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Molecular Diversity Studies in Cotton (*Gossypium hirsutum* L.) using SSR Markers

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A B S T R A C T

The present investigation was to study the molecular diversity of twelve cotton genotypes, in order to select the suitable divergent parent for heterosis breeding. Totally, 55 primers were used to diversify these genotypes and 40 pairs showed clear, scorable and unambiguous bands. Out of 40, 25 primer pairs (62.55 %) were found to be polymorphic and 15 found to be monomorphic. The average number of alleles per locus was 1.8. The similarity coefficient ranged from 0.56 to 1.00 with high dissimilarity coefficient of 0.44 for the genotypes TCH 1777 with MCU 13 and TSH 0499 with SVPR 4. A high similarity coefficient of 0.92 was observed among the parents BGDS 1063 with ARBC 19 and TSH 0499 with TSH 1819. The results of cluster analysis grouped twelve genotypes into seven clusters. The largest cluster was cluster A, which had four genotypes and cluster B had two genotypes. The cluster C, D, E, F and G had one genotype type each. Hence, selecting the genotypes for hybridization from different and distant clusters will paves the way to create variability and could be utilized in varietal or hybrid development programme.

Introduction

India occupies unique position in the global cotton scenario due to several distinct features. India has the largest cotton growing area, possibly the only cotton growing country to grow hybrid cotton involving different species of cotton. It is the native home of old world cultivated cotton and wide diversity in agro-climatic conditions. Cotton has 9 different genomes, 46 wild, 4 cultivated species and probably enriched with sufficient variability. Due to utilization of narrow genetic material in hybridization, cotton yield and quality declined. Thus, evaluation of genetic diversity related to these characters is important for

sustainable production of cotton as well as cotton breeding. The Assessment of genetic variation among genotypes may help to identify genetically diverse parental lines to exploit heterosis. Although agronomical characterization provides useful information to users, these characteristics are normally subjected to environmental influences and must be assessed during a fixed vegetative phase of the crop. In case of molecular markers, it reveals differences of natural sites at the DNA level. These variations are not seen in phenotype and each might be a single nucleotide differences in a gene or a piece of repetitive DNA. Genetic diversity analysis using molecular markers have been superior over conventional breeding (Melchinger *et al.,* 1991).

Simple Sequence Repeat has become one of the most powerful genetic markers in biology with potential applications for plant breeding programmes (Gupta and Varshney, 2000). SSR markers are abundant among the genomes and show high level of polymorphisms (Powell *et al.,* 1996). The present investigation was to study the molecular diversity of twelve cotton genotypes using SSR markers, in order to select the suitable divergent parent for heterosis breeding.

Materials and Methods

Genotypes

A total of twelve cotton genotypes were assessed for polymorphism using SSR markers for studying the extent of genetic diversity among parents. The details of the genotypes were presented in Table 1. Genomic DNA was isolated from the cotton leaves by the procedure suggested by Zhang and Stewart (2000) and the quantity and quality of the DNA was checked for polymerase chain reaction (PCR).

SSR markers

A total of 55 SSR primer pairs were used to assess the extent of genetic diversity. Among them, 40 showed distinct clear band and the list of primers along with its annealing temperature were furnished in Table 2.

Band scoring

Data were scored on the basis of the presence or absence of the bands obtained. If a band was present in a genotype, it was designated as '1' and if absent; it was designated as '0'. The data were maintained in the spread sheet format for further analysis. The data were entered in to binary matrix and subsequently analyzed by using NTSYS-pc version 2.02.

Data Analysis

The data were subjected to statistical analysis for the calculation of Jaccard's similarity coefficient (Jaccard, 1908). The resultant similarity matrix was entered into SAHN (sequential, agglomerative, hierarchical, and nested clustering method) clustering program, a tree matrix was generated and cluster analysis by UPGMA (unweighted pair-group method with arithmetic averages) using NTSYS-pc version 2.02 based software.

Results and Discussion

Molecular marker based genetic diversity analysis

In SSR analysis with 40 microsatellite markers, 25 primer pairs (62.55%) showed polymorphism and the rest showed monomorphism among the parents. A total of 47 alleles were produced by 25 primers. The number of alleles revealed by each marker ranged from 2 to 3. The average number of alleles per locus was found to be 1.8. 2-5 primer alleles with an average of 2.8 per locus

were recorded by Bertini *et al.*, (2006), Abdellatif *et al.*, (2012) and Mishra *et al.*, (2013). A maximum of three alleles was recorded by JESPR 292, while six markers viz., BNL 1161, HAU 940, NAU 797, TMB 2295, Gh 129 and MUSB 1316.

Similarity coefficient

The similarity index (SI) values were computed as the ratio of number of similar bands to the total number of bands in pair wise comparison of the genotypes. Genetic similarity co-efficient was estimated for each pair of 12 parents using the binary data from the polymorphic primers.

The similarity coefficient ranged from 0.56 to 1.00 with high dissimilarity coefficient of 0.44 for the genotypes TCH 1777 with MCU 13 and TSH 0499 with SVPR 4. The similarity coefficient of 0.38-0.98 was observed by Rana and Bhat (2005), 9.68-53.29 similarity detected by Patil *et al.*, (2007) and 0.49-1.00 observed by Thiyagu *et al.*, (2011). The similarity co-efficient of the 12 parents was presented in Table 3. ARBC 19 with BGDS 1063 and TSH 0499 with TCH 1819 recorded higher similarity index of 0.92. It was followed by five genotype combination *viz.*, BGDS 1063 with TSH 0499, ARBC 19 with TSH 0499, MCU 13 with ARBC 19, SVPR 4 with ARBC 19 and Surabhi with ARBC 19, which recorded second highest similarity coefficient of 0.88.

TSH 0499 had lesser similarity index (0.56) with TCH 1777. Hybridization between these genotypes will provide sufficient variability. But, the genotype TSH 0499 had higher similarity index of 0.92 with TCH 1819. These results are in accordance with the findings of Ehsan *et al.*, (2013) and Bilwal *et al.*, (2017). Hybridization between closer genotypes should not be advisable for securing greater variability.

Table.1 List of lines, testers and check used in the present investigation

S. No	Genotype	Source	Special feature
1.	TSH 0499	CRS, Srivilliputtur, Tamil Nadu	High yield with big boll size
2.	TSH 04/115	CRS, Srivilliputtur, Tamil Nadu	High yielding
3.	BGDS 1063	UAS, Bheemarayangudi, Karnataka	High yielding
4.	ARBC 19	UAS, Dharwad, Karnataka	Compact, high yielding
5.	MCU 7	Department of Cotton, Tamil Nadu Agricultural University, Coimbatore	Early duration
6.	MCU 13	Department of Cotton, Tamil Nadu Agricultural University, Coimbatore	High yielding
7.	CO 14	Department of Cotton, Tamil Nadu Agricultural University, Coimbatore	Extra long staple, Moderately Resistant to Jassid
8.	SVPR 4	CRS, Srivilliputtur, Tamil Nadu	Good fibre strength
9.	KC 3	ARS, Kovilpatti, Tamil Nadu	Resistant to leaf hopper
10.	Surabhi	CICR, Coimbatore, Tamil Nadu	*Verticillium* wilt Resistant
11.	TCH 1777	Department of Cotton, Tamil Nadu Agricultural University, Coimbatore	High yielding
12.	TCH 1819	Department of Cotton, Tamil Nadu Agricultural University, Coimbatore	Compact type, suitable for High density planting system, Early duration

Table.2 Details of simple sequence repeat (SSR) primer used in the study

S. No	Primer name	Annealing Temp. (°C)	S. No	Primer name	Annealing Temp. (°C)	S. No	Primer name	Annealing Temp. (°C)
1.	BNL 1161	46.75	**15.**	Gh 610	50	**29.**	TMB 2295	55
2.	BNL 1034	55	**16.**	HAU 585	57	**30.**	CM 029	50
3.	BNL 1317	55	**17.**	HAU 940	57	**31.**	CM 066	50
4.	BNL 2732	48.5	**18.**	JESPR 127	58.20	**32.**	CIR 295	55
5.	BNL 2921	55	**19.**	JESPR 154	58	**33.**	CIR 307	46
6.	BNL 3424	55	**20.**	JESPR 292	58	**34.**	CIR 407	51
7.	BNL 3623	46.75	**21.**	MUSB 0078	55	**35.**	DPL 0075	49
8.	BNL 3806	47.5	**22.**	MUSB 1316	50	**36.**	DPL 0080	49
9.	BNL 4030	54.4	**23.**	NAU 0797	46.75	**37.**	DPL 0112	49
10.	BNL 4108	54.3	**24.**	NAU 0923	50.3	**38.**	DPL 0135	49
11.	BNL 1227	48.75	**25.**	NAU 1037	57	**39.**	DPL 0153	49
12.	Gh 129	55	**26.**	NAU 1200	49.35	**40.**	DPL 0163	50
13.	Gh 132	55	**27.**	NAU 1233	57			
14.	Gh 609	50	**28.**	TMB 471	55			

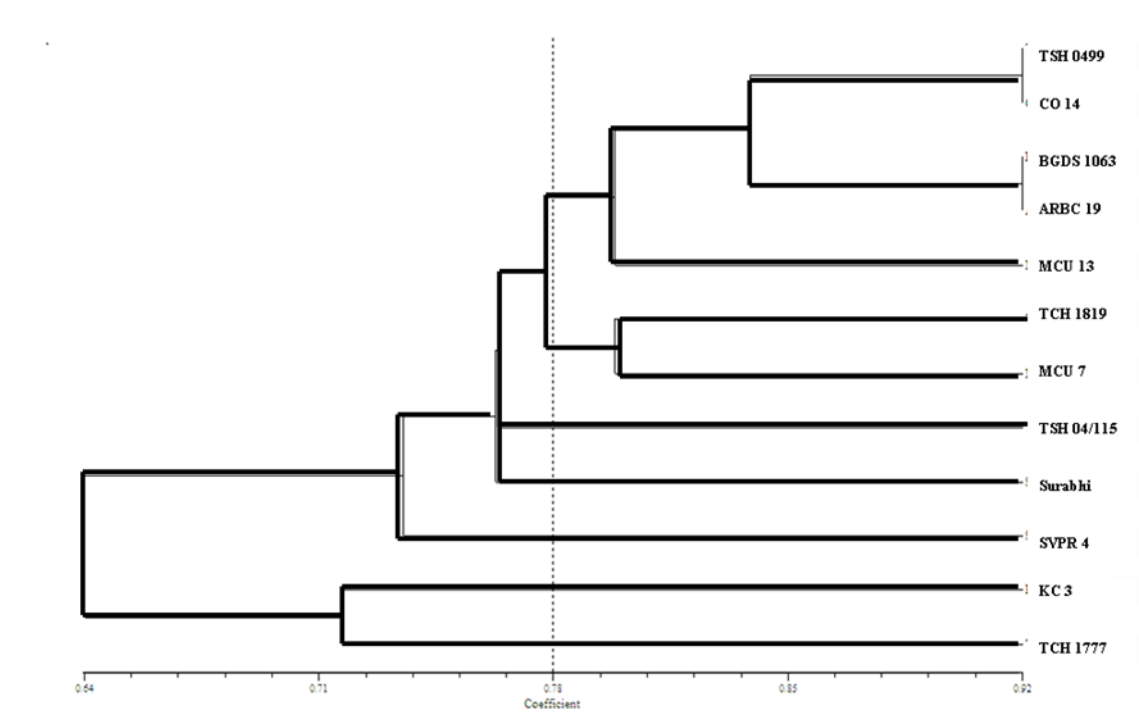
Table.3 Genetic similarity co-efficient values of 12 parental genotypes based on 25 SSR markers (*Gossypium hirsutum* L.)

Genotypes	TSH 0499	TSH 04/115	BGDS 1063	ARBC 19	MCU 13	SVPR 4	KC 3	TCH 1777	Surabhi	TCH 1819	CO 14	MCU 7
TSH 0499	**1.00**											
TSH 04/115	0.76	**1.00**										
BGDS 1063	0.88	0.80	**1.00**									
ARBC 19	0.88	0.80	0.92	**1.00**								
MCU 13	0.76	0.76	0.80	0.88	**1.00**							
SVPR 4	0.76	0.68	0.80	0.88	0.76	**1.00**						
KC 3	0.68	0.68	0.72	0.72	0.60	0.68	**1.00**					
TCH 1777	0.56	0.64	0.60	0.60	0.56	0.56	0.72	**1.00**				
Surabhi	0.76	0.76	0.80	0.88	0.76	0.76	0.76	0.64	**1.00**			
TCH 1819	0.92	0.76	0.80	0.80	0.76	0.68	0.68	0.64	0.68	**1.00**		
CO 14	0.80	0.72	0.76	0.76	0.72	0.64	0.64	0.60	0.72	0.80	**1.00**	
MCU 7	0.76	0.76	0.80	0.80	0.76	0.68	0.68	0.64	0.76	0.84	0.80	**1.00**

Table.4 Distribution of 12 parental genotypes into 5 clusters based on jaccard's coefficient

Cluster	No. of genotype	Name of the genotype
A	4	TSH 0499, CO 14, BGDS 1063, ARBC 19
B	2	TCH 1819, MCU 7
C	1	TSH 04/115
D	1	Surabhi
E	1	SVPR 4
F	1	KC 3
G	1	TCH 1777

Fig.1 Clustering of 12 cotton genotypes using Jaccard's Coefficient



Clustering based on dendrogram

All the genotypes were scored for presence and absence of bands for the specific alleles of each SSR markers. A dendrogram was made by using the unweighted pair group method of arithmetic average (UPGMA) method by NTSYSpc package version 2.02i.

The dendrogram revealed that all the genotypes were clustered into seven groups (Group A, B, C, D, E, F and G). The number of clusters along with its genotypes was presented in Table 4 and Figure 1. The group 'A' had two subgroups viz., A1 (2 accessions) with TSH 0499 and CO 14 and A2 (2 accessions) with BGDS 1063 and ARBC 19.

Whereas, the second group (B) clustered two accessions *viz.,* TCH 1819 and MCU 7. The group C, D, E, F and G had one genotype type each viz., TSH 04/115, Surabhi, SVPR 4, KC 3 and TCH 1777 respectively. The group 'A' formed the largest cluster with four accessions. The involvement of genotypes in the different cluster indicated that the varieties with the different genetic composition were falling in one cluster and this can help to conduct a hybridization programme with these genotypes.

Thus, the relatedness of the cultivars studied was efficiently established through the use of SSR markers with some differences in the positioning of some cultivars at various clusters. Dendrogram generated for SSR markers of cotton genotypes reflects the relationships among the most of the *G. hirsutum* L. cultivars depending upon their yield and fibre quality traits which can help conduct hybridization programme with distinct related genotypes.

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