



RESEARCH ARTICLE

Genetic analysis of some morphological traits in synthetic × naturally polyploid cotton derivatives

SURUCHI VIJ¹, DHARMINDER PATHAK^{1*} , PANKAJ RATHORE² and POOJA NIKHANJ¹

¹Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141 004, India

²PAU Regional Research Station, Faridkot 151 203, India

*For correspondence. E-mail: dharminderpathak@pau.edu.

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Abstract. Cotton has received attention of geneticists since more than a century. *Gossypium hirsutum*, the predominantly cultivated cotton species worldwide, has a narrow genetic base. It is important to broaden its genetic base through introgression of novel alleles from related species. Here, we report the development and characterization of a backcross population derived from the hybridization of a ‘synthetic’ (derived by crossing and chromosomal doubling of nonprogenitor *Gossypium* species) and natural tetraploid upland cotton. ‘Synthetic’ was observed to be male-sterile and thus, was used as the female parent. A total of 7434 flowers were pollinated to obtain 1868 BC₁F₁ seeds by direct and reciprocal crosses. Characterization of the experimental plant material was conducted in the field for several morphological traits such as pubescence on the stem, leaf, petiole and bract, presence/absence of petal spot, petal margin colouration and stamen filament colouration. Genetic analysis revealed that petal margin colouration phenotype was governed by a single dominant gene, whereas the petal spot and filament colouration phenotypes manifested segregation distortion. None of the BC₁F₁ plants was devoid of trichomes thus demonstrating that presence of trichomes is dominant over their absence. Modern upland cotton cultivars are usually devoid of petal spot, petal margin colouration and stamen filament colouration. These floral anthocyanin pigmentation characteristics, if fixed in the cotton germplasm, may serve as diagnostic features for the identification of cultivars during DUS testing as well as in the maintenance breeding programmes.

Keywords. segregation distortion; anthocyanin pigmentation; distinctness-uniformity-stability testing; synthetic amphiploid; *Gossypium*.

Introduction

Genus *Gossypium* of the family Malvaceae includes more than 50 species, of which four cotton species namely *G. arboreum*, *G. herbaceum*, *G. hirsutum* and *G. barbadense* are commercially cultivated. At present, *G. hirsutum* (Upland cotton/American cotton) is the predominantly cultivated cotton species occupying more than 98% of the global cotton acreage with China, India, Pakistan, USA and Uzbekistan accounting for 77% cotton area worldwide (Kranthi 2019). Although, cotton is mainly grown for lint, it is an important source of edible oil and quality proteins as well. Cotton plays a vital role in the economy of many countries. Eight genome groups (A, B, C, D, E, F, G and K) have been recognized in the diploid ($2n = 2x = 26$) cotton species, whereas all the seven allotetraploid ($2n = 4x = 52$) cotton species possess AD genomes and have monophyletic origin (Grover *et al.* 2012). More than three-fold variation among

the genome sizes of the diploid cotton species has been reported (Hendrix and Stewart 2005).

Cotton has been the subject of genetic analysis since more than hundred years. In fact, it is one of the first crops to which Mendel’s principles of inheritance were applied. Inheritance of lint colour (Balls 1906) and leaf shape (Shoemaker 1908) in cotton were reported shortly after the rediscovery of Mendel’s principles of genetics. Knowledge of genetic control of a trait is the key to its successful manipulation. However, deviations from the expected Mendelian ratios (segregation distortion) have been observed in many crop species such as maize, rice, wheat, barley, cotton, sorghum, soybean, tomato etc., especially in the populations derived from interspecific crosses. A variety of mechanisms effecting segregation distortion have been recognized. These include abortion of male and/or female gametes, pollen tube competition, competitive/preferential fertilization, zygotic selection, reduced fertility etc. Besides,

factors like cytoplasmic, physiological and environmental have been reported to contribute to segregation distortion. Distorted segregation affects the level and pattern of transmission of donor chromatin in the recurrent parent genotype. It is known to affect the precision of genetic mapping due to higher/lower estimation of recombination values. Availability of genetic variation is vital for any crop improvement programme. Use and reuse of related parents in the breeding programmes has resulted in vulnerability of crop plants to various biotic and abiotic stresses due to genetic uniformity. This has prompted the use of related species in the crop breeding programmes to broaden the genetic base. In Punjab Agricultural University, some of the *Gossypium* species belonging to secondary gene pool are being used to enhance allelic diversity of upland cotton. In this article, we report the development of a backcross population derived from the cross between a ‘synthetic’ (developed from nonprogenitor A-genome and D-genome cotton species) and natural tetraploid *G. hirsutum*; and the inheritance of some morphological traits including presence/absence of anthocyanin pigmentation and trichomes.

Materials and methods

The segregating BC₁F₁ population was developed using *G. hirsutum* accession PIL 43 as the recurrent parent, and a ‘synthetic’ as the donor parent. PIL 43 is one of the parents of an intra *hirsutum* hybrid, LHH 144, developed and commercialized by Punjab Agricultural University, Ludhiana in 1998. The synthetic amphiploid was developed by J. O. Beasley (1940) by doubling chromosomes of the interspecific hybrid between *G. thurberi* Tod. × *G. arboreum* var. *neglectum* Hutchinson and Ghose. The descendent of synthetic amphiploid was kindly provided by Dr J. F. Wendel, Distinguished Professor and Chair, Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, USA. In the present study, the initial cross was developed by hybridizing ‘synthetic’ as the female parent (as it was observed to be male-sterile) and PIL 43 as the male parent. The resulting F₁ plants were backcrossed to the recurrent parent PIL 43 during kharif 2016 to develop BC₁F₁ seeds. Both direct (F₁ × PIL 43) and reciprocal (PIL 43 × F₁) crosses were attempted. A total of 7434 flowers were pollinated and 1868 BC₁F₁ seeds (1609 seeds from reciprocal crosses and 259 seeds from direct crosses) were obtained. The BC₁F₁ seeds were sown in polythene bags, of which 296 seeds germinated, thus registering a germination percentage of 15.85. About three-week old seedlings were transplanted in the field. A total of 194 BC₁F₁ plants could be established, of which 158 produced flowers (table 1). Characterization of the experimental plant material was conducted in the field for several morphological traits such as pubescence on the stem, leaf, petiole and bract, presence/absence of petal spot, petal margin colouration and stamen

Table 1. Number of BC₁F₁ plants derived from individual F₁ plants.

Pedigree of BC ₁ F ₁ progenies*	Seeds planted	Seeds germinated	Number of BC ₁ F ₁ plants established	Number of plants flowered
PIL 43 × F ₁ # 1	22	1	1	–
PIL 43 × F ₁ # 2	19	3	2	2
PIL 43 × F ₁ # 3	309	55	25	20
PIL 43 × F ₁ # 4	89	15	13	12
PIL 43 × F ₁ # 5	340	51	35	2
PIL 43 × F ₁ # 6	91	11	4	1
PIL 43 × F ₁ # 7	22	4	4	3
PIL 43 × F ₁ # 8	7	–	–	–
PIL 43 × F ₁ # 9	790	129	93	85
PIL 43 × F ₁ # 10	0	–	–	–
PIL 43 × F ₁ # 11	80	12	9	9
PIL 43 × F ₁ # 12	9	1	1	–
PIL 43 × F ₁ # 13	74	10	3	2
PIL 43 × F ₁ # 14	16	4	4	2
PIL 43 × F ₁ # 15	0	–	–	–
PIL 43 × F ₁ # 16	0	–	–	–
Total	1868	296	194	158

*Includes both direct and reciprocal crosses.

filament colouration during kharif 2017. The chi-square tests were used for genetic analyses.

Results and discussion

The ‘synthetic’ and natural upland cotton accession PIL 43 differed from each other for several morphological characters. Synthetic possessed a characteristic petal spot, petal margin colouration, filament colouration (figure 1a) and was devoid of trichomes on different plant parts (stem, leaves, petioles and bracts). On the contrary, the male parent PIL 43 did not have petal spot, petal margin colouration and filament colouration (figure 1b); and possessed trichomes on stem, leaves, petioles and bracts.

Petal spot

Petal spot is the pigmented region at the base of flower petal which is considered to attract the insects for pollination. Majority of the cotton species possess pigmented petal spots. However, modern upland cotton varieties are devoid of petal spots, although presence of such spots is not uncommon in the primitive cottons or race stocks (Fryxell 1984). Of the 16 F₁ plants derived from the cross between synthetic and PIL 43, majority plants registered the petal spot phenotype and resembled the female synthetic parent. It is important to mention that our unpublished results show that the synthetic parent is triploid carrying $2n = 3x = 39$ chromosomes. The triploid hybrid is the result of fusion of normal gamete



Figure 1. (a) Flower of ‘synthetic’ showing presence of petal spot, colouration of petal margin and stamen filament and (b) flower of *Gossypium hirsutum* Accession PIL 43 manifesting contrasting phenotype for these traits.

carrying 26 chromosomes with the gamete having half (13) the chromosomes. The loss of chromosomes may be due to ‘affinity’, in which centromeres of the same ancestral origin have been proposed to travel to the same pole during first meiotic division (Wallace 1959 and references therein; Wallace 1960). Further experimentation is required to ascertain the identity of the lost genome/chromosomes in the triploid cotton synthetic. Therefore, it is probable that the F_1 plants lacking petal spot are devoid of chromosome carrying allele conferring petal spot phenotype. Complete dominance of petal spot phenotype in the F_1 hybrids resulting from crosses between upland cotton mutants with petal spot and RS 2013 (lacking petal spot) has been reported by Ahuja and Dhayal (2007). The presence/absence of petal spot is conditioned by a single gene and has been mapped to chromosome 7 (Endrizzi *et al.* 1985; Song *et al.* 2005; Lacape *et al.* 2009; Wang *et al.* 2016; Erpelding 2020). According to the single gene model, the number of plants with and without petal spot in a BC_1F_1 population should segregate in a Mendelian ratio of 1:1. In the present investigation, of the 85 BC_1F_1 plants (derived from the cross between PIL 43 and a single F_1 plant number 9 possessing petal spot and petal margin colouration), majority (72) did not exhibit petal spot phenotype. Petal spot was observed in the flowers of only 13 BC_1F_1 plants. The chi-squared value for the presence versus absence of petal spot was 40.96, which significantly deviated from the expected Mendelian segregation ratio (table 2). Similar results of appearance of more number of plants lacking petal spot than expected were obtained in the F_2 population of two intra-*hirsutum* crosses by Wilson (1987). In a recent study, distorted segregation for petal spot trait in the F_2 population derived from an interspecific cross between *G. hirsutum* and *G. darwinii* was reported (Zhang *et al.* 2016). In this case also, more number of plants bearing flowers without petal spot than expected were observed. On the other hand, petal spot phenotype was found to be skewed towards *G. barbadense* parent (with petal spot) in an F_2 population of *G. hirsutum* \times *G. barbadense* cross (Yu *et al.*

2007). It has been speculated that viability of the gametes carrying the allele conditioning petal spot phenotype or the resulting zygotes (homozygous for this allele) might be low resulting in segregation distortion (Zhang *et al.* 2016).

Petal margin colouration

In the present study, the manifestation of petal margin colouration was found to be dominant over its absence. The results are consistent with the findings of Ahuja *et al.* (2006) in upland cotton. In the present investigation, the number of plants with and without petal margin colouration was observed to be 36 and 49, respectively in the BC_1F_1 generation. The perusal of goodness of fit chi-square test revealed that the observed numbers did not differ significantly from the expected numbers for monogenic control (table 2). Thus, the petal margin colouration was found to be governed by a single dominant gene. Further, F_1 plants that manifested petal margin colouration also displayed petal

Table 2. Chi-square test of goodness of fit for various morphological traits in BC_1F_1 generation.

Phenotype	Observed number	Expected number	$\chi^2 = (O - E)^2/E$
Petal spot			
Plants with petal spot	13	42.5	20.48
Plants without petal spot	72	42.5	20.48
Total	85		$\sum = 40.96^*$
Petal margin colouration			
Plants with petal margin colouration	36	42.5	0.99
Plants without petal margin colouration	49	42.5	0.99
Total	85		$\sum = 1.98^{NS}$

*Significant at 0.05 level of significance;
^{NS} nonsignificant differences.

spot phenotype like its maternal parent ‘synthetic’. Similarly, the F₁ plants lacking petal margin colouration were also devoid of petal spot (like the pollen parent PIL 43), thus suggesting linkage between these traits. A chi-square test of independence was conducted to determine if the petal spot and petal margin colouration traits were assorting independently (table 3). The calculated chi-square value (20.86) is larger than the table chi-square value given for one degree of freedom indicating that genotypes are not in the proportions that would be expected if independent assortment were taking place. Thus, it is concluded that petal spot and petal margin colouration traits are not assorting independently and must be linked.

Filament colouration

Anthocyanin pigmentation of the stamen filaments is not common among the modern upland cotton cultivars and can be used as a morphological marker for cultivar identification. Parents ‘synthetic’ and ‘PIL 43’ possessed pink and cream stamen filaments, respectively. Some of the F₁ plants had flowers with pink filaments whereas, others possessed flowers with cream filaments. Variation is expected among the F₁ plants as each of them might have received different set of chromosomes from the triploid synthetic parent ($2n = 3x = 39$). Pink filament colour is dominant over cream filament colour. This has been unambiguously demonstrated by Ahuja *et al.* (2006) in the cross involving American cotton AKH-0308 mutant P1 having dark pink stamen filaments with RS-2013 (with cream filaments). In the present investigation, all the 133 BC₁F₁ plants derived from crosses of F₁ (with cream filaments) × PIL 43 were observed to have flowers with cream filaments. Of the 25 BC₁F₁ plants obtained after hybridizing F₁ (with pink filaments) with PIL 43, two plants produced flowers having pink filaments, rest 23 were observed to have cream filament flowers. Two incompletely dominant genes have been reported to govern pink filament inheritance (Wilson 1987). Considering the predominant appearance of BC₁F₁ plants with the recessive cream filament phenotype (11.5 cream filament plants : 1 pink filament plants), no valid inference about the inheritance of this trait can be drawn. However, it can be reasonably concluded that filament colouration phenotype is manifesting segregation distortion. Chi-square test of independence revealed that filament colouration phenotype was linked to both petal spot and petal margin colouration traits (table 3).

Pubescence

Trichomes are the epidermal outgrowths present on almost all aerial parts of the cotton plant. These are considered key trait for imparting various degrees of resistance/susceptibility to insect-pests. For example, glabrous leaf characteristic in cotton has been related to reduced oviposition by

Table 3. Chi-square test of independence for various morphological traits in BC₁F₁ generation.

Phenotype	Number observed	Number expected	$\chi^2 = (O - E)^2/E$
Petal spot and petal margin colouration			
Both petal spot and petal margin colouration present	13	5.51	10.18
Petal spot present and petal margin colouration absent	0	7.49	7.49
Petal spot absent and petal margin colouration present	23	30.49	1.84
Both petal spot and petal margin colouration absent	49	41.51	1.35
Total	85		$\sum = 20.86^*$
Petal margin colouration and filament colouration			
Both petal margin colouration and filament colouration present	2	0.5	4.5
Petal margin colouration present and filament colouration absent	4	5.5	0.41
Petal margin colouration absent and filament colouration present	0	1.5	1.5
Both petal margin colouration and filament colouration absent	18	16.5	0.14
Total	24		$\sum = 6.55^*$
Petal spot and filament colouration			
Both petal spot and filament colouration present	2	0.33	8.45
Petal spot present and filament colouration absent	2	3.67	0.76
Petal spot absent and filament colouration present	0	1.67	1.67
Both petal spot and filament colouration absent	20	18.33	0.15
Total	24		$\sum = 11.03^*$

*Significant at 0.05 level of significance.

Heliothis species (Treacy *et al.* 1986; Hassan *et al.* 1990). A positive correlation between young leaf trichome density and whitefly population has been reported in *desi* cotton (Grover *et al.* 2016). Exploitation of pubescence has been suggested as one of the strategies for insect-pest management. All the 16 F₁ plants of the cross ‘synthetic’ × PIL 43 were observed to possess pubescence on the stem, leaves, petiole and bract, indicating the dominant nature of the trait. As mentioned earlier, F₁ plants were backcrossed with PIL 43 (hairy) to develop the BC₁F₁ population. None of the 194 BC₁F₁ plants was devoid of trichomes thus reinforcing that the presence of trichomes is dominant over their absence. This finding is consistent with many of the previous reports in cotton (Lacape and Nguyen 2005; Desai *et al.* 2008; Pathak *et al.* 2011; Niu *et al.* 2019).

Modern upland cotton cultivars usually do not possess petal spot, petal margin colouration, and stamen filament

colouration. Given the inherent limitations of morphological markers in terms of number and polymorphism, these floral anthocyanin pigmentation characteristics, if fixed in the cotton germplasm, may serve as diagnostic features for the identification of cultivars during DUS testing as well as in maintenance breeding programmes.

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