# Tille: Exploitation of Apomixis and TGMS System in Hybrid Collon Seed Production

| | | |
|---|---|---|
| **Code No.** | TMC/MMI/1.6 | |
| **Year** | 2007-08 | |
| **Name of the PI and Lead centre** | Dr. B. M. Khadi<br>CICR, Nagpur | |
| **Name of the CCPI and centre** | Dr. S. M. Palve /<br>Dr. Vinita Gotmare /<br>Dr. V, Santhy<br>CICR, Nagpur | Dr. Bhatade<br>MAU, Nanded |
| | Dr. Rajesh Patil /<br>Dr. I. S. Katageri<br>UAS Dharwad | Dr. S. Lakshman<br>ANGRAU, Mudhol |

## INTRODUCTION

Apomixis is a phenomenon where there is formation of seed from the maternal tissues of the ovule, avoiding the process of meiosis and fertilization leading to embryo development.. Under X plan TMC MM -I programme few lines obtained from tri-species cross *(hirsutum* x *barbadense)* x *arboreum)* were showing apomictic behaviour producing uniform plants inspite of having abnormal chromosome number. This needed further confirmation for which the project was initiated. Similarly a Temperature sensitive male sterile mutant was obtained from G. *arboreum* variety DS-5 as well as G. *hirsutum* variety Renuka which needed to be confirmed and stabilised.

## OBJECTIVES

1. To confirm and study apomixis in tetraploid Cotton.
2. To stabilize apomixis in promising genotypes of Cotton.
3. To utilize apomixis forfixing heterosis
4. To confirm and study TGMS system in diploid & tetraploid Cotton.
5. To stabilize TGMS lines
6. To utilize TGMS in Hybrid seed production in cotton
7. Molecular analysis of Apomictic and TGMS plants

## ACTIVITIES

**Confirmation of Apomictic phenomena in Tetraploids**
Emasculation & selfing
Removal of Style & stigma
Crossing with dominant marker genotypes
Crossing with CMS A-line.

**To study the nature of apomixis phenomenon**
Type of Apomixis - Facultative or Obligatory
Crossing with Dominant marker genotype
Crossing with CMS A-line

**Confirmation of TGMS System**
Raising the plants
Tagging the sterile lines after observing the flower
Observing the lines for pollen fertility and correlating with meteorological data

**Stabilization of TGMS lines**
Raising the selfed progenies(plant to row) of identified lines
Observing for expression of pollen sterility
Roguing of fertile plants
Observing and tagging of lines expressing pollen fertility
Collection of selfed seeds from single plants

## EXECUTIVE SUMAMRY

For confirmation of apomixis, three apomictic lines viz. IS-181-7-1, IS-244-4-1 and IS-244- 4-2 were used at Nagpur and Nanded using four methods: Emasculation and selfing, removal of style and stigma, crossing with dominant marker and crossing with CMS A line. Boll setting could be observed in all the three lines at both locations in the first two methods i.e. emasculation and selfing as well as removal of style and stigma. F1s obtained in the cross between apomictic line and ems line showed very low level of pollen fertility, and the F2 seeds got after selfing have been sown in this season for further screening. Regarding Thermosensitive genetic male sterility system in diploids (G. arboreum), five lines have been stabilized after raising boll to row progenies and observing for

flower fertility/sterility. The critical fertility temperature has been found to be around 18°C based on correlation of minimum temperature with percentage boll set. TGMS in tetraploids was studied at Mudhol and Nagpur, which revealed 40°C as critical sterility temperature. The inheritance study done in these showed the trait to be monogenic recessive and segregated in 3:1 ratio.

## SALIENT FINDINGS

### Confirmation of Apomictic phenomenon in Tetraploids

**Emasculation and selfing :**

Three apomictic lines IS-181-7 -1, IS-244-4-1 and IS-244-

4-2 was sown for confirmation of apomictic phenomenon. Two bolls were set out of

75 buds emasculated at Nagpur (Fig-1 a) and 4 out of 400 buds at Nanded. But no seed were obtained in these bolls (Table 1).

**Removal of style and stigma:**

In the three apomictic lines 120 buds were used for removal of style and stigma and boll setting was found in only three buds (Fig-1 b) at Nagpur and 2 out of 50 at Nanded.

Fig. 1a : **Emasculation and Selling (EMS)**

**Fig. 1b : Removal of Style and Stigma (RSS)**

**Crossing with dominant marker:**

At Nagpur, four apomictic lines namely NHH 01, NAP 5, NAP 7, and NAP 10 were crossed with a dominant marker B. Narma (Red) (Table 2). Boll to row progenies will be grown during 2008 for confirmation of apomictic nature of lines At Nanded, dominant marker genes viz., plants with pigmented staminal column and plants with okra leaf type were used for crossing with the introgressed genotypes (Table 3)

**Crossing with CMS A -line**

At Nagpur, ratoons of following F1's obtained from the cross between male sterile x Apomictic lines cross is being maintained in the species garden:

1. JCMS2xBulkAP5-2
2. C117AxAP 3-4
3. IAN 579 xAP 3-4
4. B 59-1684 x AP 2-8
5. B59 xAP4-15
6. B59xAP1-4
7. MCU10xAP1-4
8. Buri Nectariless xAP 2-4
9. H777 x BulkAP4-4
10. PKV081 xBulk4-4
11. Vishnu (G cot1 00) x AP2-8
12. DWRA-1 xAP2-1
13. NCM4-34x181/7/1
14. IC 244 x 4/2/2

Some selfed collected. Crossed with pigmented genotype BN Red and few seeds have been obtained. At Nanded, the introgressed lines IS 244-4-2, IS-244-4-3 and IS-181-7-1 using as a male parent were crossed with CMS 'A' line and selfing was done. Twenty crossed bolls were obtained. Boll to progeny will be grown during ensuing season for further study

Table-1 Confirmation of Apomixis using Emasculation and Selfing(EMS) and Removal of Style and stigma(RSS) methods.

| Sr.No. | Entry | EMS | RSS | Bolls Set |
|---|---|---|---|---|
| 1 | IS 244-7-1 | 1 | | 1 |
| 2 | IS 244-4-2 | 1 | 1 | 2 |
| 3 | 4/1 bulk | | 1 | 1 |
| 4 | 4/2 | 1 | 1 | 2 |
| 5 | IS-181-7 -1 | 1 | 2 | 3 |
| 6 | IS-244-4-1 | 1 | | 1 |
| 7 | IS-244-4-2 | | 1 | 1 |
| 8 | BG-4424-P1 | 1 | | 1 |
| 9 | BG-4424-P2 | | 2 | 2 |
| 10 | 7/1 P1 | 2 | 4 | 6 |
| 11 | 1044 | 1 | 3 | 5 |
| 12 | 4/2 abm | | 1 | 1 |
| 13 | 1060 | | 1 | 1 |
| 14 | NAP-10 | 5 | 4 | 9 |
| 15 | Bt NHH-01 | 2 | 1 | 3 |
| 16 | NAP-5 | | 3 | 3 |
| 17 | NAP-7 | 1 | 1 | 2 |

Table 2 : Bolls Set in Apomictic Genotypes Crossed with B.Nerma Red

| Sr.No. | Apomictic Genotypes | Bolls Set |
|---|---|---|
| 1 | Bt-NHH-01 | 4 |
| 2 | NAP-5 | 6 |
| 3 | NAP_7 | 5 |
| 4 | NAP-10 | 6 |

Table 3 : Bolls Set in Apomictic Genotypes Crossed with other dominant markers

| Sr. No. | Line | No. of crossed bolls obtained | |
|---|---|---|---|
| | | Pigmented staminal column | Okra leaf |
| 1 | 15-244-4-2 | 59 | 6 |
| 2 | 15-244-4-3 | 137 | 24 |
| 3 | 15-181-7-1 | 82 | 10 |
| | **Total** | **278** | **85** |

**TGMS (Diploid)**

**To stabilize the TGMS system and correlate with meteorological data.**

Plants raised from boll to row were thoroughly monitored for alterations in fertility/ sterility and it was found that these were stable with respect to TGMS trait.. Two stable lines i.e 1-1 & 9-1were subjected to daily flower observations starting from the month of September i.e with the onset of flowering. The percentages of partial fertile/ fertile flowers were estimated. In addition to that the flowers were also selfed and selfed boll set % was calculated (Table: 4).

It was observed that percentage of fertile flowers was zero during the month of September and the percentage boll set was also zero. During October maximum percentage of fertile flowers along with high boll set percentage was observed in both the lines. This could be well correlated with the decrease in mean minimum temperature to 18°c (Fig: 2).

**Fig 2: Alteration of anthers from sterility to fertility in diploid TGMS lines**

In the subsequent months, in spite of high percentage of flower fertility, the boll set percentage was very less probably due to the variations in fertility levels ranging from very low amount of fertile pollen to high amount of fertile pollen in the fertile flowers (Fig: 3)

Low fertile Mediumfertile CompletelyFertile

**Fig 3 : Changes in fertility levels**

Table 4 : Correlation of temperature and pollen fertility Line No: 1-1

| Month | Temperatures (Maxi, Min) | Sterile | Partial fertile | Fertile | % of F & PF | % of boll set |
|---|---|---|---|---|---|---|
| Sept | 30°c / 22.4°c | 25 | 0 | 0 | 0% | 0 |
| Oct | 32° c / 18°c | 0 | 20 | 19 | 51%PF,48%F | 43.6 |
| Nov | 30.6°c / 15°c | 4 | 51 | 11 | 77% PF,16% F | 10 |
| Dec | 29.32°c / 13.15°c | 0 | 53 | 0 | 100% PF | 6 |
| Jan | 29.4rc / 12.9r c | 0 | 30 | 0 | 100% PF | 8 |
| Feb | 30.6°c / 14.4°c | 0 | 19 | 8 | 67%PF,29%F | 16 |
| March | 35.rc / 20.1 °c | 7 | 32 | 3 | 76%PF, 7% F | 20 |
| April | 41.3°c /21.0°c | 10 | 0 | 0 | | 0 |

**Understanding the TGMS phenomenon**

Inheritance studies: Crosses between EGMS and fertile lines

Three individual plants from five stable lines viz. 1-1, 8-1, 9-1, 13-1, 15-1 were crossed with commercial arboreum variety PA255 as well as performed reciprocal crosses for further studies on inheritance and molecular tagging of gene. These F1s along with parents have been planted in off season for raising F2's and BCl1 s for inheritance studies as well as molecular tagging

**To study the stability of EGMS line of G. *hirsutum.***

At ANGRAU, Mudhol, three hundred EGMS lines were sown during Summer 2007 and their stability for expression of sterility was studied and 250 single plants expressing male sterility were tagged and continued upto kharif 2007 and their reversibility to fertility was also studied. All the 250 plants reverted back to fertility during kharif period of 2007. The same plants were pruned and irrigated during January 2008 and fresh growth and flowering was obtained during Summer of 2008. Expression of sterility was again recorded in April, 2008. All the 250 plants reverted back to male sterility during April, 2008. (Fig-4).

The EGMS plants were crossed with two normal male fertile lines i.e. DHY 286 and 382266 during kharif 2007. The $F_1$ of these two crosses was raised during rabi 2007 and $F_2$ seed was

**Fig: 4 : Sterile and fertile flowers in TGMS tetraploid**

obtained. During summer 2008 parents of above two crosses along with $F_1$ & $F_2$ population (35 $F_2$ plants per each cross) were raised and expression of sterility was recorded.

Inheritance studies indicated that DHY 286, 382266 and $F_1$ of two crosses (EGMS X DHY 286 and EGMS X 382266) were male fertile while EGMS line was male sterile. $F_2$ population of two crosses segregated in 3 fertile: 1 sterile ratio (Table 5 ) indicating that the male sterility is being controlled by single recessive gene.

Table 5: Inheritance studies of EGMS gene in cotton *(G. hirsutum)*

| | Fertile plants | | Sterile plants | |
|---|---|---|---|---|
| | EGMS X DHY 286 | EGMS X 382266 | EGMS X DHY 286 | EGMS X 382266 |
| Observed value | 253 | 250 | 82 | 103 |
| Expected value | 251 | 264 | 84 | 88 |

(EGMSXDHY286) $X^2 1, .05 = \frac{(253-251)2}{251} \quad \frac{(82-84)2}{84} = 0.064$

(EGMS X382266) $= t1, .05 = \frac{(250 - 264)2}{264} + \frac{(103 - 88)2}{88} = 0.91$

$X^2 1, .05$ tabulated value = 3.84.

As $X^2 1, .05$ calculated values of both crosses (0.064 for the cross EGMS X DHY 286 and 0.91 for the cross EGMS X 382266) are smaller than X2 1.05 tabulated value (3.84) the data fits in ratio of 3:1 clearly indicating that the male sterility is controlled by single recessive gene.

Five EGMS lines were sent to CICR, Nagpur for testing the expression of male sterility.

Expression of male sterility during Summer 2008 was also recorded at CICR, Nagpur both at field level and lab testing. At Nagpur, boll to row progenies were raised for these lines. The plants which show fertility were tagged and others removed. Selfed seeds (Boll wise) were collected from selected labeled plants. The parent plants were further continued during summer as rattoons and their reversion to sterility was confirmed (Fig-5).

Four Tetraploid EGMS lines obtained from ANGRAU, ARS station, Mudhol :

EGMS-H - 2-8-4-3

EGMS-H-5-7 -3-5

EGMS-H-3-7 -5-8

EGMS-H-5-12-8-6

Sterility at Nagpur was observed at above 40°C temperatures. When fertile, selfing was done and selfed seeds collected. Screening for summer fertility will be done.

Boll

4n TGMS Plant

**Fig 5 : TGMS lines of Tetraploid Cotton grown at CICR, Nagpur**