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## Research Article

### Crossability relationship between wild cotton *Gossypium armourianum* and *Gossypium gossypoides* with American cotton

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#### Abstract

The goal of the current study was to assess the possibility of interspecific hybridization for transfer of jassid-resistant genes from the two wild cotton species *Gossypium armourianum* (Kearney (2n=2x=26) D<sub>2</sub>A) and *Gossypium gossypoides* (Standley (2n=2x= 26) D<sub>6</sub>) into the genotypes of cultivated *Gossypium hirsutum*. The study demonstrated that seeds were successfully produced from crosses between *G. hirsutum* and *G. armourianum* using the cultivated tetraploid as a female parent. The reciprocal cross did not produce any seed. None of the crosses were successful in combination with *G. gossypoides*. Most of the boll shedding in reciprocal cross was between 2-6 days after pollination. MCU5 x *G. armourianum* had the highest germination percentage whereas; CO17x *G. armourianum* had highest survival percentage of the seedlings. With further backcrosses or chromosome doubling, the segregants can be further utilized for the introgression of different relevant traits like sucking pest resistance against leafhopper and whitefly along with resistance to other insect pests.

**Keywords:** Wide hybridization, Crossability, *Gossypium armourianum*, *Gossypium hirsutum*

#### INTRODUCTION

Cotton also referred to as “White gold” and “King of fibers,” is a significant fiber crop (Smith and Cothren, 1999) produced in more than 80 nations across the world. India generates 21% of the world’s cotton production and shares around 36% of the cotton zone. Concerning the cultivation alone, cotton supports labor for about 200 man-days/ha; provides enormous employment in processing, marketing, and other subsidiary industries throughout the year (Rajendran *et al.*, 2018).

Cotton crop is very much prone to a wide range of insect pest starting from Lepidopteran insects to the sucking

pest complexes. In the Indian scenario, about 166 insects were identified as cotton crop pests (Ayyar, 1932; Khan and Rao, 1960; Ingram, 1981; Puri *et al.*, 1999) belonging to group of the sucking insects, foliar and stem feeders, and boll feeders. Recently, the sucking pest problem has become very severe and to fight against them, development of resistance variety with sound resistance is a sustainable solution. These insects evolve very fast and tend to surpass the given resistance level in the variety quickly. Hence, the previously used resistance genes need more addition and backing up by other genes (Rajendran *et al.*, 2018).

The amount of genetic variability in the cultivated species is limited as far as the resistance against disease and pests is concerned (Manickam and Prakash, 2014). Hence there is an immense urge to widen the genetic base and developing insect-resistant varieties. Numerous instances showed how beneficial genes can be successfully introgressed from wild species to crop plants including wheat, oats, rice, mustard, tomatoes, etc. (Brar *et al.*, 2017). To develop novel plant types, incorporate new traits from concomitant wild species into the concerned crop, wide hybridization has been a tool of choice for plant breeders in the present and the future (Woo *et al.*, 2018). Botanically, out of the 50 species of cotton, only four are cultivated and rests of the specieses are wild. All the wild species are rich sources of many resistance genes (Khadi *et al.*, 2002). In India, wild species like *G. tomentosum* and *G. anomalum* have been used in prebreeding program at JNKVV, Indore and PKV, Akola to develop varieties like Badnawar 1, and PKV 081 respectively (Gotmare *et al.*, 2000). The wild diploid *G. armourianum* shows resistance against many insect pests like resistant to jassids, bollworm, and whitefly. Apart from that, the species *G. gossypoides* also exhibit a high level of resistance to jassid (Narayan and Singh, 1994; Mehetre, 2010). Given the sound level of resistance of the two wild species *G. armourianum* and *G. gossypoides*, these two species was chosen to develop prebreeding lines.

These wild species belong to different genome groups and have different ploidy levels as compared to the cultivated cotton. Hence combining them together in one background may yield many random and uncertain outcomes in the context of success in crosses, seed production, plant survivability and further progress to subsequent generations. The current study is attempted to assess all these aspects of the wide hybridization of the two wild species, *G. armourianum* and *G. gossypoides* with *G. hirsutum* along with the feasibility of developing and progressing in prebreeding activity in cotton.

## MATERIALS AND METHODS

The experiment was performed using two wild species

and three American cotton varieties. The details of the parents used in the experiment are described in **Table 1**. Hybridization was done using Doak's method (1934) by hand emasculation and dusting at Department of Cotton, TNAU, Coimbatore. The interspecific hybridization was carried out in a pattern similar to that of line x tester design during summer 2021, *kharif* 2021, and summer 2022. A total of 12 crosses were made in both direct and reciprocal manner. In direct crosses, the wild species were used as male parents and cultivated tetraploid as female and *vice versa*. The selfed seed of the cultivated varieties was collected from germplasm collection maintained at Department of Cotton, CPBG, TNAU, Coimbatore. Wild species parents are maintained at the wild species garden in the Department of Cotton, TNAU, Coimbatore. The cultivated parents were raised in crossing blocks with proper spacing. Proper agronomic practices were followed for a healthy crop. For an uninterrupted supply of pollen from cultivated species, they were raised in three staggered crops with a time gap of 15 days. Unlike the cultivated species, the pollen of the wild species matures a little late in the morning hence dusting for direct crosses was done around 9.30 -10:00 AM. The developed F<sub>1</sub> seeds were raised in evaluation block and their germination along with survivability were observed. For cytological analysis, the root tips were collected and further processing and mitotic observations done as per Maru *et al.* (2021). For observing pollen fertility, the mature flowers were collected from field early in the morning and the pollen was dusted on a slide with addition of 1% KI solution. After a gentle mix, the slide observed under microscope to count the fertile pollen. Five microscopic fields were taken into consideration for each entry for calculating the fertility percentage.

## RESULTS AND DISCUSSION

Direct and reciprocal inter-specific crosses were done in all three seasons. A total of 12,387 pollination events were performed across 12 crosses, the average number of crosses made for direct and reciprocal crosses were 1560 and 538 respectively. The extent of crossing was varying depending upon the availability of the wild species flowers

**Table 1. Details of the plant material used for the experiment**

Name of the species	Chromosome number	Ploidy	Genome group	Important characters
<i>G. hirsutum</i> (MCU5)	2n= 52	Tetraploid	(AD) <sub>1</sub>	Multicross derivative, Extra long staple cotton
<i>G. hirsutum</i> (CO14)	2n=52	Tetraploid	(AD) <sub>1</sub>	Derived from (MCU 5 x TCH 92-7) x MCU5-1, high yielding, good fiber quality, long staple cotton
<i>G. hirsutum</i> (CO 17)	2n=52	Tetraploid	(AD) <sub>1</sub>	Erect stature, medium staple cotton, suitable for high density planting system
<i>G. armourianum</i>	2n=26	Diploid	D <sub>2-1</sub>	Diploid wild sp. double palisade, smooth, caducous bract, resistant to leafhopper, bollworm and whitefly.
<i>G. gossypoides</i>	2n=26	Diploid	D <sub>6</sub>	Resistance to leafhopper

and their pollen load which differed significantly in different seasons throughout the year. The pollen load was very low for *G. gossypoides*. The sound flower availability and ample pollen load made it possible to make more number of crosses in *G. armourianum* combinations.

Out of the 12 crosses attempted, only three cross combinations were successful viz., MCU5x *G. armourianum*, CO17x *G. armourianum*, and CO14 x *G. armourianum* (Table 2). The direct crosses involving wild species *G. gossypoides* did not yield any crossed boll. This may be because of the action of fertilization barriers preventing the successful boll set in the above cross. The presence of pre-fertilization barriers has been demonstrated by Ganesh Ram *et al.* (2008) while utilizing the wild species *G. davidsonii* and *G. thurberi* in wide crosses. The successful production of viable seeds in all three direct combinations indicated the compatibility of *G. armourianum* with that of *G. hirsutum* (Table 2). Successful direct crosses using *G. armourianum* as male parent has also been reported by Kaur *et al.* (2016), Imtiyazahmed *et al.* (2020), Muthuraj *et al.* (2019).

The highest rate of boll set was obtained in the cross MCU 5 x *G. armourianum* with 16 bolls surviving till maturity whereas, for CO17 x *G. armourianum*, and CO14 x *G. armourianum*, 15 and 12 bolls were obtained, respectively. The size of the individual boll was comparatively smaller than that of the normal boll from the selfed one. It was also observed in all crosses with *G. gossypoides* that, bolls did set initially, but after 5-7 days of crossing, their further growth stopped.

In the crossed bolls, the seed were smaller in size and

morphologically deformed in many cases. Many locules inside boll were observed to have shriveled seeds; hence very less number of seeds per boll could be obtained. On an average, MCU 5 x *G. armourianum*, CO14 x *G. armourianum* had highest (16 seeds/ boll) and lowest (13 seeds/ boll) number of set seeds per boll respectively. Very less number of seeds, as low as four, were observed in few crossed bolls. The lower seed set might be due to the off-season weather parameters including wide temperature variation and high rain along with high humidity in summer and *kharif* season respectively.

Setting percentage gives the idea about the probability of getting success in any cross. As evident from the results obtained, the setting percentage was very low (0-1.33%) in all three crosses. The highest boll set was observed in the cross between MCU 5 and *G. armourianum* (1.33%) followed by cross between CO17 and *G. armourianum* (1.11%).

In direct crosses, on an average, 106 developing bolls were retained per cross after 1 day after crossing and 78 bolls were retained 2 days after crossing. The highest amount of boll drop was observed from 4-10 days after crossing and then a gradual reduction in the rate of dropping was observed. On other hand, for the selfing event performed in the cultivated parents, comparatively less boll drop was observed between 0-6 days after selfing (Table 3). Among the direct crosses during the 1<sup>st</sup> week of crossing, the highest boll retention was observed in CO17x *G. armourianum* followed by MCU5 x *G. armourianum*, whereas lowest retention was in the crosses involving *G. gossypoides* followed by CO 14 x *G. armourianum* (Table 3).

**Table 2. Crossability relationship between two wild diploid and tetraploid *G. hirsutum* cotton varieties**

S.No.	Name of the cross	Number of crosses made	Number of boll set (survived till maturity)	Number of seeds/boll	Setting percentage
<b>Direct crosses</b>					
A.	MCU5 x <i>G. armourianum</i>	1200	16	20	1.33
B.	MCU5 x <i>G. gossypoides</i>	1630	0	-	0
C.	CO 14 x <i>G. armourianum</i>	1656	12	13	0.68
D.	CO 14 x <i>G. gossypoides</i>	1750	0	-	0
E.	CO 17 x <i>G. armourianum</i>	1356	15	16	1.11
F.	CO 17 x <i>G. gossypoides</i>	1765	0	-	0
<b>Reciprocal crosses</b>					
G.	<i>G. armourianum</i> x MCU5	600	0	-	0
H.	<i>G. armourianum</i> x CO 14	580	0	-	0
I.	<i>G. armourianum</i> x CO 17	500	0	-	0
J.	<i>G. gossypoides</i> x CO14	535	0	-	0
K.	<i>G. gossypoides</i> x CO14	530	0	-	0
L.	<i>G. gossypoides</i> x CO17	485	0	-	0

Table 3. Number of bolls retained at different day intervals after pollination

Name of the cross *DAP	0 <sup>th</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	4 <sup>th</sup>	6 <sup>th</sup>	8 <sup>th</sup>	10 <sup>th</sup>	14 <sup>th</sup>
<b>Direct crosses</b>								
MCU5 x <i>G. armourianum</i>	150	140	118	59	23	15	8	5
MCU5 x <i>G. gossypoides</i>	150	112	53	8	0	0	0	0
CO 14 x <i>G. armourianum</i>	150	145	127	18	15	8	3	3
CO 14 x <i>G. gossypoides</i>	150	100	62	5	0	0	0	0
CO 17 x <i>G. armourianum</i>	150	138	87	45	45	32	6	4
CO 17 x <i>G. gossypoides</i>	150	108	24	12	0	0	0	0
<b>Reciprocal crosses</b>								
<i>G. armourianum</i> x MCU 5	150	108	46	31	25	12	0	0
<i>G. armourianum</i> x CO14	150	118	51	37	37	28	10	0
<i>G. armourianum</i> x CO 17	150	123	56	56	56	6	6	0
<i>G. gossypoides</i> x MCU5	150	111	35	5	0	0	0	0
<i>G. gossypoides</i> x CO14	150	135	72	3	0	0	0	0
<i>G. gossypoides</i> x CO17	150	48	12	6	0	0	0	0
<b>Wild parents</b>								
<i>G. armourianum</i>	150	142	133	123	115	112	112	112
<i>G. gossypoides</i>	150	120	45	0	0	0	0	0
<b>Cultivated parent</b>								
MCU 5	150	142	124	116	110	110	110	110
CO 14	150	145	121	112	112	112	112	112
CO17	150	138	113	106	101	101	101	101

\*DAP- Days after pollination

The results of crossability tests between cultivated *hirsutum* and wild diploid species indicated a close relation between A and D genomes leading to successful boll growth and viable seed recovery. Cytological investigations by previous workers like Pushpam and Raveendran (2006) also supported the crossability behaviour. They also mentioned the close homology between chromosomes of *G. armourianum* and *G. hirsutum*. The homology among the genome aids in the production of desirable recombinants despite minor cytological disturbances. Hence, D genome-based wild species can be used successfully in gene transfer with novel techniques to overcome fertilization barriers.

In the case of the reciprocal crosses, none of the combinations gave a successful seed set at maturity. In these crosses, different level of boll retention was observed. Large numbers of boll drops were seen in the 1st week after the pollination. Then, a comparatively lesser drop was observed in the later stage. Still, none of the bolls were retained in the plant after two weeks. Out of all reciprocals, the largest sized boll was developed in the case of CO14 x *G. armourianum* (2 cm in height and 1.5 cm in width).

It is previously stated in reports that the lowest ploidy

parent should be employed as the pollen source for interspecific hybrids in *Gossypium* (Beasley, 1941). In the current investigation, direct crosses between *G. armourianum* and *G. hirsutum* cultivars only produced bolls whereas, no success was obtained in reciprocals where the lower ploidy wild species were used as male which also showed similar pattern of success in the wide hybridization attempted.

After one day of pollination, out of the 150 samples in reciprocal cross, more than a hundred developing bolls were retained for all of them except *G. gossypoides* x CO17. On the 2nd day, almost half of the bolls retained fell across the crosses. In the crosses involving *G. gossypoides*, no more crossed bolls were found on the plants after 6th day. In both crosses, *G. armourianum* x CO14 and *G. armourianum* x MCU5, few crossed bolls were retained on the plant till the 10<sup>th</sup> day after crossing, but none of them survived till maturity (Table 3). Previous reports have indicated maximum retention of boll up to 0.7% when a tetraploid was used as a female parent in a tetraploid-diploid cross, and only 0.1% when a diploid was used as a female (Feng, 1935; Amin, 1940). Furthermore, Vijayalaxmi (1998) also reported low boll retention of 0.8% and 0.4% in direct and reciprocal crosses of tetraploid-diploid combination.

Gill and Bajaj (1984) and Pushpam and Raveendran (2006) have suggested the use of growth hormones to enhance boll retentivity. In the present study, growth hormones like NAA and GA<sub>3</sub> were applied continuously from the opening of the flower to three days after crossing, which enhanced the boll retention for 3-4 more days but again the bolls dropped.

Since successful F<sub>1</sub> could not be developed in the reciprocal crosses, and early stage dropping of boll was observed, embryo rescue can be attempted for further survival of the embryo. Umbeck and Stewart (1985) also suggested that the doubling of interspecific hybrids is necessary to restore plant fertility.

Out of the 12 crosses made, only three direct crosses were successful, all of them having *G. armourianum* as one of the parents. In the F<sub>1</sub> crop, a total of 202 seeds of three hybrids were sown. Out of which, the highest percent of germination was observed in MCU5 x *G. armourianum* followed by CO17 x *G. armourianum*. Up to 56% of seed germination was observed in MCU5 x *G. armourianum*, while in CO14 x *G. armourianum* only 35.21% of seed germinated. Out of the 42 seeds germinated giving rise to seedlings in MCU5 x *G. armourianum*, only 28 made it to maturity with a seedling survivability of 66.67%, whereas better seedling survival was seen in the rest two F<sub>1</sub> progenies. *G. armourianum* progeny with CO14 had a survival percentage of 72 % and those with CO17 had a 77.78% survival (Fig. 1). The F<sub>1</sub> hybrids took a longer time to germinate compared to the cultivated parents.

All these plants produced profuse flowering almost throughout the year with a low level of fertility to complete sterility. The fertility level of all the three F<sub>1</sub> is depicted in Fig 2. The fertility level of the three *G. armourianum*

based hybrids ranged from 6.13 % to 14.07 %. Highest fertility was observed in CO14x *G. armourianum* (14.07%) followed by MCU5x *G. armourianum* (11.90%). Pushpam and Raveendran (2006) have also reported an average low fertility level of 9.04% in *G. hirsutum* x *G. armourianum* hybrid. Similar results were also observed by Kaur et al. (2016).

Cytological study of the root tips has showed the presence of 39 chromosomes undergoing mitosis in all the F<sub>1</sub>s studied confirming the triploid nature of the hybrid (Fig. 3). Previously, Manickam and Prakash (2014) have also observed presence of 39 chromosome and the triploid condition in the interspecific hybrid produced from cross between *G. armourianum* with *G. hirsutum*. In case of the triploids, since three sets of one chromosome are present, usually they tend to pair randomly. In this case, the triploid nature of the hybrid might also be associated with abnormal pairing, possibly resulting in poor fertility of F<sub>1</sub>. In the experiment also, it was noticed that, even after a meticulous effort to develop F<sub>2</sub> seeds, no success was obtained. Here, the triploid condition with genomic imbalances may be the reason for the unsuccessful seed set. The synthesis of a sterile interspecific F<sub>1</sub> and doubling of chromosomal complement to attain fertility are the possible methods proposed for circumventing the ploidy barrier (Stewart, 1995).

Chromosome pairing revealed a close resemblance between the D sub genome of *G. hirsutum* and *G. armourianum* (D<sub>2-1</sub>) (Pushpam and Raveendran, 2006). Other reports by Skovsted (1937) and Webber (1939) has also showed that the genomes of A and B are more homologous than those of B and D, which may be taken into consideration while progressing further with prebreeding activity.

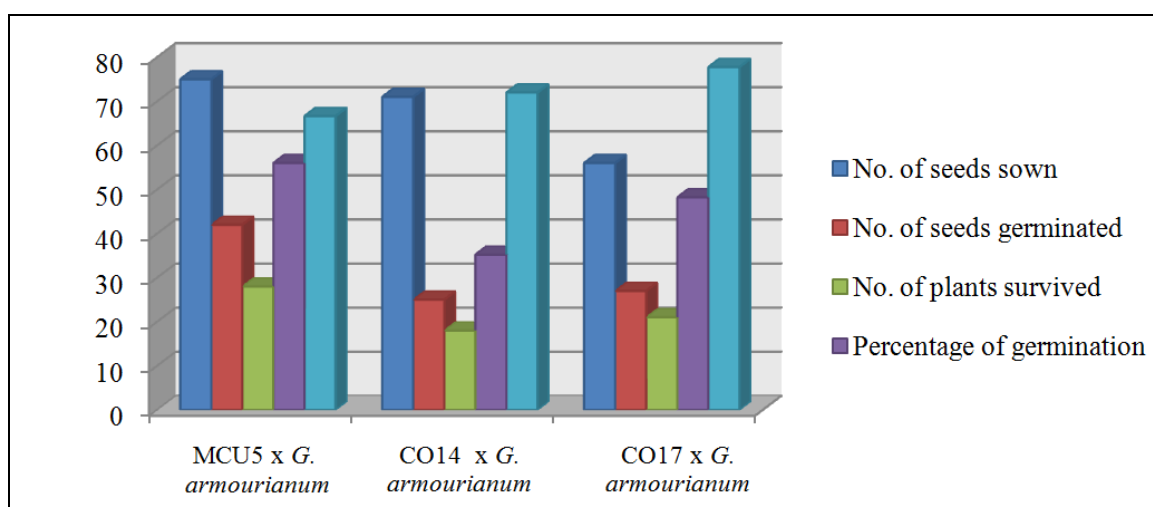


Fig. 1. Survivability of the F<sub>1</sub> hybrids



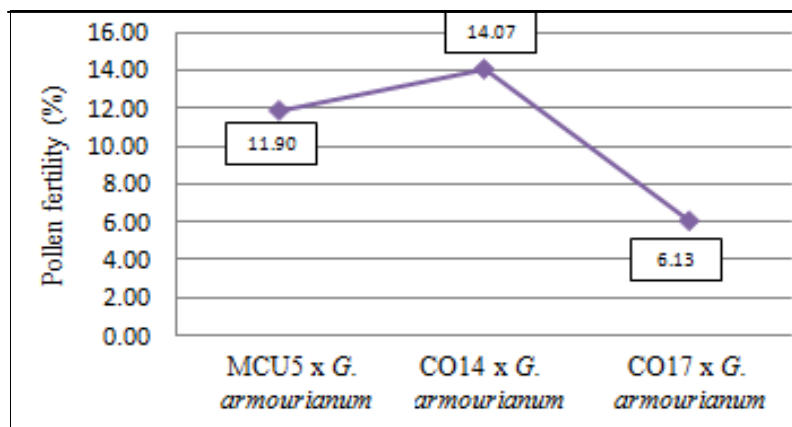


Fig. 2. Pollen fertility level of the three F<sub>1</sub> hybrids

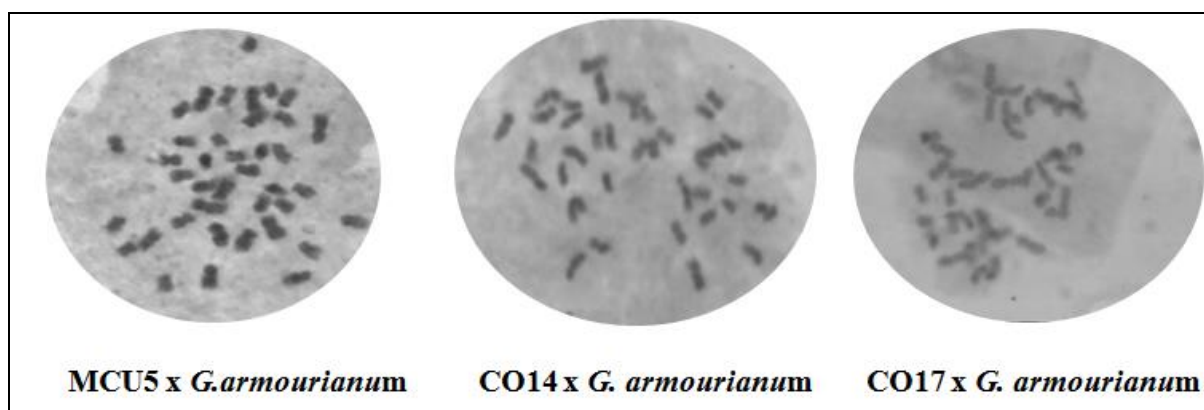


Fig. 3. Mitotic metaphase chromosome of the three F<sub>1</sub> hybrids

Though the direct crosses gave some amount of success in the wide hybridization, the reciprocals and further progress of F<sub>1</sub> to next generation was not possible. Nevertheless, the lines developed in the direct crosses are very important genetic material concerning the introgression of the resistance genes against not only the insects but also for other biotic stresses such as bacterial blight. They need to be evaluated properly and further special techniques like embryo rescue can be applied for recovering immature/abortive embryo in early stage. Doubling chromosomes of the diploid parent and further crossing may also be attempted. With further efforts, the prebreeding lines developed has immense potential to be used in the breeding process for introgressing the useful characteristics.

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