TMC MM 1.6: Exploitation of apomixis and TGMS system in hybrid cotton seed production

INTRODUCTION

Apomixis, an asexual method of reproduction through the seed, provides unique opportunities for developing superior cultivars in the future. It occurs at low levels in some cultivated species and can be found in closely related species of many cultivated species After extensive screening of available apomictic lines derived from cross involving three cultivated species of cotton *(G.arboreum, G.hirsutum* and *G.barbadence)* which are aneuploid, few apomictic cotton lines with a low frequency of apomixis were found, but their frequency was too low. These identified lines are: IS 244-4-1, IS 244-4-2-27, IS 181-7-1-19, AP 2-1, AP 3-2 and AP1-4. The boll formation following flower emasculation and removal of style and stigma was observed in these lines and seeds have been harvested. However, it was observed that boll setting was higher in apomictic lines at UAS, Dharwad compared to Nanded and Nagpur centre. The crosses between these lines and pigmented dominant genotype Bikaneri Narma (Red) showed presence of both pigmented and non-pigmented plants in F1. Cytological studies of lines IS 244 -4-1, IS 244-4-2 and IS 181-7-1 showed presence of aneuploidy. In both the diploid (Thermo sensitive Genetic Male Sterility) and tetraploid (Environment sensitive Genetic Male Sterility) cotton, male sterility was controlled by one pair of recessive genes. The identified lines EGMS-35, EGMS-18 (tetraploid) and 1-1 and 9-1 (diploid) will also be studied for stability over the years for their use in hybrid seed production. Exploitation of some of these lines to develop cotton hybrids has been undertaken. At NRCPB Phytotron studies of diploid cotton TGMS line showed plants were completely male sterile at 30°C/25°C day/night temperature regime. The minimum night temperature was lowered step wise (2-3°C steps) up to 15°C. Each of the new temperature regimes was maintained for 8-10 days and newly opening flowers were checked for pollen viability. At the day temperature 30°C and night temperature 1$^{\circ}C$ more than 90% of the flowers are completely fertile and the night temperatures more than 20°C the flowers are completely sterile. A minimum of three days exposure at 1$^{\circ}C$ was necessary for shift from sterile to fertile flowers.

OBJECTIVES

- To study apomixis in tetraploid and TGMS system in diploid & tetraploid cotton.
- To stabilize apomixis and TGMS in promising genotypes of Cotton.
- To utilize apomixis for fixing heterosis and TGMS in hybrid seed production in cotton.

SALIENT FINDINGS

1. Confirmation of apomictic phenomena through emasculation and selfing in identified apomictic lines.

At C. I. C. R., Nagpur, ten apomictic lines identified during the year 2008-09 were grown for confirming boll setting using emasculation and selfing (EMS) and removal of style and stigma (RSS). In four lines namely 1060 (B), AP1-4, AP 4-15 and AP 5-10 boll setting was observed following methods of EMS and RSS during 2009-10. However, the frequency of boll setting was very low. The details are given in table 1.6.1.

Table 1.6.1: Emasculation and selfing (EMS) and removal of style and stigma (RSS) in apomictic lines grown of CICR Nagpur and ARS Nanded

S. No.	Genotype	No. of boll set (EMS)	Percentage of boll set (%)	No. of boll set (RSS)	Percentage of boll set (%)
1.	IS-244-4-1	1	1.79	-	4.35
2.	IS-244-4-2-4	1	1.92	-	
3.	AP-1-4	1	2.86	-	
4.	AP-4-15	-	-	1	1.32
5.	AP-5-10	-	-	1	1.30
6.	AP-1-5	1	1.92	-	-
7.	1060 (B)	-	-	3	4.35
8.	AP-2-1	-	-	1	5.0
9.	IS-244-4-2-27 -1-1-7	-		1	1.59
10.	IS-244-4-3-35-1-1-4	-		1	1.33
11.	IS-244-4-3-35	1	1.22	1	1.69
12.	AP 2-8	-		2	2.44
	ARS, Nanded				
13.	IS 244-4-2-19-1-1-2-1-1	-	-	2	4.65
14.	IS 244-4-3-5-1-1-2	-	-	1	2.56
15.	IS 244-4-2-27-1-1-7	1	0.93	-	-
16.	IS 181-7-1-19-1	-	-	1	0.78
17.	IS 244-4-3-5-1-1-1-1	3	5.17	2	6.87

Boll to row progenies of seeds obtained from EMS and RSS were grown for studying frequency of boll setting. In general, the progenies were dwarf and normal boll setting was very less. This indicated unstable nature of apomictic lines over the location and years.

At ARS, Nanded, the genotype IS-244-4-2-19-1-1-2-1-1 formed two bolls by removal of style and stigma (RSS) method with a frequency of 4.65 %. The same genotype had shown boll formation to the extent of 0.41 % by emasculation and selfing (EMS) method during last year (2008-09) conformation testing. The genotypes IS-244-4-3-5-1-1-2 and IS-181-7-1-19-1 showed 2.56 % and 0.78 % boll formation, respectively by removal of style and stigma (RSS) methods. The genotype IS-181-7 -1-19-1 also had shown boll formation by RSS to the extent of 0.78 %.

The genotype IS-244-4-2-27-1-1-7 showed boll formation by emasculation and selfing method with a frequency of 0.93 %. This genotype showed boll formation to the extent of 0.40% during 2008-09 by EMS method. The genotype IS-244-4-3-5-1-1-1-1 showed boll formation by both method EMS and RSS to the extend of 5.17 % and 6.87%. respectively.

2. Crossing with dominant marker to study the nature of apomictic phenomenon.

At CICR. Nagpur, apomictic lines NAP 5, NAP 7 and IS 244-4-2 when crossed with BN Red (Bikaneri Narma Red) dominant marker, non-pigmented plant (maternal plant) were observed in the F_1. However, these plants could not set any seed following methods of emasculation and selfing and removal of style and stigma. This also indicates the unstable nature of these apomictic lines

During 2008-2009, out of 21 F_1 crosses (between apomictic lines and okra leaf type of PH 330) tested at ARS. Nanded. and five crosses showed normal leaf characters (Table 1.6.2). Seeds from these F_1 lines of normal plants were sown for testing their F_2behavior during 2009-2010. The F_2behavior of cross IS-244-4-3-16-2-1 x PH-330 showed all the plants having normal leaves but variation in normal leaf type i,e. dense hairy leaves in nine plants; Semi dense hairy leaves in four plants and leathery normal leaves in five plants.

The F_2behavior of cross IS-181-7-1-19 x PH-330-showed all the plants having normal leaves but variation in flower type i,e. plants with flower type yellow petal & buff anther and plants with flower type cream petal & cream anther. All the plants had uniform bushy spreading growth habit.. The F2behavior of cross IS-181-7 -1-89-2-1 x PH-330-1 showed uniformity with normal leaves development and flower uniformity (yellow petal and buff anther). The bolls observed on these plants were relatively big size. The F2behavior of cross IS-244-4-2-27 x PH-330-1 also showed uniformity with normal leaves development and flower uniformity (cream petal and yellow anther).

Table 1.6.2 : Number of plant showing okra and normal leaves in F, and F_2 population

8r. No.	Cross	Total no. of plants Observed 2008-09(F1)	plants showing leaf		% of normal type leaves	% of okra type	Total no. plants observed 2009-10(F,)	plant showing . leaf		% of normal type	% of okra type
			Normal	Okra				Normal	Okra		
1,	18-244-4-3-8 x PH-330	17	04	13	25,53	74.47	--	--	--	--	--
2.	18-244-4-3-16-2-1 x PH-330	13	01	12	07.69	92.31	18	18	00	100	00
3.	18-181-7-1-19 x PH-330	14	01	13	7.14	92.86	20	20	00	100	00
4.	18-181-7-1-89-2-1 x PH-330-1	14	11	03	78.57	21.43	20	20	00	100	00
5.	18-244-4-2-19-1-1-2 x PH-330	3	03	00	100.00	0.00	--	--	--	--	--
6.	18-244-4-2-27 x PH-330-1	17	01	16	5.88	94.12	17	17	00	100	00

At UAS, Dharwad, 238 plants were raised from the 33 entries in polythene covers and these plants were compared with the mother (female) plant in the field. 46 plants showed the characteristics of the mother plant. These plants *have* been potted and flowers will be selfed to confirm the apomictic behavior in 201 O.

3: Cytological studies of identified apomictic lines

Cytology was done on two lines AP 4-15 and AP 5-10 plants which showed the aneuploidy nature at metaphase and telophase of chromosome configuration. In addition, cytological studies were also taken up on IS 244-4-1 and IS 244-4-2.

In normal microsporogenesis, at the end of second meiotic phase, there should be tetrad formation. But in the lines namely, 1060 B, IS 244-4-1 and IS 244-4-2, abnormal behavior of chromosomes was observed which resulted in triad formation instead of tetrad (Fig 1.6.1 & 1.6.2).Also in metaphase I, univalents were seen.

4. Evaluation of experimental hybrids of tetraploid EGMS lines:

Eight EGMS lines namely 145, EGMS 132, EGMS 108, EGMS 18, EGMS 35, EGMS 36, 08093-10R and EGMS 3822266 were evaluated for their stability and seed cotton yield under rainfed conditions at CICR, Nagpur. EGMS line EGMS-35 recorded the highest seed cotton yield of 2474 kg/ha followed by EGMS 08093-1 OR (1558 kg/ha) and EGMS 18 (1477 kg/ha). Among three hybrids only one hybrids EGMS x GP-9 recorded the highest seed cotton yield of 1087 kg/ha.

Six EGMS lines were sown during summer 2008-09 at ARS, Mudhol and their stability for expression of sterility was studied. All the six EGMS lines showed stability for expression of male sterility. Plant to row progenies of 20 EGMS lines were sown during February 2009 and their stability for expression of sterility was studied. Out of these 20 progenies, 6 progenies were found more uniform and stable for expression of male sterility. These 6 progenies were continued in the field upto kharif 2009 and selfing was done to obtain selfed seed. Selfed seed of these 6 EGMS lines is again sown during January 201 0 in observation plots to confirm stability of sterility.

Twenty experimental hybrids were evaluated in replicated trial during kharif 2009-10 at Agricultural Research Station, Mudhol.. Out of 20 hybrids evaluated, *five* hybrids were found statistically at par with check Mallika Bt. The yield data is given in the Table 1.6.3.

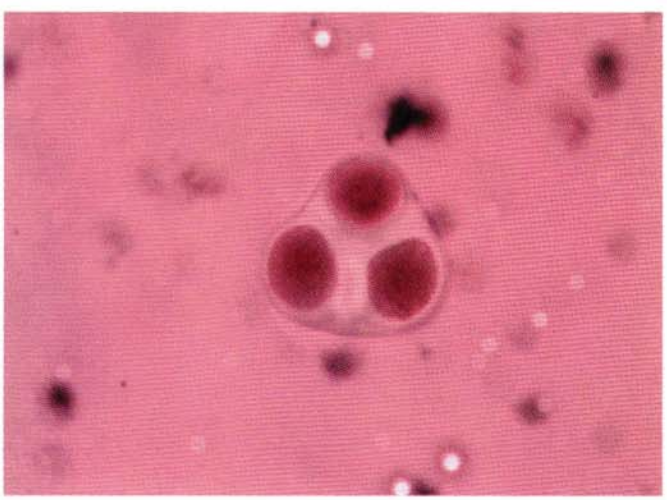

Fig. 1.6.1 : Microsporogenesis in 1060 B

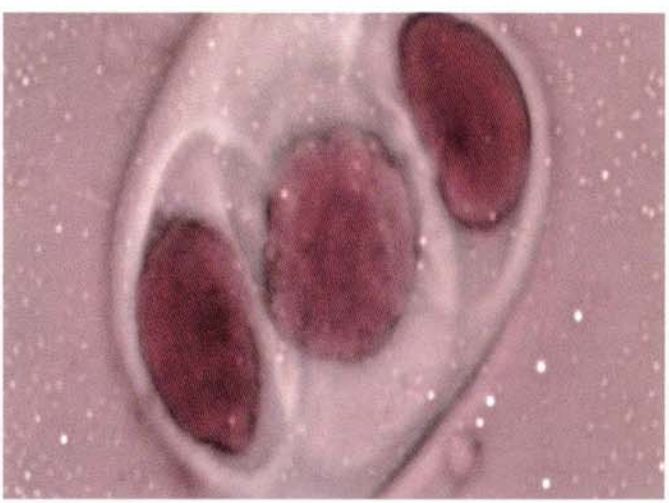

Fig. 1.6.2 : Microsporogenesis in IS 244-4-2

Table 1.6.3: Experimental hybrids trial at ARS, Mudhol during 2009-10

Sl.No.	ExperimentalHybrids	SeedCotton Yield(Kg/ha)
1	EGMSx158458	3112
2	EGMSx 33892	3127
3	EGMSx CAT4207	3113
4	EGMSx CAT207 BB	2955
5	EGMSx SA- 234	2909
6	MallikaBt.©	2770
7	EGMSx SA992	1847
8	EGMSx CNH120MB	1780
9	EGMSx 477990	1693
10	EGMSx 200759	1636
11	EGMSx 141993	1581
12	EGMSx SA- 496 MB	1516
13	EGMSx EC 170339	1509
14	EGMSx 382266	1506
15	EGMSx 3 WAY45 S.P.	1479
16	EGMSx 200767	1467
17	EGMSx SA. 1016	1354
18	EGMSx L.S. LINE	1332
19	EGMSx L.S. LINE- 1	1190
20	EGMSx CAT2894	1078
21	EGMSx EC450617	1072
	SEm	130
	CD. (0.05)	370
	C.v.%	12.19

Phytotron studies

At NRCPB, New Dehli, tetraploid lines grew vigorously and showed fertility at normal and high temperature up to 36°C under Phytotron conditions. As the plants touched the roof of the chamber, further raising of temperature affected the growth and hence higher temperatures could not be evaluated.

5.Evaluation of experimetal hybrids ofTGMS Diploid

At CICR, Nagpur, a total number of 158 F_2plants from cross TGMS 1-1 x PA 255 were raised to study the inheritance pattern by phenotyping as well as to perform genotyping. Phenotyping was done by daily flower observations in individual plants and sterility/fertility behavior was recorded. The leaf samples of individual plants were collected for DNA extraction and genotyping was done by the NRCPB, New Delhi Centre.

The three experimental hybrids of TGMS were sown along with released and popular hybrids, AKDH-7, CISA-2 and popular varieties, AKA8401, PA402 and PA-255 to evaluate the performance of new hybrids.

The Statistical analysis of the total seed cotton yield

Table1.6.4 : Evaluation of experimental hybrids for seed cotton yield at CICR, Nagpur

S. No.	Nameof the Hybrid Yield(Kg/ha)	Seed Cotton
1	9-1 x 255	1789
2	1-1 x PA 255	1320
3	13-1x PA255	1084
4	AKDH-7	2730
5	CISA2	1647
6	AKA8401	1472
7	PA402	1597
8	PA255	1434
C.D 0.05	616.37	

revealed significant difference among the genotypes. All the three experimental hybrids showed lower seed cotton yield than the released GMS hybrid of central zone (Table 1.6.4). However, the seed cotton yield of one experimental hybrid TGMS 9-1 x PA 255 was higher compared to the yields of CISA-2, the popular desi hybrid released for north zone and the seed cotton yields of popular varieties of central zone viz. PA-402, PA-255 and AKA8401.

The pollen from flowers formed during complete fertility at temperature i.e. below 18°C was observed for viability under microscope. The pollen grains were regular and stained in completely fertile flowers where as presence of irregular shaped and non stained pollen grains were observed in sterile flowers.

At UAS, Dharwad, the hybrid vigor realized in the hybrids is very encouraging. The hybrids, TGMS 1-1 (Plant 11) x DDHC-11 and Plant 11 x ARBHA 35 have got enormous potential as revealed by their yield levels. The F1 seed could not be produced in sufficient quantities as the time period of sterility being influenced by temperature regims is variable.

6. Molecular marker studies in diploid and tetraploid TGMSlines

At NRCPB, New Delhi, studies were conducted on SSR and RAPD polymorphism in TGMS and normal fertile lines of diploid and tetraploid cotton for further use in tagging ofTGMS locus.

RAPD analysis

Out of 428 RAPD primers tested, 31 and 60 primers were polymorphic in diploid (TGMS and PA-255) and tetraploid (EGMS, OHM, 382266) lines, respectively (Fig. 1.6.3 and 1.6.4).

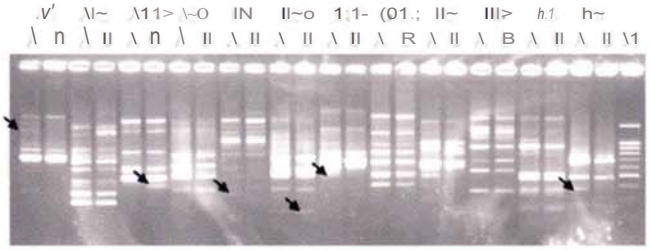

Fig 1.6.3: RAPD patterns of diploid cotton genotypes TGMS (A) and PA-255 (8) with different Operon primers. (M-1 kb ladder). Arrows indicate the polymorphic amplicons.

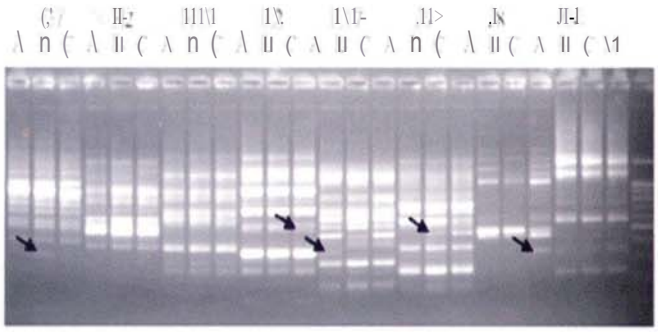

Fig 1.6.4: RAPD patterns of diploid cotton genotypes TGMS (A) and PA-255 (B) with different Operon primers. (M-1 kb ladder). Arrows indicate the polymorphic amplicons.

SSR analysis

A total of 172 BNL SSR primers were used to study parental polymorphism in diploids. Only 19 primers showed polymorphic amplification pattern in cotton diploid cotton lines. TGMS and PA-255 (Fig. 1.6.3).

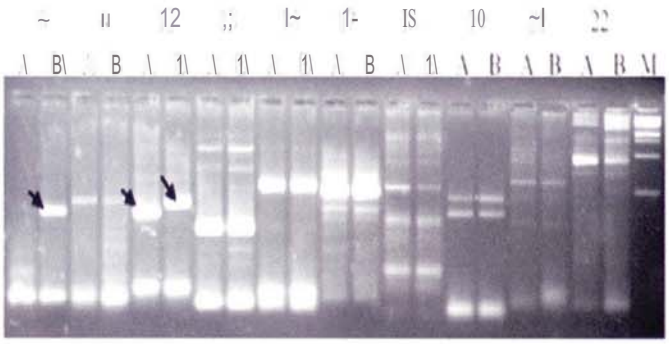

Fig 1.6.5: SSR amplification pattern of diploid cotton genotyps TGMS (A) and PA-255 (B)with different BNL SSR primers (M-1 kb ladder). Polymorphic bands or indicated by arrow

Cloning of polymorphic RAPO amplicons for development of SCAR markers

Three polymorphic RAPD amplicons (M9600, S41000 and W13680) from tetraploid lines were cloned and sequenced. Based on the sequence information, new primers were designed and used to test the SCAR.. However, no polymorphic amplification pattern was observed with SCAR primers between the cotton lines.

Bulked segregant analysis in diploid TGMS lines

DNA was isolated from 159 F_2 individuals of the cross TGMS 1-1 x PA255 raised at CICR, Nagpur. Bulk segregant analysis was performed using 31 RAPD and 19 SSR markers which were earlier identified as polymorphic between the parents. Initially, two bulks, sterile and fertile, were prepared by mixing equal quantity of DNA from 10 individual plants of each category. PCR reactions were set up using DNA from the parents, and sterile and fertile bulks. Of the 31 RAPD primers tested, three primers OPA06, OPV08 and OPN03 showed polymorphism between parents and sterile and fertile bulks (Fig. 1.6.6).

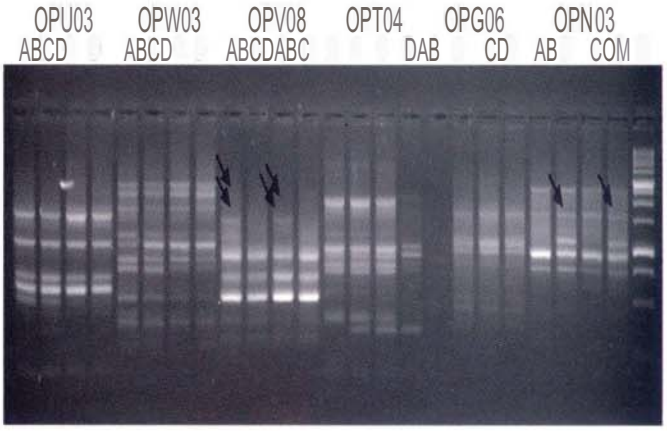

Fig.1.6.6: RAPO patterns of diploid cotton genotypes TGMS (A), PA-255 (B), sterile bulk (C) and fertile bulk (0) with polymorphic RAPO primers. Arrows indicate the polymorphic amplicons (M-1 kb ladder)

Similarly, three SSR primers namely, SSR95, SSR102 and SSR 167, gave polymorphic amplification between the bulks (Fig. 1.6.7).

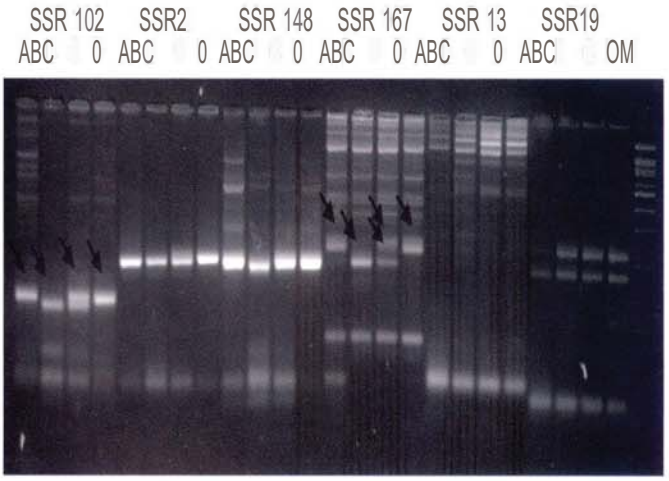

Fig.1.6.7 : SSR amplification patterns of diploid cotton genotypes PA-255 (A), TGMS (B), sterile bulk(C) and fertile bulk (0) with polymorphic SSR primers. Arrows indicate the polymorphic amplicons. (M-1 kb ladder)

Next, individual F_2 plants were genotyped using the above six primers. One SSR marker was found to co-segregate with the TGMS. Detailed analysis of linkage of markers and the trait is in progress.

Cloning of polymorphic RAPO amplicons for development of SCAR markers

Two polymorphic RAPD amplicons (V81100 and V81500) from OPV8 primer were cloned and sequenced. Based on the sequence information, new primers were designed and used to test the SCAR.. However, no polymorphic amplification pattern was observed with SCAR primers between the cotton lines.