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Short Communication

Microsatellite based genotyping and assessment of genetic divergence in upland cotton (*Gossypium hirsutum)*

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Abstract

Nineteen upland cotton genotypes were evaluated for genetic divergence for development of desirable hybrids. Based on twelve phenotypic markers D^2 analysis identified five clusters containing one to ten genotypes. Prinipal Component Aanalysis reduced the twelve variables into four principal components explaining 82.2% variability that clearly indicated the genotypes, H1236, H1472, CSH 3075, ISR 12, H1465 and H1117 were high yielder and H 1236, H 1472, H 1156 and H1476 were having better seed indices traits. Out of 49 SSR primers, 42 were polymorphic and amplified 173 alleles with PIC value ranged from 0 to 0.948. The NTSYS-pc UPGMA analysis clustered into seven distinct groups.

Keywords: Genetic diversity, D^2 analysis and SSR primers

Upland cotton (*Gossypium hirsutum* L.) being the cash crop meets 90% of world's cotton demands. India is pioneer country in genetic improvement and development of hybrids using the conventional technique as well as genetic male sterility system (Bakhtavar et al. 2015). The choice of good genotypes having broader genetic base (genetic divergence) to breed the genotypes with high yield and fiber quality traits is crucial in breeding programs to obtain the desirable gene combination. Therefore, the study was planned to study the genetic vaiability in cotton genotypes using different statistical parameters such as D^2 and Principal Component Analysis (PCA), and molecular markers, Simple Sequence Repeats (SSRs).

The experimental material comprised of nineteen genotypes including four testers (Table 1). The material was planted in randomized block design with three replications during *kharif*, 2014 at CCSHAU, Hisar. The material was sown in 6 rows of 7.2 m and spacing at 100cm and plant to plant distance of 45cm. Observations were recorded on five plants for days to first flower, plant height, monopods per plant, sympods per plant, bolls per plant, boll weight, seeds per boll, seed cotton yield, lint yield, ginning out turn, seed index, lint index. PCA was done using SPSS software. D^2 analysis was used for clustering the genotypes. For molecular analysis, a set of 49 SSR markers were used. Genomic DNA was isolated by CTAB method (Saghai-Maroof et al. 1984; Xu et al. 1994). PCR amplified DNA products were resolved and visualized on PAGE. The molecular data 0/1 matrix was used to calculate similarity index, genetic distance using 'simqual' subprogram of software NTSYS-PC.

D^2 cluster analysis identified five clusters (Table 1). Cluster I contained 10 genotypes while clusters IV and V had one genotype in each. Maximum inter-cluster distance was observed between clusters II and III (42.38) followed by clusters III and V (38.02) (Table 2). The crosses between the genotypes belonging to distantly located clusters are likely to produce better heterosis as also suggested earlier (Xian Tao et al. 2011; Kulkarni et al. 2012; Akter et al. 2019). The first four principal components (PC) having eigen values more than one cumulatively explained 82.27% variability (Table 3). Factor loading of different characters clearly indicated high loading of seed cotton

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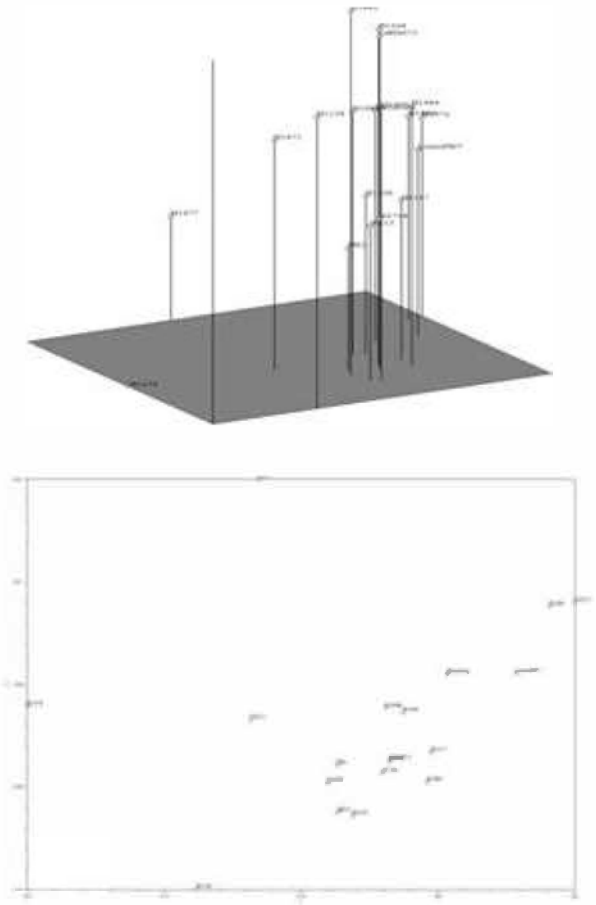
Table1. Cluster membership of cotton genotypes based on phenotypic observations and molecular markers

	Genotypes	No.
	Based on phenotypic observations	
Group I	H1471, H1476, H1226, H1472, H1156, Luxmi PKV, AC726, H1465, H1464, H1117	**10**
Group II	CSH3075, H1236, H1098-I	**3**
Group III	Delta Pine, H1470, H1477,HR1	**4**
Group IV	H1463	**1**
Group V	ISR12	**1**
	Based on molecular markers	
Group I	H1156	**1**
Group II	ISR12, AC726 and HR1	**3**
Group III	Luxmi PKV, H1472, H1465, Deltapine, H1464, H1117 and H1236	**7**
Group IV	H1463, H1470, CSH3075, H1226 and H1098-i	**5**
Group V	H1471	**1**
Group VI	H1477	**1**
Group VII	H1476	**1**

Table 2. Inter and Intra cluster distances in cotton genotypes (morpho)

Cluster No.	I	II	III	IV	V
I	10.44				
II	28.18	6.01			
III	18.80	**42.38**	6.28		
IV	21.02	14.17	**32.05**	0.00	
V	22.62	18.64	**38.02**	23.84	0.00

yield (0.923), lint yield (0.930), seeds/boll (0.896), boll weight (0.886) and bolls/plant (0.691) on the first PC and this can be regarded as seed yield factor. The second PC showed high loadings of ginning outturn (0.615), seed index (0.915) and lint index (0.938) and can be regarded as better seed development factor. High loadings of different traits in a PC indicated strong association among them and can be used as selection criteria in breeding programmes to improve yield with better seed development. The results are supported by Nazir et al. (2013) who estimated genetic diversity for earliness and fibre quality in *Gossypium hirsutum*.



Figs. 1 and 2. 2D and 3D PCA scaling of 19 upland cotton genotypes based on 49 SSR markers

On the basis of Principal Factor scores genotypes were plotted for PC 1 and PC 2 which accounted for 60% variation differentiating genotypes according to their cluster membership. The genotypes, H1236, H1472, CSH 3075, ISR 12, H1465 and H1117 were superior for higher yield while genotypes H 1236, H 1472, H 1156 and H 1476 were having better seed indices traits. Similar results were found by Kantartzi and Stewart (2015) who investigated the trait associations in *G. arboretum*. Genetic diversity was also assessed using 49 SSR primers out of which 42 amplified the DNA revealing total 173 bands (Fig. 3). The overall size of PCR amplified products ranged from 80 bp (GH5) to 450 bp (MGH ES18, DPL0039 and NAU2083). Polymorphic information content (PIC) value ranged from 0 to 0.9. In the present findings, 0 to 7 bands per genotypes with the mean of 4.10 alleles per allele indicating its occurrence in both A and D genomes of cotton were observed similar to that found in cotton by Ashraf et al. (2016) and Gutiérrez et al. (2017). The SSR diversity data grouped nineteen

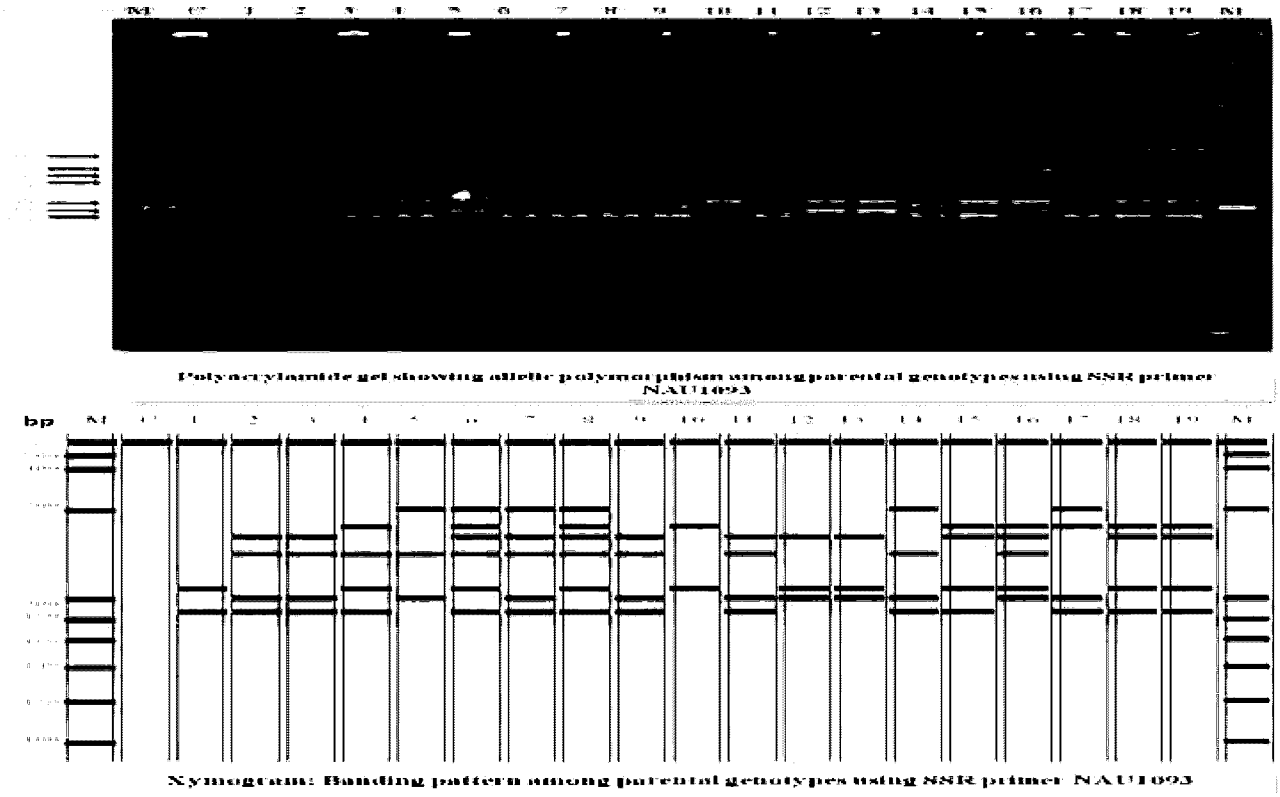


Fig. 3. Polymorphism in nineteen genotypes of cotton by using primer NAU1093. 1- H1156, 2- ISR 12, 3- HR1, 4- Luxmi PKV, 5- AC 726, 6- Delta Pine, 7- H 1472, 8- H 1465, 9- H 1463, 10- H 1464, 11- H 1470, 12- H 1471, 13- H 1476, 14- H 1477, 15- CSH 3075, 16- H 1226, 17- H 1098-I, 18- H 1117, 19- H 1236

Table 3. Total variance explained by different principal components

Principal component	Extraction sums of squared loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.974	41.452	41.452	4.577	38.146	38.146
2	2.249	17.744	60.196	2.182	18.187	56.332
3	1.509	12.57	72.775	1.698	14.153	70.485
4	1.139	9.493	82.268	1.414	11.783	82.268

genotypes into seven groups. Cluster III was the largest one comprising 7 genotypes followed by IV (5) and II (3) with similarity index varying from 0.69 to 0.92. The genetic relationships determined by NTSYS PCA 2-D and 3-D scaling of nineteen genotypes shown in Figs. 1 and 2. The two-dimensional analysis showed thatthe lines were scattered in two major groups, which were further divided into different subgroups. The present findings of grouping the germplasm lines are in accordance with the earlier reports of Ilaj et al. (2013), Abbas et al. (2015) and Ashraf et al. (2016) in cotton. Clustering pattern obtained through molecular and morphological analysis of the genotypes was not similar to each other. It may be because of the reason that the morphological traits were more affected by the environment and further the clustering was based only on a set of traits related to yield, however, molecular analysis diversified the genotypes based on entire variability and there is no effect of environment. The second possible reason for this is that SSR primers were able to scan only 173 alleles as also observed by Ijaj et al. (2013). However, Santhy et al. (2019) studied genetic diversity in 48 popular varieties of tetraploid cotton from each cultivated zone

of India using 68 SSR markers distributed across linkage groups. They reported only a total of 144 alleles with an average of 2.19 per locus. They further reported the narrow genetic base in cotton. These results indicated that the meager screening of genome and information thus obtained is not sufficient to define actual genetic distinctness for genotypes. However, both types of analysis revealing wide range of variability among these genotypes. It is expected the genotypes, HR1,H1236, Deltapine, H1098-1, CSH3075 and H1477 identified in the present study may be suitable to produce heterotic hybrids.

Authors' contribution

Conceptualization of research (SuSS, SSSi); Designing of the experiments (SuSS, SSSi); Contribution of experimental materials (SuSS, SSSi); Execution of field/lab experiments and data collection (SSSi, Su); Analysis of data and interpretation (Su, SSSi); Preparation of the manuscript (SSSi, Su).

Declaration

Authors declare no conflict of interest.

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