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Development and Characterization of *Gossypium hirsutum* and *Gossypium armourianum* Interspecific Hybrids

L. Mahalingam*, N. Premalatha, K. Senguttuvan, P. Latha and M. Kumar

[1]*Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, India*

**Corresponding author*

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A B S T R A C T

Interspecific triploid hybrid was developed between tetraploid cultivated species *Gossypium hirsutum* genotypes MCU 5 and TCH 1819and diploid wild species *Gossypium amourianum*. The F_1 hybridity was confirmed through morphological and cytological studies. The ploidy level of interspecific F_1 hybrids was triploid and male sterile. The maternal parents MCU 5 and TCH 1819 have exhibited erect growth habit, green stem, hairy stem and leaves, palmate, thick and prominent leaf veins, cream petals and embedded stigma. Both the *hisutum* genotypes MCU5 and TCH 1819 were showed similar expression in all morphological features except anther colour in which the MCU 5 showed dense yellow colour anther while dense creamy anther was observed in TCH 1819. The paternal parent diplpod wild species *Gossypium armourianum* had spreading growth habit, reddish green stem, cordate leaves, thin leaf veins, yellow petals, medium dense yellow anthers and protruded stigma. The growth habit, petal colour, leaf shape and size of interspecific F_1 hybrids were intermediate. Plant stem colour and hairiness, leaf pubescence, anther colour and stigma protrusion of *Gossypium armourianum* were observed to be dominant and the 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 present in MCU 5 and TCH 1819. Variable expression of petal spot, anther colour and filament colour was observed in the F_1 hybrids. *Gossypium hirsutum* genotypes MCU 5 and TCH 1819 had 52 chromosomes, *Gossypium armourianum* had 26 chromosomes and 39 chromosomes was observed in the interspecific F_1 hybrids of MCU x *Gossypium armourianum* and TCH 1819 x *Gossypium armourianum* through mitotic chromosomal study. Significant differences were observed between pollen size, pollen fertility of parents and their hybrids. TheF_1interspecific hybrids having more than 97 percent of sterile pollen grains. This F_1may be utilized as a pre breeding genetic resource for transfer of cotton jassid resistance to American cotton in breeding

Introduction

The genetic diversity of genus *Gossypium spp* is exclusively wide with diverse geographical and ecological niches (Fryxell, 1992).The genus *Gossypium* belongs to the family *Malvaceae* and contains more than 45 diploid species and five well documented allotetraploid species. Species of this genus 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; Percy and Wendel,1999). Based on the chromosomal uniformity the diploid D genome species of the New World cotton include 26 chromosomes. Some hybrids within the genome are fertile and the chromosomere combination during meiosis. However, hybrids across genomes are generally infertile and have a few bivalents at meiosis as a result progeny plant 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 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 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). The most of the genetic variation available in wild *Gossypium* species has to be exploited.

Materials and Methods

The crossing block comprises of two *hirsutum* genotypes *viz.*, MCU 5 and TCH 1819raised during summer 2019 season. The male parent *Gossypium armourianum* is maintained at Cotton wild species garden. Crosses were effected by using Doak's method of hand emasculation and pollination. The F_1hybrids along with their parents were evaluated during winter 2019. The male parent *Gossypium armourianum* are being maintained in cotton wild species garden. The observations were recorded both in F_1 hybrids and parents for various morphological and cytological studies (Plate 1).

Eighteen 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 and position of stigma were observed on both the parents and the F_1 hybrids in order to confirm the hybridity status of the F_1 hybrids. Biometrical traits *viz.*, 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 in 1% Potassium Iodide solution and viewed under a

compound microscope. The large, darkly stained and circular pollen grains only 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 with 2-3 cm length in quick succession between 9.00 am to 10.00 am on bright sunny days and pre-treated in paradichloro 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 minutes and washed thoroughly. 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.

Results and Discussion

Morphological features of parents MCU 5, TCH 1819, *Gossypium armourianum* and their corresponding F_1 hybrids of MCU 5 x *Gossypium armourianum* and TCH 1819 x *Gossypium armourianum* were compared and presented in Table 1 and 2. Interspecific F_1 hybrids exhibited 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. The maternal parents MCU 5 and TCH 1819 had deep leaf incision and *Gossypium armourianum* had no leaf incision whereas, the F_1 hybrids had shallow leaf incision. Leaf shape of MCU 5 and TCH 1819 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(Plate 2). Pushpam and Raveendran (2006) and Kaur *et al.*, (2016) 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 and Noor, 2011) and *Gossypium herbaceum*x *Gossypium australe*(Liu *et al.*, 2015). 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 of 92.55, 93.83, 97.22and 0.30 - 1.09 % in MCU 5, TCH 1819, *Gossypium armourianum* and F_1 hybrids respectively (Tabl 3 and 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 Kaur *et al.,* (2016) in *Gossypium hirsutum*x *Gossypium armourianum* hybrids. The average pollen size of MCU 5, TCH 1819, *Gossypium armourianum* and F_1 hybrid was determined to be 39.51,39.93, 34.34 and 21.37 - 25.29µ 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 TCH 1819 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 Kaur *et al.,* (2016) in *Gossypium hirsutum cv.,* 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 and Noor (2011) and 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 Kaur *et al.,* (2016) in the F_1 hybrids of *Gossypium hirsutum cv.* F 1861 x *Gossypium armourianum*.

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

S.No	Characters	MCU 5	MCU 5 x *G.armourianum*	*G.armourianum*
1	Growth habit	Annual, erect	Perennial,semi spreading	Perennial, semi spreading
2	Stem colour	Dark green with brown	Brownish purple green	Brownish purple
3	Stem pubescence	Sparsely pubescent	Glabrous	Glabrous
4	Petiole colour	Greenish brown	Brownish green	Brownish green
5	Leaf shape	Palmate with 3-5 lobes	Palmate with slight lobes	Cordate
6	Leaf colour	Green	Dark green	Green
7	Leaf incision	Shallow to slighty deep	Shallow	Shallow
8	Leaf veins	Thick and prominent	Thick and prominent	Thin
9	Leaf texture	Medium smooth	Smooth	Smooth
10	Leaf hairiness	Sparsely hairy	Glabrous	Glabrous
11	Bract size	Medium	Small	Caducous bract
12	Corolla colour	Creamy white	Light yellow	Yellow
13	Petal size	Medium	Medium	Medium
14	Petal spot	Absent	Present	Present
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	White to creamy white	White to creamy white
18	Position of stigma	Embedded	Protruded	Protruded

Table.2 Morphological traits of parents and F_1 hybrid of TCH 1819 x *G. armourianum*

S.No.	Characters	TCH 1819	TCH 1819 x *G.armourianum*	*G.armourianum*
1	Growth habit	Annual shrub	Perennial, semi spreading	Perennial, semi spreading
2	Stem colour	Dark green	Brownish purple green	Brownish purple
3	Stem pubescence	Sparsely pubescent	Glabrous	Glabrous
4	Petiole colour	Green	Greenish purple	Brownish green
5	Leaf shape	Palmate with 2-4 lobes	Palmate with slight lobes	cordate
6	Leaf colour	Light 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	Thin smooth	Smooth
10	Leaf hairiness	Sparsely hairy	Glabrous	Glabrous
11	Bract size	Medium	Small	Caducous bract
12	Corolla colour	Creamy white	Light yellow	Yellow
13	Petal size	Medium	Medium	Medium
14	Petal spot	Absent	Present	Present
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	White to creamy white	White to creamy white
18	Position of stigma	Embedded	Protruded	Protruded

Table.3 Biometrical traits of parents and F_1 hybrid of MCU 5 x *G. armourianum*

S.No	Characters	MCU 5	MCU 5 x *G.armourianum*	*G.armourianum*
1	Number of bracterial teeth	9.66**	6.58**	-
2	Bracterial length(cm)	3.78**	2.43**	-
3	Bracterial breadth(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^a	$2,22^b$	1.40^a
9	Petal length(cm)	3.87**	4.31**	4.97**
10	Petal breadth(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^a	3.07^b	3.50^b
14	Gossypol gland density	11.00^a	8.50^b	11.25^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 parents and F_1 hybrid of TCH 1819 x G.*armourianum*

S.No	Characters	TCH 1819	TCH 1819 x *G.armourianum*	*G.armourianum*
1	Number of bracterial teeth	10.00**	7.16**	-
2	Bracterial length(cm)	4.27**	2.42**	-
3	Bracterial breadth(cm)	1.95	1.73	-
4	Petiole length(cm)	12.21**	5.80**	1.28**
5	Leaf length(cm)	14.97**	8.91**	3.56**
6	Leaf breadth(cm)	14,80**	8.57**	3.45**
7	Leaf area(cm^2)	157.73**	49.84**	7.09**
8	Pedicel length(cm)	1.57^a	3.07^b	1.53^a
9	Petal length(cm)	4.62**	4.49**	4.97**
10	Petal breadth(cm)	3.91^a	4.62^b	4.98^b
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 gland density	6.50**	9.00**	11.25**

Plate.1 Parents used for crossing

MCU 5 **TCH 1819**

Plate.2 Morphological features of parents and hybrids

Plate.3 Floral morphology of parents and hybrids

MCU 5TCH 1819

MCU 5 x *G.armourianum*TCH 1819 x *G.armourianum*

*G.armourianum**G.armourianum*

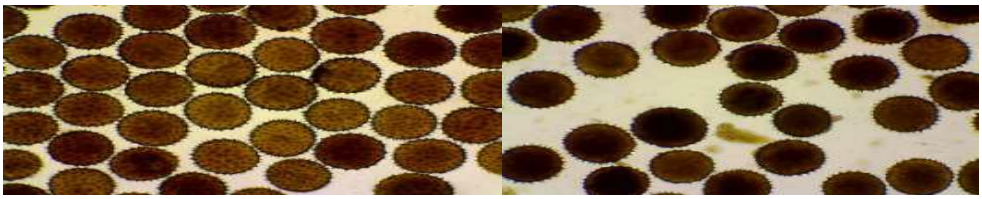
Plate.4 Petal morphology of parents and hybrids

MCU 5TCH 1819

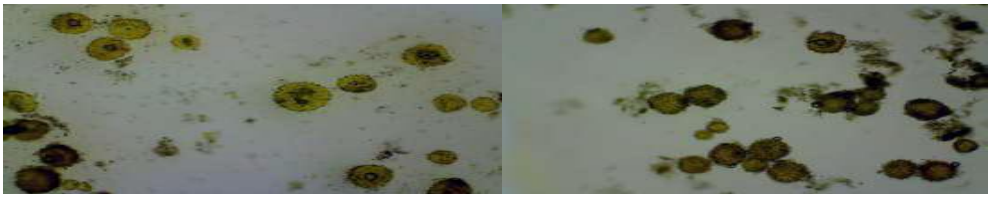
MCU 5 x *G.armourianum* T CH 1819 x *G.armourianum*

G.armourianumG.armourianum

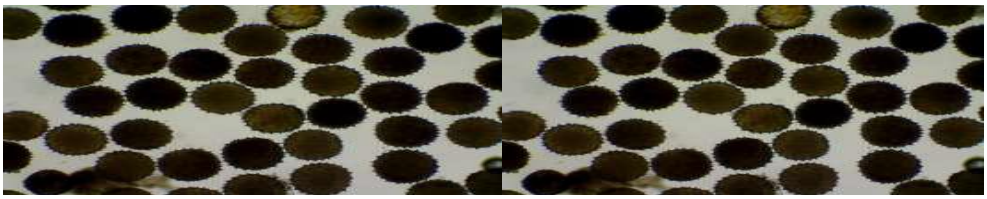
Plate.5 Pollen fertility of parents and hybrids



MCU 5 TCH 1819

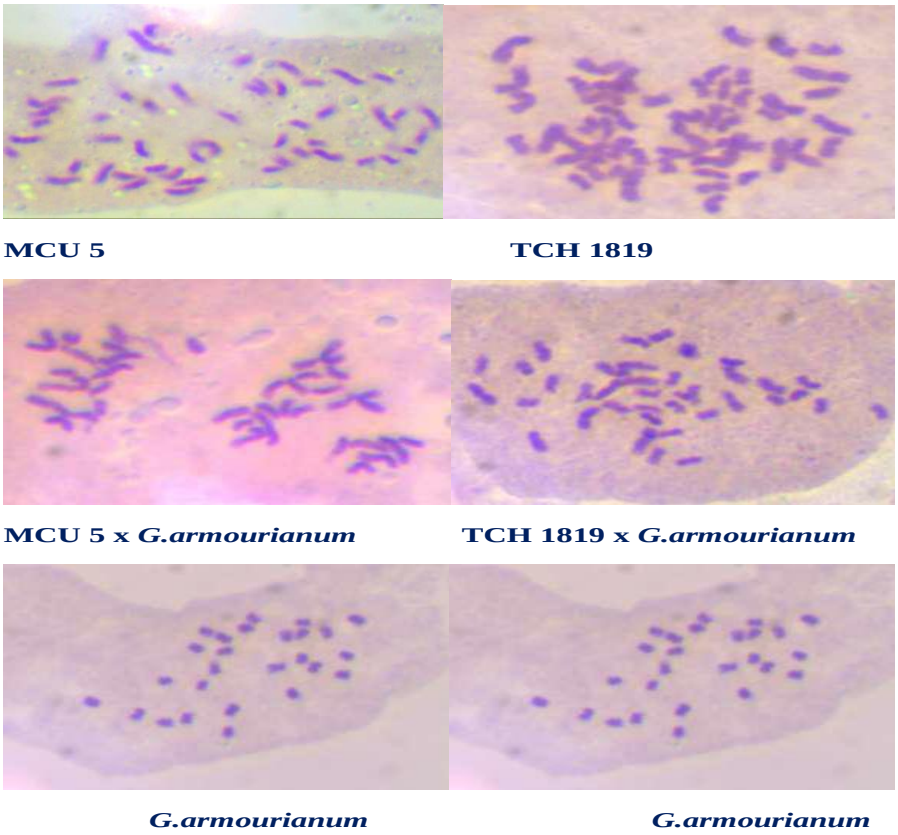


MCU 5 x *G.armourianum* TCH 1819 x *G.armourianum*



G.armourianumG.armourianum

Plate.6 Cytological study in parents and hybrids



Filament colour of boththe 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). 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 Kaur *et al.*, (2016) in the F_1 hybrid of *Gossypium hirsutum cv*. F 1861 x *Gossypium armourianum*.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). 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, TCH 1819 and *Gossypium armourianum* had the leaf area of 154.85 cm2, 157.73cm^2 and 7.09cm^2 respectively, whereas the leaf area of F_1 hybrids MCU 5 x *Gossypium armourianum*

and TCH 1819 x *Gossypium armourianum* were 36.62 $cm^2$and 49.84cm^2 respectively which are intermediate between both the parents. Petiole length of MCU 5 and *Gossypium armourianum* was 11.75 cm and 1.28cm respectively, whereas the F_1 hybrid exhibits the intermediate length of 5.44 cm. Petiole length of TCH 1819 and *Gossypium hirsutum* was 12.21 cm and 1.28cm respectively, whereas the F_1 exhibits the intermediate length of5.80cm.Mitotic metaphase counts revealed that the presence of 52 chomosomes in *Gossypium hirsutum* genotypes MCU 5 and TCH 1819, 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 TCH 1819 x *Gossypium armourianum* (Plate 6). 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.

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