Molecular diversity analysis among elite genotypes of American cotton (*Gossypium hirsutum* L.)

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ABSTRACT: Cotton is the most important fiber crop globally, grown over tropical and subtropical regions world. The narrow genetic base of the cotton germplasm being used in breeding programs is one of the factors in failing to achieve appreciable amount of progress in improving fiber as well as yield contributing traits. Molecular markers have been successfully applied to estimate genetic diversity, besides their use in advancing the breeding cycle. ISSR markers were used to assess molecular diversity among nine elite genotypes in upland cotton in this investigation. Primers ISSR 32 and UBC 842 were recorded with maximum PIC value (0.68) indicating maximum contribution of these primers for diversity analysis. The NTSYS-pc UPGMA tree cluster analysis resulted that nine genotypes were quite distinct and were grouped in four clusters at 69% similarity index. The use of two genotypes from different clusters inhybridization could produce feasible heterotic combinations in forthcoming breeding programmes.

Key words: Cluster analysis, ISSR, molecular diversity, PIC

Cotton the most important fibre crop globally. Development of new varieties and hybrids according to the necessity has exploited the most of the germplams available worldwide. Most of the heterosis has been exploited for yield and fibre quality by conventional breeding. Development of the varieties and hybrids as per requirement has brought monotony of the few of the genotypes and further development of crop only at the base of conational breeding is becoming tedious. Therefore, understanding of genetic diversity will help in utilization of promising genotypes in breeding programme. Molecular markers based diversity analysis will provide more reliable results to the breeder. Among molecular markers ISSR markers are PCR based reliable, quick, and reproducible. Therefore, the present study was conducted using ISSR markers, with the purpose to find out the genetic diversity among cotton genotypes, estimation of relatedness and to characterize genotypes with distinct fingerprints.

MATERIALS AND METHODS

Nine genotypes of upland cotton *viz*., H 1098i, H 1300, H 1316, H 1353, H1465, H 1476, H 1226, H 1490 and H 1464 used for diallel cross in our previous study (Sagar *et al.,* 2019) were subjected for molecular diversity analysis. These genotypes were grown in *kharif* 2017 in Cotton Research Area, Department of Genetics and Plant Breeding, CCSHAU, Hisar. Fresh leaves were collected from the juvenile plants of each genotype and genomic DNA was isolated using CTAB Method of Saghai-Maroof *et al.* (1984). The quality of the extracted DNA of these nine genotypes was tested by 0.8% agarose gel.

PCR amplification reaction was carried out in Benchtop Themocycler. The optimization of PCR reaction was done by using varying concentrations of template DNA (25, 50, 75, 100 ng), dNTPs mix (100 µM, 150 µM, 200 µM, 250 µM, 300 µM), $MgCl_2$ (2 mM, 2.5 mM), primers (0.1 µM, 0.2 µM, 0.3 µM, 0.4 µM, 0.6 µM, 0.8 µM) and *Taq* DNA Polymerase (1 unit, 2 unit, 3 unit) in a reaction volume of 20 µl. The optimized reaction mixture (20 µl) contained 75 ng of DNA template, 250 µM of dNTPs mix, 2.5 mM of $MgCl_2$, 0.4 µM of primers and 2 units of *Taq* DNA Polymerase. A control was also included in each PCR reaction set up, in which sterilized distilled water was used in place of the template DNA, keeping the other reagents concentration and conditions same. A total of 21 ISSR markers selected from previous cotton studies were used for PCR amplification of the template DNA (Table 1).For each marker banding pattern was scored visually for all nine cotton genotypes. The presence and absence of an amplified band was scored as 1 and 0, respectively. Gel scoring was done by placing the gel on the light box immediately after running it. The size (in nucleotides base pairs) of the amplified bands was determined based on its migration relative to standard 20 bp/ 100 bp DNA ladder.

Polymorphic information content (PIC) was calculated for each ISSR marker vides estimates of the discriminatory power of locus by taking into account the numbers of alleles that are expressed (Anderson *et al.,* 1993). PIC value was calculated as:

$$PIC = 1-\Sigma Pij2$$

Where *Pij*2 is the frequency of the *ith* allele.

Allele molecular weight data were also used to export the

data in binary format (allele presence = "1" and allele absence = "0") and entered into a matrix. Based on the matrix of (GD) values, NTsys 2.1software was used to obtain the dendrograms depicting genetic relatedness of the cultivars.

RESULTS AND DISCUSSION

Molecular diversity among parents using ISSR markers

A total of 21 ISSR markers were screened to evaluate the molecular variation among the parent genotypes out of which 3 did not amplify, 3 were monomorphic and remaining 15 were polymorphic which were used for molecular analysis as given in Table 2.

Among these 15 polymorphic ISSR primers a total of 460 alleles were observed with an average of 11.80 bands per primers. The number of alleles ranged 27-37 with primer IS10 (18) with lowest number of alleles and primer IS 18 (37) with highest number of alleles. The polymorphic band ranged from 4-19 with primer UDC 826 (4) having lowest number of polymorphic bands and primer ISSR 12

Table 1: A brief description of ISSR primers used during the present investigation

S. No.	Primer	Sequence (5'-3')	Annealing Temperature (°C)	GC %	References
1	ISSR 32	AGAGAGAGAGAGAGAC	39.9	50.0	Ali *et al.,* 2012
2	IS 4	AGCAGCAGCAGCGC	41.0	71.4	Dongre *et al*., 2011
3	ISSR 8	GACGACGACGACG	37.0	69.2	Ali *et al.,* 2012
4	ISSR 15	ACGACGACGACGAAC	56.2	60.0	Abdi *et al.,* 2012
5	IS 9	CACACACACACAGA	35.0	50.0	Dongre *et al*., 2011
6	ISSR 12	GAGAGAGAGAGAGAGAGA	46.8	50.0	Bilwal *et al.,* 2017
7	UDC 817	CACACACACACACACAA	52.8	47.0	Bardak *et al*., 2012
8	IS 2	AGCAGCAGCAGCGG	41.0	71.4	Dongre *et al*., 2011
9	UBC 842	GAGAGAGAGAGAGAGAAYG	52.3	47.3	Tripathi *et al*., 2012
10	IS 10	CACACACACACAAA	33.0	42.8	Dongre *et al*., 2011
11	IS 8	CACACACACACAGC	37.0	57.1	Dongre *et al*., 2011
12	ISSR 5	AACAACAACGC	25.0	45.4	Abdi *et al.,* 2012
13	IS 13	GTGTGTGTGTGTCA	35.0	50.0	Dongre *et al*., 2011
14	UDC 826	ACACACACACACACAC	46.9	50.0	Bardak *et al*., 2012
15	IS 18	GCTGAGAGAGAGA	33.0	53.8	Dongre *et al*., 2011
16	IS 3	AGCAGCAGCAGCGT	39.0	64.2	Dongre *et al*., 2011
17	IS 17	CAGGAGAGAGAGA	33.0	53.8	Dongre *et al*., 2011
18	IS 12	GTGTGTGTGTGTG	35.0	50.0	Dongre *et al*., 2011
19	CIR 084	CAAAAAAAATAAAAAAA	45.2	5.8	Nguyen *et al.,*2004
20	JESPR 292	CTTCTTCTTCTTCTTCTTCTT	52.9	33.3	Bardak *et al*., 2012
21	CIR 398	ACATTTTTCAAAAAAA	45.1	12.5	Nguyen *et al.,*2004

Table 2: Evaluation of nine parental genotypes of upland cotton using ISSR primers with annealing temperature, amplification range (bp), total number of alleles generated by parents, polymorphic bands and PIC values

ISSR locus	Annealing temperature (°C)	Amplification Range (bp)	Total Number of alleles generated by parents	Polymorphic bands	PIC value
ISSR 32	39.9	220-620	30	10	0.68
IS 4	41.0	210-900	29	15	0.07
ISSR 8	37.0	180-600	29	12	0.07
ISSR 15	56.2	270-620	35	10	0.49
IS9	35.0	300-1100	28	10	0.03
ISSR 12	46.8	140-800	29	19	0.34
UDC 817	52.8	160-700	32	11	0.18
IS 2	41.0	200-850	31	12	0.29
UBC 842	52.3	240-600	32	10	0.68
IS 10	33.0	140-600	27	14	0.12
IS 8	37.0	150-600	33	13	0.59
ISSR 5	25.0	220-600	31	11	0.44
IS 13	35.0	180-580	29	11	0.59
UDC 826	46.9	220-1000	28	4	0.53
IS 18	33.0	180-800	37	15	0.62
Total			460	177	5.72
Average			30.66	11.80	0.38

(19) having highest number of polymorphic bands.

In the present study, size of the alleles of DNA fragment amplified varied from 140bp to 1100bp. Similar results were obtained by Ullah *et al.* (2012). The PIC value for 15 polymorphic markers among 9 parent genotypes ranged from 0.03 to 0.68 with an average of 0.38. The primer IS 9 was recorded with minimum PIC value (0.03) while, primer ISSR 32 and UBC 842 were recorded with maximum PIC value (0.68).Similarly, Boopathi *et al.* (2008) observed average PIC value 0.39, Noormohammadi *et al.* (2013) also observed average PIC value 0.35 in cotton genotypes.

Cluster analysis using ISSR markers

Similarity (Sm) matrix was developed using binary (0/1) molecular data for UPGMA based clustering (Fig 1.) using NTSYS 2.02 software of selected parent genotypes. The genotypes were clustered into four groups at level of GS= 0.69 (Table 3.) Cluster-1 was considered as major group and included five parents H 1098i, H 1353, H 1226, H 1316 and H 1490 whereas, followed by cluster-3 with two parents *viz.,* H 1465 and H 1464, where cluster -2 and cluster-4 had one genotype each H 1300 and H 1476, respectively. Similarly 3 cluster were obtained by Dongre *et al.*(2004) and 7 clusters by Ashraf *et al.* (2016) on the basic of ISSR Markers.The results of present molecular clustering showed that the significant genomic variation is present among the selected parents which can be exploited in breeding programmes.

Table 3: Distribution of nine parental genotypes of upland cotton in different clusters based on ISSR markers

Major cluster	Genotypes	Number of genotypes
Cluster-1	H 1098i, H 1353, H 1226, H 1316 and H 1490	5
Cluster-2	H 1465, H 1464	2
Cluster-3	H 1300	1
Cluster-4	H 1476	1

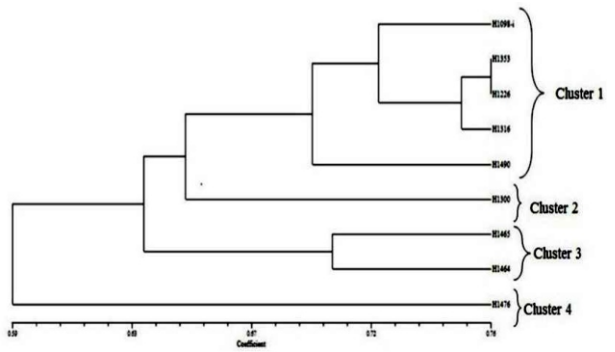

Fig 1: UPGMA dendrogram showing relationship among nine upland cotton genotypes based on similarity matrix data using ISSR markers

CONCLUSION

Primer ISSR 32 and UBC 842 were recorded with maximum PIC value and showing that these primers contributed maximum for diversity analysis.The NTSYS-pc UPGMA tree cluster analysis resulted that nine parental genotypes were quite distinct. These genotypes formed four clusters at 69% similarity index. The use of genotypes from these different clusters in hybridization is assuring to produce feasible heterotic combinations in forthcoming breeding programmes.

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