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In vivo inter specific cross recovery among different species of cotton (*Gossypium* spp.)

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Abstract

Inter-specific crosses, *viz.*, Jayadhar (2x) × BCS-23 (4x), A82-1-1 (2x) X BCS-23 (4x), Jayadhar (2x) × *G. australe* (2x), Jayadhar (2x) × *G. gossypoides* (2x) and Abadhita (4x) × *G. australe* (2x) were attempted. Plant growth hormones like Indole acetic acid, Naphthalene acetic acid and Gibberellic acid with three concentrations were applied to crossed buds to prevent shedding. Very low frequency (0.047-0.15 %) of *in vivo* cross recovery between cultivated diploid and tetraploid and cultivated and wild species, respectively, indicated the presence of post-zygotic incompatibility. Application of PGRs particularly GA_3 @ 0.1 % in 2x × 4x crosses prevented abscission of crossed buds at the highest frequency. There was 36.69 % boll retention as against only 2.70 in control. Phenotyping revealed the intermediate nature of hybrids for most of the morphological characters. Presence of multiple nuclei at tetrad stage (pentad and hexad) was observed cytologically due to abnormal segregation of laggards.

Key words: Cotton, inter-specific hybridization, incompatibility, plant growth hormones, phenotyping, multiple nuclei.

Introduction

The genus *Gossypium* consists of 50 species of which, only four are cultivated cotton and remaining are wild species. Based on their geographical distribution and cross compatibility they are divided under 8 different genomic groups. *Gossypium herbaceum*, L. and *Gossypium arboreum* L. are diploids with 13 as 'n' number of chromosomes. They are linted cottons and considered as old world cotton with Central Asia and Hindustan centers as their centre of origin respectively. *Gossypium hirsutum* L. and *G. barbadense*, L. are allotetraploids. Their origin was due to natural crossing between cultivated diploids, *G. herbaceum* and American wild, non-linted *G. raimondii* (n = 13) followed by natural doubling of chromosomes to yield amphidiploid. They are superior to cultivated (linted) diploids in fibre properties and seed cotton yield, lint yield etc. Cultivated diploids posses the resistance to jassids and drought. In the diploid species *G. herbaceum* and *G. arboreum* and *G. raimondii, G. armourianum* and in tetraploids *G. tomentosum* resistance to sucking pest is associated with leaf pubescence, toughness of leaf veins, thickness of leaf lamina and length and angle of insertion of leaf hairs. Wild species have recorded several desirable characteristics such as high ginning and fibre maturity along with gossypol free seed character in case of *G. asutrale*, sucking pest resistance in *G. gossypoides*. This indicates the richness of genetic diversity in cotton. There are several successful stories of inter-specific hybridization in cotton itself. Fibre characters like fibre strength were transferred from *G. thurberi* into Egyptian barbadense. Jassid resistant *G. hirsutum* cultures called as co-ano types were obtained from cross between cultivated jassid susceptible *G. hirsutum* (Co-types) and wild jassid resistant *G. anomalum* species. Drought resistant character from wild species *G. stocksii* was successfully transferred into cultivated species (*G. arboreum* strain 5975). The crosses between *G. hirsutum* and *G. barbadense* have been commercially exploited in India. Hybrids like Varalaxmi and Jayalaxmi made India capable of exporting 80s count cotton. The extra long fibre characters of *G. barbadense* and high seed cotton yield potentiality of *G. hirsutum* was exploited to the best in their interspecific hybrids. The cross recovery between cultivated tetraploid and diploid was around 1.0 and 0.5 percent when tetraploids and diploids were maternal parent respectively [1-2]. *In vivo* low cross recovery in distant hybridization indicates the presence of post-zygotic barrier. The incompatibility reactions between diploid and tetraploid cottons are known to be post zygotic [3]. There are successful crosses for introgression of characters.

In the present investigation, an attempt was made to obtain distant crosses to transfer desirable fibre characters of *G. barbadense* (4x) into cultivated diploid spp and also gossypol free seed character from *G. australe* and sucking pest tolerance from *G. gossypoides* into cultivated species.

Materials and methods

Genotypes: In the present study all the four cultivated *G. herbaceum* (Jayadhar, 2x), *G. arboreum* (A82-1-1, 2x), *G. hirsutum* (Abadhita, 4x), *G. barabadense* (BCS-23, 4x) and two wild (*G. australe* and *G. gossypoides*) diploid species of cotton were used.

Crossing Technique: The cultivated diploid and tetraploid species were grown at Agricultural Research

Station, Dharwad farm during 2000-01. The wild accessions were used from established wild garden of this station. Hand emasculation and pollination method of crossing was followed.

Application of plant growth regulator to crossed buds: IAA (indole acetic acid), NAA (Napthalene acitic acid) and GA_3 (Gibberlic acid) were applied to the base of the pedicel by placing the cotton swab dipped in the solutions of growth regulators immediately after crossing and the swab was kept moist for nearly 8 hrs a day and upto 10 days even after pollination.

Cytological studies:Flower buds of crosses (F_1) harvested from 7.00 am to 3.00 pm with an half an hour interval were fixed in carnoys-II fixative. Two per cent acetocarmine stain was used to stain meocytes obtained from immature anthers present in the buds. The anthers were smeared in 2 per cent acetocarmine on a glass slide.

Pollen fertility studies: Pollens obtained on the day of anthesis from crosses were stained with 2 per cent acetocarmine on a glass slide. They were allowed to take stain for about 45 minutes before observation.

Statistical analysis: All the studies were done in completely randomised design (CRD) with equal number of replications. Data in percentage was transferred into angular transformed values and analysis was done under three factorial CRD using DRYSOFT software package.

Results and discussion

(a) In vivo cross recovery between cultivated species (Jayadhar × BCS-23 and A82-1-1 × BCS-23): The percent cross recovery based on number of bolls retained in crosses between Jayadhar (2x) and BCS 23 (4x) and A-82-1 (2x) and BCS 23(4x), was respectively 2.5 and 2.74 per cent. But, based on the ratio between numbers of seeds harvested to expected number of seeds percent cross recovery was 0.39 and 0.29 per cent respectively. Finally the cross recovery was also calculated based on the ratio between plants established to expected number of seeds @ 25 seeds/ boll and it was only 0.12 and 0.15 per cent respectively in two crosses (Table 1). The decrease in cross recovery success from number of bolls retained to seed recovery and from seed recovery to plants establishment indicates the presence of incompatibility at different stages.

(b) Role of plant growth regulators in inter-specific cross recovery of Jayadhar × BCS-23 and A82-1-1 × BCS-23: Various growth hormones have been found to stimulate pollen growth and development. They also prolong the receptivity of stigma and prevent early abscission of pollinated flowers [4]. Similarly, increased enzymatic activity of pectinase and cellulase, near the pedicel dissolves the middle lamella and weaken the cells of abscission zone and result in boll drop [5-6] and among the growth regulators auxin inhibits abscission while GA_3 and cytokinins have variable effect. Further, application of GA_3 to maternal plant, two days before and after pollination is a routine procedure for producing interspecific and intergeneric hybrids in Triticum and Hordeum, and GA_3 in general increases both seed setting and embryo development [4].

In cotton increased ethylene production in young bud, boll or in neighbouring tissue is observed and when it exceeds threshold level it induces production of an enzyme B, 1-4 glucanhydrolase in the cells of abscission zone. This and other enzymes dissolve cellulose in the abscission zone and result in shedding. Application of plant growth regulators maintains higher ratio between auxins and ethylene in plant tissues which prevents synthesis of cellulose dissolving enzymes and thereby reduction in shedding. Thus in the present study three plant growth regulators were applied to the base of the pedicel by putting the cotton dipped in solution of PGRs. It was continued upto 10 days from the day of pollination and care was taken to keep swab wet for 8 hours in daytime. Irrespective of crosses and concentrations of growth regulators, significantly highest boll retention (30.75%) was observed with GA3 application followed by NAA (23.42%) and IAA (21.54%). Between 3 concentrations, irrespective of growth regulators, 0.1 per cent showed highest boll retention (29.05%) followed by 0.2 per cent (Table 2). The level/concentration of PGRs needed may vary from cross to cross, since the level of ethylene production vary between genotypes as evident from the present study where the cross recovery in Jayadhar × BCS-23 was highest (38.38%) due to application of GA_3 (0.1%) while it was (35.0%) in the cross A82-1-1 × BCS-23. Presence of differential response between genotypes was also supported from earlier studies [6]. Thus the role of either GA_3 or Auxins in prevention of boll

Table 1. *In vivo* cross recovery between cultivated diploid and tetraploid *Gossypium* spp.

Cross	Total No. of pollinations	No. of drop	Percentage retention	Expected no. of seeds	Seeds harvested		Total	No. of seedlings established	Percentage success
					Bold	Shrivelled			
1. Jayadhar (2x) × BCS-23 (4x)	10,018	9,770	2.50	2,50,450	673 (0.26%)	310 (0.12%)	983 (0.39%)	305	0.12
2. A 82-1-1 (2x) × BCS-23 (4x)	7,088	6,894	2.74	1,77,200	307 (0.17%)	217 (0.12%)	524 (0.29%)	269	0.15

Table 2. Interaction effect of crosses, plant growht regulators and their concentrations

Treatments	Cross 1	Cross 2	Mean
T_1 IAA (0.05)*	16.49 (23.96)**	11.62 (19.90)	14.05 (21.93)
T_2 IAA (0.10)	25.15 (30.07)	22.85 (28.58)	24.00 (29.32)
T_3 IAA (0.20)	27.43 (31.56)	25.71 (30.45)	26.57 (31.00)
Mean	**23.03 (28.53)**	**20.06 (26.31)**	**21.54 (27.42)**
T_4 NAA (0.05)	19.14 (25.69)	17.46 (24.70)	18.30 (25.19)
T_5 NAA (0.10)	29.02 (32.57)	23.91 (29.25)	26.46 (30.91)
T_6 NAA (0.20)	25.42 (30.05)	25.64 (30.40)	25.53 (31.22)
Mean	**24.52 (30.10)**	**22.33 (28.11)**	**23.42 (29.10)**
T_7 GA_3 (0.05)	22.15 (28.10)	20.40 (26.86)	21.27 (27.48)
T_8 GA_3 (0.10)	38.38 (38.29)	35.00 (36.26)	36.69 (37.27)
T_9 GA_3 (0.20)	35.29 (36.45)	33.33 (35.25)	34.31 (35.85)
Mean	**31.94 (34.28)**	**29.57 (31.79)**	**30.75 (33.53)**
Mean	**26.49 (30.97)**	**23.99 (29.07)**	

	SEm±	CD at 5%
1. Treatment (growth regulators)	0.247	0.708
2. Genotypes (crosses)	0.202	0.578
3. Concentration	0.247	0.708
4. Treatment × genotype × concentration	0.605	NS

* - Concentration in percentage; ** - Figures in parenthesis indicate angular transformed values; NS - Non significant

as male parent and crossed to Jayadhar (A genome) to resynthesize the tetraploid cotton (AADD). Wild species belonging to the Australian group, *viz.*, *G. bickii*, *G. sturtianum* and *G. australe* possess the glandless seed character. Gossypol free seed character was transferred from *G. sturtianum* into cultivated upland cotton [10-11]. In the present study (Table 3), *G. australe* was used as source of Gossypol free seed character to transfer into cultivated diploid and tetraploid species to obtain plant with gossypol glands in the vegetative part for resistance to pest and disease and gossypol free seed to exploit cotton as cheap source of edible oil as there is no need of purification alone or even extraction is not required for cotton seed oil to be free from gossypol which is an alkaloid compound being hazardous to health to non-ruminants. *G. australe* also possesses other economic characters such as resistance to drought, high ginning and fibre maturity. On the basis of ratio between seeds harvested and expected seeds in crosses between Jayadhar (2x) and *G. australe* (2x) and Abadhita (4x) and *G. australe* (2x) the cross recovery was respectively 1.4 and 0.14 but based on the ratio between plants established in field (19 and 2) to expected number of seeds, the cross recovery was 0.19 and 0.09 per cent respectively (Table 3). Similarly, the cross recovery of 7.19 per cent was observed on the basis of ratio between seeds harvested to expected number of seeds in cross between Jayadhar and *G. gossypoides* and based on the ratio of plants established to expected number of seeds, 0.047 per cent was the cross recovery. The low frequency of cross recovery in this study is in agreement with that during the inter-specific hybridization involving *G. gossypoides* as one of the parents, the hybrids die at various stages of development from embryonic development to the reproductive phase of F_1 plants.

Table 3. *In vivo* cross recovery between cultivated and wild cotton species

Cross	No. of pollinations	No. of drop	Expected no. of seeds	Total no. of seeds harvested	Percentage cross recovery	Seedlings established	Percentage cross recovery
G. herbaceum cv. [Jayadhar (2x)] × *G. australe* (2x)	383	187	9575 @ 25 seeds/boll	134	1.4	19	0.190
G. herbaceum cv. [Jayadhar (2x)] × *G. gossypoides* (2x)	253	73	6325 @ 25 seeds/boll	455	7.19	3	0.047
G. hirsutum cv. [Abadhita (4x)] × *G. australe* (2x)	74	73	2072 @ 38 seeds/boll	3	0.14	2	0.090

shedding may be due to the suppression of ethylene concentration, thereby inhibiting the synthesis of cellulase which is expected to dissolve cellulose in the abscission zone.

(c) Wide Hybridization: *G. herbaceum* (A_1) and *G. raimondii* (DD) are the possible proginators of the New World Cotton [7-8]. However, from the studies of Wendel [8] *G. gossypoides* appears to be the closest living descent of ancestral D-genome parent of the New World Cotton. Zhao [9] also supported the Wendels proposal by reporting that, A-genome dispersed repeats has spread to D-genome and correlated it with *G. gossypoides*. Therefore, *G. gossypoides* (DD) was used

(d) Phenotyping: Phenotyping was carried out based, on the typical morphological characters to confirm the introgression. The F_1 of the cross between Jayadhar (2x) × BCS-23 (4x) resembled the female parent in characters like leaf and stem characteristics (Table 4). The hybrid was intermediate for stigma protrusion, sinus, petal colour, petal spot, pedicel length and leaf size (Fig. 1). But, also there were three plants with characters intermediate between plant hairiness, bract size and in

nature of attachment to developing bolls. In the crosses like Jayadhar × *G. australe* and Jayadhar × *G. gossypoides*, the hybrids resembled female parent for leaf lobing, stem and leaf hairiness. Most of the hybrids resembled the female parent (2x) in the cross between cultivated diploid and tetraploides. However, the leaf size increased in the F_1 compared to both parents. Similar reports of vigorous and broader leaves were observed [12] from *G. herbaceum* × *G. anomalum* and *G. hirsutum* × *G. armourianum* crosses.

The hybrid from the cross between Jayadhar and *G. australe* was intermediate for the stem and leaf characters but resembeled the male parent for bracts and floral characters (Fig. 1) and between Jayadhar and *G. gossypoides* the F_1 resembled the male parent by having pigmented woody stem with short hairs and bright green colour leaves, petiolate, 7-9 nerved with three deep lobes but wider in nature and pubescent (short and thick hairs) on lower surface of the leaves (Plate 1). The F_1, from the cross between *G. hirsutum* and *G. australe* resembled female parent for leaf colour and shape but it resembled the male parent for teeth/serrations of the bract and petal spot. However, the hybrid did not resemble either of parents in petal size, petal colour, leaf lobe and length of style. Petal colour was intermediate between colourless and pink, leaf lobing was also intermediate, size of the petals and length of style was bigger than either of parents (Fig. 1).

(e) Cytological studies: Meiotic studies and fertility status of inter-specific hybrids revealed the extent of homology between parents involved in the cross and, subsequently this information helps to determine breeding strategies to incorporate desirable characters.Meiosis of crossed buds from, Abadhita × *G. australe* and Jayadhar × BCS-23 crosses was carried out. Multiple nuclei in diad and tetrad stage due to improper segregation of univalents/laggards were observed (Fig. 1). The frequency of pentads and hexads resulted in instability of inter-specific hybrid [13-15].

(f) Pollen fertility studies: Pollen fertility studies were carried out to measure the fertility status in inter-specific Abadhita × *G. australe*, Jayadhar × BCS-23 and A82-1-1 × BCS-23 crosses. Sterility to the extent of 94.5 per cent was observed in Abadhita × *G. australe* cross. It was due to presence of all three different genomes (ADG) in single copy number in F_1. Between cultivated diploid and tetraploid crosses (Jayadhar × BCS-23 and A82-1-1 × BCS-23), pollen sterility of 38.62 and 31.64 per cent was observed (Fig. 1). Although the hybrids were triploid in nature they were partially fertile because they shared a common genome, A from both the parents. Thus there are two copies of A genome and one copy of D genome. Thirty six percent per cent sterility was observed in the cross (*G. arboreum* × *G. herbaceum*) [16], twenty five per cent sterility from cross (*G. hirsutum* × *G. stocksii*) [14] and 42 per cent sterility between *G. arboreum* (male sterile) and *G. anomalum* [17].

Introgression of desirable characters between cultivated and wild species is possible but hinderd by post-zygotic incompatibility barriers, which is evident from the present study. The frequency of cross recovery can be increased by application of plant growth regulators to crossed buds. Rescuing the crosses by *in vitro* embryo axes culture can be achieved [18-20].

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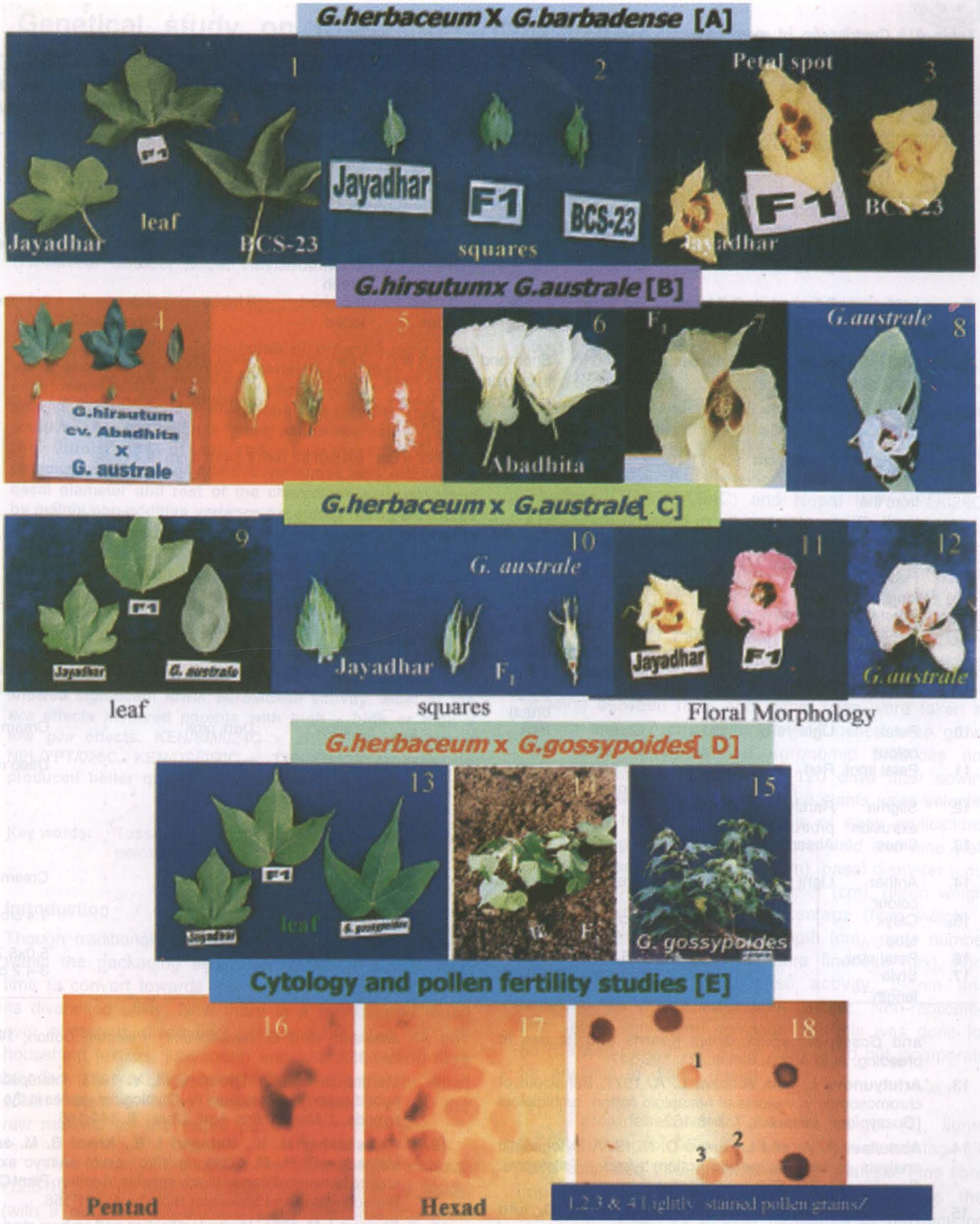

Fig. 1 (Cross A-D): A - Jayadhar BCS-23 Cross (1) Intermediate leaf size and lobing F_1 ; (2) Increase in size of the square teeth and intermediate square in F_1; (3) Intermediate flower size and higher intensity of petal spot in F_1. B. Abadhita x *G. australe* (1). Partial velveting of leaf in F_1; (2) Fregobractness in F_1; (3). Light yellow petal with spot in F_1; C. Jayadhar x *G. australe*: (1) Intermediate leaf shape of F_1; (2) Square formation [P_1, F_1, P_2]-intermediate square size; (3) Pink coloured petals of F_1. D. Jayadhar x *G. gossypoides* : (1) Intermediate leaf lobing F_1; (2) F_1 plantlet; (3) *G. gossypoides*. E. Cytological and Pollen fertility studies: (1&2) Pentad and Hexads; (2) Hexad; (3) Sterile pollens lightly stained in Acetocarmine

Table 4. Comparison of morphological characters of parents and their hybrids

S. No.	Character	Jayadhar (2x)	BCS-23 (4x)	*G. hirsutum* Abadhita (4x)	*G. australe* (2x)	*G. gossypoides* (2x)	F_1 (Jayadhar × BCS-23)	F_1 (Jayadhar × *Australe*)	F_1 (Jayadhar × *Gossypoides*)	F_1 (Abadhita × *Hirsutum*)
1.	Stem hairyness	Hairy	Glabrous	-	Perennial woody stem thick and short hairs	Perennial woody and pigmented	Hairy to glabrous	Thick hairs, velvet in nature	Pigmented stem and woody in nature	-
2.	Leaf colour	Parrot green	Dark green	Parrot green	Ash green	Dark and bright green	Dark green to parrot green	Ash green	Bright green	Ash green
3.	Leaf shape	3-5 short lobed	3-7 lobed	3-5 lobed	Entire	3 deep lobes	3-5 short lobed	3 lobes	3 deep lobes and wide	2-3 lobed
4.	Leaf hairyness	Hairy	Glabrous	-	Short and thick hairs velvet in nature	Short and thick hairs on lower surface of the leaf	Hairy to glabrous	Short and thick velvet in nature	Short and thick on lower surface	-
5.	Leaf size 3rd or 5th from the top (L×B)	7.9×8.1 cm	9.7×11.10	-	7.0×4.1 cm	9.4×7.4 cm	8.45×8.82 cm	7.54×8.4 cm	9.1×7.86	-
6.	Square size	Medium	Big	-	-	-	Small to big	-	-	-
7.	Pedicle length	Small	Long	-	-	-	Medium to long	-	-	-
8.	Bract size	Medium	Big	-	-	-	Small to big	-	-	-
9.	Bract type	Open	Closed	3 normal moderately serrated	3 free and deeply serrated (frego bract)	-	Open to closed	3 free and deeply serrated (Fregobract)	-	3 free deeply serrated (Fregobract)
10.	Petal colour	Light yello	Yellow	Cream	Pink	-	Yellow	Dark pink	-	Cream
11.	Petal spot	Red	Deep red	No petal spot	Red	-	Red to deep red	Deep red	-	Deep red
12.	Stigma extrusion	Rarely protruded	Protruded	-	-	-	Protruded	-	-	-
13.	Sinus	Absent	Present	-	-	-	Absent to present	-	-	-
14.	Anther colour	Light yellow	-	Cream	Pink	-	-	Pink	-	Cream
15.	Calyx tube	-	-	1 cm	0.6 cm	-	-	-	-	1.4 cm
16.	Petal size	-	-	4.5 × 2.7	7.0×4.1 cm	-	-	-	-	5.0×5.7 cm
17.	Style length	-	-	1.9 cm	1.9 cm	-	-	-	-	3-4.2 cm

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