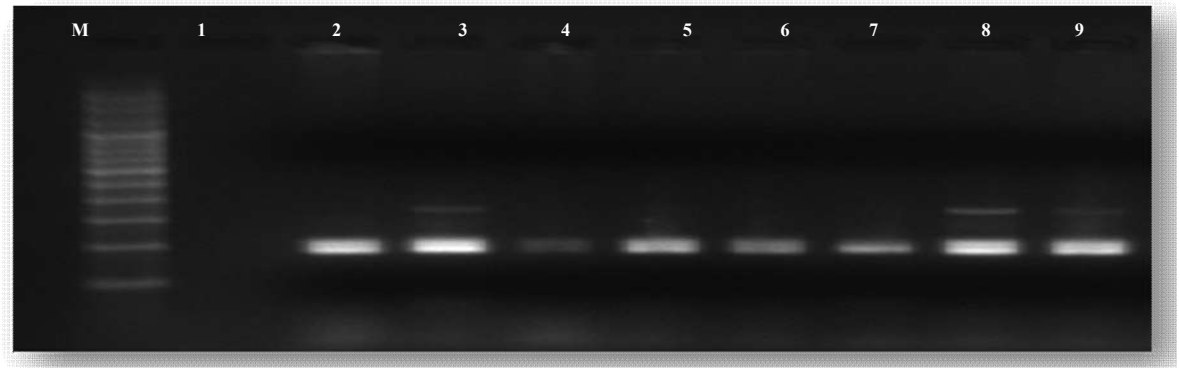


Fig. (1C).

M: Marker, 1: GSHV-97/13, 2: GJHV-337, 3: AET-5, 4: GN. Cot-22, 5: GSHV-97/1016, 6: GISV-8/1029, 7: DELTA-15, 8: EC-10/786 and 9: KH-119.

Fig. 1-RAPD, ISSR and SSR profile of parental lines of cotton amplified by using primers RPI 5, primer ISSR 14 and SSR primer SRT 62.

Table 1. Primer sequence, the total number of amplicons, polymorphic amplicons, and the percentage of polymorphism as revealed by RAPD analysis.

Primers	Accession Numbers	Total number of bands	Number of polymorphic bands	Per cent Polymorphism
RPI 1	AM765819	9	5	55.55
RPI 2	AM750044	8	7	87.50
RPI 3	AM773310	9	8	88.88
RPI 4	AM773769	12	10	83.33
RPI 5	AM773770	8	3	37.50
RPI 6	AM773771	6	4	66.66
RPI 7	AM773312	8	8	100.00
RPI 8	AM773773	8	0	00.00
RPI 9	AM773315	5	3	60.00
RPI 10	AM750045	9	7	77.77
RPI 11	AM911709	3	2	66.66
RPI 12	AM773316	5	5	100.00
RPI 13	AM750046	8	6	75.00
RPI 14	AM773774	8	5	62.50
RPI 15	AM773775	8	0	00.00
RPI 16	AM773776	9	9	100.00
RPI 17	AM911710	8	6	75.00
RPI 18	AM765830	9	9	100.00
RPI 19	AM773777	11	9	81.81
RPI 20	AM773317	8	7	87.50
RPI 21	AM765820	12	7	58.33
RPI 22	AM911711	8	8	100.00
RPI 23	AM911712	14	11	78.57
RPI 24	AM765821	11	7	63.63
RPI 25	AM750054	14	10	71.42
Total		**171**	**156**	**91.22**

Polymorphism as detected by ISSR analysis:

Twenty two ISSR primers were employed to investigate the genetic polymorphism as presented in Table 2, the number of alleles/locus ranged from 5 (ISSR 15 and ISSR 16) to 15 (ISSR 4 and ISSR 22), while the number of polymorphic alleles varied from 2 (ISSR 10, ISSR 16, ISSR 17 and ISSR 18) to 11 (ISSR 22) and the average level of polymorphism was 67.94 per cent (Fig. 1B). [11] investigated the genetic diversity of the cotton *Gossypium hirsutum* L., widely cultivated in India, by ISSR analysis. They found that of the 19 polymorphic markers analyzed, the value of similarity coefficients of dendrogram calculated by ISSR markers ranged from 0.39 to 0.98. The average polymorphic values for ISSR were found to be 76.55 per cent indicating that ISSR marker is more informative. [12] studied the genetic diversity among six sources of cotton germplasm with different parental origins basis of 19 ISSR primers generated a total of 65 alleles of which 45 alleles were found to be polymorphic, resulting in 69.23% polymorphism. A total of 142 alleles with 67.94 per cent polymorphism were detected from 22 ISSR primers. [13] ISSR marker used by to generate DNA profiles of 19 genotypes of cotton (diploid and tetraploid). The results of cluster analysis indicated not only the separation of genotypes of the two major species (*G.arboreum* and *G.hirsutum*) into separate groups, but also the separation of the genotypes among each group. The similarity coefficient based on ISSR and microsatellite markers ranged from 0.59 to 0.93. Thus suggesting considerable genetic variation between the cotton species studied.

Table 2. Primer sequence, the total number of amplicons, polymorphic amplicons, and the percentage of polymorphism as revealed by ISSR analysis.

Primer	Primer sequence(5'-3')	Total number of bands	Number of polymorphic bands	Per cent polymorphism
ISSR 1	AGCAGCAGCAGCAGCGA	8	7	87.50
ISSR 2	AGCAGCAGCAGCAGCGG	10	10	100.00
ISSR 3	AGCAGCAGCAGCAGCGT	9	7	77.77
ISSR 4	AGCAGCAGCAGCAGCGC	15	9	60.00
ISSR 5	CACACACACACACAAT	11	10	90.90
ISSR 6	CACACACACACACAAC	9	9	100.00
ISSR 7	CACACACACACACAGT	9	7	77.77
ISSR 8	CACACACACACACAGC	11	8	72.72
ISSR 9	CACACACACACACAGA	12	5	41.66
ISSR 10	CACACACACACACAAA	6	2	33.33
ISSR 11	GTGTGTGTGTGTGTTA	6	6	100.00
ISSR 12	GTGTGTGTGTGTGTTG	7	4	57.14
ISSR 13	GTGTGTGTGTGTGTCA	11	6	54.54
ISSR 14	GTGTGTGTGTGTGTCT	9	3	33.33
ISSR 15	GTGTGTGTGTGTGTAT	5	5	100.00
ISSR 16	GTGTGTGTGTGTGTAC	5	2	40.00
ISSR 17	CAGGAGAGAGAGAGAGA	7	2	28.57
ISSR 18	GCTGAGAGAGAGAGAGA	7	2	28.57
ISSR 19	GCAGAGAGAGAGAGAGA	10	10	100.00
ISSR 20	GTGTGTGTGTGTGTCT	14	8	57.14
ISSR 21	GTGTGTGTGTGTGTAT	13	9	69.23
ISSR 22	GTGTGTGTGTGTGTCT	15	11	73.33
Total		**209**	**142**	**67.94**

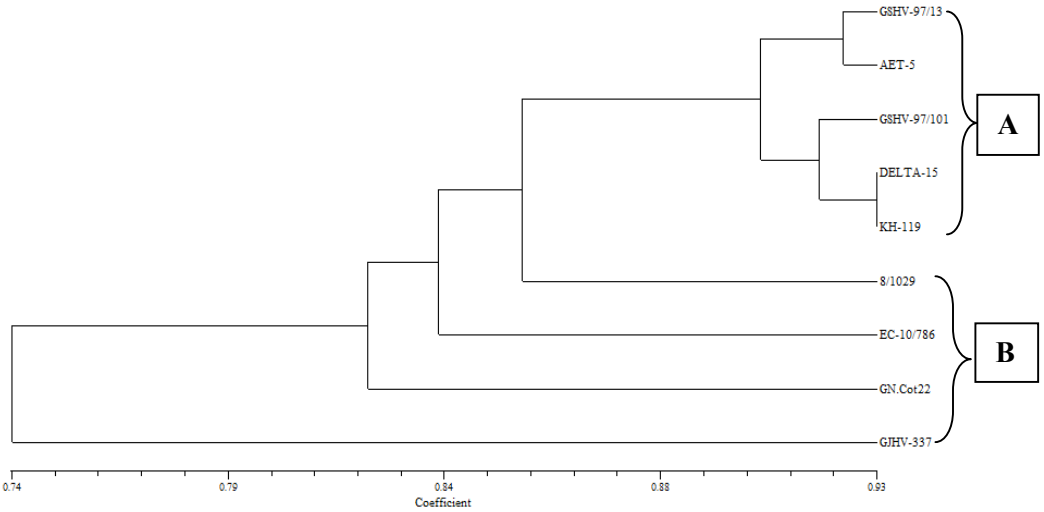


Figure 1: Dendrogram generated by NTYSIS-pc UPGMA cluster analysis method using pooled data of RAPD, ISSR and SSR primers for nine parental line of cotton (*G. hirsutum* L.)

Polymorphism as detected by SSR analysis:

Sixteen SSR primers were employed to investigate the genetic polymorphism as presented in Table 3, the number of alleles/locus ranged from 1 to 4, while the number of polymorphic alleles varied from 1 to 2 and the average level of polymorphism was 72.72 per cent (Fig. 1C). [14] investigated the genetic diversity of the Asian cotton *Gossypium arboreum* L., widely cultivated in China, by microsatellite analysis. They found that of the 358 microsatellite markers analyzed, 74 primer pairs detected 165 polymorphic DNA fragments among 39 *G. arboreum* accessions examined. [15] also studied the genetic diversity among 43 sources of upland cotton germplasm with different parental origins, breeding periods, and ecological growing areas in China on the basis of SSR markers. Genetic diversity in 50 representative Pakistani cotton cultivars was studied using 70 SSR primer pairs by [16] generated a total of 241 SSR alleles, of

which 147 (60 %) were found to be polymorphic, resulting in 57.5 per cent polymorphism, and the average number of polymorphic alleles per primer was observed to be 2.10. [17] investigated the genetic diversity and relationship among 23 cultivar of cotton using 10 SSR primers, which produced 22 amplicons. The number of polymorphic amplicons was found five resulting into a polymorphism equivalent to 22.7. The genetic similarities ranged from 54 to 96 per cent.

Table 3. Primer, primers sequence, the total number of amplicons, polymorphic amplicons, and the percentage of polymorphism as revealed by SSR analysis.

Primer	Forward sequence (5'--3')	Reverse sequence (5'--3')	Total number of amplicons	Number of polymorphic amplicons	Per cent polymorphism
SRT 48	GGAGTTGGGTTACAAAAGG	GCATTGACAAACGTTGAGAT	2	2	100.00
SRT 49	GAACCGATTGATATCCCAA	ACCTTTCCAGCGTTTAGAT	1	1	100.00
SRT 56	GAGCCTCTTTTTCCTTTCTT	GCGAACTATTACGGTGATATCA	2	2	100.00
SRT 57	CTCTGAAAAGTCCCAAGTTG	AAATGGTTTGACCATCTCC	2	1	50.00
SRT 58	CTCCCAACTTACACAATGCA	CCCTCCAAAATAGAAACGTG	2	2	100.00
SRT 59	CGGGTCTATCGCTATCCA	ATTTGACGGGATACTAGTGTG	3	2	66.66
SRT 60	CCTGACGTCACCTATGGAT	CAGCAAGTTGAATATCACCC	2	2	100.00
SRT 61	CGATCATAAACATCACGGTT	GTGGGCGTTGTTAATAACAA	1	1	100.00
SRT 62	GTCGATCTTCGAATACAACG	GGAGTCCATCTAAATCCAGC	3	2	66.66
SRT 63	CGAACAAATCAGCGAAGATC	TACTCCCTCCTCAACGCTC	1	1	100.00
SRT 64	AATTCGCACGAGGCCCTT	CCAAAATATAAGCCGGGTCAA	4	2	50.00
SRT 86	TCGCTTGACTTTCCATTTCC	AACCCTCGGGATTATCGTCT	2	1	50.00
SRT 87	CGACTCCTCGACTCGCTATT	GCGCCACATACATCTCTCC	2	1	50.00
SRT 88	CATCATGGCTTTCCGTTTTT	CCAGGATTGGTAAACCCGTA	1	1	100.00
SRT 89	CAGGGGAGCCATTGTTAGAA	CAGGGGTCCTGTGTTTCAGT	3	2	66.66
SRT 90	GAGGAGGCTGTGGTTGAAGA	ATGGTGACCCTGCTTACACC	2	1	50.00
Total			**33**	**24**	**72.72**

Table 4: Jaccard's similarity coefficient values generated using pooled data of RAPD, ISSR and SSR primers in parental lines of cotton

Parents	GSHV-97/13	GJHV-337	AET-5	GN.Cot.-22	GSHV-97/1016	GISV-8/1029	DELTA-15	EC-10/786	KH-119
GSHV-97/13	1.00								
GJHV-337	0.77	1.00							
AET-5	0.92	0.75	1.00						
GN.Cot.-22	0.84	0.74	0.82	1.00					
GSHV-97/1016	0.91	**0.72**	0.91	0.83	1.00				
GISV-8/1029	0.85	**0.72**	0.85	0.80	0.84	1.00			
DELTA-15	0.92	**0.72**	0.91	0.82	0.92	0.87	1.00		
EC-10/786	0.84	0.74	0.83	0.80	0.84	0.80	0.85	1.00	
KH119	0.89	**0.72**	0.91	0.82	0.92	0.86	**0.93**	0.85	1.00

Polymorphism as detected by RAPD, ISSR and SSR markers:

The Jaccard's similarity matrixes were calculated based on RAPD, ISSR and SSR combine data (Table 4). Jaccard's similarity index shown highest similarity between DELTA-15 and KH119 (0.93) while highest distance between GJHV-337 and GSHV-97/1016, GISV-8/1029, DELTA-15 and KH119 (0.72) respectively. The dendrogram (Fig. 2) showed genetic similarity among the nine cotton cultivars the ranged from 0.72 to 0.93 and revealed two distinct clusters A and B. Maximum number (5 out of 9) of cultivars fell in cluster A. Cluster A contained genotype DELTA-15 and KH119 as well as GSHV-97/13 and AET-5 were found to be genetically similar and could not be distinguished from each other and GSHV-97/1016 was diverted genotype in cluster A. On the other hand least similarity genotypes demonstrated by cluster B, GJHV-337 and GSHV-97/1016, GJHV-337 and GISV8/1029 and GJHV-337 and DELTA-15. In cluster B not close relationship genotypes to each other were cluster hence in B cluster all four genotypes highly diverted each other based combined molecular data. The genetic diversity and similarity were investigated by [18] in nine elite parental lines of cotton using 25 RAPD, 3 ISSR and two isozyme markers.

Based on Jaccard's similarity coefficient, the similarity index was in the range of 0.11 to 1.00 among nine parental lines. The lowest similarity (11 %) was found among LRA-5166, G.Cot.10 and 76 IH-20. The dendrogram of cotton lines showed two major clusters. The Parental line LRA-5166 was found in one cluster and the rest eight parental lines were found in different sub-sub cluster of another cluster. Similar results also reported by [19] employing thirty one SSR markers. Out of thirty one primers, six SSR primers were found polymorphic at different SSR loci and genetic similarity coefficients ranged from 0.47 to 0.97. The highest dissimilarities (0.47) was observed between genotype 298-F and CIM-496 and maximum similarity coefficient of 0.97 per cent.

CONCLUSION

The result obtained on genetic relatedness using pooled molecular makers data in the present study will be helpful in further breeding of cotton cultivars, the selection of parents for crossing and will be helpful in widening the genetic bases of breeding materials. Therefore, the results of the present investigation confirmed the efficiency of the molecular markers in detecting polymorphism among cotton genotypes, estimation of relatedness and identifying genotypes and species by unique fingerprints.

REFERENCES

1. Pillay M and Myers GO. (1999). Genetic diversity in cotton assessed by variation in ribosomal RNA genes and AFLP markers. *Crop Science;* 39(6):1881-1886.
2. Preetha S and Raveendren TS. (2008.) Molecular marker technology in cotton. *Biotechnology and Molecular Biology*; 3 (2), pp. 032-045.
3. Percy RG and Kohel RG. (1999). Cotton qualitative genetics. In: Cotton (ed. C.W. Smith and J.T. Cothren), John Wiley & Sons; NY, pp. 319-360.
4. Rana MK, Singh VP and Bhat, KV. (2005). Assessment of genetic diversity in Upland Cotton breeding lines by using amplified fragment length polymorphism (AFLP) markers and morphological characteristics. *Genetic Resources and Crop Evol*ution; 52: 989-97.
5. Gostimsky SA, Kokaeva ZG and Konovalov FA. (2005). Studying plant genome variation using molecular markers. *Russian Journal Genetics*; 41: 378-88.
6. Doyle JJ, Doyle JL. (1990). Isolation of plant DNA from fresh tissue. Focus; 12: 13-15.
7. Rohlf, FJ. (1998). NTSYS-pc: Numerical taxonomy and multi variety analysis system. *Exeter Publishing*, Setauket. Pp. 119.
8. Hussein, Ebtissam HA, Amina AM, Soltan AM and Adawy A. (2006). Molecular characterization and genetic relationship among cotton genotypes 1- RAPD, ISSR, and SSR analysis. *Arab Journal of Biotech*nology; 9: 222-29.
9. Khan SA, Hussain D, Askari E, Stewart JM, Malik KA and Zafar Y. (2000). Molecular phylogeny of *Gossypium sp*. by DNA fingerprinting. *Theoretical and Applied Genetics* 101: 931-938.
10. Bhartakumar C, Patil RS, Kumar KS, Akhileshwari SV and Khadi BM. (2014). Molecular divergence in upland cotton (*Gossypium hirsutum* L.). *Environments & Ecology*; 32(1): 178-183.
11. Dongre AB, Raut MP, Bhandarkar MR and Meshram KJ. (2011). Use of RAPD and ISSR for determining genetic diversity of cotton (*Gossypium hirsutum* L.). Working sample *Journal of Cotton Research and Development;* 25(2): 137-143.
12. Patel DH, Patel DU, Jha S, Rajkumar BK and Kumar V. (2014). Genetic diversity assessment in cotton genotypes (*Gossypium hirsutum* and *G. barbadense*) using PCR based markers. *Green Farming*; 5(6): 1016-1019.
13. Dongre AB, Bhandarkar M and Jee SB. (2007). Genetic diversity in tetraploid and diploid cotton using ISSR and SSR DNA markers. *Indian journal of Biotechnology*; 6: 349-353.
14. Liu D, Guo Z, Lin Y and Nie X. (2005). Genetic diversity of Asian cotton (*Gossypium arboreum* L.) in China evaluated by microsatellite analysis. *Genetic Resources and Crop Evol*ution; 53: 1145-52.
15. Chen G and Du, XM. (2006). Genetic diversity of source germplasm of Upland cotton in China as determined by SSR marker analysis. *Yi Chuan Xue Bao*; 33: 733-45.
16. Dahab AA, Saeed M, Mohamed BB, Ashraf MA, Puspito AN, Bajwa KS, Shahid AA and Husnain T. (2013). Genetic diversity assessment of cotton (*Gossypium hirsutum* L.) genotypes from Pakistan using simple sequence repeat markers. *Australian Journal of Crop Sci*ence; **7**: 261-267.
17. Tyagi S, Verma SK, Nehra K and Bajya DR. (2015). Molecular marker based genetic diversity analysis in cotton using RAPD and SSR marker. *Journal of Cotton Research and Development*; 29(2): 232-236.
18. Mahatma MK, Khandelawal, V Jha, SK Kumar V and Shah RR. (2009). Genetic diversity analysis of elite parental lines of cotton using RAPD, ISSR and isozyme markers. *Indian Journal of Physio*logy; 14: 105-110.
19. Ehsan B, Haque A, Younas M, Shaheen T, Huma T, Sattar S and Iqbal Z. (2013). Assessment of genomics diversity of cotton (*Gossypium hirsutum* L.). *International Journal of Plant Science*; 2: 53-56.

CITATION OF THIS ARTICLE

Bilwal, B. B., Vadodariya, K.V., Rajkumar, B. K and Lahane, G. R.Genetic diversity of parents using RAPD, ISSR and SSR molecular markers in upland cotton (*Gossypium hirsutum* L.). Bull. Env. Pharmacol. Life Sci., Vol 6 [8] July 2017: 51-57