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Introgression of Traits from Wild Diploids into Cultivated Tetraploids: A Pragmatic Analysis Using Genetic and Cytogenetic Tool

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A B S T R A C T

Interspecific triploid hybrid was developed between tetraploid cultivated species *Gossypium hirsutum cv.* MCU 5 and CO 14 and diploid wild species *Gossypium anomalum*. The F_1 hybridity was confirmed by morphological and cytologicalstud. The ploidy level of interspecific F_1 hybrid was triploid and male sterile. Maternal parents MCU 5 and CO 14 had erect growth habit, green stem, palmate leaves, thick and prominent leaf veins, cream petals and embedded stigma. MCU5 was with dense yellow anthers while CO 14 dense creamy anthers, whereas male parent *Gossypium anomalum* has spreading growth habit, dull violet petals, pale brownish green stem, creamy white, thin leaf veins, embedded stigma and strongly hairyness plant body. The growth habit, leaf shape and petal colour and petal size of interspecific F_1 hybrids were similar to the paternal parent. Plant stem colour, hairiness, leaf pubescence and anther colour of *Gossypium anomalum* were observed to be dominant as hybrid fully resembled *Gossypium anomalum* for these characters. Petal spot was observed in *Gossypium anomalum* and in F_1 hybrids while petal spot was not observed in MCU 5 and CO 14. Variable expression of petal spot, anther colour and filament colour was observed in the F_1 hybrids. The mitotic study revealed that the maternal parent MCU 5 and CO 14 with 52 chromosomes, *Gossypium anomalum* had 26 chromosomes and the interspecific F_1 was with 39 chromosomes. Significant differences were observed between pollen size, pollen fertility of parents and their hybrids. The F_1 interspecific hybrids having more than 97 percent of sterile pollen grains. This F_1 may be utilized as a pre breeding genetic resource for transfer of cotton jassid resistance to American cotton in breeding programme.

Introduction

The genus *Gossypium* encompasses 50 species (45 diploids and five allopolyploids) which were distributed in tropical and subtropical region of the world (Fryxell, 1992). Out of the four cultivated species *Gossypium hirsutum* L.($2n=4x=52,A_1D_1$) is

contributing 90 per cent of the cotton production worldwide. The other three cultivated species are the African diploid *Gossypium herbaceum* (2n=2x=26, A_2), the Asian and Indian Diploid *Gossypium arboretum* (2n=2x=26, A_1), and the New World tetraploid *Gossypium barbadense* (2n=4x=52, A_2D_2). Diploid *Gossypium* species fall into eight cytological groups designated A-G and K based on the chromosomal pairing relationship and the geographical distribution (Wendel, 1989; Percival and Wendel, 1999). Wild species of cotton represent a significant genetic repository for potential exploitation by cotton breeders who have long recognized the beneficial effect of exotic genes (Heitholt and Manney, 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, 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). Despite of these successes the most of the genetic variation in wild *Gossypium* species remains to be exploited.

Gossypium anomalum is a wild species belonging to the B_1 genome group. *Gossypium anomalum* grows in Southwest Africa and along the southern fringes of Sahara almost from the Atlantic to the Redsea (Fryxell, 1920). As a member of Subsection of *Anomalo* Todaro, *Gossypium anomalum* possess several desirable characters such as extremely fine fibres, good strength, low fibre weight, resistance to insect pests, immunity to the diseases black arm and bacterial blight and tolerance to water deficit(Ganesh *et al.,* 2013).Some efforts have been made to introduce desirable characters from *Gossypium anomalum*to the cultivated cotton (Liu *et al.,* 1992; Mehetre, 2010). *Gossypium anomalum* represents an inestimable source of genes that can potentially be transferred to the cultivated cotton gene pool.

Materials and Methods

The crossing block has been raised during summer 2019 season in the field number C3 comprising of two maternal parents *viz.,* MCU 5 and CO14. The male parent *Gossypium anomalum* is maintained at Cotton Wild Species Garden. Crosses were effected by using Doak's method of hand emasculation and pollination and the crossed bolls were collected. The hybrids along with their female parents *viz.,* MCU5 and CO 14 were raised in field C3 field during Winter 2019 and the male parent *Gossypium anomalum* is being maintained at Cotton Wild Species Garden. The various morphological and cytological observations recorded in parents and F_1 hybrids (Plate 1 and 2).

Nineteen morphological characters *viz.,* 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 were recorded for hybridity confirmation. A total of 14 biometrical traits namely number of bracterial teeth, bracterial length, bracterial breadth, petiole length, leaf length, leaf breadth, leaf area, pedicel length, petal length, petal breadth, pollen size diameter, pollen fertility (%), length of pistil and gossypol gland density were observed on both 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 averaged. Flowers were collected in morning on the day of anthesis between 10.00 am to 11.00 am for pollen fertility study. Pollen fertility was recorded by dusting pollen grains in1% KI solution 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.

The mitotic metaphase chromosome study was carried out by using root tips as explant to find out the ploidy level of F_1 hybrids and their parents. Seeds of parents and their F_1hybrids soaked for overnight and germinated in the germination paper. The roots were collected from the germinated seeds with 2-3cm length in quick succession between 9.00 am to10.00 am on bright sunny days and pretreated in paradichloro benzene to accumulate metaphase cells. After 2 hours the pretreated root tips washed thorouhgly in running tap water and fixed in the ethanol: glacial acetic acid (3:1) fixative. After keeping the fixed material under low temperature (4^o C) for a minimum period of four hours, the roots were thoroughly washed in the distilled water and stored in 70 % ethanol. The roots were hydrolysed at 60^o C for 5 minutes and washed thoroughly. Then the root tips are treated in a 0.25% pectinase solution for 15 minutes in dark and putit 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 1%acetocarmine. After maceration the slide covered with cover slip and heated gently over a sprit lamp. The excess stain was removed by giving gentlepress 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

Morphological characters of parents (MCU 5, CO14 and *Gossypium anomalum*) and F_1 hybrids (MCU 5 x *Gossypium anomalum* and CO 14 x *Gossypium anomalum*) were compared and presented in Table 1 and 2. MCU 5 and CO 14 had annual plant growth habit, whereas the F_1 hybrids and pollen parent *Gossypium anomalum* exhibited perennial shrub growth habit. The pollen parent and both the F_1interspecific hybrids recorded pale brownish green colour stem whereas MCU 5 and CO 14 exhibited greenish brown stem colour. Sparsely pubescent stem were noticed in both female parents (MCU 5 and CO 14) while, the male parent and two F_1 hybrids viz., MCU 5 x *Gossypium anomalum* and CO 14 x *Gossypium anomalum* showed stem with strong pubescence. The leaf veins in maternal parents (MCU 5 and CO 14) and in two interspecific F_1 hybrids (MCU 5 x *Gossypium anomalum* and CO 14 x *Gossypium anomalum*) were thick and prominent in nature whereas the pollen parent *Gossypium anomalum* showed thin nature of veins. The medium smooth leaf texture was observed in maternal parents (MCU 5 and CO 14) but in male parent (*Gossypium anomalum*) and F_1 hybrids velvety nature of leaf texture were noticed. Leaf with strong hairiness was observed in F_1 hybrids and *Gossypium anomalum* whereas, the maternal parents showed sparsely hairy nature of leaf. The corolla colour of maternal parents(MCU 5 and CO 14) were creamy white but dull violet coloured corolla were observed in F_1 hybrids

(MCU 5 x *Gossypium anomalum* and CO 14 x *Gossypium anomalum*).

The maternal parents, F_1 hybrids and paternal parent *Gossypium anomalum* exhibited same expression for petalsize, anther colour, filament colour, position of stigma and nectar gland traits (Plate 3,4 and 7).

Out of 19 qualitative characters observed, three characters viz., growth habit, leaf incision and corolla colour were showed intermediate between both parents in the F_1hybrids.Similar results were reported by Kaur *et al., (*2016) in the F_1 hybrids *Gossypium hirsutum x Gossypium armourianum* for the characters like growth habit and petal colour. Manickam and Prakash (2014) also reported the intermediate leaf and flower morphology in *Gossypium hirsutm x Gossypium armourianum* hybrid. Petal colour of *Gossypium arboreum x Gossypium hirsutum* hybrid and its reciprocal crosses were found be intermediate (Ahmad *et al.,* 2011; Tahir *et al.,* 2011). Kale *et al*., (2007) reported as the F_1 hybrid of *Gossypium arboretum x Gossypium thurberi*had showed dull violet petal colour and which is similar to the female parent. The F_1 hybrid of *Gossypium herbaceumx Gossypium raimondii* exhibited dominance for the anther colour and it is identical to the maternal parent (Wu *et al.,* 2017).

The morphological traits namely stem colour, stem pubescence and hairiness observed in MCU 5 x *Gossypium anomalum* and CO 14 x *Gossypium anomalum* hybrids found to be dominant and resembled with male parent *Gossypium anomalum.* Both the hybrids(MCU 5 x *Gossypium anomalum* and CO 14 x *Gossypium anomalum*) were showed dominant expression for leaf texture and leaf hairiness and which was more similar to its male parent *Gossypium anomalum*. Kaur *et al.,* (2016) also reported similar dominant expression for stemcolour, leaf pubescence and leaf hairiness as fully resembled *Gossypium armourianum* in the *Gossypium hirsutum cv.,* 1861 *x Gossypium armourianum* F_1 hybrid. Average leaf length, leaf breadth and leaf area of MCU 5 x *Gossypium anomalum* and CO 14 x *Gossypium anomalum* hybrids were observed to be intermediate between both parents. Highly significant differences were observed between male and female parents as well as between the parents and hybrids. This results were in agreement with Kaur *et al.,* (2016) and Wu *et al., (*2017).

The female parents MCU 5 and CO 14 and the male parent *Gossypium anomalum* recorded the average pollen fertility of 92.55%, 93.33% and 94.10% respectively. F_1 hybrids MCU 5 x *Gossypium anomalum* and CO 14 x *Gossypium anomalum*were highly sterile with the pollenfertility of 2.17% and 0.7% respectively (Plate 5). Kaur *et al.,* (2016) noticed 2.19 % of average pollen fertility in the F_1 hybrid of *Gossypium hirsutum* cv., 1861 x *Gossypium armourianum*. Pushpam and Raveendran (2006) reported that the average pollen fertility of 9.04% and 9.67% in *Gossypium hirsutum x Gossypium armourianum* and *Gossypium hirsutum x Gossypium raimondii* respectively

Mitotic metaphase counts revealed that the presence of 52 chromosomes in *Gossypium hirsutum cv*. MCU 5 and CO 14, 26 chromosomes in *Gossypium anomalum and,* 39 chromosomes in corresponding F_1 hybrids and confirmed the hybridity and triploid status of the F_1 hybrids developed from cross between MCU 5 x *Gossypium anomalum* and CO 14 x *Gossypium anomalum* (Plate 6). This results in agreement with Manickam and Prakash (2014).

Table.1 Morphological traits of parents and F_1 hybrid of MCU 5 x *Gossypium anomalum*

S. No	Characters	MCU 5	MCU 5 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
3	Leaf colour	Green	Green	Dull green
4	Leaf incision	Shallow to slightly deep	Medium to deep	Deep
5	Leaf shape	Palmate with 2-3 lobes	Palmate with 5 lobes	Palmate with 5 lobes
6	Leaf texture	Medium smooth	Velvety	Velvety
7	Leaf veins	Thick and prominent	Thick and prominent	Thin
8	Petiole colour	Greenish brown	Greenish brown	Dull green with brown
9	Leaf hairyness	Sparsely hairy	Strongly hairy	Strongly hairy
10	Stem pubescence	Sparsely pubescent	Strongly pubescent	Strongly pubescent
11	Corolla colour	Creamy white	Dull white	Dull violet
12	Anther colour	Yellow	Yellow	Creamy white
13	Anther density	Dense	Dense and compact	Dense and compact
14	Bract size	Medium	Medium	Very small
15	Petal size	Medium	Medium	Medium
16	Petal spot	Absent	Present (dark red spot)	Present (dark red spot)
17	Filament colour	White to creamy white	White to creamy white	White to creamy white
18	Position of stigma	Embedded	Embedded	Embedded
19	Nectar gland	Present	Present	Present

Table.2 Morphological traits of parents and F_1 hybrid of CO 14 x *Gossypium anomalum*

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

Table.3 Biometrical traits of MCU 5 x *Gosspium anomalum* hybrid and their parents

S.No	Characters	MCU 5	MCU 5 x *G.anomalum*	*G.anomalum*
1	Number of bracterial teeth	9.66**	6.20**	2.60**
2	Bracterial length (cm)	3.78**	3.52**	1.62**
3	Bracterial breadth (cm)	2.77**	2.20**	0.46**
4	Petiole length (cm)	11.75**	7.52**	4.28**
5	Leaf length (cm)	13.91**	10.22**	5.68**
6	Leaf breadth (cm)	13.25**	10.34**	5.00**
7	Leaf area (cm^2)	154.85**	62.99**	15.16**
8	pedicel length (cm)	1.27^b	1.22^b	0.48^a
9	Petal length (cm)	3.87**	4.12**	3.28**
10	Petal breadth (cm)	3.50**	4.02**	3.44**
11	Pollen size diameter(µ)	39.51**	25.12**	35.56**
12	Pollen fertility (%)	92.55**	2.17**	94.10**
13	Length of pistil (cm)	2.27^b	2.24^b	1.74^a
14	Gossypol gland density	11.00^b	8.50^a	9.75^a

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

Table.4 Biometrical traits of CO 14 x *G. anomalum* hybrid and their parents

S.No.	Characters	CO 14	CO 14 x *G. anomalum*	*G.anomalum*
1	Number of bracterial teeth	10.00**	5.40**	2.60**
2	Bracterial length (cm)	4.27**	3.44**	1.62**
3	Bracterial breadth (cm)	1.95**	2.14**	0.46**
4	Petiole length (cm)	12.21**	8.02**	4.28**
5	Leaf length (cm)	14.97**	11.16**	5.68**
6	Leaf breadth (cm)	14.80**	10.74**	5.00**
7	Leaf area (cm^2)	157.73**	60.51**	15.16**
8	pedicel length (cm)	1.57^b	1.20^b	0.48^a
9	Petal length (cm)	4.62**	4.44**	3.28**
10	Petal breadth (cm)	3.91**	4.42**	3.44**
11	Pollen size diameter(µ)	39.93**	18.00**	35.56**
12	Pollen fertility (%)	93.83**	0.70**	94.10**
13	Length of pistil (cm)	2.20^b	2.66^b	1.74^a
14	Gossypol gland density	6.50^a	8.25^b	9.75^b

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

Plate.1 Parents used for crossing

MCU 5

CO 14

Gossypium anomalum

Plate.2 Morphological features of parents and hybrids

MCU 5 **CO 14**

MCU 5 x *G. anomalum* **CO 14 x *G. anomalum***

G.anomalum *G.anomalum*

Plate.3 Floral morphology of parents and hybrids

Plate.4 Petal morphology of parents and hybrids

MCU 5

CO 14

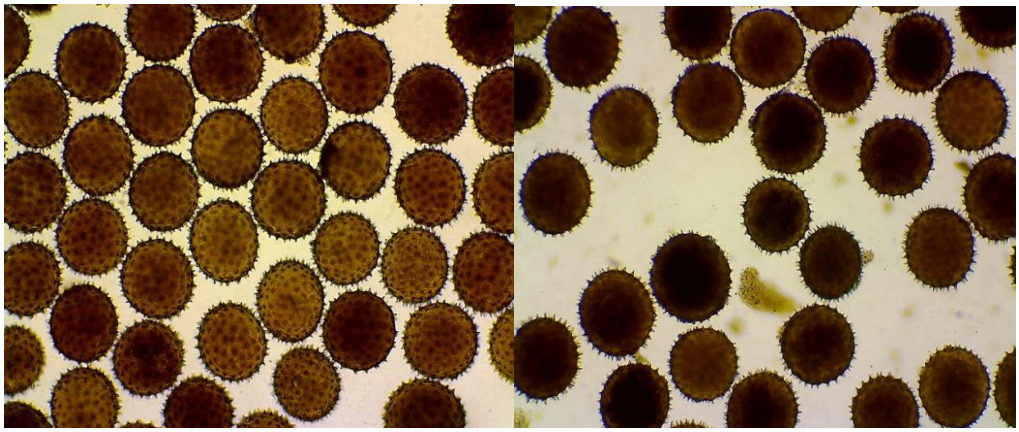
MCU 5 x *G.anomalum*

CO 14 x *G.anomalum*

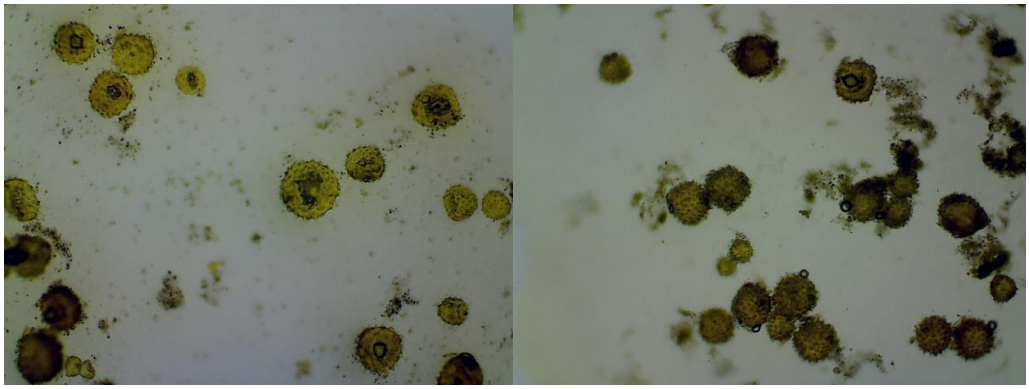
G.anomalum

G.anomalum

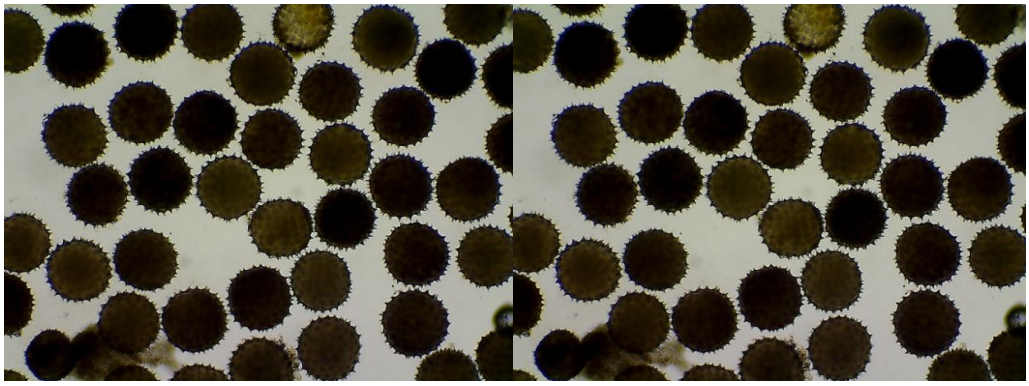
Plate.5 Pollen fertility of parents and hybrids



MCU 5 **CO 14**

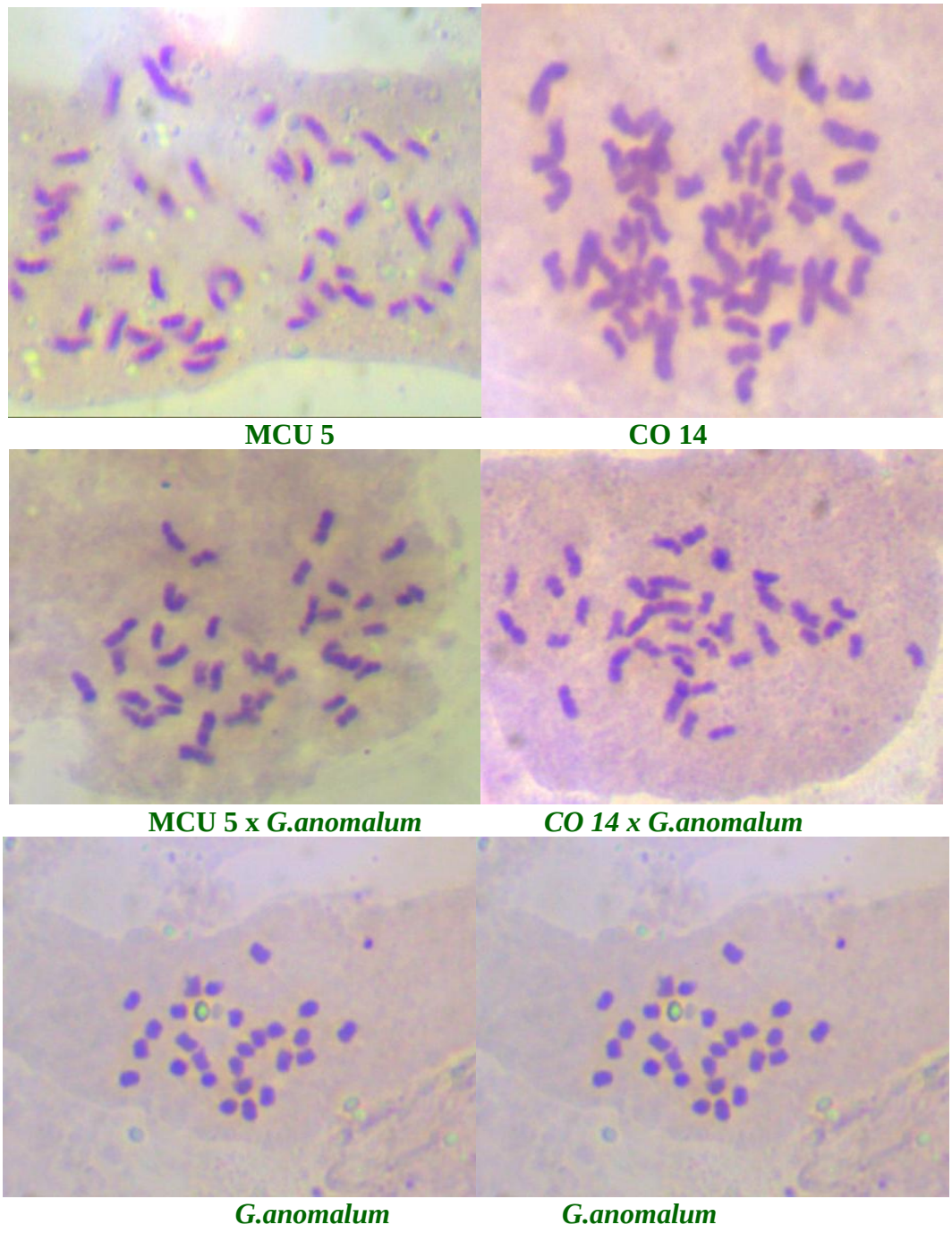


MCU 5 x *G.anomalum* **CO 14 *x* *G.anomalum***



G.anomalum ***G.anomalum***

Plate.6 Mitotic chromosomal study in parents and hybrids



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