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Molecular characterization of cotton genetic male sterile lines using RAPD, ISSR and SRAP markers

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Abstract : In the present study, RAPD, ISSR and SRAP markers were used to characterize genic male sterility(GMS) lines in cotton. The molecular markers has generated RAPD (25.88%), ISSR (13.45%) and SRAP (9%) polymorphism in GMS lines. Total 49 primers out of 74 primers in combined analysis had generated 19.45 per cent polymorphism among GMS. RAPD primers have shown highest polymorphism in GMS lines. The molecular characterization found to develop unique marker and polymorphism among GMS lines. The molecular phylogeny has revealed the near and distant lines. In GMS, most breeding lines are restorers, so it is easy to combine any elite line to produce strong heterosis. This observation will assist in cotton crop breeding programme to select and combine inter GMS lines to obtain high vigor combinations.

Key words: Cotton, GMS, ISSR, PCR, PIC value, polymorphism, RAPD, SRAP

Male sterility has been applied to cotton crop as an effective and economical pollination control system. The advantage of using CMS system is that it can generate a complete male sterile population economically. GMS has many advantages. Firstly, GMS involves only 2 lines and is transferred feasibly among parental lines, which may result in a shortened breeding cycle. Secondly, for GMS, most breeding lines are restorers, so it is easy to combine any elite lines to produce strong heterosis. Thirdly, GMS does not have the negative cytoplasmic effect on yield as CMS might do. But GMS system has its limitation of being difficult to derive a complete male sterile population. About 50 per cent male fertile plants must be removed from the female lines during hybrid seed production. Development of a complete genic male sterile population with a temporary maintainer Bline of GMS was a breakthrough to overcome the previous limitation. Consequently, several GMS based hybrids have been released commercially worldwide. Use of molecular markers provide an accurate approach to encase genetic diversity and unique markers (Mandaliya *et al.*, 2010a, 2011).

In GMS, most breeding lines are restorers, so it is easy to combine any elite lines to produce strong heterosis. Thus, aim and objective of this study was molecular characterization of GMS lines based on RAPD, ISSR, and SRAP analysis to develop phylogeny, unique marker, and polymorphism among GMS lines. This molecular study could be useful to produce strong heterosis and higher vigour cotton crop by combining GMS breeding lines in crop breeding programme.

MATERIALS AND METHODS

Plant material and PCR amplification : GMS lines(1) G203, (2) G217, (3) G205 and (4) G209 were obtained and grown at Botanical Garden, Saurashtra University, Rajkot. DNA extraction of GMS lines was carried out according to Mandaliya *et al.*, (2010b) and was subjected to RAPD (OPA and OPB series), ISSR,and SRAP analysis. Total 30 ISSR primers were used among which 24 primers selected from UBC series and 7 ISSR primers selected from literature (Dongre *et al.*, 2004) specific to cotton: I1: (AGC)5GC, I2: (CA)7AC, I3: (GT)7AC, I4: GCA(GA)7, I5: (GA)9C, I6: (GA)9A, I7: (CG)8C. SRAP primes were selected from cotton SRAP markers studies :

me1: TGAGTCCAAACCGGATA,
me2: TGAGTCCAAACCGGAGC,
em1: GACTGCGTACGAATTAAT,
em2: GACTGCGTACGAATTTGC.

The SRAP combination were designed as reverse and forward primer either of used: SRAP-A: me1+em1, SRAP-B: me1+em2, SRAP-C: me2+em1, and SRAP-D: me2+em2.Primer details were given into either in Table 1 or wherever respective results discussed. Each PCR reaction mixture (Mandaliya *et al.*, 2010c) consists of total 12.5 µL for RAPD, ISSR and 25 µL for SRAP. Electrophoresis of samples was carried out on 2 per cent per centagarose gel for RAPD, ISSR and 3 per cent for SRAP.

Statistical analysis: The band products were scored according to Vafaie-Tabar *et al.*, (2004) as presence of band scored (1) and absence of band scored (0) for each primer. The per cent polymorphism and monomorphism among MS lines were calculated (Mandaliya *et al.*, 2010c) based on RAPD, ISSR, SRAP, and combine (RAPD+ISSR+SRAP) analysis. Unique markers for each line were identified from agarose gel electrophoresis amplification pattern. The programme PICcalc of University of Pannonia Georgion, Hungary was used for calculation of polymorphic information content (PIC) and heterozygosity (H) (http://w3.georgikon.hu/pic/english/default.aspx). Jaccard's similarity coefficient values, and coefficient matrix was constructed with the help of Free Tree software. This matrix was subjected to Unweighted Pair Group Method for Arithmetic averages analysis (UPGMA) to generate dendrograms (Hussein *et al.*, 2007) using CLC Main Workbench5.

RESULTS AND DISCUSSION

Genic male sterility (GMS) is an inherited trait that prevents the production of functional pollen, but maintains female fertility. It has been widely used in breeding programmes for F1 hybrid seed production in cotton (*Gossypium hirsutum* L.). The remarkable advantages of GMS are complete sterility, wide source of recovery, and ease of obtaining high vigour combinations (Ma *et al.*, 2007). Molecular characterizations by RAPD, ISSR and SRAP have been performed on cotton GMS lines same as CMS lines. This molecular characterization will assist in crop breeding programme to select and combine inter GMS lines to obtain high vigor combinations.

A total of 731 reproducible bands were generated in whole study (Table 1). Total amplicon, total amplicon/primer, and per cent polymorphism were calculated for molecular markers. The PCR product of GMS lines amplified by RAPD, ISSR and SRAP primers were shown in Fig. 1.

Per cent polymorphism of GMS lines : Optimum recordable 365 RAPD bands ranging from 100 to 5000 bp length were amplified by total 25 primers among all the lines (Table 1). The RAPD amplification profile generated 316 monomorphic and 49 polymorphic bands that correspond to 74.12 per cent monomorphism and 25.88 per cent polymorphism (Table 2). The average 4.56 amplicons/ primers were generated. The maximum number of amplified product was 38 (OPA 2) and minimum 1 (OPB 10). Amongst the 30 primers studied 20 ISSR primers generated reproducible 265 bands (Table 1). UBC803, UBC826, UBC862, UBC872, UBC873, UBC881, UBC885, UBC888, UBC889, and I7 have not generated any band in GMS lines. The polymorphic 31 and 234 monomorphic bands were observed thus, percentage of polymorphic bands observed 13.45 and of monomorphic 86.55 (Table 2). The average 4 amplicon/ primer was observed. The maximum amplified product was 33 (I1) and minimum 2 (I3). SRAP primers were generated total 101 bands, among which 92 were monomorphic and 9 were polymorphic (Table 1). It had shown 9 per cent polymorphism. The average 7.75 amplicon/primer was observed (Table 2). The maximum number of amplified product was 29 (combination D) and minimum 18(combination A). Thus, molecular markers were able to encase polymorphism in GMS lines.

Heterozygosity (H) and polymorphic information content (PIC) value of GMS lines: RAPD, ISSR and SRAP primers based heterozygosity (H) and polymorphic information content (PIC) value calculated for GMS lines

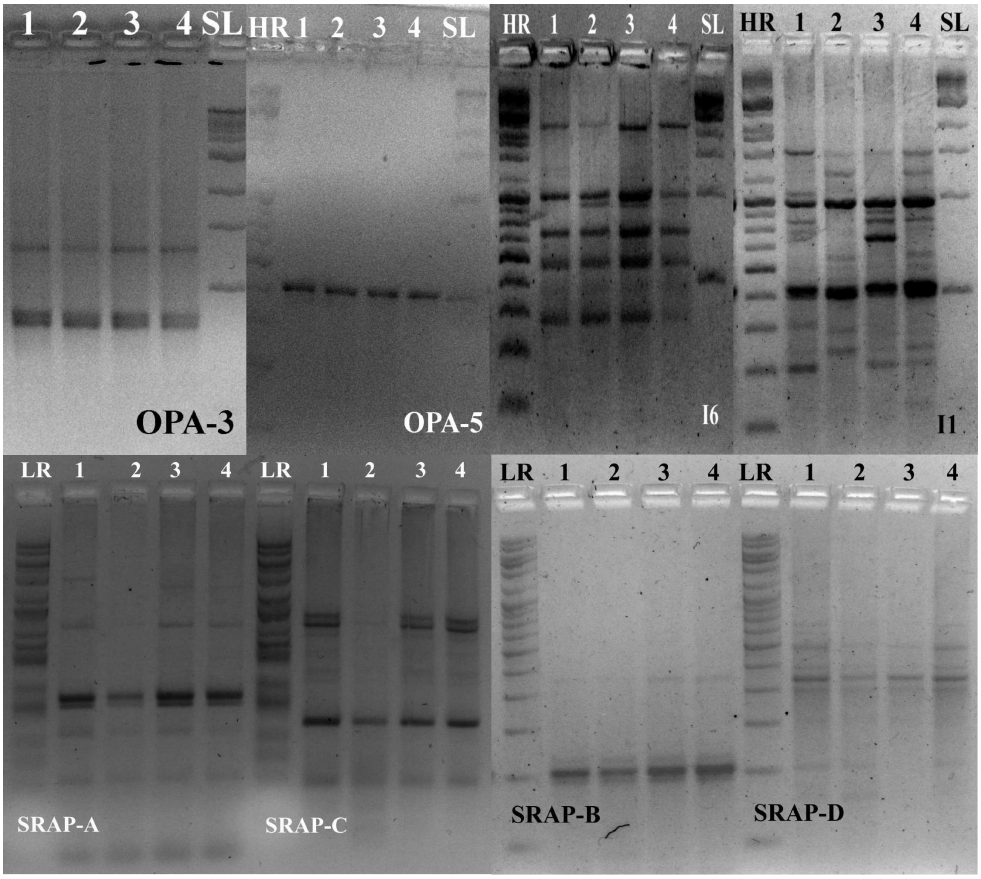

Fig 1. Amplification pattern of GMS lines in the presence of selected RAPD (OPA3, OPA5), ISSR (I1, I6) and SRAP (A, B, C and D combination) primers. [1-4: GMS lines (1) G203, (2) G217, (3) G205 and (4) G209; SL: Supermix Ladder, HR: High Range Ruler and LR: Low Range Ruler from Merck Genei, Banglore].

(Table 1). The heterozygosity (H) for RAPD primers ranged from 0.00 to 0.50 with a mean of 0.24 and PIC varied from 0.00 to 0.38 with a mean of 0.19. The H value for ISSR varied from 0.0to 0.50 with a mean of 0.15 and PIC varied 0.00 to 0.38 with a mean of 0.12. In case of SRAP, H value ranged from 0.18 to 0.40 with a mean of 0.28, PIC value ranged from 0.16 to 0.32 with a mean of 0.24. In total, 49 primers together shown that H value ranged from 0.00 to 0.50 with a mean of 0.20, PIC value ranged from 0.00 to 0.38 with mean of 0.17.

Unique markers for GMS lines: Unique markers for GMS lines were identified in PCR analysis. The highest unique markers were identified for G217 and lowest for G205 (Table 2). There were several other reports on various markers studied in GMS lines (Table 2). The molecular markers were proven to develop unique markers in GMS lines.

UPGMA relationship of GMS lines: The Jaccard's distance/similarity matrixes were calculated based on RAPD, ISSR, SRAP, and combine data (Table 3). In RAPD, Jaccard's similarity index shown highest similarity between G203 and G205 (0.84), while highest distance between G203 and G209 (0.69). In ISSR, highest similarity and distance observed between G205 and G209lines (0.84), and G203 and G217lines (0.76) respectively. In case of

Table 1. Amplicon profile, per cent polymorphism, H and PIC value of GMS lines

No.	Primer	Total amplicon	Poly-morphic amplicon	Total band	Poly-morphic band	Per cent mono-morphism	Per cent poly-morphism	H value	PIC value
1	OPA1	10	2	33	2	93.94	6.06	0.289	0.247
2	OPA2	10	1	38	2	94.74	5.26	0.095	0.090
3	OPA3	3	0	12	0	100.00	0.00	0.000	0.000
4	OPA7	5	1	17	2	88.24	11.76	0.255	0.222
5	OPA8	3	1	8	2	75.00	25.00	0.444	0.346
6	OPA9	12	1	37	1	97.30	2.70	0.353	0.291
7	OPA10	4	0	16	0	100.00	0.00	0.000	0.000
8	OPA12	6	2	19	3	84.21	15.79	0.330	0.275
9	OPA15	4	4	4	4	0.00	100.00	0.375	0.305
10	OPA16	6	1	20	1	95.00	5.00	0.278	0.239
11	OPA19	2	0	6	0	100.00	0.00	0.375	0.305
12	OPB1	2	0	8	0	100.00	0.00	0.000	0.000
13	OPB2	1	0	3	0	100.00	0.00	0.375	0.305
14	OPB3	3	2	8	4	50.00	50.00	0.444	0.346
15	OPB4	5	0	20	0	100.00	0.00	0.000	0.000
16	OPB5	1	0	4	0	100.00	0.00	0.000	0.000
17	OPB6	6	0	24	0	100.00	0.00	0.000	0.000
18	OPB8	3	0	12	0	100.00	0.00	0.000	0.000
19	OPB10	1	1	1	1	0.00	100.00	0.375	0.305
20	OPB11	4	1	14	2	85.71	14.29	0.219	0.195
21	OPB14	2	2	2	2	0.00	100.00	0.375	0.305
22	OPB15	4	4	8	8	0.00	100.00	0.500	0.375
23	OPB17	7	0	28	0	100.00	0.00	0.000	0.000
24	OPB18	7	6	14	14	0.00	100.00	0.500	0.375
25	OPB19	3	1	9	1	88.89	11.11	0.375	0.305
26	UBC808	2	0	8	0	100.00	0.00	0.000	0.000
27	UBC814	4	3	8	5	37.50	62.50	0.500	0.375
28	UBC817	7	0	28	0	100.00	0.00	0.000	0.000
29	UBC822	3	0	12	0	100.00	0.00	0.000	0.000
30	UBC834	4	1	16	0	100.00	0.00	0.000	0.000
31	UBC840	4	0	16	0	100.00	0.00	0.000	0.000
32	UBC864	3	0	10	0	100.00	0.00	0.278	0.239
33	UBC867	1	0	4	0	100.00	0.00	0.000	0.000
34	UBC868	1	0	4	0	100.00	0.00	0.000	0.000
35	UBC876	6	2	20	4	80.00	20.00	0.278	0.239
36	UBC880	3	0	12	0	100.00	0.00	0.000	0.000
37	UBC884	3	0	12	0	100.00	0.00	0.000	0.000
38	UBC886	2	0	8	0	100.00	0.00	0.000	0.000
39	UBC891	2	0	8	0	100.00	0.00	0.000	0.000
40	I1	15	9	33	14	57.58	42.42	0.495	0.372
41	I2	3	0	12	0	100.00	0.00	0.000	0.000
42	I3	1	1	2	2	0.00	100.00	0.500	0.375
43	I4	4	2	10	3	70.00	30.00	0.469	0.359
44	I5	6	1	20	1	95.00	5.00	0.278	0.239
45	I6	6	1	22	2	90.91	9.09	0.153	0.141
46	A	8	2	27	3	88.89	11.11	0.264	0.229
47	B	5	1	18	2	88.89	11.11	0.180	0.164
48	C	8	0	27	0	100.00	0.00	0.264	0.229
49	D	10	3	29	4	86.21	13.79	0.399	0.319
	Total	225	56	731	89				
	Average	**4.59**	**1.14**			**80.57**	**19.43**	**0.204**	**0.166**

SRAP, highest similarity observed between G205 and G209 (0.89), and highest distance between G209 and G217 (0.62). The combined data shown, highest similarity between G203 and G205 (0.83), and highest distance between G209 and G217 (0.72) (Table 4).

The dendrogram were constructed based on UPGMA relationship of RAPD, ISSR, SRAP, and combined data (Fig.2). RAPDUPGMA analysis has shown that G203 and G205 lines were most near lines in UPGMA relationship and G209line was most diverted. ISSR UPGMA analysis also has shown that G203 and G205 lines were most near lines and G217 line was most diverted. SRAP UPGMA analysis has shown G205 and G209 lines were most near lines and G217 line was diverted

Table 2. RAPD, ISSR, and SRAP combine analysis of GMS lines

No.	Markers	Total amplicon	Total amplicon/ primer	Total band	Mono-morphic band	Poly morphic band	Per cent mono-morphism	Per cent poly-morphism
1	RAPD	114	4.56	365	316	49	74.12	25.88
2	ISSR	80	4	265	234	31	86.55	13.45
3	SRAP	31	7.75	101	92	9	91	9
4	Combine	225	4.59	731	642	89	80.57	19.43

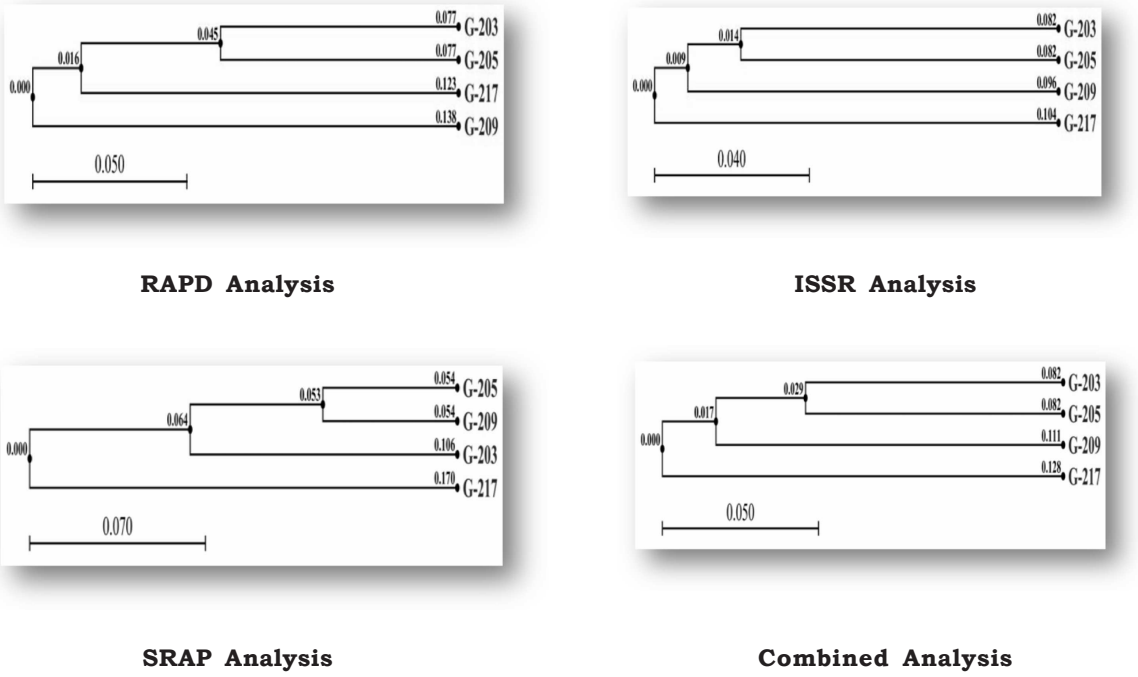

Fig. 2. UPGMA analysis of GMS lines

Table 3. Unique markers identified for GMS lines

GMS lines	Unique markers
G217	$OPA1_{800}$, $OPA1_{700}$, $OPA9_{4000}$, $OPA12_{4000}$, $OPA15_{2000}$, $OPA15_{1815}$, $OPA15_{1700}$, $OPA15_{1500}$, $OPB14_{1185}$, $UBC814_{550}$, and $I1_{400}$
G203	$OPA16_{4000}$, $OPB14_{1000}$, $I1_{1100}$, $I1_{800}$, $I1_{500}$, SRAP-A_{1500}, and SRAP-D_{1200}
G209	$OPB18_{3000}$, $OPB19_{4000}$, $OPB10_{1500}$, $I5_{1400}$, and SRAP-D_{350}
G205	$I1_{950}$ and $I1_{750}$

Table 4. Jaccard's distance/similarity matrix based on RAPD analysis among GMS lines

Markers	RAPD				ISSR				SRAP				Combine			
Lines	G203	G217	G205	G209	G203	G217	G205	G209	G203	G217	G205	G209	G203	G217	G205	G209
G203		0.78	0.84	0.69		0.76	0.84	0.78		0.71	0.80	0.77		0.77	0.83	0.73
G217	0.78		0.72	0.69	0.76		0.81	0.81	0.71		0.64	0.62	0.77		0.74	0.72
G205	0.84	0.72		0.79	0.84	0.81		0.84	0.80	0.64		0.89	0.83	0.74		0.82
G209	0.69	0.69	0.79		0.78	0.81	0.84		0.77	0.62	0.89		0.73	0.72	0.82	

than others. Lastly, combined analysis was performed that shown that G203 and G205 lines were most nearest lines and G217 line was most diverted. In the present study, highest similarity between G203 and G205 (0.8350) and the UPGMA analysis also revealed same that shown that G203 and G205 lines were most near lines and G217 was most diverted from other all GMS lines.

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