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Short Communication

Molecular characterization of genetic male sterile genotypes in cotton (*Gossypium* spp.)

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Cotton, the white gold is the hub of textile industry in the world. Cotton, being often cross pollinated crop, is amenable for heterosis breeding; system of male sterility is of great practical significance as it avoids labor intensive process of hand emasculation that greatly aids in hybrid seed production. Apart from easy hybrid seed production, the hybrid seeds produced through male sterility system have high genetic purity. In cotton, GMS is preferred over Cytoplasmic Genetic Male Sterile (CGMS) system as it overcomes the drawbacks of cytoplasmic effect and problem of fertility restoration. GMS character in tetraploids is conditioned by double homogeneous recessive alleles, ms_5 ms_5 ms_6 ms_6 and other genes have also been identified whereas in diploids it is controlled by single recessive alleles, ams_1 ams_1.

The Random Amplified Polymorphic DNA (RAPD) technique [1] provides an unlimited number of markers, which can be used for various purposes. High level of polymorphism based on RAPD technique has been reported among the genotypes of cotton [2]. RAPD markers have been successfully used for the estimation of genetic similarities and the cultivar analysis of various plant species. These have been used to discriminate intra and interspecific genotypes in cotton [3]. Diploid cottons have several useful traits that could be transferred to present day tetraploid cottons [4]. Selecting useful traits for inter specific transfer depends on the genetic diversity in the parental material [5]. In this context, reliable assessment of diversity in Indian diploid and tetraploid cottons at the molecular (RAPD) and morphological levels assumes substantial importance, and hence attempted to characterize GMS lines through molecular markers.

Near Isogenic lines (NIL) derived from repeated back crossing of both diploids (20 nos.) and tetraploids (14 nos.) were included in the investigation (Table 1).

Table 1. List of GMS genotypes used for the molecular characterization

S.No.	Sterile	S.no.	Fertile
	Diploid genotypes		
1	RGMS-3 (S)	2	RGMS-3 (F)
3	GAKA-15 (S)	4	GAKA-15 (F)
5	GAKA-26 (S)	6	GAKA-26 (F)
7	GAK-20A (S)	8	GAK-20A (F)
9	SGMS-13 (S)	10	SGMS-13 (F)
11	GAKA-8615 (S)	12	GAKA-8615 (F)
13	GAKA-423 (S)	14	GAKA-423 (F)
15	Million-GMS (S)	16	Million-GMS (F)
17	RGMS-2 (S)	18	RGMS-2 (F)
19	DS-5 GMS (S)	20	DS-5 GMS (F)
	Tetraploid genotypes		
1	DGMS-1 (S)	2	DGMS-1 (F)
3	IAN-579-1 (S)	4	IAN-579-1 (F)
5	G-67 GMS (S)	6	G-67 GMS (F)
7	Laxmi GMS (S)	8	Laxmi GMS (F)
9	SRT-1 GMS (S)	10	SRT-1 GMS (F)
11	GAK-32 (S)	12	GAK-32 (F)
13	SHGMS-9 (S)	14	SHGMS-9 (F)

The DNA was extracted from the leaf samples by following CTAB extraction method with certain modifications[6]. Commercial kits of random decamer

primers obtained from Operon Technologies Inc. Alamedas, USA were used. Polymerase chain reaction (PCR) was performed in 25 µl volume containing primer, deoxyribonucleotides, KCl, Tris-HCl (pH 8.3), $MgCl_2$ and *Taq* DNA polymerase. Products from PCR reaction were resolved by electrophoresis in 1.2% agarose gel.

Pairwise genetic similarities (Sij) between genotypes were estimated by DICE similarity co-efficient. Clustering was done using the symmetric matrix of similarity co-efficient and cluster obtained based on unweighted pair group arithmetic mean (UPGMA) using SAHN module of NTSYS-PC version 2.02.

A total of 119 random decamer primers were used to screen diploid near isogenic lines, in which 8 primers did not show any amplification and 29 primers produced monomorphic bands. The remaining 82 primers with ten pair of near isogenic lines generated 314 fragments of which 187 were polymorphic. The level of polymorphism ranged from 16.66 to 100 per cent. The number of amplified fragments varied from one to seven with an average of 3.83 fragments per primer of which 2.28 were polymorphic.

Pairwise similarity coefficient values for 20 near isogenic lines ranged from 0.70 to 0.98. The dendrogram (Fig. 1) revealed six main distinct clusters. All fertile lines made independent clusters (I & III) with similarity index 0.94 and 0.96 respectively. Similarly all sterile lines made another independent cluster (II & IV). Together they formed a single cluster at similarity co-efficient of 0.88. The fertile and sterile plants of the diploid genotypes RGMS-2 and Million GMS made separate clusters (V & VI) at similarity co-efficient of 0.87 and 0.83 respectively.

Eighty eight (88) random decamer primers were used to study genetic diversity in 14 near isogenic lines of tetraploid genotypes including 7 sterile and 7 fertile lines. As many as 310 amplicons were amplified, out of which 190 were polymorphic with an average of 3.33 polymorphic fragments per primer. Total number of fragments amplified ranged from one to thirteen, with an average of 5.48 fragments per primer. The polymorphism per primer ranged from 10 per cent to 100 per cent.

The similarity co-efficient ranged from 0.76 to 0.98.The dendrogram (Fig. 2) revealed three distinct clusters. The sterile and fertile plants of the genotype DGMS-1 made an independent cluster (I) with Sij 0.96. The sterile plants and fertile plants of the tetraploid genotype GAK-32 and SHGMS-9 also made a separate cluster (III) with Sij 0.87. The other genotypes including sterile and fertile plants made a separate big cluster (II) with Sij 0.94.

Since *hirsutum* and *arboreum* groups have been improved independently, these form separate clusters, depicting enormous variation among them despite having one genome common.Molecular markers linked to particular gene of interest are useful in identification of phenotypes in any population of genotypes. A variety of options are available for identifying the desired gene which could be useful in further breeding programme species. So, the present investigation was aimed at identifying and developing a marker linked to fertility/ sterility in GMS lines.

Ability of individual primers to fingerprint the genotypes was assessed, based on their ability to produce polymorphism in the near isogenic GMS lines. To differentiate between fertile and sterile plants of each GMS genotype RAPD markers were used. The primers OPZ14 and OPB04 showed 25 per cent polymorphism in all pairs of near isogenic diploid GMS lines In case of tetraploid GMS lines, the primer OPB04 showed polymorphism in fertile and sterile plants of all the genotypes.

Greater diversity among diploids was observed as compared to tetraploids. Despite the strong homology exhibited by many of the cotton genotypes, it is now possible to differentiate very closely related species and near isogenic lines using molecular markers. Lines considered as near isogenic lines based on the morphological characters, showed the polymorphism to the extent of 0-100 per cent for different primers. It speaks limitedness of morphological markers and the higher strength of molecular markers in genetic diversity analysis. Primers like OPB04 and OPZ14 stored genetic diversity between fertile and sterile plants of all the diploid GMS genotypes. Similarly in tetraploid GMS lines also primer OPB04 has showed diversity between fertile and sterile in all the genotypes. So they can be considered as putative markers to study them for linkage studies.

Twenty near Isogenic diploid Genetic Male Sterile (GMS) lines in commercial cotton were screened using 119 random decamer primers. Out of 314 amplicons amplified, 187 were found to be polymorphic, with an average of 3.83 fragments per primer of which 2.28 were polymorphic. Genetic diversity analysis among fourteen

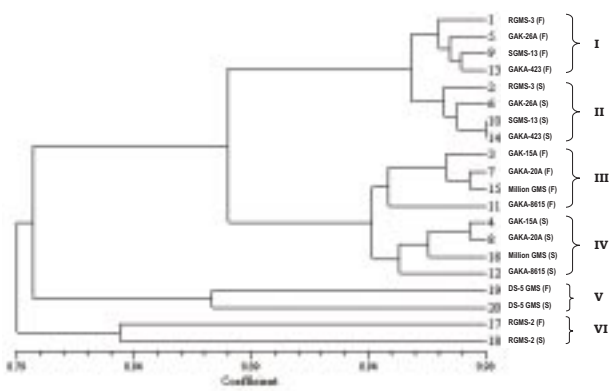

Fig. 1. Dendrogram generated from pooled data of RAPD profile using UPGMA analysis in the diploid near isogenic GMS lines

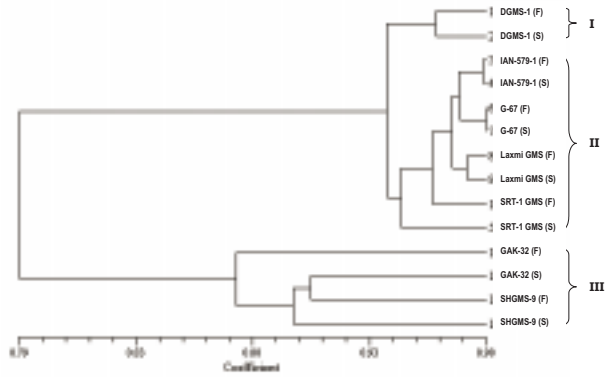

Fig. 2. Dendrogram generated from pooled data of RAPD profile using UPGMA analysis in the tetraploid near isogenic GMS lines

near isogenic tetraploid GMS lines was carried out using 88 random decamer primers. Out of 310 fragments amplified, 190 were found to be polymorphic, with an average of 5.44 fragments per primer of which 3.33 fragments were polymorphic. The sterile and fertile plants of different genotypes made independent clusters indicating their divergence and providing an opportunity for tagging either fertile or sterile gene. Primers like OPB04 and OPZ14 showed genetic diversity between fertile and sterile plants of all the diploid GMS genotypes. Similarly in tetraploid GMS lines also primer OPB04 showed diversity between fertile and sterile in all the genotypes. So they can be considered as putative markers for further genetic studies.

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