Int.J.Curr.Microbiol.App.Sci (2021) *10*(03): 324-331

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 03 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1003.043

Molecular Characterization of Germplasm Accessions of *G. hirsutum* using SSR Markers

Mayuri Tamboli, Sushil Mawle, M. Saravanan, S. S. Mahajan and V. N. Waghmare*

ICAR-Central Institute for Cotton Research, Nagpur, Maharashtra, India

**Corresponding author*

Keywords

Cotton, *G. hirsutum*, Molecular characterization, SSR markers

Article Info

Accepted:
04 February 2021
Available Online:
10 March 2021

ABSTRACT

The present investigation studied genetic diversity of ninety four cotton germplasm accessions, in order to improve genetic base of the present cultivars. Out of 48 primer pairs surveyed, 20 (42%) were showing polymorphism. The average number of alleles per locus was 2.45. The polymorphism information content (PIC) varied from 0.0735 to 0.6461 with an average value of 0.2978. The major allele frequency ranged from 0.3352 to 0.9602 with the mean value of 0.7304. The observed heterozygosity values for the marker loci varied from 0.000 to 0.702 with the mean value of 0.146. Three SSR loci namely MUSS0013, GH0499 and BNL2960 did not show heterozygosity/any heterozygotes while remaining loci showed the values ranging from 0.0118 to 0.7024. The expected heterozygosity (gene diversity) values varied from 0.0764 to 0.7046. NJ tree showed three major genotypic clusters within the collection of 94 accessions with bootstrap support. Cluster 1 (G1) was a major group consisting of 42 accessions with several subgroups. Cluster 2 (G2) included 33 accessions and cluster 3 (G3) included 19 accessions.

Introduction

Cotton is an important fibre cash crop and also referred as white gold because of its importance in the global economy particularly India. India is the only country to grow all four cultivated cotton species. The two cultivated species namely, *G. hirsutum* and *G. barbadense* belongs to tetraploid (2n=4x=52) and *G. arboreum* and *G. herbaceum* belongs to diploid species (2n=2x=26) of cotton. Among them, *G. hirsutum* has high yielding potential and wider adaptability. Due to domestication, selection process and use of few germplasm accessions in the breeding programme has led to the loss of genetic diversity in commercial cultivars over time (May *et al.,* 1995; Bowman *et al.,* 1996; Wendel *et al.,* 1992; Brubaker et al.1999). The narrow genetic base results into vulnerability to biotic and abiotic stresses and can be broadened by using diverse germplasm accessions in breeding program. Molecular characterization of germplasm accessions has been carried out by using PCR-based markers such as random amplified polymorphic DNA (RAPD) (Tatineni *et al.,* 1996; Xu *et al.,* 2001; Lu and Myers 2002, Sapkal et.al 2011), amplified fragment length polymorphism (AFLP) (Abdalla *et al.,* 2001; Alvarez and

Wendel 2006) and simple sequence repeats (SSRs) (Liu *et al.,* 2000; Zhu *et al.,* 2003, Santhy *et al.,* 2019). Among these markers, SSRs are most abundant, robust, co-dominant type, easy to use, hyper-variable i.e., they are highly polymorphic (Reddy *et al.,* 2001). The molecular markers facilitate in the marker assisted breeding for the improvement of economic traits through exploitation of genetic diversity in cotton (Mishra *et al.,* 2013). The aim of the present study was to determine the genetic diversity present in the available cotton germplasm accessions and to identify the genetically diverse accessions that could be potentially used in the breeding program for cotton improvement with broad genetic base.

Materials and Methods

A total of 94 germplasm accessions of *Gossypium hirsutum* were selected for studying the genetic diversity analysis (Table 1). The genomic DNA was extracted from fresh leaf samples of seedling by rapid method (Paterson *et al.,* 1993). The DNA samples were quantified using Nanodrop Spectrophotometer (ND1000).The quality was assessed through 0.8 % agarose gel electrophoresis. All samples were adjusted to a uniform DNA concentration of 10 ng/µl using sterile distilled water. A set of 48 SSR markers covering majority of AD genome were selected from the publicly available CottonGen (http://www.Cottongen.org) and CottonMarker (http://www.Cottonmarker.org) databases (Table 2). PCR amplification was carried out in 20 µl reaction volume containing 1X PCR buffer with 2.5 mM $MgCl_2$, 0.2 mM each of dNTPs, 0.4 µM each of forward and reverse primer, 0.5 U Taq polymerase and 2 µl of DNA (10 ng/µl) as Template. Genomic regions pertaining to SSR markers were amplified under the following PCR cycle conditions: 94ºC for 4 minutes for initial denaturation, 30 cycles comprising of three steps viz., denaturation at 94ºC for 1 minute, annealing at 50-68ºC for 30 seconds (vary as per primer annealing temperature) and extension at 72ºC for 1 minute. Reaction was finally ended with incubation at 72º C for 5 minutes and followed by hold at 4º C using a thermal cycler (Biorad iCycler). The PCR products were resolved using vertical gel electrophoresis system using 8.5% PAGE in 1X TBE buffer and polymorphism was visualized by staining with ethidium bromide and documented using a Gel Documentation system (AlphaImager). The amplicons were scored across the lanes comparing their respective molecular weights. Presence of a band was scored as “1”and absence of a band as “0” for further analysis.

Data analysis

Genetic diversity estimates

Level of polymorphism with respect to each marker on allele frequencies, observed heterozygosity (Ho), gene diversity (expected heterozygosity, He) and polymorphism information content (PIC) was estimated using PowerMarker version 3.25 (Liu and Muse, 2005).

Genetic relatedness

The pairwise dissimilarity co-efficients (simple matching) was calculated and a neighbour joining tree was constructed (Perrier *et al.,* 2003) using DARwin (Dissimilarity Analysis and Representation for windows) 6.0.012 from SSR allelic data (Perrier and Jacquemod Collet 2006). This helped to understand the relationship among the genotypes, using the Sokal and Michener index. Bootstrap dissimilarity matrices were calculated by drawing 1000 entries. A factorial analysis, Principal coordinates analysis (PCoA) based on the pairwise distance matrix was performed to visualize

the overall representation of diversity of germplasm accessions.

Results and Discussion

A total of 48 primer pairs were used to amplify genomic DNA of 94 germplasm accessions. Out of 48 primer pairs surveyed, 20 (42%) were polymorphic. The sample profile exhibiting polymorphism for the SSR markers DPL0752 and MUSS0004 were given in Figure 1. These primer pairs produced a total of 49 alleles across all the accessions. The number of alleles per locus varied from 2 to 4 with a mean of 2.45 (Table 3). Similar kind of results are obtained by several investigators such as, 2.20 alleles per locus (Zhang *et al.,* (2005); 2.13 alleles per locus (Bertini *et al.,* (2006) etc. Some of the studies reported higher number of alleles per locus such as, 5.61 (Lacape *et al.,* 2007); 5.73 (Kaur *et al.,* 2017) and 7.9 (McCarty *et al.,* 2018). Majority of the primer pairs (12) were biallelic/Co-dominant. The major allele frequency ranged from 0.335 to 0.960 with the mean value of 0.730. The observed heterozygosity values for the marker loci varied from 0.000 to 0.702 with the mean value of 0.146. Three SSR loci did not show heterozygosity or any heterozygotes while remaining loci showed the range of values from 0.012 to 0.702. The expected heterozygosity (gene diversity) values ranged from 0.076 to 0.705. The polymorphism information content (PIC) values were ranged from 0.074 to 0.646 with an average value of 0.298. The most informative markers in this investigation were NAU5411 and GH0433 with PIC values of 0.5398 and 0.646, respectively. The mean PIC value of 0.31 and 0.55 is reported by Liu *et al.,* 2000 and Lacape *et al.,* 2007, respectively.

Table.1 List of germplasm accessions used for the present study

Sr. No.	Accession numbers	Sr. No.	Accession numbers
1	IC360028	48	IC359918
2	IC360028 SuperOkra	49	H70
3	IC359866	50	N187
4	IC359898	51	IC356841
5	IC359953	52	IC357644
6	IC360030	53	IC359909
7	IC359745	54	IC359762
8	IC359772	55	IC356226
9	IC359787	56	IC356543
10	IC359789	57	IC356626
11	IC359804	58	IC356696
12	IC359811	59	IC356894
13	IC359812Okra	60	IC357042
14	IC359812BLL	61	IC356720
15	IC359822	62	IC356761
16	IC359822Okra	63	IC359069
17	IC359883	64	IC356840
18	IC359892	65	IC356857
19	IC359900	66	IC356900
20	IC359913	67	IC359797
21	IC359887	68	IC356516
22	IC356750	69	IC356538

23	IC356784	70	IC356540
24	IC356954	71	IC356554
25	IC357078	72	IC356568
26	IC358603	73	IC356655
27	IC358781	74	IC356897
28	IC359895	75	IC357361
29	IC359944 CB	76	IC357727
30	IC359976	77	IC356692
31	IC359978	78	IC356846
32	IC360001	79	IC356941
33	IC360070	80	IC356943
34	IC357945	81	IC357052
35	IC358146	82	IC357460
36	IC359945	83	IC357463
37	DCI230	84	IC357606
38	DCI214	85	IC357740
39	MGB286	86	IC357856
40	N70	87	IC358244
41	N99	88	IC358535
42	IC357191	89	IC359662
43	IC357554	90	IC356573
44	IC359028	91	IC356700
45	IC359932	92	IC356975
46	IC359048	93	IC357025
47	IC359083	94	IC357652

Table.2 List of primers used in the genetic diversity analysis

S. No.	**Name of Markers**	**S. No.**	**Name of Markers**	**S. No**	**Name of Markers**
1	DPL0752	17	BNL2960	33	NAU2894
2	NAU5411	18	TMB2962	34	NAU2126
3	CIR0320	19	TMB1356	35	DPL0600
4	NAU1248	20	GH0132	36	GH0277
5	CIR0347	21	GH0316	37	BNL3649
6	BNL0530	22	NAU2640	38	TMB0043
7	BNL2572	23	TMB2557	39	GH0330
8	JESPR0065	24	JESPR0153	40	JESPR0220
9	CIR0267	25	CIR0228	41	DPL0079
10	GH0433	26	GH0295	42	NAU5189
11	MUSS0004	27	GH0002	43	NAU5335
12	MUSS0013	28	GH0684	44	NAU2292
13	NAU1037	29	NAU5120	45	DPL0799
14	NAU5357	30	DPL0910	46	NAU0928
15	GH0499	31	TMB2295	47	GH0629
16	NAU3888	32	DPL0143	48	TMB0083

Table.3 Diversity measures of SSR loci for characterization of *G. hirsutum* germplasm accessions

Marker	Major Allele Frequency	Allele No.	Gene Diversity	Observed Heterozygosity	PIC*
GH0316	0.9602	2.0000	0.0764	0.0795	0.0735
TMB2557	0.9366	3.0000	0.1201	0.1268	0.1155
NAU5357	0.9295	2.0000	0.1311	0.1410	0.1225
NAU2640	0.9217	3.0000	0.1452	0.1566	0.1363
CIR0328	0.8605	2.0000	0.2401	0.2791	0.2113
CIR0267	0.8512	2.0000	0.2533	0.0119	0.2212
BNL0530	0.8424	2.0000	0.2655	0.3152	0.2303
CIR0228	0.8269	2.0000	0.2862	0.2436	0.2453
GH0295	0.8294	3.0000	0.2849	0.0118	0.2475
MUSS0013	0.7692	2.0000	0.3550	0.0000	0.2920
GH0499	0.7647	3.0000	0.3776	0.0000	0.3334
JESPR0065	0.6875	2.0000	0.4297	0.0500	0.3374
NAU1037	0.6369	2.0000	0.4625	0.0119	0.3556
MUSS0004	0.6294	2.0000	0.4665	0.0824	0.3577
NAU3888	0.6488	3.0000	0.4598	0.7024	0.3594
BNL2960	0.5714	2.0000	0.4898	0.0000	0.3698
JESPR0153	0.5511	2.0000	0.4948	0.0114	0.3724
DPL0752	0.5372	3.0000	0.5068	0.0957	0.3887
NAU5411	0.5181	3.0000	0.6108	0.5783	0.5398
GH0433	0.3352	4.0000	0.7046	0.0227	0.6461
Mean	**0.7304**	**2.4500**	**0.3580**	**0.1460**	**0.2978**
Maximum	**0.9602**	**4.0000**	**0.7046**	**0.7024**	**0.6461**
Minimum	**0.3352**	**2.0000**	**0.0764**	**0.0000**	**0.0735**

*PIC-Polymorphism Information Content

Fig.1 The banding pattern of polymorphic markers DPL0752 and MUSS0004

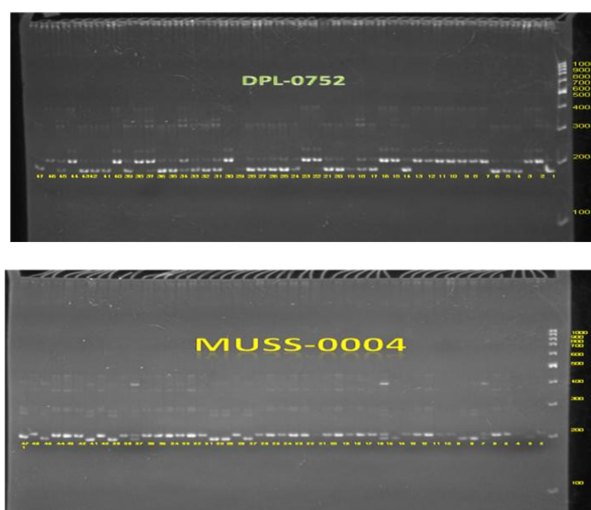


Fig.2 NJ tree based hierarchical dendrogram depicting genetic relationships among 94 germplasm accessions of *G. hirsutum*

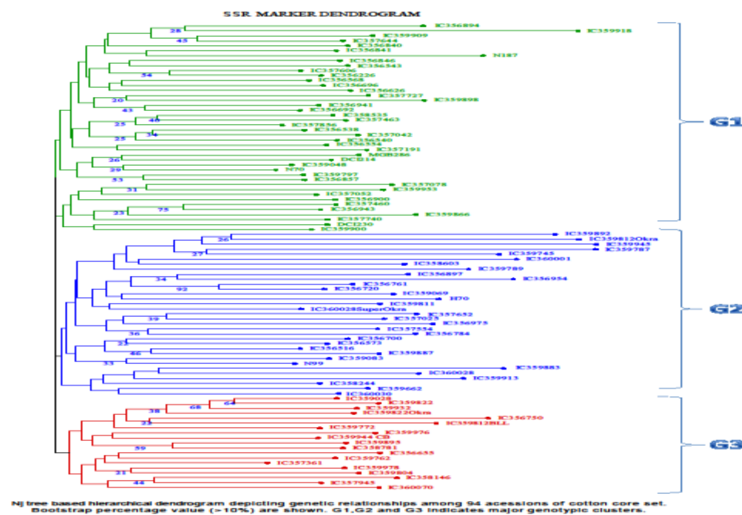
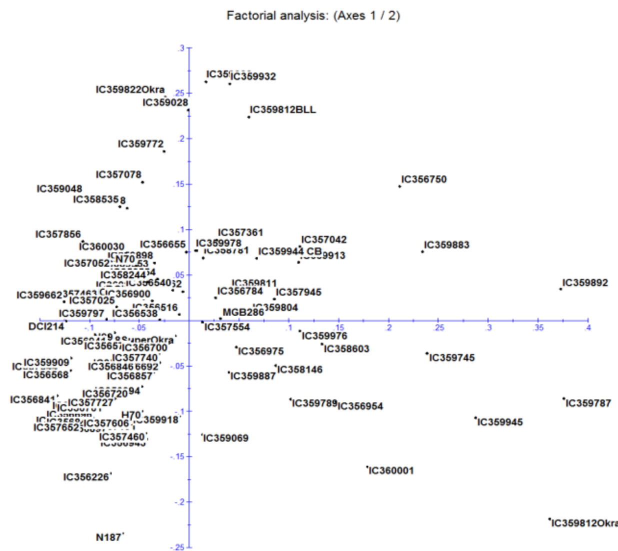


Fig.3 Factorial analysis (Principal Coordinate Analysis) of germplasm accessions of *G. hirsutum*



NJ tree showed three major genotypic clusters within the collection of 94 accessions with bootstrap support (Fig. 2). Cluster 1 (named as G1) was a major group consisting of 42 accessions with several subgroups. Cluster 2 (G2) included 33 accessions and cluster 3 (G3) included 19 accessions. Principal Coordinates Analysis (PCoA) was carried out to visualize the overall representation of genetic diversity (Fig. 3).

Narrow genetic base in the available cotton varieties makes them vulnerable to abiotic and biotic stresses causing yield stagnation. So, there is an urgent need to widen the genetic base of the parental material in the breeding programme. For achieving this, identification of diverse genetic materials is essential. The SSR markers are the preferred markers for estimation of genetic diversity at the molecular level. A total of 48 primer pairs were used to analyse the genetic diversity of 94 samples of *G. hirsutum*. The number of alleles per locus ranged from 2 to 4 (mean value of 2.45). The polymorphism information content (PIC) values from 0.074 to 0.646 with an average value of 0.298. This result indicates moderate level of diversity present in the tested germplasm accessions. Based on dendrogram and genetic relationships, these diverse cotton accessions can be identified and used in breeding programme for improving seed cotton yield and mitigate the problems posed by biotic and abiotic stresses.

Acknowledgment

The authors thank the Director, ICAR-CICR and Head, Division of Crop Improvement for providing lab facilities to undertake this research program.

References

Abdalla, A.M., Reddy, O.U.K., El-Zik, K.M. and Pepper, A.E. 2001. Genetic diversity and relationships of diploid and tetraploid cottons revealed using AFLP. *Theor Appl Genet* 102:222-229. Doi:10.1007/s001220051639.

Alvarez, I. and Wendel, J.F. 2006. Cryptic interspecific introgression and genetic differentiation within *Gossypium aridum* (Malvaceae) and its relatives. *Evol Int J Org Evol* 60:505-517.

Bertini, C.H., Schuster, I., Sediyama, T., Barros, E.G.D. and Moreira, M.A. 2006. Characterization and genetic diversity analysis of cotton cultivars using microsatellites. *Genet.Mol.Biol*, 29:321-329.

Bowman, D.T., May, O.L. and Calhoun, D.S. 1996. Genetic base of upland cotton cultivars released between 1970 and 1990. *Crop Sci* 36:577–581.

Brubaker, C.L., Bourland, F.M. and Wendel, J.F. 1999. The origin and domestication of cotton. In: Smith CW, Cothren JT (eds) Cotton: Origin, History, Technology, and Production. Wiley, New York, pp 3–32.

Kaur, B. P., Tyagi, P. and Kuraparthy, V. 2017. Genetic Diversity and population structure in the landrace accession of *Gossypium hirsutum*. *Crop. Sci* 57:2457-2470.

Lacape, J.M., Dessauw, D., Rajab, M., Noyer, J.L. and Hau, B. 2007. Microsatellite diversity in tetraploid *Gossypium* germplasm: assembling a highly informative genotyping set of cotton SSRs. *Mol Breed* 19(1):45-58.

Liu, K. and Muse, S.V. 2005. Power Marker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21: 2128-2129.

Liu, S., Cantrell, R.G., McCarty, J.C.J. and Stewart, J.M.D. 2000. Simple sequence repeat-based assessment of genetic diversity in cotton race stock accessions. *Crop Sci* 40:1459-1469.

Lu, H.J. and Myers, G.O. 2002. Genetic relationships and discrimination of ten influential Upland cotton varieties using RAPD markers. *Theor Appl Genet* 105:325-331. Doi: 10.1007/s0012-002-0947-8.

McCarty, J.C., Deng, D.D., Jenkins, J.N. and Geng, L. 2018. Genetic diversity of day-neutral converted landrace *Gossypium hirsutum* L. accesions. *Euphytica* 214: 173.

May, O.L., Bowman, D.T. and Calhoun, D.S. 1995. Genetic diversity of U.S. upland cotton cultivars released between 1980 and 1990. *Crop Sci* 35:1570–1574.

Mishra, K.K. and Fougat, R.S. 2013. Genetic

relationship among different species of cotton as revealed by SSR markers for fiber quality traits. *International Journal of Pure and Applied Bioscience.* 1(3): 81-93.

Paterson, A.H., Brubaker, C. and Wendel, J.F. 1993. A rapid method of extraction of cotton (*Gossypium* spp.) genomic DNA suitable for RFLP or PCR analysis. *Plant Mol. Biol. Rep.* 11:122-127.

Perrier, X. and Jacquemoud-Collet, J.P. 2006. DARwin software http://darwin.cirad.fr/

Perrier, X., Flori, A. and Bonnot, F. 2003. Data analysis methods in: Hamon P, Segin M, Perrier X, Glaszmann JC (ed) genetic diversity of cultivated tropical plants. Science Publishers, Montpellier, Enfield, pp,43-76.

Reddy, O.U.K., Pepper, A.E., Abdurakhmonov, I., Saha, S., Jenkins, J.N. and Brooks T.D. *et al.,* 2001. New dionucleotide and trinucleotide microsatellite marker for cotton genomic research *J. Cotton Sci.* 5, 103-113.

Santhy, V., Meshram, M., Santosh, H.B. and Kranthi, K.R. 2019. Molecular diversity analysis and DNA fingerprinting of cotton varieties of India. *Indian J. Genet,* 79(4):719-725.

Sapkal, D.R., Sutar, S.R., Thakre, P.B., Patil, B.R., Paterson, A.H. and Waghmare, V.N. 2011. Genetic diversity of maintainer and restorer accessions in Upland cotton (*Gossypium hirsutum* L.) *J. Plant Biochem. Biotechnology* 20, 20-28.

Sethi, K., Siwach, P and Verma, S.K. 2015. Assessing genetic diversity among six populations of *Gossypium arboreum* L. Using microsatellites markers. *Physiol Mol Biol Plants* 21(4):531-539.

Singh, A. K. 2016. Revisiting the Status of Cultivated Plant Species Agrobiodiversity in India: An Overview. *Proc Indian Natn Sci Acad* 83: 151-174.

Tatineni, V., Canterell, R.G. and Davis, D.D. 1996. Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. *Crop Sci* 36:186-192.

Watt, S.G. 1907. The wild and cultivated cotton plants of the world. First Indian Edition (1989) P.1-885.

Wendel, J.F. and Cronn, R.C. 2003. Polyploidy and the evolutionary history of cotton. *Adv Agron* 78:139–186.

Wendel, J.F., Brubaker, C.L. and Percival, A.E. 1992. Genetic diversity in *Gossypium hirsutum* and origin of upland cotton. *Am. J. Bot.* 79:1291-1310.

Xu, Q.H., Zhang, X.L. and Nie, Y.C. 2001. Genetic diversity evaluation of cultivars (*G. hirsutum* L.) from the Changjiang River valley and Yellow River valley by RAPD markers. *Acta Genet Sin* 28: 683-690.

Zhang, J., Lu, Y., Cantrell, R.G. and E. Hughs. 2005. Molecular marker diversity and field performance in commercial cotton cultivar evaluated in the south western USA. *Crop. Sci*. 45: 1483-1490.

Zhu, L.F., Zhang, X.L. and Nie, Y.C. 2003. Analysis of genetic diversity in upland cotton (*Gossypium hirsutum* L.) cultivaars from China and foreign countries by RAPDs and SSRs. *J Agric Biotechnol* 11:450-455.

How to cite this article:

Mayuri Tamboli, Sushil Mawle, M. Saravanan, S. S. Mahajan and Waghmare, V. N. 2021. Molecular Characterization of Germplasm Accessions of *G. hirsutum* using SSR Markers. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 324-331. doi: https://doi.org/10.20546/ijcmas.2021.1003.043