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# Cytogenetic Investigations of Triploid and Hexaploid Derivative Involving *Gossypium hirsutum* Var. MCU 12 and *Gossypium arboreum* Var. PA 255

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**Abstract:** An experiment was designed to get novel biotic and abiotic stress tolerant derivatives involving tetraploid and diploid cottons. The interspecific derivatives of *Gossypium hirsutum* and *Gossypium arboreum* were triploid and sterile. The triploid was then doubled with colchicine to get hexaploid. Morphological interpretation revealed that leaf lobe, length of calyx tube, petal eyespot, poor pollen fertility and abortive ovarian system were found to be dominant in the triploid. The doubled triploid possesses leaves with larger veins, larger epicalyx, larger petals and shriveled anthers and reduced pollen fertility as compared to triploid and parents. The normal meiotic behaviour was witnessed among parents while abnormal meiotic system as evidenced from the formation of trivalents and quadrivalents was noticed among hexaploid. Abnormal sporads also recorded among triploid and hexaploid leading to formation of sterile pollen grain.

**Key words:** *Gossypium hirsutum, Gossypium arboreum*, triploid, hexaploid

## INTRODUCTION

Cotton is an important crop, which provides raw material in the form of lint to the textile industry. The contribution of cotton to export income is to the tune of Rs. 36000 crores[1]. The loss in lint and seed yield because of narrow genetic base and repeated use of genetically related material in breeding increased vulnerability to new strains of pathogens and pests. The genus has got rich genetic diversity. Deficiencies in crop cultigens for desirable characters such as resistance to pests and diseases, male sterility etc. and then availability in distant relatives are now well documented. Polyploid formation has played a major role in the evolution of many plant and animal genomes. Because a large number of plant lineages include polyploid species, the evolution of plant polyploidy may have had major effects on the interaction structure of terrestrial communities[2]. Although triploid bridge, alone, may not account for the evolution of autotetraploidy, it probably contributes to the prevalence of mixed-ploidy populations and therefore, in contrast to hybrids in homoploid species, triploids may actually facilitate rather than diminish the fixation of tetraploids by enhancing the rate of formation[3]. This has evolved keen interest in introgessive breeding for affecting the transfer of desirable genes from widely distant species into cultivated species.

## MATERIALS AND METHODS

The experiment was conducted during 2002-2004 at Tamil Nadu Agricultural University, Coimbatore. *Gossypium arboreum* used as a male parent in crossing with *Gossypium hirsutum* to evolve the triploids. The triploid being sterile was then doubled with colchicines to get hexaploid. The triploid and hexaploid obtained were studied for traits of major morphological significance. For cytological investigation, young flower buds of these plants and their respective parents were fixed in Carnoy's fluid (6:3:1) and squashed in 1% aceto-carmine. The cytological analysis of chromosome behaviour in PMCs was made from temporary mounts[4]. Pollen sterility was tested with differential stain.

## RESULTS AND DISCUSSION

Significant differences in leaf lobe, leaf shape, calyx tube, petal eyespot, pollen fertility and nature of ovary were noticed among parents (diploids and tetraploids), triploids and colchicine induced hexaploids (Table 1).

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Triploids differed from their parents in habit, leaf texture, leaf shape, bract shape, bract size, length of staminal column and pollen fertility. Plant habit, plant height and monopodia production were some of the traits that triploids resembled the tetraploid parent. Larger leaves, flowers, enlarged size of petals, petal spot, longer staminal column, fertile anthers and increased pollen fertility than triploids were the distinguishing traits for colchicine-induced hexaploids (Table 1).

In all the pollen mother cell studied, the course of meiosis was normal and regular. There were 26 and 13 bivalents respective in MCU 12 and PA 255 at metaphase I of meiosis as reported by Skovsted[5]. During anaphase I, the chromosome separation was equal and normal and tetrad analysis also showed normal behaviour (Table 2).

The cross, MCU 12 x PA 255 showed 2n=39 chromosomes in meiosis. At metaphase I, the formation of univalents, trivalents and quadrivalents along with bivalents was noticed. The mean chromosome association was $1.61_{IV} + 2.83_{III} + 7.39_{II} + 9.28_{I}$ with a maximum and minimum association of $3_{IV}+5_{III}+4_{II}+4_{I}$ and $2_{III}+11_{II}+11_{I}$, respectively. The bivalents ranged from 4 to 11 at metaphase I of meiosis in MCU 12 x PA 255. The anaphase I analysis revealed the unequal and irregular chromosome distribution to opposite poles as 18+18+3 and 22+14+3 along with laggards in MCU 12 x PA 255. Normal tetrad formation was also influenced by the formations of diad, triad and pentad formation (Table 3). Similar reports were also made by Deshpande *et al.*[6].

The studies on meiotic behaviour in Col. (MCU 12 x PA 255) revealed the abnormal and irregular chromosome behaviour with formation of varied number of bivalents ranging from 11 to 20 along with univalents, trivalents and quadrivalents. The maximum and minimum chromosomal association was recorded as $6_{IV} + 7_{III} + 11_{II} + 11$ and $1_{IV} + 8_{III} + 20_{II} + 10_{I}$, respectively with mean chromosomal association of $3.21_{IV} + 5.79_{III} + 15.36_{II} + 17.07_{I}$ at metaphase I of meiosis (Table 2-4). The findings of the present study agree with the reports of Niyazov and Ruban[7] and Narayanan[8]. Deshpande *et al.*[6] reported that allotetraploids produced by doubling the chromosome number in $F_1$ hybrid of *Gossypium arboreum* x *Gossypium hirsutum* were ordinarily male sterile but rarely flowers had viable pollen. The occasional occurrence of trivalent and quadrivalents in hexaploids appeared chain like indicating short segment involved in heterozygote

Table 1: Comparative morphological characters of *Gossypium arboreum, Gossypium hirsutum, Gossypium hirsutum* x *Gossypium arboreum* and *Col. (Gossypium hirsutum* x *Gossypium arboreum)*

| Characters | Tetraploid (MCU 12) | Diploid (PA 255) | Triploid (MCU 12 x PA 255) | Hexaploid Col. (MCU 12 x PA 255) |
|---|---|---|---|---|
| Plant height (cm) | 80 | 136 | 96 | 122 |
| No. of monopodia | 1-2 | 2-3 | 1-2 | 3-4 |
| First sympoda (node No.) | 3-4 | 3-4 | 2-3 | 4-5 |
| Leaf | 3-1 lobed | 5 lobed | 3-4 lobed | 2-3 lobed |
| Calyx tube length (cm) | 0.80 | 0.90 | 0.96 | 1.1 |
| Corolla | Creamy, no eyespot | Yellow petal, dark red eyespot | Yellow, light red eyespot | Creamy colour with no eyespot |
| Pollen colour | Creamy yellow | Dark yellow | Dark yellow | Light yellow |
| Pollen fertility (%) | 94.23 | 92.41 | 28.4 | 52.26 |
| Tetrad (%) | 92 | 95 | 62 | 58 |
| Ovary | 4 locules | 4 locules | Abortive | Abortive |

Table 2: Tripolar separation of chromosome during anaphase I in cross involving MCU 12 and PA 255

| | No. of PMC's | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Entries groups | 26+26 | 13+13 | 18+18+3 | 22+14+3 | 20+20+5 | 22+20+3 | 40+30+8 | 42+32+4 |
| MCU 12 | 10 | - | - | - | - | - | - | -- |
| PA 255 | - | 8 | - | - | - | - | - | - |
| MCU 12 x PA 255 | - | - | 2 | 1 | - | - | - | - |
| (MCU 12 x PA 255) x MCU 12 | - | - | - | - | 4 | 2 | - | - |
| Col. (MCU 12 x PA 255) | - | - | - | - | - | - | 2 | 2 |

Table 3: Status of abnormal sporads at telophase I in cross involving MCU 12 and PA 255

| | No. of PMC's | | | | |
|---|---|---|---|---|---|
| Abnormal sporads | Dyad | Triad | Tetrad | Pentad | Polyad |
| MCU 12 | - | - | 13 | - | - |
| PA 255 | - | - | 12 | - | - |
| MCU 12 x PA 255 | 8 | 4 | 6 | 3 | - |
| (MCU 12 x PA 255)x MCU 12 | 4 | 1 | 7 | 3 | - |
| Col. (MCU 12 x PA 255) | 3 | 2 | 10 | 1 | 1 |

Table 4: Details of chromosomal association in crosses involving MCU 12 and PA 255

| | Chromosomal association | | | | No. of Pollen Mother Cells (PMC's) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| S. No. | IV | III | II | I | MCU 12 x PA 255 | (MCU 12 x PA 255)x MCU 5 | Col. (MCU 12 x PA 255) | PA 255 | MCU 12 |
| 1 | - | 4 | 10 | 7 | 2 | - | - | - | - |
| 2 | 1 | 2 | 8 | 13 | 4 | - | - | - | - |
| 3 | 3 | 5 | 4 | 4 | 1 | - | - | - | - |
| 4 | - | 2 | 11 | 11 | 3 | - | - | - | - |
| 5 | 3 | 3 | 4 | 10 | 6 | - | - | - | - |
| 6 | 2 | 3 | 10 | 2 | 2 | - | - | - | - |
| 7 | 4 | 3 | 8 | 4 | - | 1 | - | - | - |
| 8 | 2 | 5 | 10 | 2 | - | 3 | - | - | - |
| 9 | - | 7 | 10 | 4 | - | 2 | - | - | - |
| 10 | 1 | 4 | 6 | 17 | - | 4 | - | - | - |
| 11 | 2 | 8 | 4 | 5 | - | 1 | - | - | - |
| 12 | 1 | 5 | 11 | 4 | - | 5 | - | - | - |
| 13 | 6 | 7 | 11 | 11 | - | - | 3 | - | - |
| 14 | 4 | 6 | 18 | 8 | - | - | 1 | - | - |
| 15 | 1 | 8 | 20 | 10 | - | - | 4 | - | - |
| 16 | 2 | 5 | 12 | 31 | - | - | 2 | - | - |
| 17 | 4 | 2 | 16 | 24 | - | - | 3 | - | - |
| 18 | 3 | 6 | 12 | 24 | - | - | 1 | - | - |
| - | - | - | 13 | - | - | - | - | 12 | - |
| - | - | - | 26 | - | - | - | - | - | 10 |
| Total PMC's | | | | | 18 | 16 | 14 | 12 | 10 |

Mean association of: MCU 12 x PA 255 $- 1.61_{IV} + 2.83_{III} + 7.39_{II} + 9.28_{I}$; (MCU 12 x PA 255) x MCU 12 $- 1.31I_{V} + 5.06_{III} + 8.81_{II} + 6.94_{I}$; Col. (MCU 12 x PA 255) $- 3.21_{IV} + 5.79_{III} + 15.36_{II} + 17.07_{I}$

translocation affecting a pair of chromosomes. In the above hexaploids, occurrence of bivalents indicates the allosyndetic pairing between the chromosomes of the parental species[9].

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