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# Development and hybridity confirmation of $F_1$ interspecific hybrids between *Gossypium barbadense* and *Gossypium anomalum*

S. IMTIYAZAHMED, L. MAHALINGAM*, N. PREMALATHA, K. SENGUTTUVAN, V.P. SOBHAKUMARI AND M. KUMAR

[1]Department of Cotton, Tamil Nadu Agricultural University, Coimbatore-641003
**Email: rmdmahal@yahoo.co.in*

**ABSTRACT :** The interspecific triploid hybrid was developed between tetraploid cultivated species *Gossypium barbadense* cv. Suvin and TCB 37 and diploid wild species *Gossypium anomalum*. The $F_1$ hybridity was confirmed by morphological, cytological and molecular approaches. The ploidy level of interspecific $F_1$ hybrid was triploid and male sterile. The female parents *viz.*, Suvin and TCB 37 had erect growth habit, green with brown colour stem, sparsely pubescent nature of stem, dark green colour leaves, bright yellow colour petal, petals with dark red petal spot and creamy anther. The $F_1$ interspecific hybrids of Suvin x *Gossypiumanomalumand* TCB 37 x *Gossypium anomalum* exhibited perennial, shrub growth habit, pale brownish green colourstem, leaves with strongly pubescent nature, velvety nature of leaves texture, dull violet colour and protruded stigma. The male parent Gossypium anomalum perennial and shrub growth habit, pale brownish green coloured stem,leaves with strongly pubescent nature, velvety nature of leaves texture, dull violet colour and protruded stigma which were resembled with both $F_1$ interspecific hybrids. The female parents *viz.*, Suvin and TCB 37 showed 52 chromosomes at mitotic metaphase stage. 26 chromosomes was observed in male parent *Gossypium anomalum* whereas the $F_1$ interspecific triploid hybrids exhibited 39 chromosomes and the hybridity status was confirmed. Significant differences were observed between pollen size, fertility of the parents and their $F_1$ hybrids. The pollen fertility ranged from 1.67 to 2.13 per cent in the $F_1$ interspecific triploid sterile hybrids. This hybridity may serve as a useful genetic resource for transfer of leaf hopper resistance to Egyptian cotton.

**Key words:** Gossypium, triploid, wide hybridization

The genus *Gossypium* belongs to the family *Malvaceae* and contains more than 45 diploid species and five well documented allotetraploid species. The genetic diversity of genus *Gossypium* sppis exclusively wide with diverse geographical and ecological niches (Fryxell, 1992). The *Gossypium* species are grouped into nine genome groups and designated as AD, A, B, C. D, E, F, G and K based on the similarities in chromosome size, structure and success of chromosomal pairing (Wendel, 1989; Percival *et al.*, 1999). The diploid D genome species of the New World include 26 chromosomes based on the chromosomal uniformity. Some hybrids within the genome are fertile due to chromosome recombination during meiosis. However, hybrids across genomes are generally infertile and have a few bivalents at meiosis as a result, progenies survival from the interspecific crosses is sometime low. The allotetraploid cotton *Gossypium hirsutum* ($AD_1$) and *Gossypium barbadense* ($AD_2$) of the New World cotton dominate natural fibre production. Wild *Gossypium* species represent a significant genetic repository for potential exploitation by cotton breeders who have long recognized the beneficial effect of exotic genes (Heitholt and Mauney, 2010). The introduction of alien genetic variation into upland cotton from the chromosome of the wild species is a valuable and proven technique for cotton improvement. The most successful examples of the use of wild species during the history of cotton breeding include *Gossypium harknessii* as a source of cytoplasmic male sterility (Meyer, 1975) and *Gossypium thurberi* as a source of fibre quality (Culp and Harrell, 1973; Culp *et al.*, 1979). More

recently, the other important traits such as nematode resistance and low gossypol plant traits were successfully introduced from diploid species into upland cotton using various strategies (Sacks and Robinson, 2009; Benbouza *et al.*, 2010).

## MATERIALS AND METHODS

The crossing block has been raised during summer 2019 comprises of two female *Gossypium barbadense* parents *viz.*, Suvin and TCB 37 and the male parent wild species [1]*Gossypium anomalum* (Plate 1) was maintained in cotton wild species garden established in the Departtment of Cotton, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University Coimbatore. Crosses were effected by using Doak's method (Doak, 1934) of hand emasculation and pollination and crossed bolls were obtained and collected for further evaluation. The two $F_1$ hybrids along with their parents *viz.*, Suvin and TCB 37 were raised in during winter 2019. The male parent *Gossypium anomalum* is being maintained in cotton wild species garden. $F_1$ hybrids along with parents was used for recording data on various morphological, biometrical and cytologicalanalysis (Plate 2).

## MORPHOLOGICAL STUDY

Nineteen (19) morphological characters *viz.*, plant growth habit, stem colour, stem pubescence, petiole colour, leaf shape, leaf colour, leaf incision, leaf veins, leaf texture, leaf hairiness, bract size, corolla colour, petal size, petal spot, anther colour, anther density, filament colour, position of stigma and nectar glands and 14 biometrical traits namely, bracterial teeth, bracterial length, bracterial breadth, petiole length, leaf length, leaf breadth, leaf area, pedicel length, petal length, petal breadth, pollen size (dia.), pollen fertility (%),gossypol gland density and length of pistil were studied to discriminate the parents and $F_1$ hybrids. Fourth fully matured and expanded leaves from the top of the plant were taken and their maximum length and breadth was recorded. Leaf area was measured from 5 fully expanded, matured leaves of both parents and $F_1$ hybrids using Leaf Area Meter and an average used for recording observations. Flowers were collected on the day of anthesis between 10.00 am to 11am for pollen fertility study. Pollen fertility was recorded by dusting pollen grains in Potassium Iodide solution (1%) and viewed under a compound microscope. Only large, darkly stained and circular pollen grains were considered as fertile. In both parents and $F_1$ hybrids. Four microscopic fields were taken to find out the pollen fertility percentage and averaged for further evaluation.

## CYTOLOGICAL STUDY

In cytological analysis, mitotic metaphase chromosome study was carried out by using root tips to confirm the ploidy level of $F_1$ hybrids and their parents. Seeds of parents and their $F_1$ were soaked overnight and then germinated in the germination paper. The roots were collected from the germinated seeds with 2-3 cm length in quick succession between 9 to 10 am on bright sunny days and pre-treated with Para-Dichloro Benzene to accumulate metaphase cells. After 2 hours the pre-treated root tips were washed thoroughly in running tap water to remove excess Para-Dichloro Benzene and fixed in the Ethanol: Glacial Acetic Acid (3:1) fixative. After keeping the fixed material under low temperature (4°C) for a minimum period of four hours, the roots were thoroughly washed in the distilled water and stored in Ethanol 70%. The roots were hydrolysed at 60°C for 8 minutes and washed thoroughly in running tap water and then the root tips are treated in a (0.25%) Pectinase solution for 30 minutes in dark and put it in basic Fuchsin stain for 30 minutes in dark. The darkly

stained extreme tip portion of the roots were excised out and macerated in a drop of aceto-carmine (1%). After maceration the slide covered with cover slip and heated gently over a sprit lamp. The excess stain was removed by giving gentle press with thumb between two layers of filter paper. The slide was temporarily sealed using wax and observed under the Olympus system microscope @ 1000X magnification. The chromosomes were counted from the metaphase cells and recorded pictorially.

## RESULTS AND DISCUSSION

Nineteen (19) qualitative parameters were recorded in Suvin, TCB 37, *Gossypium anomalum* and their corresponding $F_1$ hybrids of Suvin x *Gossypium anomalum* and TCB 37 x *Gossypium anomalumin* (Table 1 and 2). Out of 19 qualitative characters two characters *viz.*, leaf incision and corolla colour were showed intermediate expression between both the parents in the $F_1$ hybrids. These results are in agreement with (Kaur *et al.*, 2016) in $F_1$ hybrid of *Gossypium hirsutum* cv., 1861 x *Gossypium armourianum* for petal colour whereas (Manickam and Prakash, 2014) had reported intermediate leaf and flower morphology in *Gossypium hirsutum* x *Gossypium armourianum* hybrid. Tahir and Noor (2011) reported that intermediate expression of petal colour in *Gossypium arboreum* x *Gossypium hirsutum* hybrid and its reciprocal cross. $F_1$ hybrid of *Gossypium arboreum* x *Gossypium thurberi* exhibited dominance was reported for petal colour as like that of female parent (Kale *et al.*, 2007) and dominance expression for anther colour was observed in the $F_1$ hybrid of *Gossypium herbaceum* x *Gossypium raimondii* (Wu *et al.*, 2017). Dominance expression were observed in both $F_1$ hybrids *viz.*, Suvin x *Gossypium anomalum* and TCB 37 x *Gossypium anomalum* for growth habit (Plate 3.), stem colour, stem pubescence, leaf texture and leaf hairiness and its resembled with male parent *Gossypium anomalum.* This result are in accordance with the work of Kaur *et al.*, (2016) which showed dominance for leaf texture and leaf hairiness, similar to its male parent *Gossypium anomalum* and also reported dominant characters for stem colour, stem pubescence and leaf hairiness of *Gossypium hirsutum* cv., 1861 x *Gossypium armourianum* $F_1$ hybrid as it fully resembled *Gossypium armourianum*. Also *Gossypium herbaceum* x *Gossypium* austral hybrid showed dominance for leaf hairiness and stem hairiness as that of male parent. Saravanan *et al.*, (2007) observed dominant expression for leaf texture, petiole colour and leaf hairiness in *Gossypium hirsutum* x *Gossypium raimondii* cross as that of pollen parent. Leaf characters *viz.*, leaf colour and leaf veins in both $F_1$ hybrids expressed dominance and identical to their female parents, whereas leaf shape was dominant as that of its male parent with intermediate leaf size. Kaur *et al.*, (2016) and Wu *et al.*, (2017) have reported intermediate leaf shape in their respective $F_1$ hybrids of *Gossypium hirsutum* cv., 1861 x *Gossypium armourianum* and *Gossypium herbaceum* x *Gossypiu mraimondii*, respectively. The $F_1$ hybrids (Suvin x *Gossypium anomalum* and TCB 37 x *Gossypium anomalum*) had dominant characteristics for bract size which was resembled with maternal parents (Plate 2). These are in accordance with bract size of *Gossypium herbaceum* x *Gossypiu mraimondii* hybrid which seems as female parent reported by (Wu *et al.*, 2017). $F_1$ hybrids of Suvin x *Gossypium anomalum* as well as TCB 37 x *Gossypium anomalum* recorded intermediate expression for corolla colour and this results in accordance with the results of Kaur *et al.*, (2016) and they have reported intermediate expression for corolla colour in the $F_1$ hybrid of *Gossypium hirsutum* cv., 1861 x *Gossypium armourianum*. Wu *et al.*, (2017) have reported $F_1$ hybrid of *Gossypium herbaceum* x *Gossypium raimondii* exhibited dominance for petal colour and identical to its maternal parent. Petal

**Table 1.** Morphological traits of Suvin x *G. anomalum* hybrid and the parents

| S. No | Characters | Suvin | Suvin x *G.anomalumm* | *G.anomalum* |
|---|---|---|---|---|
| 1 | Growth habit | Annual, erect | Perennial, shrub | Perennial, shrub |
| 2 | Stem colour | Dark green with brown | Pale brownish green | Pale brownish green |
| 3 | Stem pubescence | Sparsely pubescent | Highly pubescent | Highly pubescent |
| 4 | Petiole colour | Greenish brown | Brownish green | Brownish green |
| 5 | Leaf shape | Palmate with 5 lobes | Palmate with 5 lobes | Palmate with 5 lobes |
| 6 | Leaf colour | Dark green | Green | Green |
| 7 | Leaf incision | Deep | Medium to deep | Medium |
| 8 | Leaf veins | Thick and prominent | Thick and prominent | Thin |
| 9 | Leaf texture | Medium smooth and thick | Velvety | Velvety |
| 10 | Leaf hairiness | Very sparse | Strongly pubescent | Strongly pubescent |
| 11 | Bract size | Large | Medium | Small |
| 12 | Corolla colour | Bright yellow | Dull violet | Dull violet |
| 13 | Petal size | Medium | Medium | Medium |
| 14 | Petal spot | Dark red | Light to dark red | Light to dark red |
| 15 | Anther colour | Creamy | Creamy | Creamy white |
| 16 | Anther density | Dense | Dense | Dense |
| 17 | Filament colour | White to creamy white | White to creamy white | White to creamy white |
| 18 | Position of stigma | Protruded | Protruded | Embedded |
| 19 | Nectar gland | Present | Absent | Absent |

**Table 2.** Morphological traits of TCB 37 x *G. anomalum* hybrid and the parents

| S. No. | Characters | TCB 37 | TCB 37 x *G.anomalum* | *G.anomalum* |
|---|---|---|---|---|
| 1 | Growth habit | Annual, erect | Perennial, shrub | Perennial, shrub |
| 2 | Stem colour | Dark green with brown | Pale brownish green | Pale brownish green |
| 3 | Stem pubescence | Sparsely pubescent | Highly pubescent | Highly pubescent |
| 4 | Petiole colour | Greenish brown | Brownish green | Brownish green |
| 5 | Leaf shape | Palmate with 5 lobes | Palmate with 5 lobes | Palmate with 5 lobes |
| 6 | Leaf colour | Dark green | Green | Green |
| 7 | Leaf incision | Deep | Medium to deep | Medium to deep |
| 8 | Leaf veins | Thick and prominent | Thick and prominent | Thin |
| 9 | Leaf texture | Medium smooth and thick | Velvety | Velvety |
| 10 | Leaf hairiness | Very sparse | Strongly pubescent | Strongly pubescent |
| 11 | Bract size | Large | Medium | Small |
| 12 | Corolla colour | Bright yellow | Dull violet | Dull violet |
| 13 | Petal size | Medium | Medium | Medium |
| 14 | Petal spot | Dark red | Dark red | Dark red |
| 15 | Anther colour | Creamy | Creamy | Creamy white |
| 16 | Anther density | Medium | Dense | Dense |
| 17 | Filament colour | White to creamy white | White to creamy white | White to creamy white |
| 18 | Position of stigma | Protruded | Protruded | Embedded |
| 19 | Nectar gland | Present | Absent | Absent |

**Table 3.** Biometrical traits of Suvin x *G.anomalumhybrid* and the parents

| S. No | Characters | Suvin | Suvin x *G.anomalum* | *G.anomalum* |
|---|---|---|---|---|
| 1. | Number of bracterial teeth | 9.00** | 6.00** | 3.00** |
| 2. | Bracterial length (cm) | 4.70b | 3.73b | 1.67a |
| 3. | Bracterial breadth (cm) | 2.17b | 2.02b | 0.53a |
| 4. | Petiole length (cm) | 7.60** | 6.52** | 4.22** |
| 5. | Leaf length (cm) | 8.97** | 8.10** | 5.66** |
| 6. | Leaf breadth (cm) | 10.77** | 9.34** | 5.20** |
| 7. | Leaf area (cm2) | 62.87** | 43.67** | 16.32** |
| 8. | Pedicel length (cm) | 2.80a | 1.25a | 0.57a |
| 9. | Petal length (cm) | 5.70b | 4.22b | 3.36a |
| 10. | Petal breadth (cm) | 4.90** | 4.03** | 3.43** |
| 11. | Pollen size diameter (µm) | 40.87** | 25.23** | 32.16** |
| 12. | Pollen fertility (%) | 96.67a | 2.13b | 95.30a |
| 13. | Length of pistil (cm) | 2.73 ** | 2.52** | 1.76** |
| 14. | Gossypol gland density | 12.33** | 8.60** | 9.75** |

** Significant difference at P<0.01 using Duncan's Multiple Range Test
The letters with same alphabet are considered as non-significant.

spot is present in both *Gossypium barbadense* and *Gossypium anomalum* at base of the petals which is dark red but the spot is slightly larger in male parent. $F_1$ hybrids of both the crosses were dominantly expressed with dark red spot at base of the petals which resembles more like *Gossypium anomalum* (Plate 4). No petal spot variation in intensity was not noticed as that of hybrids from *Gossypium armourianum*. The anther colour of both $F_1$ hybrids was more likely towards its corresponding maternal parents Suvin and TCB 37 (Plate 4). These results agree with works of Wu *et al.*, (2017). The anther colour of *Gossypium herbaceum* x *Gossypium raimondii* hybrid (Wu *et al.*, 2017), *Gossypium herbaceum* x *Gossypium australe* hybrid (Liu *et al.*, 2015) resembled maternal parent and *Gossypium hirsutum* x *Gossypium armourianum* hybrid (Kaur *et al.*, 2016) anther colour seems like that of its paternal parent. The position of stigma resembled maternal parent with protruded stigma were male parent had embedded stigma and in case of nectar gland and it resembled male parent with absence of nectar glands but the female parent had nectar glands. These results were not in accordance with (Kaur *et al.*, 2016) and (Wu *et al.*, 2017), respectively.

Bracterial traits *viz.*, number of bracterial teeth, bracterial length and bracterial breadth were observed (Table 3,4 and Plate 4). In which, all the characters showed intermediate expression in both $F_1$ hybrids. There was highly significant difference observed between the parents and between parents and $F_1$ hybrids. But no significant differences were observed between male parent and $F_1$ hybrids and between parents, while significant differences were observed between female parents and respective $F_1$ hybrids. The intermediate expression was noticed in interspecific $F_1$ hybrids Suvin x *Gossypium anomalum* and TCB 37 x *Gossypium anomalum* for leaf parameters *viz.*, leaf length, leaf breadth and leaf area. The average leaf length of female parent Suvin and male parent *Gossypium anomalum* was 8.97 and 5.66 cm respectively, whereas in $F_1$ hybrid it was 8.10 cm which is almost intermediate of average leaf length of parents. Significant differences were observed between the parents and $F_1$ hybrid and also between the male and female parents for this character. TCB 37 and *Gossypium anomalum* showed average leaf length of 9.10 and 5.66 cm respectively, while the $F_1$ hybrid expressed intermediate average leaf length of 8.22 cm. Significant differences were observed between the parents and between the parents and $F_1$ hybrids. Suvin and *Gossypium anomalum* recorded the average leaf breadth of 10.77 and 5.20 cm respectively, whereas $F_1$ hybrid recorded intermediate average leaf breadth of 9.34 cm significant differences were observed between the parents as well as between the $F_1$ hybrid. Average leaf breadth of TCB 37, *Gossypium anomalum*

**Table 4.** Biometrical traits of TCB37 x *G.anomalum* hybrid and the parents

| S. No | Characters | TCB 37 | TCB 37 x *G.anomalum* | *G.anomalum* |
|---|---|---|---|---|
| 1. | Number of bracterial teeth | 9.00** | 6.00** | 3.00** |
| 2. | Bracterial length (cm) | 3.97b | 3.65b | 1.67a |
| 3. | Bracterial breadth (cm) | 2.97b | 1.87b | 0.53a |
| 4. | Petiole length (cm) | 5.60** | 5.65** | 4.22** |
| 5. | Leaf length (cm) | 9.10** | 8.22** | 5.66** |
| 6. | Leaf breadth (cm) | 7.93** | 7.15** | 5.20** |
| 7. | Leaf area (cm2) | 56.81** | 44.57** | 16.32** |
| 8. | Pedicel length (cm) | 1.80b | 1.36b | 0.57a |
| 9. | Petal length (cm) | 3.77** | 3.47** | 3.36** |
| 10. | Petal breadth (cm) | 2.40a | 3.07b | 3.43b |
| 11. | Pollen size diameter (µm) | 41.60** | 26.25** | 32.16** |
| 12. | Pollen fertility (%) | 97.67a | 2.30b | 95.3a |
| 13. | Length of pistil (cm) | 2.77** | 2.46** | 1.76** |
| 14. | Gossypol gland density | 9.33** | 8.90** | 9.75** |

** Significant difference at P<0.01 using Duncan's Multiple Range Test
The letters with same alphabet are considered as non-significant

and their $F_1$ hybrid was 7.93, 5.20 and 7.15 cm, respectively. Both the parents as well as $F_1$ hybrid showed significant difference. Average leaf area of the Suvin x *Gossypium anomalum* hybrid was 43.67 cm2 whereas the female and male parents recorded average leaf area of 62.87 cm2 and 16.32 cm2 which clearly showed that the $F_1$ hybrid expressed intermediate leaf area (Table 3, 4 and Plate 2). Significant differences were noticed between the parents and $F_1$ hybrids and also between the male and female parents. TCB 37 and *Gossypium anomalum* recorded average leaf area of 56.81 and 16.32 cm2 respectively, whereas in $F_1$ hybrids it was 44.57 cm2 which is almost intermediate of average leaf area of parents. Significant differences were observed between the parents as well as between the parents and $F_1$ hybrids. Kaur *et al.*, (2016) and Wu *et al.*, (2017).also reported the intermediate leaf length, leaf breadth and leaf area in the interspecific $F_1$ hybrids of *Gossypium hirsutum* and *Gossypium armourianum*. The Suvin x *Gossypium anomalum* and TCB 37 x *Gossypium anomalum* interspecific $F_1$ hybrids showed intermediate expression for mean pedicel length and no significant difference were observed between male and female parent while significant difference was found between female parent and its corresponding $F_1$ hybrids. The intermediate expression was observed in Suvin x *Gossypium anomalum* and TCB 37 x *Gossypium anomalum* for the petal parameters namely petal length and petal breadth. No significant difference between parents and significant difference were observed between female parent and hybrid for petal length in Suvin x *Gossypium anomalum*. The mean pollen fertility of 96.67, 97.67 and 95.30 per cent were recorded in Suvin, TCB 37 and *Gossypium anomalum*, respectively while Suvin x *Gossypium anomalum* and TCB 37 x *Gossypium anomalum* hybrids exhibited the pollen fertility of 2.13 and 2.30 per cent respectively (Plate 5). No significant differences were observed between the parents and its corresponding $F_1$ hybrids while significant difference was noticed between parents. Kaur *et al.,* (2016) reported that the mean pollen fertility of 2.19 per cent $F_1$ hybrid of *Gossypium hirsutum* cv., 1861 x *Gossypium armourianum*. Pushpam and Raveendran (2006) reported mean pollen fertility of 9.04 and 9.67 per cent which were considerably higher in the $F_1$ hybrids of *Gossypium hirsutum* x *Gossypiuma rmourianum* and *Gossypium hirsutum* x *Gossypium raimondii*. The average Pollen size diameter of Suvin, TCB 37, *Gossypiuma nomalum* and their $F_1$ hybrid was recorded as 40.87, 41.60, 32.16 µm and 25.23 - 26.25 µm, respectively were found to be less than that of parents. The results showed intermediate size and significant differences were observed. This result was in accordance with results of Kaur *et al.*, (2016). Pollen diameter of the F1 hybrids showed more variations as comparable with respective of maternal and paternal parents. The two $F_1$ interspecific hybrids of Suvin x *Gossypium anomalum* and TCB 37 x *Gossypium anomalum* and their respective parents have exhibited significant differences for pistil length trait. Mean gossypol gland density of Suvin x *Gossypium anomalum* (8.60) and TCB 37 x *Gossypium anomalum* (8.90) had expressed less gossypol gland density than both the parentsSuvin (12.33), TCB 37 (9.33) and *Gossypium anomalum* (9.75) in both the hybrids. There was significant difference between the parents as well as between parents and their respective hybrids.

The hybridity status of $F_1$ interspecific triploid sterile hybrid had confirmed through mitotic study. Mitotic metaphase chromosome counts revealed that the presence of 52 chromosomesin tetraploid *Gossypium barbadense* genotypes Suvin and TCB 37 while 26 chromosomes observed diploid wild species *Gossypium anomalum* (male parent) and the corresponding $F_1$ hybrids Suvin x *Gossypium anomalum* and TCB 37 x *Gossypium anomalum* (Plate 5). These results are in agreement the with reports of Manickam and Prakash (2014) were


**Plate 1. Parents used for crossing**


**Plate 2. Morphological features of parents and hybrids**

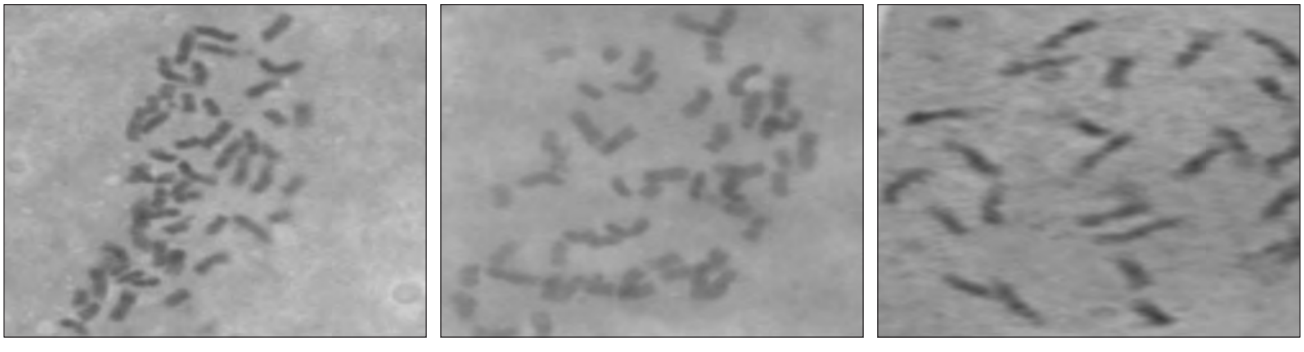
**Suvin** **TCB 37** **G.anomalum** **Suvin x G.anomalum** **TCB 37 x G.anomalum**

**Plate 3. Flower morphology of parents and hybrids**

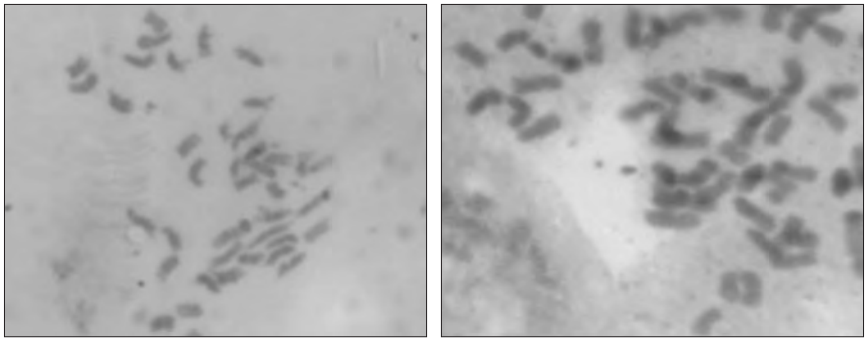
**Suvin** **TCB 37** **Suvin x G.anomalum**

**TCB 37 x G.anomalum** **G.anomalum**

**Plate 4. Petal morphology of parents and hybrids**



**Suvin** **TCB 37** **G.anomalum**



**Suvinx G.anomalum** **TCB 37 x G.anomalum**

**Plate 5. Mitotic chromosomal study in parents and hybrids**

the triploid $F_1$ hybrid of the cross *Gossypium hirsutum* cv. Anjali x *Gossypium aridum* had 39 chromosomes, while the parents *Gossypium hirsutum* and *Gossypium aridum* had 52 chromosomes and 26 chromosomes, respectively, and also reported similar results of 39 chromosomes in hybrid which is intermediate between *G.hirsutum* (2n = 4x = 52) and *G.armourianum* (2n = 2x = 26).

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