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Sheetal S Modi
M. Sc. Student, Institute of Life Sciences, School of Science and Technology, Ahmedabad University, Ahmedabad, Gujarat, India

DH Patel
Associate Research Scientist, Main Cotton Research Station, NAU, Suart, Gujarat, India

Rajkumar BK
Assistant Research Scientist, Main Cotton Research Station, NAU, Suart, Gujarat, India

Preeti R Parmar
Assistant Research Scientist, Main Cotton Research Station, NAU, Suart, Gujarat, India

Corresponding Author:
DH Patel
Associate Research Scientist, Main Cotton Research Station, NAU, Suart, Gujarat, India

Characterization of cotton germplasm through morphological characters and PCR based molecular markers

Sheetal S Modi, DH Patel, Rajkumar BK and Preeti R Parmar

Abstract
The present study was carried out to determine genetic variability through DNA markers and yield contributing traits as well as seed cotton yield in 26 cotton germplasm *viz*.,123-CO2-13, 125-CO2-18, 170-CO2-2-126-1, 170-CO2-10-1(H), 134-CO2-C-W-21, 134-CO2-M-21-4(1471), ISC-67-1- 2(450), ISC-67-B-7, BC-68-24, B-P52-M-B-2, AP-17-2-2, H-148-63, SH-1311, 108F, 320F (Ganganagar), UK-53, C-29, TH-49-Indore, 19:94-1-5-3, C-1579, Arkot-2-1, C.M.D.E, EC-138569, C2-3, BC-68xBC-229 and BN-Red. The germplasm 170-CO2-10-1(H) recorded higher seed cotton yield per plant (86.4g), boll weight (4.20g) and plant height (143.3cm). Thus, it shows that plant height and boll weight appeared to be the most important yield contributing characters. Knowledge of genetic diversity and relationships among breeding material at molecular level has significant impact on cotton improvement. The genetic variability and relationships among 26 cotton germplasm were investigated using RAPD, ISSR and SSR markers. Eleven RAPD, 19 ISSR and 17 SSR primers generated a total of 66, 43 and 36 alleles, of which 64, 42 and 30 alleles were found to be polymorphic, resulting in 96.96%, 97.67% and 83.33% polymorphism, respectively. The similarity coefficient based on RAPD, ISSR and SSR markers ranged from 0.36 to 0.92 with an average of 0.43, thus suggesting considerable genetic variation between the cotton germplasm studied. Dendrogram was generated by UPGMA cluster analysis based on Jaccard's similarity coefficient, grouped 26 germplasm lines into two distinct clusters comprising of cluster A and cluster B. The results obtained suggests that combined molecular markers data are efficient for measuring genetic diversity and relatedness and also aid in selection of diverse outstanding lines to be used in future breeding programs to develop new cotton cultivars.

Keywords: Cotton, genetic diversity, random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), and simple sequence repeat (SSR)

Introduction
Cotton is a major fibre crop of global importance and is grown commercially in the temperate and tropical regions worldwide. Cotton production in India has a wide impact on the livelihood of the farmers and its product account for 30% in foreign exchange earning and 3% in GDP of the country. The knowledge of genetic diversity among commercial cotton genotypes will potentially aid cotton breeding strategies and facilitate utilization of promising germplasm. Genetic diversity can be evaluated with morphological characteristics, isozymes and DNA markers. To have a reliable estimate of genetic diversity and genetic relationships, molecular markers are efficient in assessing polymorphism. Among these marker techniques, DNA based markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Microsatellite or Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) are of utmost significance for crop improvement.

RAPD is the most widely used molecular method owing to its inexpensiveness, technical simplicity and speedy process. RAPD analysis has also been utilized for DNA fingerprinting, hybrid identification and assessment of genetic diversity in cotton (Asif *et al.*, 2002) [4]. Inter Simple Sequence Repeats (ISSR) is an easy and informative genetic transfer system in cotton for revealing both inter and intraspecific variations. ISSR technique uses primers that are complimentary to a single SSR and anchored at either 5' or 3' end with one- to three- base extension (Dongre *et al.*, 2007). The availability and abundance of SSR markers throughout the cotton genome coupled with the fact that they are polymorphic, codominant and are based on PCR which make them particularly useful in genetic diversity studies of cotton (Zhang *et al.*, 2007). Further, comparative study of different molecular markers (RAPD, ISSR and SSR) revealed that combined molecular data could be reliable tools to evaluate genetic diversity of *Gossypium* species (Dongre *et al.*, 2004 [9], Rana *et al.*, 2007 [13], Sapkal *et al.*, 2011) [18].

Therefore, the present study was focused on the characterization of genetic diversity among 26 germplasm of cotton at both morphological and molecular level.

Materials and Methods

Morphological analysis: The morphological traits measured to assess the genetic diversity include plant height (cm), monopodia per plant, sympodia per plant, number of bolls per plant, boll weight in gram (BW), seed cotton yield/plant (g) (SCY/P), ginning outturn percent (G.P), seed index (g) (S.I.). Mean of five selected plants were used to determine the morphological variations.

Molecular analysis: DNA was extracted from 26 selected cotton germplasm by CTAB method proposed by Murray and Thompson (1986) [12] with minor modification. Quality and quantity of DNA were estimated by measuring O.D. at 260/280nm in UV Spectrophotometer. Intactness of genomic DNA was checked by gel electrophoresis. PCR was performed in a programmable gradient thermocycler and total of 11 RAPD, 19 ISSR and 17 SSR primers were screened for genetic analysis 26 cotton gerplasm. PCR for RAPD, ISSR and SSR was carried out in of 25 ul volume containing buffer MgCl2, primer, deoxynucleotides mixtures and Taq polymerase (Bangalore GeNei, India, GeNei TM), and genomic DNA. The amplified RAPD products were electrophoresed on 1.2% agarose gels, while SSR loci were resolved on 2.5% agarose gels. Horizontal electrophoresis system was used and after electrophoresis, finely resolved PCR products were visualized under UV light and photographed. 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 germplasm were displayed as dendrogram based on NTSYs data analysis programme (Rohlf, 1998) [16].

Result and Discussion

Morphological parameters: The estimates of morphological parameters computed to evaluate relative performance of different germplasm are presented in Table 1. The plant height ranged from 143.3cm [170-CO2-10-1(H)] to 68.3cm (EC-138569), the line 134-CO2-M-21-4(1471), ISC-67-B-7, B-P52-MB-2 and AP-17-2-2 recorded maximum monopodia per plant (2.3) compared to overall mean (0.9). Whereas, AP-17-2-2 (23.0) recorded highest and BN-Red (10.6) recorded least sympodia per plant as against overall mean of 19.9. Amongst germplasm, AP-17-2-2 stood first with 28.3 bolls per plant, which was higher as compared to overall mean (15.4). The boll weight was highest in 170-CO2-10-1(H) (4.20g) followed by C-1579 (4.10g) and 134-CO2-C-W-21 (3.90g) and the least boll weight was of 1.80g was registered by 320F (Ganganagar). The overall mean for seed cotton yield per plant was 39.8g, and the highest seed cotton yield per plant was registered by 170-CO2-10-1(H) (86.4g) followed by 134- CO2-C-W-21 (85.8g) The maximum ginning outturn was recorded by C-1579 (45.2%) which was higher than that of overall mean value (34.9%) and the least ginning outturn was 32.3% (C.M.D.E). The germplasm 125-CO2-18 and H-148-63 possessed higher seed index (8.5g) than that of overall mean value (7.0g).

From the morphological parameter studied it can be inferred that the germplasm 170-CO2-10-1(H) recorded higher seed cotton yield per plant (86.4), boll weight (4.20) and plant height (143.3). Thus, it seems that plant height and boll weight appeared to be most important yield contributing character. The results suggested that selection for a promising cotton germplasm for high seed cotton yield may be accomplished with boll weight and plant height. Akbar *et al.* (1994) [2] reported similar results for boll weight and plant height. Rauf *et al.* (2004) and Salahuddin *et al.* (2010) [15, 17] also showed boll weight as the most important yield contributing character.

Table 1: Mean performance for yield attributing traits in cotton germplasm

Germplasm	Plant height (cm)	Number of monopodia	Number of Sym podia	Number of bolls per plant	Boll weight (g)	Seed cotton yield/plant (g)	G.P (%)	S.I (g)
123-CO2-13	109.3	1.0	17.3	17.6	3.15	50.4	34.6	8.0
125-CO2-18	110.0	1.6	22.6	18.0	3.10	51.8	33.6	8.5
170-CO2-2-126-1	115.0	0.0	14.3	16.3	3.05	47.7	41.1	7.5
170-CO2-10-1(H)	143.3	1.3	20.3	22.0	4.20	86.4	33.9	7.0
134-CO2-C-W-21	95.0	0.6	21.6	23.3	3.90	85.8	41.0	8.0
134-CO2-M-21-4(1471)	110.3	2.3	17.6	16.3	2.35	35.0	38.2	6.0
ISC-67-1-2(450)	109.6	1.0	16.0	7.3	3.00	22.9	37.3	7.5
ISC-67-B-7	100.0	2.3	22.6	13.6	2.50	30.0	35.4	8.0
BC-68-24	70.6	1.3	16.0	12.3	2.20	48.3	40.7	7.5
B-P52-M-B-2	87.0	2.3	12.0	18.0	1.85	30.7	40.9	6.0
AP-17-2-2	109.0	2.3	23.0	28.3	1.85	45.3	38.2	5.5
H-148-63	117.6	0.6	20.0	14.0	3.00	39.8	36.5	8.5
SH-1311	78.3	1.0	18.6	15.6	3.45	46.5	39.2	8.0
108F	99.0	1.0	15.6	11.3	2.50	23.2	37.8	5.0
320F (Ganganagar)	112.6	2.0	18.6	13.3	1.80	18.9	40.6	6.5
UK-53	126.0	0.6	17.0	9.6	2.90	24.5	36.5	7.5
C-29	133.6	0.6	20.3	17.0	3.50	50.5	40.5	7.0
TH-49-Indore	116.6	0.3	19.0	14.3	1.90	20.1	42.0	6.0
94-1-5-3	94.0	0.0	17.6	14.6	2.20	26.9	36.4	7.5
C-1579	71.6	0.0	10.3	13.3	4.10	48.4	45.2	6.0
Arkot-2-1	111.6	1.0	17.0	9.6	2.40	18.8	38.6	7.0
C.M.D.E	105.0	0.0	15.3	19.0	3.45	58.5	32.3	7.0
EC-138569	68.3	0.3	11.3	12.3	2.40	22.6	37.5	7.0
C2-3	80.6	0.0	16.0	16.0	2.45	30.2	36.6	7.0
BC-68xBC-229	88.6	0.0	15.0	12.0	3.40	36.0	33.5	7.0
BN-Red	81.6	0.0	10.6	16.0	2.85	37.6	38.8	7.0
Mean	101.6	0.9	19.9	15.4	3.05	39.8	34.9	7.05

Genetic diversity through molecular markers

The amplification profiles of the 26 cotton germplasm produced by the 11 decamers revealed a total number of 66 bands, 64 of them were polymorphic that corresponds to the level of polymorphism of 96.96%.Out of 11 RAPD markers, nine markers showed polymorphism to an extent 100% *viz.*, RPI 1, RPI 3, RPI 4, RPI 5, RPI 6, RPI 7, RPI 8, RPI 9 and RPI 10. Maximum 12 bands were produced by RPI 10, while minimum of 3 bands were produced by RPI 1 and RPI 6. The polymorphic information content (PIC) of RAPD primers ranged from 0.63 (RPI 6) to 0.91 (RPI 10) and total PIC value for RAPD markers screened in this was 7.74. Earlier, Esmail *et al.* (2008) [10] reported 84.95% polymorphism in 21 cotton germplasm using RAPD primer whereas, Rana *et al.* (2005) [14] detected 89.1% polymorphism in 23 cotton germplasm comprised of *G. arboreum* and *G. hirsutum* using 26 RAPD primers. Thus, these findings support the results obtained in the present research programme.

Nineteen ISSR primers were employed to investigate the genetic polymorphism, of which ten ISSRs showed reproducible and sharp bands. Except ISSR 3 (75%) the eight markers *viz*., ISSR 1, ISSR 2, ISSR 5, ISSR 6, ISSR 7, ISSR 9, ISSR 10, ISSR 13 and ISSR 14 showed 100% polymorphism. Maximum of 8 bands produced by ISSR 1and single band was produced by ISSR 14. The PIC of ISSR primer ranged from 0.48 (ISSR 13) to 0.84 (ISSR 1) and the total PIC value for ISSR markers screened was 6.34. The varied percent of polymorphism for diversity using ISSR primers were reported by earlier studies. Soliman *et al.* (2012) [1] reported of 91.4% polymorphism and Mahatma *et al.* (2009) [11] reported 66.66% polymorphism and Dongre *et al.* (2011) [8] reported 76.55% polymorphism.

Table 2: The effectiveness of RAPD, ISSR and SSR markers in detecting polymorphism in cotton germplasm

Particulars	RAPD	ISSR	SSR
Number of primers	11	19	17
Total number of bands	66	43	36
Polymorphic bands	64	42	30
Percent polymorphism	96.96	97.67	83.33
Average of polymorphism	8.81	5.14	4.90
Total PIC value	7.74	6.37	6.52

Among 17 SRT primers, 12 primers showed 100% polymorphism. Maximum 4 bands were produced by SRT 49 and SRT 90, while single band was produced by SRT 48, SRT 57 and SRT 60. The total number bands amplified using SSR primers were 36 from which 30 bands were polymorphic accounting 83.33% polymorphism. The PIC of SRT primer ranged from 0.13 for the primer pair SRT 62 to 0.74 for the primer pair SRT 90; and the total PIC value scored for SSRs in this study was 6.52. Overall SSR showed 4.90 average polymorphism compared with 8.81 and 5.14 in RAPD and ISSR, respectively. While the percentage of total polymorphism detected was 96.96%, 97.67% and 83.33% in RAPD, ISSR and SSR, respectively (Table 2). Azmat and Khan *et al.* (2010) [5] and Ali *et al.* (2011) [3] reported 88.46% and 78.6% polymorphism using SSR primers respectively. These results are in agreement with those obtained in present investigations.

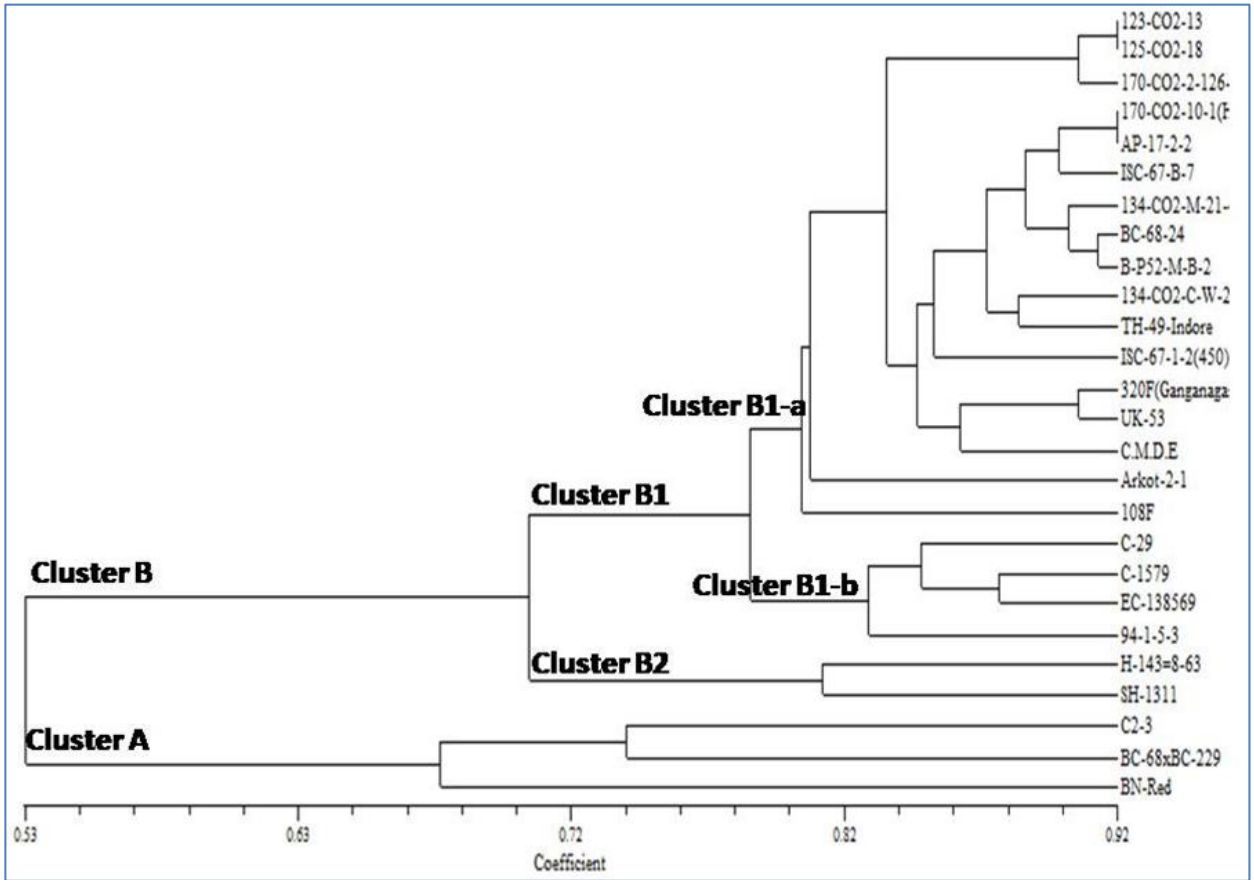

Fig 1: Dendrogram generated by UPGMA cluster analysis using pooled data of RAPD, ISSR and SSR

RAPD, ISSR and SSR

Jaccard's pair wise similarity coefficient values generated using pooled data of 11 RAPD, 10 ISSR and 17 SSR primers for twenty six cotton germplasm. The genetic similarities ranged from 0.36 to 0.92 with an average of 0.43. The highest similarity (0.92) was observed between 123-CO2-13 and 125-CO2-18 as well as 170-CO2-10-1(H) and AP-17-2-2. On the other hand least similarity of 0.36 was revealed by 170-CO2-10-1(H) and BN-Red (Figure 1). Range of genetic similarity in *G. hirsutum* germplasm was reported by Dahab *et al.* (2013) [6] was 0.1 to 0.53%.

The dendrogram was constructed by UPGMA method and the UPGMA cluster pattern of 26 germplasm using RAPD, ISSR and SSR markers clearly indicated that the two clusters A and B have similarity coefficient of 0.53. Cluster A had only three germplasm *viz*., C2-3, BC-68xBC-229 and BN-Red shares similarity of 0.68. BN-Red was distinctively clustered due to plant genotype (red), and the three germplasm *viz.*, C2-3, BC-68xBC-229 and BN-Red grouped in Cluster A had yield ranged from 37.6 to 30.2. Cluster B consisted of 23 germplasm and subdivided in two clusters *viz*., sub-cluster B1 and sub-cluster B2 having similarity coefficient of 0.7. Sub-cluster B1 consisted of 21 germplasm and Sub-cluster B2 consisted of only two germplasm *viz.*, SH-1311 and H-148-63. The sub-cluster B1 further subdivided into two sub-cluster *viz*., sub-cluster B1-a and sub-cluster B1-b sharing 0.79 similarity coefficient. Sub-cluster B1-b had 4 germplasm, while sub-cluster B1-a had 17 germplasm. In sub-cluster B1-a germplasm 108F and Arkot-2-1 were distinctively clustered separately from the rest of 15 germplasm. However, Arkot-2-1 was least yielder (SY/P=18.8g). Further, the germplasm *viz*., 123-CO2-13, 125-CO2-18, 170-CO2-2-126-1 and 170-CO2-10-1(H) were grouped near to each other as they were from the similar genetic origin and they were the ever first efforts of crossing between Indian and American cultivars.

Conclusions

The genetic diversity assessed through morphological and molecular markers in 26 cotton germplasm suggests the presence of useful genetic diversity among the studied germplasm to be used as resource for plant breeding program. Further, cluster analysis revealed interesting facts that good yielder clustered together; while BN-Red is distinctly clustered as it is red plant type. To improve resolution of grouping the germplasm need to be characterized with functional markers like EST-SSR and SNP which reflect finer molecular profiling of germplasm to be used in further cotton breeding programme.

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