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Crossability Relationship between Wild Cotton *G. anomalum* and *G. aridum* with Upland Cotton (*G. hirsutum*)

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ABSTRACT

Background: Utilizing two wild cotton species, *Gossypium anomalum* [Wawra and Peyritsch (2n=2x=26) B_1] and *Gossypium aridum* [Skovsted (2n=2x=26) D_4], extensive wide hybridization event carried out with upland cotton to assess the feasibility and ease of delivering genetic variation responsible for resistance to various stresses.

Methods: The crossing was carried out using the wild species and the cultivate varieties of upland cotton in 2021-2022 at TNAU, Coimbatore. Total of 12 crosses were attempted and the various aspect regarding the feasibility success of different crosses was studied.

Result: In contrast to their reciprocals, viable offspring were generated in the direct crosses with tetraploid parents employed as seed parents with both wild species. A huge bulk of bolls dropped during the first seven days of pollination and no matured boll set was observed in reciprocals. Most *G. anomalum* hybrids produce F_1 offspring with thick and long leaf hairs which help the plant attain resistance against sucking pests. Through successive backcrosses or chromosome duplication, such genetic material potentially can be utilized furthermore in plant breeding to provide new beneficial resistance traits apart from other significant and relevant features.

Key words: American cotton, Crossability, Interspecific hybridization, Wild diploid cotton.

INTRODUCTION

Cotton is the king of fibers and its tremendous economic value has earned it the title "white gold." This crop is commonly grown in more than 80 different nations around the globe (Sethi, 1960). India generates more than one-fifth of the overall cotton used worldwide out of the 36% of the area dedicated to cotton farming. It offers several job prospects and work opportunity all year around (Rajendran *et al*., 2018).

More than a thousand insects damage the cotton crop in varying degrees by eating the leaves, bolls, or other components of the plant (Hargreaves, 1948). About 150 plus pests were identified to be common in the Indian setting out of them (Khan and Rao, 1960; Puri *et al*., 1999). These make cotton less sellable and have a poor market value. Farmers frequently employ pesticides to control them, which is highly expensive and causes significant ecological damage.

Utilizing the available sources, numerous resistant types have been created, but as a result of the recurrent exploitation of the same resistance base, the biological origin and genetic basis have become much more restricted. Additionally, the amount of diversity in the genome is extremely low (Manickam and Prakash, 2014). This results in the quick disintegration of resistance and the resurrection of new pests. Therefore, it is imperative to explore new genes and incorporate them into the cultivated background (Rajendran *et al*., 2018). When it comes to many major field crops like rice, wheat, oats and others, wild species and their relatives have been a significant source of a large array of resistance genes (Brar *et al*., 2017). Wild *Gossypium* are rich source of the important traits which are required to be carefully explored (Khadi *et al*., 2002).For creating distant hybrids between B, D and AD genomes in this experiment, great care has been taken in this experiment. Given that the D genome is one of the cotton *hirsutum* parent genomes, it appears to be a reasonable likelihood that it would produce successful progeny.

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G. anomalum belongs to the B_1 genome group and diploid in nature with its origin in Africa. It has high level of fiber strength, fiber length and longer elongation with high fiber yield. Other than this good fiber character, it also showcases higher level of resistance against bollworms, jassid, mites and diseases like bacterial blight, drought resistance. In other hand, *G. aridum* falls in D_4 genomic group originating from America. This is one of the species of choice for prebreeding activity for multiple useful traits. It has been used for transfer for the transfer of the characters like cytoplasmic male sterility, drought resistance *etc*. (Narayan and Singh, 1994; Mehetre, 2010).

MATERIALS AND METHODS

This investigation into interspecies hybridization involved the wild cotton species *G. anomalum* and *G. aridum* and three well-known *hirsutum* types, MCU5, CO14 and Co17. CO14 is a high-yielding variety that produces long-staple cotton of good quality, whereas MCU5 is a multi-cross product that yields extra long staple cotton. The erect type CO17 variety employed in the study has a high yield and is ideal for planting in large densities.

Hand emasculation and crossing were performed at the Department of Cotton, TNAU, Coimbatore. Emasculation at the candle stage was performed the evening before the day of crossing and the very next morning dusting was conducted. The crossing is carried out over three seasons the summer of 2021, the *kharif* of 2021 and the summer of 2022. Tetraploid and diploid were employed as females in 12 direct (tetraploid × diploid) and reciprocal (diploid × tetraploid) crosses, respectively. In the wild species garden in the Department of Cotton, TNAU, Coimbatore, all wild species are raised and preserved. The *hirsutum* varieties were raised in crossing blocks using proper agronomic management techniques with three staggering in each season spaced 15 days apart.

RESULTS AND DISCUSSION

The plan of work in the current breeding programme in all three seasons involved direct and reciprocal inter-specific crosses. The average number of pollination made in direct crosses is 874, while the average number of crosses in reciprocals is 338. This results in a total of 7279 pollination events across 12 crosses. The flowering of the wild species and the availability of pollen varied greatly throughout the year according to the seasons hence, becoming the key factors in the degree of pollination events for a particular cross. The crossability relation between the diploid wild species and the upland cotton has been Table 1.

For *G. aridum*, the pollen load was very marginal and had few blooms throughout the year. Regarding combinations comprising the *G. anomalum*, a greater number of crosses were possible because of the high and abundant pollen load.

Direct crosses

Only five effective cross combinations *viz*. MCU5 × *G. anomalum*, MCU5 × *G. aridum,* CO14 × *G. aridum*, CO17 × *G. anomalum* and CO14 × *G. aridum* were produced out of the 12 crossings endeavored. The majority of direct combinations producing viable seeds showed that the wild species *G. anomalum* and*G. aridum* were compatible with American cotton,*G. hirsutum*.

Previous reports suggest, when a tetraploid was employed as a female parent in a tetraploid-diploid cross, maximum boll retention was up to 0.7%, but only 0.1% when a diploid was utilized as a female (Feng, 1935; Amin, 1940). In addition, Vijayalaxmi (1998) evidenced that direct and reciprocal tetraploid-diploid crossings had low boll retention of 0.8% and 0.4%, respectively. According to Beasly (1941), using a lower-ploidy male parent increases the likelihood of succeeding in interspecific hybrids in the *Gossypium* genus.

Table 1: Crossability relationship between wild diploid and tetraploid *G. hirsutum* cotton varieties.

Name of the cross	No. of crosses made	No. of boll set (survived till maturity)	No. of seeds/boll	Setting percentage
Direct crosses				
MCU 5 × *G. anomalum*	1120	8	13	0.71
MCU 5 × *G. aridum*	425	5	8	1.17
CO 14 × *G. anomalum*	1600	0	-	0
CO 14 × *G. aridum*	400	7	10	1.75
CO 17 × *G. anomalum*	1230	5	10	0.41
CO 17 × *G. aridum*	472	3	10	0.63
Reciprocal crosses				
G. anomalum × MCU 5	555	0	-	0
G. anomalum × CO14	540	0	-	0
G. anomalum × CO17	450	0	-	0
G. aridum × MCU 5	168	0	-	0
G. aridum × CO14	163	0	-	0
G. aridum × CO17	156	0	-	0

No. of boll set

Among the crosses involving *G. anomalum*, the highest rate of boll set was observed for MCU 5 × *G. anomalum* where 8 bolls survived until maturity as opposed to CO17 × *G. anomalum* with 5 bolls. The CO17 × *G. aridum* cross had three mature bolls, whereas the MCU5 × *G. aridum* and CO14 × *G. aridum* crosses only produced five and seven successful crossing bolls, respectively. No crossed bolls produced in the direct crosses. Compared to the typical boll from the selfed one, the individual boll from the inter-specific crossed plants is relatively small in size. Additionally, all crosses show that bolls did set initially, but after 7-10 days, their further growth ceased.

No. of seeds/boll

The seed in crossed boll were slightly smaller and some are partially malformed ones. Although many bolls appeared healthy from the outside, they did not contain adequately developed seeds. Few seeds were collected from each boll because most of the locules inside the boll had very small to shriveled seeds. In nutshell, MCU 5 × *G. anomalum* yielded the most seeds per boll (13 nos.) and MCU 5 × *G. aridum* produced the fewest seeds (8). Rest three fruitful crosses (CO14 × *G. aridum*, CO17 × *G. anomalum* and CO17 × *G. aridum*) contained ten bolls each.

In cotton, the main obstacle limiting inter-specific hybridization is the lesser retention of fertilized bolls and the failure of survival of mature bolls. The outcomes of tests on crossability among cultivated *hirsutum* and wild species revealed a strong relationship between the A and D genomes, resulting in good boll growth and viable seed recovery.

Setting percentage

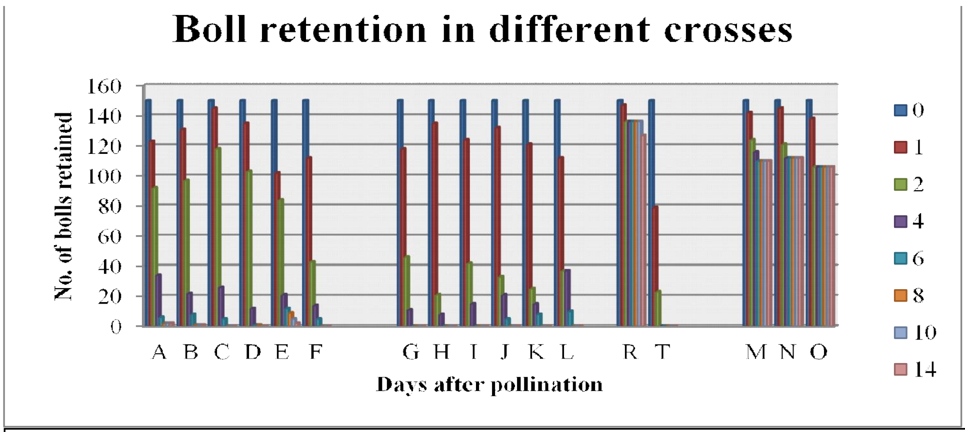
In any cross, the likelihood of success can be judged by the parameter setting percentage. The setting percentage in all five crosses ranged from 0.41 to 1.75%, as is evident from the figures. The hybrid between CO14 and *G. aridum* had the highest rate of boll set, measuring 1.75%. In this type of crossing programme, attaining boll set was quite challenging. Multiple seasons crossing performed to achieve this purpose. The offseason weather conditions, which included significant temperature volatility, heavy rainfall and high relative humidity in the summer and *kharif* seasons, may have contributed to the reduced seed set.

No. of bolls retained at different day intervals from crossing

On average, 124 developing bolls from direct crosses survived after the first day after crossing but, only 89 of them remained on the plant after the second day of pollination. However, after 4 days of crossing, merely 21 of the 150 bolls remained with the plant. The highest boll drop happened between 4 and 10 days after crossing. Then, there was a steady slowdown in the rate of dropping. On the other hand, a substantially lesser boll drop was seen between the first and sixth days following the selfing event carried out in the cultivated parents (Fig 1).

The maximum boll retention was seen in CO17 x *G. anomalum* among direct crossings during the first week after crossing. In the direct crosses, after 1st week of crossing, the highest boll retention was observed in CO17 x *G. anomalum*, MCU5 × *G. anomalum* and the lowest was in the crosses involving *G. aridum* with CO17 and CO14.

With cytological studies, previous reports have validated the way of behavior of one species with another in wide hybridization (Puspam and Raveendran, 2006). The similarity between the chromosomes of *G. hirsutum* and *G. anomalum* has also been reported. As there are effective boll setups and viable seed production in the current study, the higher similarity between the A and D genomes facilitates the development of acceptable recombinants despite little cytological abnormalities. As a result, new approaches for gene transfer can be successfully applied to wild species with D genomes to overcome reproductive barriers.



A-F- Direct crosses in series same as table 1 G- L- Reciprocal crosses same as table 1 R- *G. anomalum* T- *G. aridum* M- MCU5 N- CO14 O - CO17

Fig 1: Number of bolls retained at different day intervals from crossing in parent and hybrids in direct and reciprocal manner.

A close relationship between the D subgenomes was established by observation on chromosome pairing previous reports (Puspam and Raveendran, 2006). Additionally, A and B genomes are more homologous than B and D genomes, as per Skovsted (1937) and Webber (1939). According to these experts, it might be difficult to introduce B genomic chromosomes or fragments to A_hD_h. The current findings make it evident that, despite minor cytological anomalies, the high level of homology between the A and D genomes facilitates the emergence of desired recombinants by promoting healthy boll setting and seed germination.

Reciprocal crosses

For reciprocal crossings, none of the arrangements produced mature seeds set successfully and alive till maturity. Different levels of boll retention were found in the reciprocal crosses. The first week following pollination witnessed a profusion of boll drops. Then, a relatively lesser fruit drop was observed. Even so, after two weeks, none of the bolls were kept in the plant. The highest amount of the boll formed in *G. aridum* × CO17 among all reciprocals (Fig 1).

Over a hundred growing bolls were intact on the plant in each of the 150 samples in reciprocal cross after one day of pollination whereas, only less than fifty bolls remained on the plant after days of pollination and the rest fell in one day itself. No more bolls were found on the plants in the *G. anomalum* parentage progenies after the sixth day. Few bolls remained on the plant in all three crosses involving *G. aridum* through the sixth day following the crossing, but none of them continued to grow through the eighth day.

The failure of reciprocal cross-configuration might be a result of the species having fertilization barriers. Ganesh Ram *et al*. (2008) have shown that pre-fertilization barriers exist in this kind of wide cross. Beasly (1941) suggested that the pollen source for interspecific hybrids in *Gossypium* should be the lowest ploidy plant.

Survivability of the F_1 hybrids

Five of the F_1 crosses from six direct crosses succeed. Next-generation crops are produced from the bolls harvested. A total of 128 seeds from five hybrids were sown in the F_1 crop. The CO17 × *G. anomalum* variety had the highest germination percentage, which was close to 60% and led to the growth of five F_1 plants. Furthermore, unlike when combined with CO17 (46.67%), in rest two crosses involving *G. aridum*, more than 50% seed occurred. A marginal 47.27% of the combination MCU5 with *G. anomalum* seeds germinated (Fig 2).

MCU5-*G. anomalum* progenies had a seedling establishment rate of 76.92%, resulting in 20 plants, compared to 71.43% for CO17 progeny. In *G. aridum* progenies, the combination with MCU5 had the highest progenies survival rate (75%), while CO14 and CO17 had relatively lower rates (62.5% and 57.14%, respectively). The MCU5 × *G. anomalum* progeny had the best survival rate of all the F_1s produced. Compared to their cultivated parents, the F_1 hybrids took longer to germinate. The propensity of resistance to sucking pests in F_1 progenies is due to spongy leaves with dense trichomes from the wild parent *G. anomalum*. The *G. anomalum* gene pairing with MCU5 and CO17 in the current work gave rise to progeny with prolific flowering nearly all year round, ranging from partial sterility to low fertility. The sterility and low fertility of F_1 may be due to the aberrant pairing of triploids. Because of this, despite intensive efforts to create F_2 seeds, a successful boll was not observed.

Most of the crosses faced numerous dropping of the selfed bolls after crossing. Growth hormones like NAA and GA_3 are given regularly from the flower opening until three days after selfing, which reduces boll shedding and improves the boll retentivity. Similar results were observed by Gill and Bajaj (1984); Puspam and Raveendran (2006) and others. The failure of the seed set in this instance may be due to the triploid state with genetic abnormalities. The only approaches to get around the ploidy barrier have been the creation of a sterile intergenomic F_1 and doubling chromosomal complement to achieve conception (Stewart, 1995).

As early-stage boll dropping occurred and viable F_1 and further F_2 could not be produced in the reciprocal crosses, embryo rescue may be used in order to increase the embryo's chances of survival. Moreover, Umbeck and

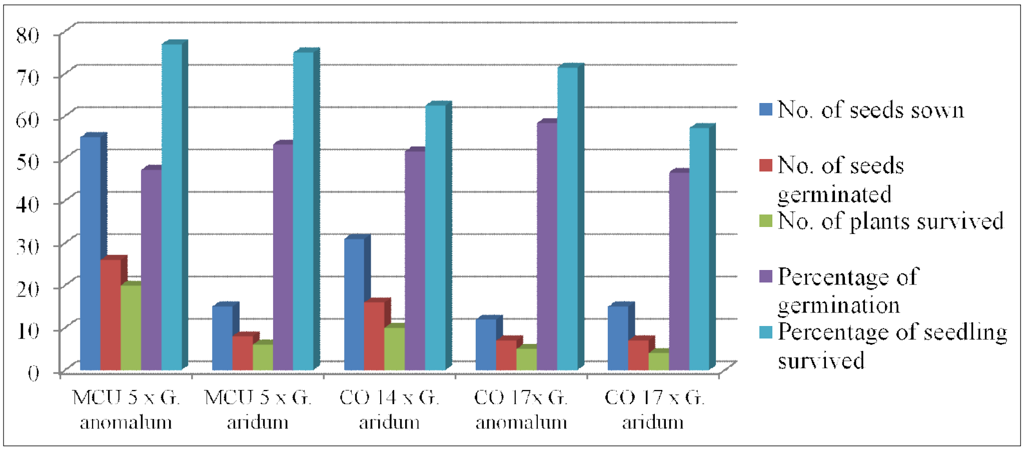


Fig 2: Survivability of thea F_1 hybrids.

Stewart (1985) recommended the doubling of interspecific hybrids for restoring plant fertility. Colchipoidy can be used for restoring F_1 fertility. The derived lines will form an important part of prebreeding germplasm and can further be utilized in the genetic enhancement breeding process to introduce jassid resistance and other beneficial traits.

CONCLUSION

Wild forms and wild relatives constitute vary important gene reservoir for any crop. Their goodness can be harnessed by use of wide hybridization and the further backcross events. But the success rate of these crosses depends on the several genetic, evolutionary and nongenetic factors. The current study has showed the success of the direct crosses where the wild species are used as male, but the reciprocals failed to give any success in cross. Embryo rescue and other tissue culture method are the probable suggested way for getting better results.

Conflict of interest: None.

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