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Genetic Diversity Analysis in Cotton (*Gossypium Hirsutum L.)* Based on Morphological Traits and Microsatellite Markers

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Abstract: **One hundred and fifty six cotton genotypes representing different plant types were studied for genetic diversity on the basis of quantitative and qualitative traits using Mahalanobis D^2 statistic and SSR marker analysis. Out of ten clusters obtained, cluster I was the largest with fifty nine genotypes, cluster II had fifty five genotypes, cluster III had ten genotypes, cluster V had 13 genotypes, cluster VI had twelve genotypes, cluster VII had three genotypes while the remaining four clusters had single genotype each. Cluster X exhibited the maximum mean values for seed cotton yield, number of bolls per plant and number of sympodia per plant.**

Two distinct groups resulted from SSR marker data analysis. Among the 49 primers, BNVB-37421, CIR081, CIR081, CIR005, CIR182, CIR238, CIR413, JESPR29, NAU1278, NAU3561, NAU1262, NAU992, NAU1037 produced maximum number of alleles. All the robust lines were clustered in cluster I (fifty nine) and all the compact lines (fifty five) were clustered in cluster II and also confirmed by molecular study. The highest contribution towards genetic divergence was recorded by boll weight (gm), in 6917 cases out of 19506 combinations, accounting for 35.46 per cent followed by number of bolls per plant and seed cotton yield. Hence, these traits may be used as selection parameters in the hybridisation programme.

Keywords: **Plant types, Genetic diversity, SSR marker.**

1. INTRODUCTION

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology. Information on genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. The analysis of genetic variation both within and among elite breeding materials is of fundamental interest of plant breeders. Diversity based on phenological and morphological characters usually varies with environments and evaluation of these traits requires growing the plants to full maturity prior to identification. DNA markers have proved to be powerful tools in assessment of genetic variation. Compared to morphological analysis, molecular markers can reveal differences among accessions at DNA level and thus provide a more direct, reliable and efficient tool for germplasm conservation and management.

In the present study, promising cotton genotypes possessing high yield coupled with good fibre quality traits was undertaken to assess genetic diversity using SSR markers. Our objective was firstly to assess the genetic diversity of large collection of hirsutum genotypes based on the morphological and molecular markers and secondly to identify the suitable parents for hybrid breeding programme.

Materials and methods: Genetic diversity is essential requirement for exploiting heterosis. Present study was aimed at assessing diversity in a large collection of hirsutum varietal lines developed and maintaining in agriculture research station, Dharwad. And to attempt characterizing molecular diversity in a set of genotypes representing this collection and to study the potentiality of hybrids developed based on selected lines representing this collection. The analysis of genetic divergence using Mahalanobis [1] D^2 statistic and grouping of genotypes was done by using Tocher's method [2]. A total of 50 random primers were utilized for SSR analysis and the sequences of each of the primer pairs were downloaded from cotton marker data base showing high PIC (polymorphic information content) values.

Electrophoresis

PCR products were confirmed for amplification on 1.2 % agarose gel before loading them in the sequencing gel. For separation of amplified DNA fragments, non-denaturing polyacrylamide gel electrophoresis (PAGE) were employed.

Scoring the amplified fragments :The amplification of DNA profiles for all the primers were compared with each other and the bands of DNA at each amplification level of every primer

were scored as present (1) or absent (0) thus generating the 0, 1 matrix.

$$\text{Per cent polymorphism (\%)} = \frac{\text{Total No. of polymorphic bands}}{\text{Total No. of bands generated by 49 primers}} \times 100$$

Analysis of SSR profiles

Pair-wise genetic similarities (Sij) between genotypes were estimated by DICE similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and cluster obtained based on unweighted pair group arithmetic mean (UPGMA) using SHAN module of NTSYS-PC version 2.0 [3], the similarity measurements were converted to genetic distance measurements as (1 – SM) × 100 [4].

2. RESULTS AND DISCUSSION

Genetic divergence among the parents

Genetic diversity based on morphological traits

The precise information about the degree of relationship between different genotypes is very much essential for an effective breeding programme. Genetic diversity between populations indicates the differences in gene frequencies. In addition to estimates of variability, knowledge of genetic diversity among genotypes is essential for selecting diverse parents for hybridization programme. The mean values of different quantitative characters were utilized for working out genetic distance between pairs of genotypes. Estimates of D^2 value was found out for 156 genotypes (varietal lines).

Mahalanobis generalized distance (D^2)

Genetic divergence analysis in general helps in assessing nature of diversity in order to identify specific parents for realizing higher heterosis for yield and other economic characters in hybrid breeding programme. Mahalanobis D^2 statistics has been utilized by several workers in a wide range of crop species including cotton to measure the genetic distance among their breeding material. In the present study, data collected on 13 characters in 156 cotton genotypes were subjected to Mahalanobis's statistical analysis to assess the genetic diversity using the concept of generalized distance (D^2).

Contribution of different characters towards divergence

The proportion of contribution by each trait to the total D^2 statistics was different. The proportion of contribution by each trait towards divergence is given in the Table 1.

Table 1: Contribution of characters towards divergence in cotton

Sl. No.	Source	Times Ranked 1st	Contribution %
1	Seed cotton yield (q/ha)	3671	18.82
2	Number of bolls per plant	4689	24.04
3	Boll weight (g)	6917	35.46
4	No. of monopodia per plant	21	0.11
5	No. of sympodia per plant	235	1.20
6	No. reproductive points	2398	12.29
7	Inter branch distance	49	0.25
8	Sympodial length at 50 %	14	0.07
9	Plant height (cm)	456	2.34
10	Ginning out turn (%)	587	3.01
11	No. of seeds per boll	234	1.20
12	Seed index (g)	156	0.80
13	Lint index (g)	79	0.41

Group constellations

Intra and inter cluster distance

All the 156 genotypes were grouped into ten clusters based on D^2 values by following Tocher's method [2]. The intra and inter cluster values were presented in Table 2.

Table 2: Average intra and inter cluster distances in cotton

Cluster Distances	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
Cluster I	6.60	11.08	9.86	8.70	11.38	14.72	22.20	17.45	9.27	14.10
Cluster II	11.08	6.85	10.53	16.22	18.90	10.64	14.26	25.95	13.51	17.97
Cluster III	9.86	10.53	6.52	11.51	14.17	16.93	20.98	22.93	15.70	20.80
Cluster IV	8.70	16.22	11.51	0.00	5.94	20.38	28.48	12.75	12.58	16.31
Cluster V	11.38	18.90	14.17	5.94	7.37	22.90	30.94	11.88	14.47	18.04
Cluster VI	14.72	10.64	16.93	20.38	22.90	8.47	13.43	27.71	12.98	15.37
Cluster VII	22.20	14.26	20.98	28.48	30.94	13.43	6.83	37.56	22.72	25.90
Cluster VIII	17.45	25.95	22.93	12.75	11.88	27.71	37.56	0.00	16.12	17.08
Cluster IX	9.27	13.51	15.70	12.58	14.47	12.98	22.72	16.12	0.00	7.05
Cluster X	14.10	17.97	20.80	16.31	18.04	15.37	25.90	17.08	7.05	0.00

Cluster mean values of quantitative traits in different clusters

The mean values for different characters for all clusters are given in Table 3. The D^2 values ranged from 8.00 to 1492.11 among the large collection of hirsutum lines, the highest D^2 values was noticed between KH 134 (robust plant type) and SC-29-29 (compact plant type) and lowest D^2 values was noticed between R-14 (102) and DH-348 (intra plant types).

Table 3: Cluster mean values of quantitative traits in different clusters

Cluster Means	SCY (q/ha)	No.of bolls/ plant	Boll wt (g)	No. of monopodia/ plant	No. of sympodia /plant	No. reproductive pionts	Internode distance (cm)	Sympodial length at 50 % Pl ht (cm)	Plant height (cm)	Ginning out turn (%)	No. of seeds /boll	Seed index (g)	Lint index (g)
Cluster I	15	22	4	2	15	3	23	37	115	41	27	8	6
Cluster II	16	20	4	2	15	3	23	37	117	41	27	8	6
Cluster III	15	20	4	2	14	3	23	35	110	40	27	9	6
Cluster IV	16	21	4	2	15	3	24	36	110	42	26	8	6
Cluster V	14	20	4	2	14	3	27	37	112	41	28	8	6
Cluster VI	14	19	4	2	14	4	23	35	108	39	27	8	5
Cluster VII	16	18	4	2	15	4	19	34	99	40	26	9	6
Cluster VIII	13	17	3	2	17	3	23	35	127	42	32	9	6
Cluster IX	11	19	4	1	15	3	26	36	122	42	35	8	6
Cluster X	18	21	4	2	16	3	23	36	138	34	25	8	4

3. MOLECULAR CHARACTERIZATION OF PARENTAL LINES USING SSR MARKERS

Analysis of microsatellites (SSR's) in 46 parental lines using 49 primers revealed a high DNA polymorphism among parental lines (Fig 1). 38 primers produced a total of 120 amplified profiles. Among these, 86 were polymorphic with an average of 59.70 per cent polymorphism. Primers *viz.*, BNVB-37421, CIR081, CIR081, CIR005, CIR182, CIR238, CIR413, JESPR29, NAU1278, NAU3561, NAU1262, NAU992, NAU1037, gave highest (100%) polymorphism. The number of bands ranged from one (primers CIR081, CIR182, CIR413, NAU1037, NAU1278 and JESPR29) to six (primers BNRACH25-17482 and BNRACH25-17408) with an average of 2.44 bands per primer. The primers *viz.,* BNVB-37531, BNRAH-21580, BNVB-37227, CIR148, CM29, CIR393, JSPR211, JSPR114 and NAU2176 (50%) showed the least polymorphism.

The diversity coefficient ranged from 21 % to 65 %. Among the parental lines, RAH-16 with RAH-337 showed lowest (0.04) similarity coefficient value. While, between RAH-370 and RAH-337 highest (0.57) similarity coefficient value was observed. All the parental lines showed diversity among themselves indicating that there is considerable amount of variation, which can be exploited through appropriate breeding programme. The dendrogram constructed from the pooled data (Fig. 2) revealed two distinct clusters. One cluster involved testers and in another all other lines were placed. It is very interesting to note that all lines and all testers, except some other genotypes which we not utilized in the crossing programme were differentiated by placing them in different cluster. This indicated the presence of high diversity between lines and testers and low diversity among lines and testers.

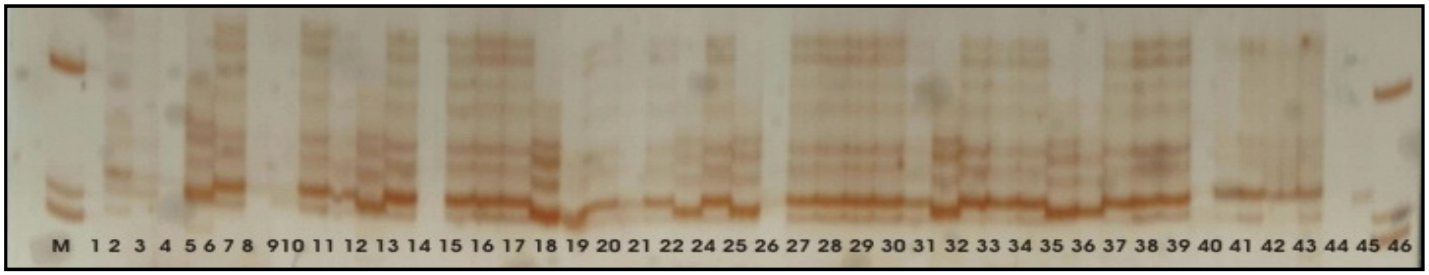

Fig 1 : DNA amplification pattern of 46 hirsutum lines

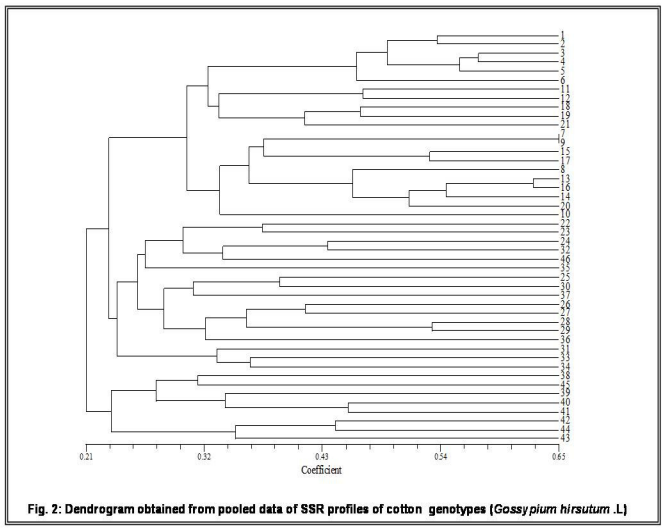

Fig. 2: Dendrogram obtained from pooled data of SSR profiles of cotton genotypes (*Gossypium hirsutum* .L)

The similarity coefficient values ranged from 0.15 to 0.46 among the lines which are involved in the line x tester study and the highest similarity coefficient values was noticed between line RAH-370 and RAH-35, It revealed that RAH-370 was closely related to RAH-35 with 46 % similarity between parents. The hybrid between RAH-370 and RAH-35 exhibited an yield of 19.76 (q/ha). Highest similarity coefficient values was noticed between line RAH-13-86 and tester RAH-35 which revealed that they are far distinct from each other. This combination exhibited an yield of 32 (q/ha), while the highest seed cotton yield (41.5 q/ha) was obtained in the cross between RAH-53 x RAH-10 and its similarity co efficient value is 0.25 indicates at the level of 75 % diversity between parent contributed highest yield.

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