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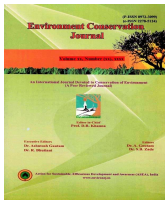

Characterization of a cotton interspecific hybrid of American cotton with wild species *G. armourianum*

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ABSTRACT

Wild species constitute a source of valuable genes for many adverse climatic conditions, disease and pests. To match up the level of quick depleting insect pest and disease resistance and fast evolving pests, it is the urge of the hour to broaden the resistance base. In order to achieve this goal in cotton, wide hybridization was performed between *G. hirsutum* (AADD) cv. MCU5, CO14 and CO17, and *G. armourianum* and interspecific hybrids developed were characterized for several morphological characters for obtaining an idea about the status of the various traits. Interspecific hybrid developed with all three *G. hirsutum* varieties are potential lines for future introgression programs of insect and disease resistance along with other useful traits. The F_1 hybrid displayed intermediate expression for most of the traits. Traits like colour of the stem, leaf colour, position of stigma, nectarines of hybrid completely resembled wild parent-and are considered as dominant in expression. The petal spot was present in the hybrid similar to that wild parent, unlike the cultivated parent; this appeared with different levels of intensity in F_1 along with other characters like colour of the anther, and filament colour. Hybrids had profuse flowering throughout the year with low pollen load and pollen of variable shape and size expressing sterility to partial fertility. Noteworthy differentiation was seen between the leaf size and size of other plant parts of the hybrid.

Introduction

Botanically, cotton belongs to the genus *Gossypium* and the family Malvaceae. About 50 species constitute this genus, including five allotetraploids (2n = 4x = 52) and 45 diploids (2n = 2x = 26) (Gotmare *et al.,* 2000). All four cultivated species are grown commercially in India (Blaise and Kranthi, 2019). Among the D genome diploid wild species counting to 13 in number, *G. armourianum,* is resilient towards a variety of insects, pests, and diseases, especially hazardous pink bollworms and jassid (Narayanan *et al.*, 2014; Pushpam and Raveendran, 2006; Kaur *et al.*, 2016). Numerous instances have demonstrated the significance of cultivated as well as wild *Gossypium* diploid genomes as reservoirs of commercially valuable genes (Mehetre *et al.*, 2002; Kebede *et al.*, 2007). To harness the useful features of the wild germplasm, wide hybridization with a further advanced tool to recover the fertile progeny constituting valuable pre-breeding lines are the method of choice by the breeders.

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The cultivated variety chosen for the study has moderate to high susceptibility to the various sucking pests such as cotton jassid, aphids and whitefly, which not only causes loss directly by sucking the sap and burning the surface of the leaf but also attract secondary infections. Differentiating the hybrids is possible at very early stage of crop growth with the presence of sufficient and strong morphological markers. Previous reports suggest the utility of various qualitative and quantitative characters of different parts like a leaf; stem flower characters in differentiating the triploid progeny from tetraploid and diploid parents (Manickam and Prakash, 2014).

As the aim of the study was to develop prebreeding lines and transfer of the useful resistance genes present abundantly in the wild species, the crosses were made and all the character starting from the leaf related to stem related, all were carefully observed. Morphological observation is given priority in this experiment considering the fact that, in wide hybridization it is rare to achieve the combinations with desirable trait. Hence, before moving for further generation and undergoing any expensive molecular breeding program for further transfer of the traits, morphological characterization can give sound amount of idea about the status of visually expressive traits.

Material and Methods

The wide hybridization was carried out between *G. hirsutum* cv.MCU5, CO14 and CO17, and *G. armourianum* during *Summer,* 2021, *Summer*, 2022, and *Kharif*, 2022. MCU5 is a multi-cross derivative famous for its extra-long staple length obtained from the germplasm maintained at TNAU, Coimbatore. The CO14 and CO17 are the improved high yielding variety of American cotton developed at the Department of Cotton, TNAU, Coimbatore. The wild species *G. armourianum* is maintained as perennial at the wild species garden at Department of Cotton, TNAU, Coimbatore. The flowers were emasculated at the candle stage, the previous day of anthesis by removing anthers by the Doak's method (Doak, 1934). Since huge numbers of crosses were to be attempted in wide hybridization, considering ease of handling, the Doak's method was followed. The next day morning, the emasculated flowers were dusted, and the buds were covered with a white butter paper bag. The dusting date along with the parentage of crosses recorded on jewel tags.

For gathering information on several morphological aspects, the F_1 hybrid and its parents were examined. The traits like growth habit of the plant, coloration, extent of hairiness of stem shape, lobe number, size, pubescence, presence of nectarines in leaf, the shape of the bract, and the number of serrations on it were observed. Apart from these traits, the other aspects of plants like colour, presence of spots in petals, pigmentation in filaments, colour and average no. of the anther, pollen size, and boll shape were also examined. All the characters were observed on fully developed plants. The presence or absence of pubescence on the stem and leaves was recorded. On the abaxial surface of the leaves, at the midrib, the number of nectarines was counted.

Results and Discussion

Table 1 depicts the morphological features of the parents, *G. hirsutum* varieties, *G. armourianum*, and their hybrid progenies. For a series of traits, hybrids displayed either dominance from one of the parents or intermediate expression. It was also revealed that in the hybrid, growth habits, petal coloration, the shape of the leaf, and leaf size and incision were all at the intermediate level (Table 1). Parallel result was obtained in the experiment performed by Mahalingam *et al.* (2020). The intermediate level of expression and availability of a wide range of trait expression for each of them shows probable polygenic nature of these traits.

All the cultivated species used in the crosses were annual and erect type in nature whereas, the diploid male being the wild species is naturally perennial and spreading in growth habit. CO17 parent had the most erect stem and occupied the least area among the cultivated ones. *G. armourianum* is a very spreading one as far as branching is concerned. It was not much tall but heavily spreading. The hybrids have unanimously shown the perennial tendency as that of the wild parent. But the plant spread showed intermediate expression and semi-spreading in nature. The wild parent had a brownish-purple stem whereas, three cultivated parents all had a greenish-brown colour. The F_1 across three crosses had a brownish to the grayish purple stem. Glabrous stem surface was there in the wild parent but, the cultivated parents varied a lot

Table 1: Characterization of the different hybrids produced in combination with *G. hirsutum*-parents and *G. armourianum*

SN	Characters	*G. armourianum*	CO14	CO14 x *G. armourianum*	MCU 5	MCU 5 x *G. armourianum*	CO17	CO17 x *G. armourianum*
1.	Growth habit	Perennial, spreading	Annual, Erect	Perennial and semi spreading	Annual, erect	Perennial, Semi spreading	Annual, Erect	Perennial shrub
2.	Stem colour	Brownish purple	Greenish brown	Brownish purple	Dark green With brown	Brownish purple	Dark green With brown	Brownish green
3.	Stem pubescence	Absent	Moderately pubescent	Moderately pubescent	Sparsely pubescent	Moderately pubescent	Sparsely pubescent	Moderately pubescent
4.	Leaf shape	Ovate	Palmate	Palmate to ovate	Palmate with 3-4 lobes	Palmate with slight lobes	Palmate with 3-4lobes	Palmate with 2-3 lobes
5.	Leaf colour	Dark green	Green	Dark green	Green	Dark green	Green Light green	Dark green
6.	Leaf lobe	No lobe	Check	1-3 lobe	Five	2-4	Five	2-4
9.	Leaf incision	Shallow	Deep	Moderate deep	Shallow to Moderate deep	Shallow	Deep	Shallow or absent
10.	Leaf veins	Thin	Thick and prominent	Thick and Prominent	Thick and prominent	Thick and prominent	Prominent	Medium thick and prominent
11.	Leaf texture	Smooth	Smooth	Moderate	Medium smooth and thin	Smooth and thick	Medium smooth and thin	Smooth and thick
12.	Leaf hairiness	Glabrous	Medium	Moderate	Sparse	Sparsely hairy	Sparse	Sparsely hairy
13.	Leaf size	Absent	Big	Intermediate	Big	Intermediate	Big	Intermediate
14.	Bract type	Caducous	Cordate	Cordate	Cordate	Cordate	Cordate	Cordate
15.	Bract size	Small	Large	Medium	Medium	Small	Medium Large	Small Medium
16.	Petal colour	Yellow	Yellow	Yellow	Creamy white	Creamy white	Yellow	Yellow
17.	Petal size	Medium	Large	Large	Medium	Medium	Large	Medium
18.	Petal spot	Dark pink	Absent	Light to dark pink	Absent	Light to dark pink	Absent	Light to dark pink
19.	Anther density	Dense	Dense	Moderate	Dense	Medium	Dense	Medium
20.	Pollen colour	Yellow	Light yellow	Creamy yellow	Yellow	Yellow	Yellow	Yellow
21.	Filament colour	Absent	Absent	Creamy white to purple	White to creamy White	Variable colouration	Absent	Variable colouration
22.	Position of stigma	Protruded	Protruded	Protruded	Embedded	Protruded	Embedded	Protruded
23.	Nectarines	Absent	Present	Absent	Present	Absent	Present	Absent
24.	Connective	Pink to purple	Colourless	Coloured variable	Colourless	Coloured variable	Colourless	Coloured Variable

for the level of pubescence on the stem. MCU5 and CO17 had sparsely pubescent stems. Combinations with MCU5 and CO17 showed a glabrous stem like wild parent whereas; the CO14 hybrid had a moderately hairy stem. Out of the three hybrids developed, the *G. armourianum* hybrid with CO14 had highest level of stem pubescence followed by MCU5 x *G. armourianm* which seemingly contributed to jassid resistance as the crop grew further. Substantial variations were noticed between each parent and the hybrid in terms of petal and leaf sizes (Fig.1, Fig. 2). In contrast to *G. armourianum*, having cordate-shaped foliage with no lobes, MCU5 has palmate leaves with 3–4 distinct lobes. The number of lobes in the palmate leaves of the F_1 hybrid varied greatly, whereas some plants showed many leaves with no to one or two leaves, some branches even showed lobation similar to that of the cultivated parent but comparatively smaller in size and depth of serration (Fig. 2).The leaves with no lobes mostly have a similar shape to that of the wild parent but are larger than it. The depth of serration in the lobes was shallower than the parent plant. Even some branches had all combinations together. The trend of the hybrid to show higher level of serration is expected to contribute further to the jassid resistance. Dark green colour leaves were found in the F_1 progenies similar to the wild parents, thus showing dominance for the trait. Thick and prominent leaf veins observed in the hybrids of all three crosses were similar to that of the *hirsutum* parent. But, the veins were not as prominent as that of the parent. Leaf nectaries were not present in the *hirsutum* parent. The hybrid progenies had similar characteristics like the wild male parent showing complete dominance for the trait.

The wild parent had glossy leaves but, the *hirsutum* parent is moderately smooth but comparatively has thicker leaves. In the progenies, they had smooth surfaces on the leaf with a thickness higher than the wild parent. CO17 x *G. armourianum* had the thickest leaves among the three hybrids. *Hisrutum* parents had a sparse amount of hair whereas; the wild diploid parent was glabrous. A moderate amount of hair was there on F_1 hybrids. The progenies with CO17 were the least hairy among the three hybrids. Trichome parameters are quite important concerning the sucking pest resistance. Since the wild parent was glabrous in nature, much improvement in trichome character was not observed among the hybrids produced for leaf hairiness. Petal colour in all three F_1 resembled their female parent mainly with a wide range of variation. The colour of the flower in the cross with MCU5 was creamy white to light yellow, whereas CO14 had yellow coloured petals. As far as the size of the petals is concerned, the progenies also had medium-sized petals.

The mature flower of the wild parent was completely devoid of bracts showing caducous nature, where bracts shed at a preliminary phase of flower development, even before the opening of the flower. Both the seed parent and hybrid had three normal bracts with teeth-like serrations. The female parental bracts were bigger than that of the hybrid (Fig.2). Petal spot, as usual, was lacking in the parent American cotton but visible in *G. armourianum*. In the F_1hybrid, various flowers from the same plant and the offspring of the same cross varied in petal spot size and intensity. The colour ranged from pale to dark pink, and the degree of development as a male parent (Fig.3). The petal spot, a crucial morphological indicator for confirming hybridity, exhibited variable expression strength. On the other hand, intra-*hirsutum* crosses between wild type and mutant strains revealed complete dominance of the petal spot (Ahuja and Dhayal, 2007).Other researchers have noted that *G. hirsutum* x *G. arboreum* hybrids had less colour intensity in their petal spots than F_1hybrids (Tahir *et al.*, 2011; Ahmad *et al.*, 2011, Kaur *et al.*, 2016). Mixed variable expression of petal spots was also observed in *G. hirsutum* and *G. barbadense* interspecific hybrids grown in India (Kaur*et al.*, 2016).The probable reason for the expression of petal spot might be because of variable expressivity for the trait. In the current investigation, an analogous response was observed in the case of filament coloration ranging from creamy white to white in both parents. The F_1 hybrids, however, had filaments that spanned from colourless to varied colours in various flowers. Within the same flower, few anthers displayed colourless anthers while others had coloured anthers. Similar results have been reported by Pushpam and Raveendran (2006), Mahalingam *et al.* (2020). Pollen colour of the F_1 was seen to be light yellow to yellow in colour, intermediate between two parents in all three crosses.

MCU5 x *G. armourianum* CO14 x *G. armourianum* CO17 x *G. armourianum*

Figure 1: Flower structure of different *G. armourianum* based hybrid with *G. hirsutum*

MCU5 x *G. armourianum* CO14 x *G. armourianum* CO17 x *G. armourianum*

Figure 2: Variation among the *G. armourianum* hybrid with different *G. hirsutum* varieties.

The connective tissue interconnecting the pollen-carrying anther and the filament were not having any colour in the female parent but was coloured in the male. However, the hybrid had both colourless and coloured connectives present in the different flower and also in same flower. Previous researchers hypothesized that the varied expression may be caused by epigenetic changes in the DNA (Kaur *et al.*, 2016). Mahalingam *et al.* 2020 has also reported similar results regarding the colour of connective tissue. The dark colouration in the connectives seems to have role in attracting the pollinators and aiding to the pollen dissemination.

The current findings regarding growth habit, petal colour, and leaf shape do not complement those of the dominant expression, because the hybrids exhibited variable and intermediate types of expression rather than a specific expression identical to one of the parents. For certain traits like plant stem colour, position of stigma, leaf colour and nectarines, the wild diploid parent was revealed to be dominant since the hybrid closely matched with it. These observations are in line with those noted by Mahalingam *et al.* (2020). But the traits like stem pubecence, pollen colour were found to be intermediate. These traits expression are in contrast to dominance as reported by previous author (Mahalingam *et al.*, 2020). Polygenic and epistatic interactions might be present in plant concerning these traits yielding intermediate expression. The current findings support data previously revealed by Pushpum and Raveendran (2006), Kaur *et al.* (2016), Muthuraj *et al.* (2019), and Mahalingam *et al.*(2020), who established that hybrids of *G. hirsutum* and *G. armourianum* had intermediate leaf shape and size. Other interspecific *Gossypium* species hybrids, including those between *G. herbaceum* and *G.australe* (Liu *et al.*, 2015), *G. hirsutum* and *G. arboreum* (Ahmad *et al.*,

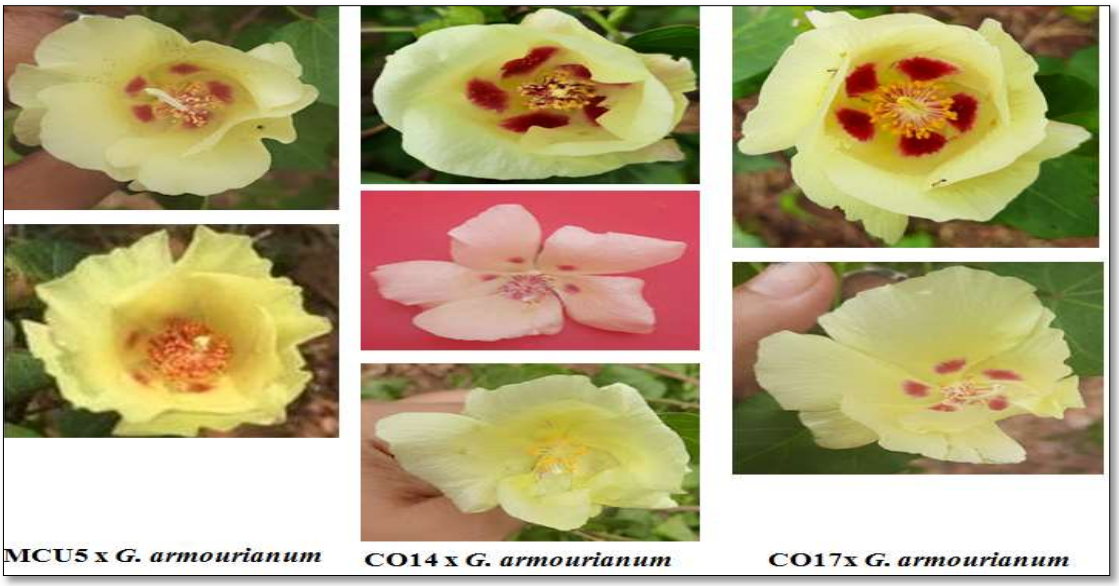

Figure 3: Variation for petal spot trait among the different *G. armourianum* hybrid

2011; Tahir *et al.,* 2011), and *G. arboreum* and *G. thurberi*, have also been reported to exhibit comparable intermediary manifestation of the growth habit of the plant, leaf size, and petal coloration (Manisha *et al.*, 2007).These characters show dominance, according to some researchers though. Saravanan *et al.* (2007)have shown the paternal parent's growth habits to be there in the triploid hybrid of American cotton with diploid wild species, *G. raimondii*. The hybrids form important genetic material for further development through different breeding methods and subsequent transfer of useful genes to cultivated background.

For the trait considered in the study, either they had dominant expression similar to one of the parent or intermediate variable in the F_1 progeny. For those traits showing complete dominance, transfer of trait may be little simpler compared to polygenic ones.

Hence while deciding the further breeding program using such lines, the program should be designed keeping in view the trait to be improvised. The wide range of variation available for each trait provide ample scope for further breeding process such as conventional backcross breeding or molecular breeding programs.

Conclusion

In the present study of characterization of the hybrids in the interspecific hybridization involving the wild cotton, *G. armourianum,* leaf shape, size, intensity of petal spot and the size of the bract were seen to have much amount of variation. Though belong to same species of cultivated cotton the three varieties used as female parent behaved substantially different in their progeny performance. These progenies constitute a valuable collection of prebreeding material which can be further harnessed for crop improvement programme.

Conflict of interest

The authors declare that they have no conflict of interest.

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