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Research Article

F_1 Interspecific hybridity confirmation in cotton through morphological, cytological and molecular analysis

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Abstract
Interspecific triploid hybrid was developed between tetraploid cultivated species *Gossypium hirsutum cv.* MCU 5 and CO 14 and diploid wild species *Gossypium armourianum*. 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. Female parents MCU 5 and CO 14 had erect growth habit, cream petals, palmate leaves, green stem, thick and prominent leaf veins, embedded stigma, hairy stem and leaves but MCU5 was with dense yellow anthers while CO 14 dense creamy anthers, whereas male parent *Gossypium armourianum* has spreading growth habit, yellow petals, cordate leaves, reddish green stem, medium dense yellow anthers, thin leaf veins, protruded stigma and glabrous plant body. The growth habit, petal colour, leaf shape and size of interspecific F_1 hybrids were intermediate. Plant stem colour and hairiness, leaf pubescence, stigma protrusion and anther colour of *Gossypium armourianum* were observed to be dominant as hybrid fully resembled *Gossypium armourianum* for these characters. Petal spot was observed in *Gossypium armourianum* 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. *Gossypium hirsutum cv.* MCU 5 and CO 14 had 52 chromosomes, *Gossypium armourianum* 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. Out of 11 SSR markers polymorphic between parents, 5 markers unambiguously confirmed the hybrid status of interspecific hybrid. These hybrids may serve as useful genetic resource for the transfer of jassid resistance gene to *hirsutum* cotton.

Key words
Interspecific Hybridization, Wild *Gossypium* species, Triploid**,** Insect resistance

Introduction

Cotton is a dicotyledon plant belonging to the Malvaceae family, genus *Gossypium* and is a highly diverse genus. This genus encompasses approximately 50 species, including 45 diploids (2n = 2x = 26) and five allotetraploids (2n = 4x = 52) which are distributed throughout most tropical and subtropical regions of the World (Shim *et al*., 2018). Among the four cultivated species, *Gossypium arboreum* and *Gossypium herbaceum* are diploids and *Gossypium hirsutum* and *Gossypium barbadense* are tetraploids. Diploid *Gossypium* species fall into eight cytological groups designated as A, B, C, D, E, F, G, and K based on their chromosomal pairing relationships and geographical distribution (Wendel and Grover., 2015). *Gossypium hirsutum* also known as American cotton, is the most widely grown cotton species which is responsible for approximately 90% of the total cotton production World wide. *Gossypium armourianum* one of 13 wild diploid D-genome species, reportedly possesses resistance to jassid (Pushpam and Raveendran., 2006), pink bollworm (Brazzel *et al.*, 1956) and white flies. Wild *Gossypium* species possess massive amount of unexplored genetic diversity that can be exploited to broaden the genetic base of cotton (Shim *et al*., 2018).

In the present study, crosses were effected between *Gossypium hirsutum* and *Gossypium armourianum* to transfer the characters for insect resistance due to D_2 smoothness trait and the caducous bract trait that could reduce the trace content of harvested seed cotton to *Gossypium hirsutum* (Manickam *et al*., 2014). The F_1 hybrids and the parents were analysed to discriminate true hybrids using molecular marker (SSR) analysis in conjugation with morphological and cytological analysis.

Materials and Methods

The crossing block has been raised during 2017 Winter season in the field number D6 comprises of two female *hirsutum* parents *viz.*, MCU 5 and CO 14. The male parent *Gossypium armourianum* is maintained at Cotton Department wild species garden. Crosses were effected by using Doak's method of hand emasculation and pollination and the crossed bolls were collected. The two F_1

hybrids along with their female parents *viz.,* MCU 5 and CO 14 were raised in field E1 during Winter 2018. The male parent *Gossypium armourianum* is being maintained in cotton wild species garden. F_1 hybrids along with parents was used for recording data on various morphological, cytological and molecular analysis.

In order to confirm the hybridity status of the F_1 hybrids, 19 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 observed on both the parents and the F_1 hybrids. A total of 14 biometrical traits namely bracterial teeth number, 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 in order to confirm the hybridity status.

Third or 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 in 1% 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 to confirm the ploidy level of F_1 hybrids and their parents. Seeds of parents and their F_1 was soaked for overnight, and germinated in the germination paper. The roots were collected from the germinated seeds with 2-3 cm length in quick succession between 9.00 am to 10.00 am on bright sunny days and pretreated in para dichloro benzene to accumulate metaphase cells. After 2 hours the pre treated 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 mins and washed thouroughly. Then the root tips are treated in a 0.25 % pectinase solution for 15 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 1% acetocarmine. 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.

Genomic DNA was isolated from three parents and their respective F_1 hybrids by the procedure suggested by Zhang and Stewart (2000). The isolated DNA was quantified using Nano Drop^{TM} 1000 spectrophotometer. DNA was amplified using 2X PCR master mix (8µL), 0.66 mM forward (1.0 µL) and reverse (1.0µL) primers, 70 ng DNA template (1µL) and deionised water (4µL) in a 15µL reaction mixture. After PCR amplification, amplified products were resolved using 3% agarose gel. The gels were viewed by UV illumination and documented using gel documentation system (Gel Doc^{TM}XR + Gel documentation system). A total of 20 SSR markers with high PIC value were selected and obtained from cotton marker database (CMD) (http://www.cottonmarker.org/) and were commercially synthesized and procured from Sai Scientific Company, Coimbatore. 2X PCR Master mixes were purchased from Bengaluru GeNei Ltd., Bengaluru, India. The list of primers used to confirm the hybridity and to identify the polymorphism between the parents and their respective F_1 hybrid were presented in Table 5.

Results and Discussion

Morphological characters of parents (MCU 5, CO 14 and *Gossypium armourianum*) and F_1 hybrids (MCU 5 x *Gossypium armourianum* and CO 14 x *Gossypium armourianum*) were compared and presented in Table 1 and 2. Interspecific F_1 hybrids showed either dominance or intermediate expression for various morphological traits. Growth habit, leaf shape, leaf size, leaf incision and petal colour of interspecific hybrid were found to be intermediate. MCU 5 and CO 14 had deep leaf incision and *Gossypium armourianum* had no leaf incision, whereas the F_1 hybrid had shallow leaf incision. Leaf shape of MCU 5 and CO 14 was palmate with 3-4 lobes, whereas *Gossypium armourianum* had cordate leaves. In case of F_1 hybrid, leaves were palmate with 3-4 lobes and reduced in size as compared to *Gossypium hirsutum* leaves. The results are in agreement with

Pushpam and Raveendran (2006); Harpreet Kaur *et al*., (2016), where they have reported intermediate leaf shape and size in hybrids between *Gossypium hirsutum* and *Gossypium armourianum*. Similar intermediate expression of plant growth habit, leaf size and petal colour have reported in other interspecific hybrids such as between *Gossypium davidsonii* x *Gossypium anomalum*, *Gossypium arboreum* x *Gossypium thurberi*, *Gossypium hirsutum* x *Gossypium arboreum* (Ahmad *et al*., 2011; Tahir *et al*., 2011) and *Gossypium herbaceum* x *Gossypium australe* (Liu *et al*., 2015). However, some workers have reported dominance for these characters. For example, *Gossypium hirsutum* x *Gossypium raimondii* triploid hybrid resembled the paternal parent in growth habit (Saravanan *et al*., 2007), Plant stem colouration and hairiness, leaf pubescence, position of stigma, anther colour of *Gossypium armourianum* were found to be dominant as hybrid fully resembled the male parent for these characters. Average pollen fertility was recorded to be 92.55, 93.83, 97.22 and 0.3 - 1.09 % in MCU 5, CO 14, *Gossypium armourianum* and F_1 hybrids respectively (Plate 4). Pollen fertility between parents and hybrids showed significant difference. Pushpum and Raveendran (2006) reported 9.04 % average pollen fertility in *Gossypium hirsutum* x *Gossypium armourianum* hybrid and 9.67 % in *Gossypium hirsutum* x *Gossypium raimondii* hybrids. 2.19 % average pollen fertility was recorded by Harpreet Kaur *et al*., (2016) in *Gossypium hirsutum* x *Gossypium armourianum* hybrids. The average pollen size of MCU 5, CO 14, *Gossypium armourianum* and F_1 hybrid was determined to be 39.51, 39.93, 34.34 and 21.37 - 25.29 micrometer respectively (100X). Pollen size of F_1 hybrids shows more variation when compared to parents. Significant differences were observed between the pollen sizes of the parents as well as between the parents and their hybrids.

Generally petal spot was not observed in *Gossypium hirsutum* while it was found in *Gossypium armourianum*. The F_1 hybrids of MCU 5 x *Gossypium armourianum* and CO 14 x *Gossypium armourianum* exhibited variation for petal spot size and intensity in different flowers of the same plant. It ranged from complete absence to dark pink colour with full size as that of male parent (Plate 1 and Plate 2). Similar results were obtained by Harpreet Kaur *et al*., (2016) in *Gossypium hirsutum cv*. F 1861 x *Gossypium armourianum*. However, complete dominance of petal spot in intra *hirsutum* crosses involving wild type x mutant strains were reported by Ahuja and Dhayal (2007). Tahir *et al*., (2011); Ahmad *et al*., (2011) have reported reduction in the colour intensity of petal spot in F_1 hybrids in case of *Gossypium hirsutum* x *Gossypium arboreum* cross. Intermediate expression of filament colour was observed in the F_1 hybrid. Similar results were reported by Harpreet Kaur *et al*., (2016) in the F_1 hybrids of *Gossypium hirsutum cv*. F 1861 x *Gossypium armourianum*. Filament colour of both the parents were colourless. But in case of F_1 hybrid, filament was either coloured or colourless in different flower bud and, even within same plant both coloured and colourless filaments were also observed (Plate 3). Harpreet Kaur *et al*., (2016) also observed similar results in *Gossypium hirsutum cv*. F 1861 x *Gossypium armourianum*. The portion that connects the filament and the anther was coloured in male parent and colourless in female parent, whereas both coloured and colourless connectives were observed in the same flower and different flowers of the same plant in the F_1 hybrids. Similar variation was observed by Harpreet Kaur *et al*., (2016) in the F_1 hybrid of *Gossypium hirsutum cv*. F 1861 x *Gossypium armourianum*.

Harpreet Kaur *et al*., (2016) viewed that the variations was observed in morphological traits of the F_1 hybrid between *Gossypium hirsutum cv*. F 1861 and *Gossypium armourianum* may be due to "Epigenetics". Rapp and Wendel (2005) considered epigenetics as the alteration of phenotypes, without change in their coding sequence of the gene or the upstream promoter region. In beginning allopolyploids are reported to be associated with variation and instability in phenotypes that cannot be accounted for by conventional Mendelian transmission genetics or chromosomal aberrations (Comai, 2000). Many causes, including increased variation in dosage regulated gene expression, altered regulatory interactions, and rapid genetics and epigenetics changes, which are probably conferred by genome wide interactions (Osborn *et al*., 2003) has been suggested for such altered expressions.

Biometrical characters of parents and F_1 hybrids are compared and presented in Table 3 and 4. Leaf area and petiole length of interspecific F_1 hybrid were found to be intermediate. MCU 5, CO 14 and *Gossypium armourianum* had the leaf area of 154.85 cm^2, 157.73 cm^2 and 7.09 cm^2 respectively, whereas the leaf area of F_1 hybrids MCU 5 x *Gossypium armourianum* and CO 14 x *Gossypium armourianum* were 36.62 cm^2 and 49.84 cm^2 respectively which are intermediate between both the parents. Petiole length of MCU 5 and *Gossypium armourianum* was 11.75 cm and 1.28 cm respectively, whereas the F_1 hybrid exhibits the intermediate length of 5.44 cm. Petiole length of

CO 14 and *Gossypium hirsutum* was 12.21 cm and 1.28 cm respectively, whereas the F_1 exhibits the intermediate length of 5.8 cm.

Mitotic metaphase counts revealed that the presence of 52 chomosomes in *Gossypium hirsutum cv*. MCU 5 and CO 14, 26 chromosomes in *Gossypium armourianum*, 39 chromosomes in corresponding F_1 hybrids and confirmed the triploid status of the F_1 hybrids developed from cross between MCU 5 x *Gossypium armourianum* and CO 14 x *Gossypium armourianum* (Plate 5).

For hybridity conformation female parents (MCU 5 and CO 14), male parent (*Gossypium armourianum*) and F_1 hybrids (MCU 5 x *Gossypium armourianum* and CO 14 x *Gossypium armourianum*) were subjected to polymorphic analysis using 20 cotton specific SSR markers. SSR markers namely BNL 3948, BNL 3955, BNL 2443, CIR 407 and CIR 413 were selected to confirm the hybridity status of interspecific hybrid as polymorphic bands from both the parents were present in the hybrid (Plate 6).

The present study revealed that the hybridity status of F_1 hybrids developed from cross between MCU 5 x *Gossypium armourianum* and CO 14 x *Gossypium armourianum* using morphological, cytological and molecular marker analysis. These F_1 hybrids are important genetic resources for cotton breeders to develop pest and disease resistant cultivars. These materials can be used as bridges for the transfer of pest and disease resistant genes from the wild species to cultivated varieties.

References

Ahmad, S., K. Mahmood, M. Hanif, W. Nazeer, W. Malik, A. Qayyum and N. Islam.2011. Introgression of cotton leaf curl virus-resistant genes from Asiatic cotton (*Gossypium arboreum*) into upland cotton (*G. hirsutum*). *Genet. Mol. Res.*, **10**(4): 2404-2414.

Ahuja, S. L and L. S. Dhayal.2007. Comparative characteristics and gene action in three petal-spotted mutants of *Gossypium hirsutum*. *J. Genet.*, **86**(1) : 81-84.

Brazzel, J. R.1956. Resistance of cotton to pink bollworm damage.

Comai, L.2000. Genetic and epigenetic interactions in allopolyploid plants. *Plant Gene Silencing*, 267-279.

Kaur, H., D. Pathak and P. Rathore.2016. Development and characterization of an interspecific *Gossypium hirsutum* x *Gossypium armourianum* hybrid. *Appl. Biol. Res.*, **18**(2) : 146-154.

Liu, Q., Y. Chen, Y. Wang, J. Chen, T. Zhang and B. Zhou.2015. A New Synthetic Allotetraploid ($A_1A_1G_2G_2$) between *Gossypium herbaceum* and *G. australe* : bridging for simultaneously transferring favorable genes from these two diploid species into upland cotton. *PLOS one*, **10**(4).

Manickam, S and A.H. Prakash.2014. Interspecific hybridization between *Gossypium hirsutum* and *G armourianum*: Morphological and Molecular Characterization of Hybrids. *Cotton Res. J.*, **6**(1) : 7-12

Osborn, T. C., J. C. Pires, J. A. Birchler, D. L. Auger, Z. J. Chen, H. S. Lee and R. A. Martienssen.2003. Understanding mechanisms of novel gene expression in polyploids. *Trends Genet.*, **19**(3) : 141-147.

Pushpam, R and T. S. Raveendran.2006. Production of interspecific hybrids between *Gossypium hirsutum* and Jassid resistant wild species *G. raimondii* and *G.armourianum*. *Cytologia*, **71**(4) : 407-418.

Rapp, R. A and J. F. Wendel.2005. Epigenetics and plant evolution. *New Phytol*, **168**(1) : 81-91.

Shim, J., P. K. Mangat and R. B. Angeles-Shim.2018. Natural variation in wild Gossypium species as a tool to broaden the genetic base of cultivated cotton. *J. Plant Sci. Curr. Res.*, *2*(005).

Saravanan, N. A., S. G. Ram, V. Thiruvengadam, R. Ravikesavan and T.S. Raveendran.2007. Production and Fertility Restoration of an Interspecific Hybrid between *Gossypium hirsutum* L. and *G. raimondii* U. *Cytologia*, **72**(2) : 195-203.

Tahir, M. S and U. I. K. Noor.2011. Development of an interspecific hybrid (Triploid) by crossing *Gossypium hirsutum* and *G. arboreum*. *Cytologia*, **76**(2) : 193-199.

Wendel, J. F and C. E. Grover.2015. Taxonomy and evolution of the cotton genus, *Gossypium*. *Cotton*, (agronmonogr57), 25-44.

Zhang, J and J. M. Stewart.2000. Economical and rapid method for extracting cotton genomic DNA. *J. Cotton Sci.*, **4**(3) : 193-201.

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

S. No	Characters	MCU 5	MCU 5 x *G.armourianum*	*G.armourianum*
1	Growth habit	Annual, erect	Perennial, semispreading	Perennial, spreading
2	Stem colour	Dark green with brown	Brownish purple green	Brownish purple
3	Stem pubescences	Sparsely pubescent	Glabrous	Glabrous
4	Petiole colour	Greenish brown	Brownish green	Brownish green
5	Leaf shape	Palmate with 3-4 lobes	Palmate with slight lobes	Palmate with no lobes
6	Leaf colour	Green	Dark green	Dark green
7	Leaf incision	Shallow to slightly deep	Shallow	Shallow
8	Leaf veins	Thick and prominent	Thick and prominent	Thin
9	Leaf texture	Medium smooth and thin	Smooth and thick	Smooth and thick
10	Leaf hairyness	Sparsely hairy	Glabrous	Glabrous
11	Bract size	Medium	Small	Caducous bract
12	Corolla colour	Creamy white	Light yellow	Bright yellow
13	Petal size	Medium	Medium	Medium
14	Petal spot	Absent	Absent/Present (light to dark	Present (dark red)
15	Anther colour	Yellow	Yellow with red spot	Yellow with red spot
16	Anther density	Dense	Medium	Medium
17	Filament colour	White to creamy white	Creamy white to dark purple	White to creamy white
18	Position of stigma	Embedded	Protruded	Protruded
19	Nectar gland	Present	Present	Absent

Table 2. Morphological traits of parents and F_1 hybrids of CO 14 x *G. armourianum*

S. No	Characters	CO 14	CO 14 x *G.armourianum*	*G.armourianum*
1	Growth habit	Annual, erect	Perennial, semi spreading	Perennial, spreading
2	Stem colour	Dark green	Brownish purple green	Brownish purple
3	Stem pubescences	Sparsely pubescent	Glabrous	Glabrous
4	Petiole colour	Light green	Greenish purple	Purple green
5	Leaf shape	Palmate with 3-4 lobes	Palmate with slight lobes	Palmate with no lobes
6	Leaf colour	Green	Dark green	Dark green
7	Leaf incision	Shallow to slightly deep	Shallow	Shallow
8	Leaf veins	Thick and prominent	Thick and prominent	Thin
9	Leaf texture	Medium smooth and thin	Smooth and thick	Smooth and thick
10	Leaf hairyness	Sparsely hairy	Glabrous	Glabrous
11	Bract size	Medium	Small	Caducous bract
12	Corolla colour	Creamy white	Light yellow	Bright yellow
13	Petal size	Medium	Medium	Medium
14	Petal spot	Absent	Absent/Present (light to dark	Present (dark red)
15	Anther colour	Creamy white	Yellow with red spot	Yellow with red spot
16	Anther density	Dense	Medium	Medium
17	Filament colour	White to creamy white	Creamy white to dark purple	White to creamy white
18	Position of stigma	Embedded	Protruded	Protruded
19	Nectar gland	Present	Present	Absent

Table 3. Biometrical traits of MCU 5 x *G. armourianum* hybrid and their parents

S.No	Characters	MCU 5	MCU 5 x *G.armourianum*	*G.armourianum*
1	Bracterial teeth number	9.66	6.58	-
2	Bracterial length (cm)	3.78	2.43	-
3	Bracterial breath (cm)	2.77	1.76	-
4	Petiole length (cm)	11.75	5.44	1.28
5	Leaf length (cm)	13.91	7.42	3.56
6	Leaf breadth (cm)	13.25	7.62	3.45
7	Leaf area (cm^2)	154.85	36.62	7.09
8	Pedicel length (cm)	1.27	2.22	1.40
9	Petal length (cm)	3.87	4.31	4.97
10	Petal breath (cm)	3.50	4.29	4.98
11	Pollen size diameter (µ)	39.51	25.29	34.34
12	Pollen fertility (%)	92.55	1.09	97.22
13	Length of pistil(cm)	2.27	3.07	3.50
14	Gossypol glands	11.00	8.50	11.25

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

S.No	Characters	CO 14	CO 14 x *G.armourianum*	*G.armourianum*
1	Bracterial teeth number	10.00	7.16	-
2	Bracterial length (cm)	4.27	2.42	-
3	Bracterial breath (cm)	1.95	1.73	-
4	Petiole length (cm)	12.21	5.80	1.28
5	Leaf length (cm)	14.97	9.08	3.56
6	Leaf breadth (cm)	14.80	8.55	3.45
7	Leaf area (cm^2)	157.73	49.84	7.09
8	Pedicel length (cm)	1.57	3.07	1.40
9	Petal length (cm)	4.62	4.49	4.97
10	Petal breath (cm)	3.91	4.62	4.98
11	Pollen size diameter (µ)	39.93	21.37	34.34
12	Pollen fertility (%)	93.83	0.30	97.22
13	Length of pistil(cm)	2.20	2.82	3.50
14	Gossypol glands	6.50	9.00	11.25

Table 5. List of primers used for hybridity confirmation

S. No	Primer name	Primer sequence (5'- 3')	Annealing Temp. (^{o}C)
1	BNL 1034	F: TTGCTTTCAATGGAAAACCC R: CGTCGCAAAGTTGAGAATCA	55
2	BNL 1161	F: CATCTCCTCTGGAAAGAGCG R: ATGAAGCAGCACATTCCATG	55.5
3	BNL 2443	F: TTTATTGGTCGGTCTTTGCC R: TTAGGGTGTTCTTTGGGCAC	51
4	BNL 3424	F: TGTGCCGTCTCAAAATGAAG R:AAGACCAATCTGTTGCCAGC	51
5	BNL 3482	F: ATTTGCCCCAGGTTTTTTTT R: GCAACACCTTTTCCTCCCTA	48.5
6	BNL 3623	F: TTAATAGAGGGACCAA AAGTGATAT R: TTAGCGTTAATATTGTATGTTCAACTC	47.2
7	BNL 3874	F: CATGTTCTAATCATATATATATGATATATATGTGT R: AAAATAACAAAAGCCAT GGAATAA	48.5
8	BNL 3948	F: GTAATGTTCAACACTTTGCTATTCC R: GTTGGTTGGGTGAGCAGAAT	51
9	BNL 3955	F: AGAGATGCAATGGGATCGAC R: ATGTGATAATGCGGGGAATG	50
10	BNL 4108	F: TCCACCATTCCCGTAAATGT R: TGGCCAAGTCATTAGGCTTT	50.2
11	CIR 407	F: GCACAGAACATCCATACA R: TCTCTCTCTCTTTCACACAC	51
12	CIR 413	F: TTAAAGCTCACACACACA R: CAACAGTAACGAAGAACAAT	46.5
13	CM 029	F: TTCCAAGTTCCAATTTCTTC R: ATCAACCACTTTGACAATGTT	44.5
14	JESPR 127	F: GATTTGGGTAACATTGGCTC R: CTGCAGTGTTGTGTTGGGTAGA	51
15	JESPR 292	F: GCTTGCAATCTCCTACACC R: GAATATGTTTCATAGAATGGC	43
16	NAU 0797	F: AGAGAGCAAAAGCACGAGAC R: CTAACAGGGGTGACATAGGG	56
17	NAU 874	F: AAATGGCGTGCTTGAAATAC R: TGTGATGAAGAACCCTCTCA	49.5
18	NAU 1200	F: CAACAGCAACAACCACAA R: CTGCCTCGAGGACAAATAGT	47
19	NAU 1369	F: TGGCAGAGATGAATGTAAGC R: GGTAACGGATGGAAAATCAC	49.5
20	NAU 2035	F: CGAGAAACTTCACTGGACCT R: GAAAAGGTAGGCTTGTTGGA	57

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MCU 5

MCU 5 x *Gossypium armourianum*

Gossypium armourianum

CO 14

CO 14 x *Gossypium armourianum*

Gossypium armourianum

Plate 1. Hybridity confirmation through morphological features

Plate 2. Floral variations observed in the F_1 hybrids of MCU 5 x *Gossypium armourianum* and CO 14 x *Gossypium armourianum*

Plate 3. Variations in the anther column in F_1 hybrids of MCU 5 x *Gossypium armourianum* and CO 14 x *Gossypium armourianum*

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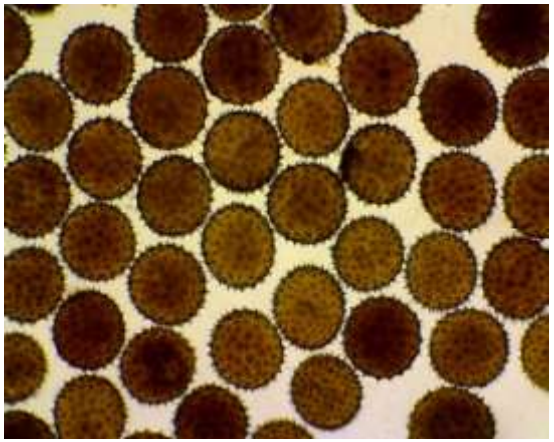
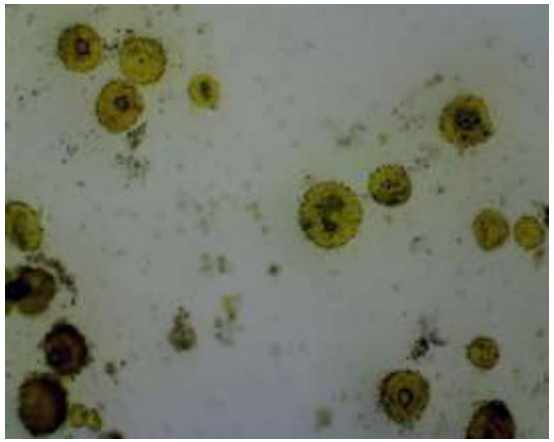
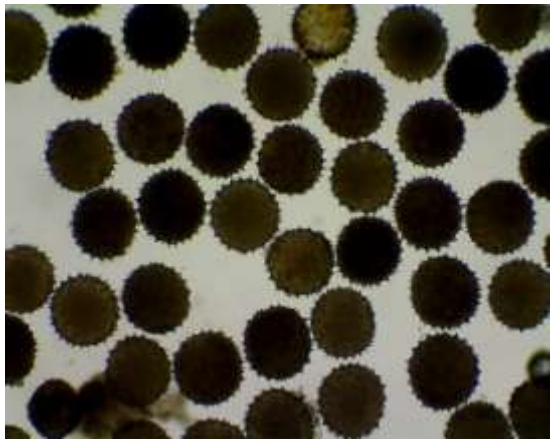


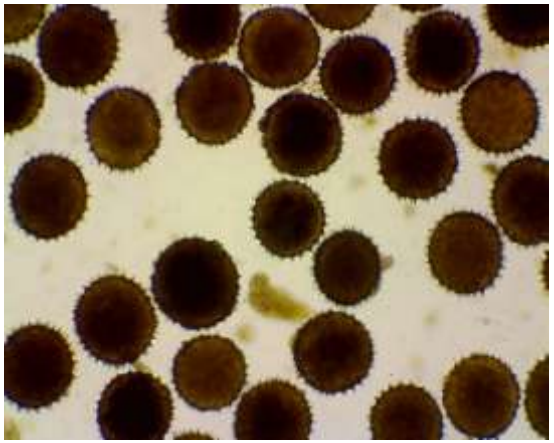
MCU 5



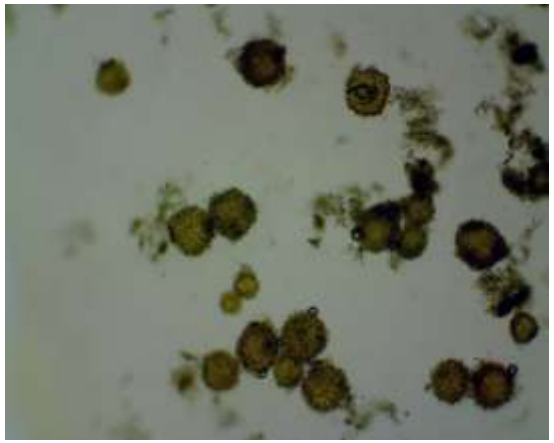
MCU 5 x *Gossypium armourianum*



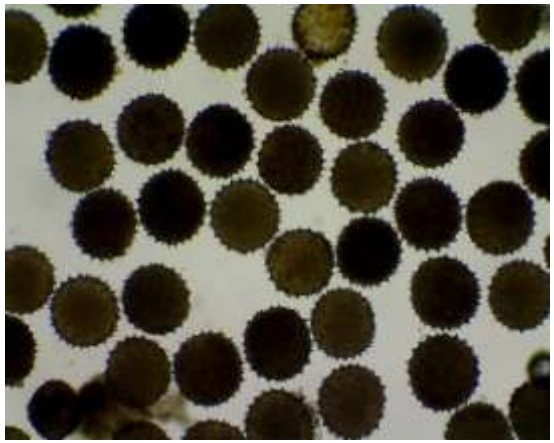
Gossypium armourianum



CO 14

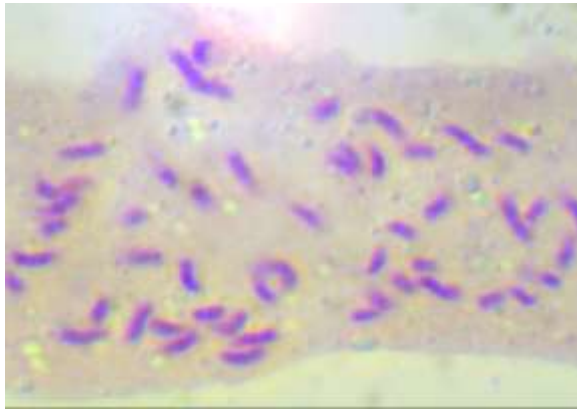


CO 14 x *Gossypium armourianum*

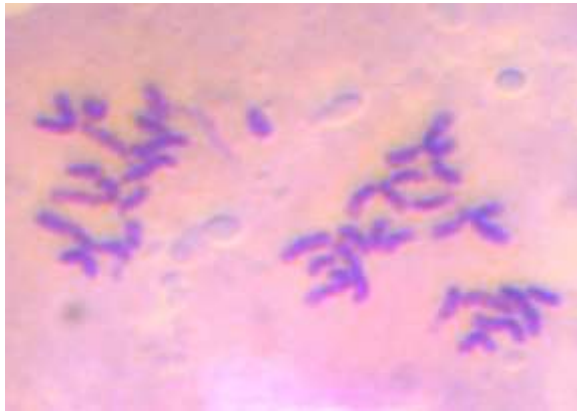


Gossypium armourianum

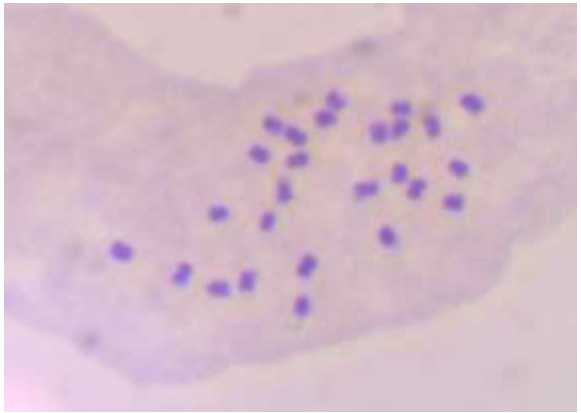
Plate 4. Hybridity confirmation through pollen studies



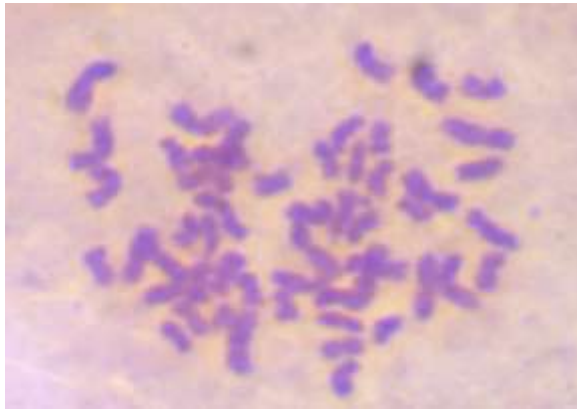
MCU 5



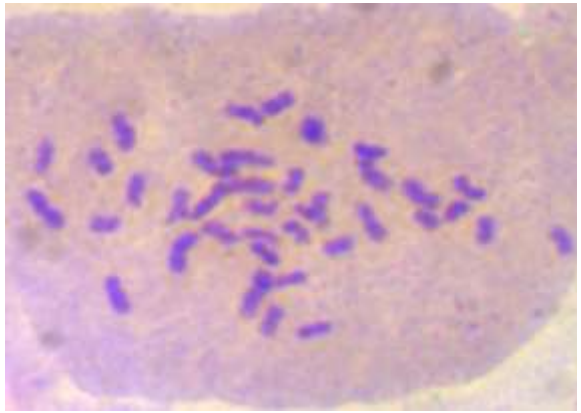
MCU 5 x *Gossypium armourianum*



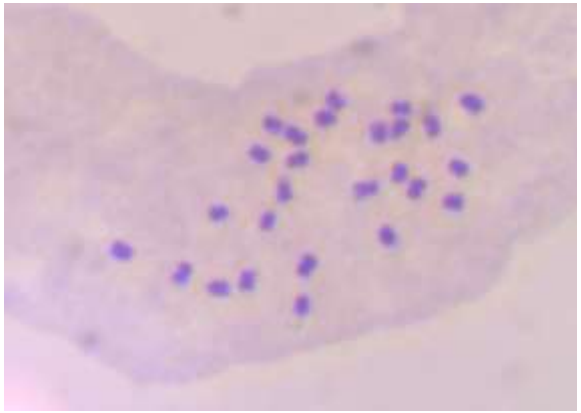
Gossypium armourianum



CO 14



CO 14 x *Gossypium armourianum*

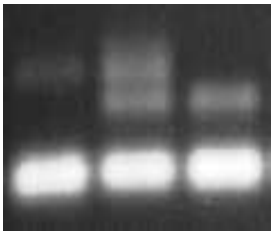


Gossypium armourianum

Plate 5. Hybridity confirmation through mitosis

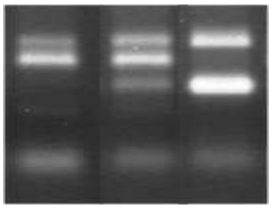
MCU 5 x *Gossypium armourianum*

P_1 F_1 P_2



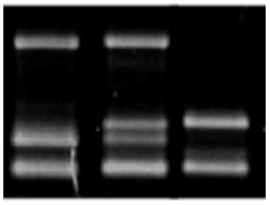
BNL 3948

P_1 F_1 P_2



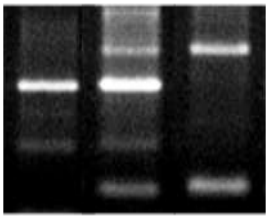
BNL 2443

P_1 F_1 P_2



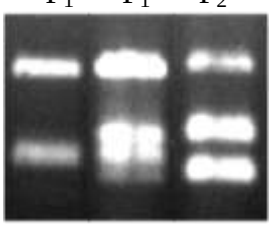
BNL 3955

P_1 F_1 P_2



CIR 413

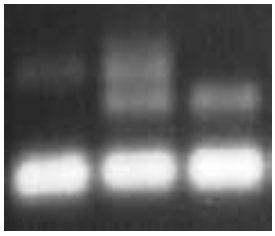
P_1 F_1 P_2



CIR 407

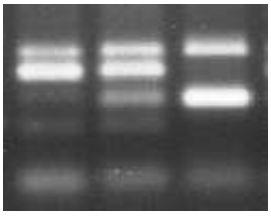
CO 14 x *Gossypium armourianum*

P_1 F_1 P_2



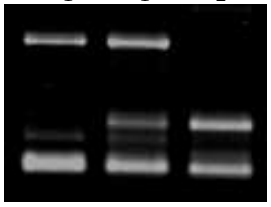
BNL 3948

P_1 F_1 P_2



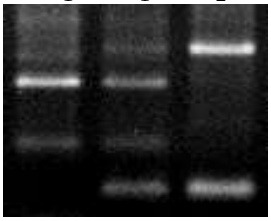
BNL 2443

P_1 F_1 P_2



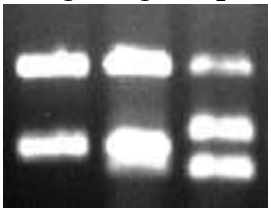
BNL 3955

P_1 F_1 P_2



CIR 413

P_1 F_1 P_2



CIR 407

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