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Cytomorphological studies in hybrid between haploid of *Gossypium hirsutum* (L.) and diploid *Gossypium arboreum* (L.)

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Abstract

A hybrid between *G. hirsutum* haploid (2n = 2x = 26; A_hD_h) and *G. arboreum* L. Var. G 27 (2n = 2x = 26; A_1A_1) was obtained. Its hybridity was confirmed on morphological and cytological basis. This hybrid is considered as a successful breakthrough in gene transfer between *G. hirsutum* × *G. arboreum* which was earlier considered as difficult and unsuccessful due to incompatibility caused by the disintegration of endosperm and failure of embryo development as well as disharmony between zygote and surrounding tissue (endosperm zygote imbalance). Restoration of such balance in haploid might make it possible for hybrid embryo to develop into normal healthy seed and adult plant. Further, utilization of this hybrid for introgression of resistance to sucking pests, drought as well as wider adaptability from *G. arboreum* to *G. hirsutum* is discussed.

Key words: *Gossypium hirsutum*, *G. arboreum*, haploid, cytomorphology, biotic and abiotic stresses

Introduction

Since most commercial cotton production involves tetraploid *G. hirsutum*, considerable interest has been generated for broadening the available genetic base. Hybrids between tetraploid and diploid *Gossypium* species are considered useful for introducing improved agronomic and quality traits into commercial cotton [1]. Cotton breeders have long been interested in producing hybrids between *G. arboreum* and *G. hirsutum* in order to combine several desirable characters [2-4]. However such hybrids are impossible to obtain *in situ* because of endosperm and embryo abortion and possibly other incompatibility factors[5] and not due to failure of pollen tube to reach the ovule[6]. Several attempts have been made to excise and grow young embryos of cotton, but limited success has been achieved [7-8]. The difficulties are attributed to sensitivity of the embryos to physical conditions and also to their complex nutrient requirements. As an alternative, ovule culture has been used with some degree of success. High salt medium (BT) for cotton gave good ovule growth when supplemented with phytohormones (BTH) [9].

Supplementation of BTP medium with 10-15mM ammonium ions supported ovule growth and germination of cotton embryos. Without extra NH_4^+, size of the embryos remained small with reduced cotyledons even after 10 weeks of incubation [10]. Extensive fertilization and embryological studies of *G. hirsutum* × *G. arboreum* and their reciprocal crosses [2-3,5] demonstrated that endospermic and embryo abortion occurred in both. Hybrid seedlings were reported from cultured ovules of cotton [5,10]. Complete plants from *G. arboreum* × *G. anomalum* and *G. arboreum* × *G. hirsutum* crosses along with their reciprocals were achieved through *in vitro* inovulo embryo culture. [10, 11]. Haploids helped in understanding the cytogenetic structure of various crop plants. Reviews of various aspects of haploids, have been reported in many plant species [12-14].

The cross *G. arboreum* × *G. hirsutum*, which is highly desirable, does not yield viable seeds. Embryo attains early dicotyledonous stage in 30 per cent of crosses but aborts subsequently. Disintegration of endosperm 15 days after pollination has been attributed to be the cause of failure of embryo development [15]. Beasley's [15] attempt to culture isolated young hybrid embryos did not meet with much success. Heart-shaped embryos resulting from a cross *G. arboreum* × *G. hirsutum* attained initial differentiation of cotyledons with a massive hypocotyls, and radicle [4]. Pundir [5] was able to obtain viable seeds containing a massive embryo, but growth of the embryo was arrested on culturing the ovules three days after pollination on a medium containing myo-inositol. Fibre development in the cultured ovules of cotton was observed in liquid medium conclusively showed that gibberellins form an essential requirement [9]. These reviewers clearly indicate that it is difficult to obtain cross between 2x × 4x in cotton.

Haploids of *G. hirsutum* cotton are fairly fertile and successful hybrids between haploids of *G. hirsutum* × *G. thurberi* (2x) and also *G. anomalum* (2n) and *G. arboreum* (2n) have already been reported [16-17]. Thus

Table 1. Morphological characteristics of haploid *G. hirsutum*, *G. arboreum* (G 27) and their hybrid

Plant part	*G. hirsutum* Haploid	Hybrid	*G. arboreum* G 27
Plant	Shrub type. Yellowish green. Branching -recemose. Free and lateral stipules. Alternate phyllotaxy	Shrub type plant. Reddish green. Branching -recemose. Free and lateral stipules. Branches and stem covered with long soft hair. Alternate phyllotaxy	Shrub type plant. Red . Branching -recemose. Free & lateral stipules. Alternate phyllotaxy
Leaf	Yellowish green. 3-4 lobes. Mid rib and veins yellowish and hairy at bottom. Nectary: prominent and blackish and only one. Sinus spot-present. Surface-smooth,glanded. Leaf venation reticulate. Upper surface : trichomes absent. Lower surface: present. Peduncle hairy and glanded	Greenish yellow. Palmate leaves. 3-5 lobes. Mid rib and veins yellowish green and having hair. Nectary small and only one. Sinus spot-present. Surface-smooth glanded. Leaf venation reticulate. Upper surface : trichomes absent. Lower surface: densely hairy. Peduncle hairy and glanded	Old leaves - Dark green to red. Young leaves. Green plamate leaves. 3-5 lobes. Leaf size varying. Mid rib and veins are prominent and red in colour. Nectary small. Sinus spot-absnt. Surface-glabrous Leaf venation reticulate
Flower i) Epicalyx or Bract	Polysepalous. Light green to green in colour. Glands: very few. Inner surface-light green. Outer surface-green	Gamosepalous. Greenish red. Veins prominent. Glands: very few and well distributed. Inner surface-light green. Outer surface-edges reddish	Gamosepalus. Reddish green in colour. Veins prominent Glands: outer surface - prominent and well distributed, inner surface-absent.
ii) Calyx tube	Yellowish green. Glands prominent and well distributed on upper and lower surfaces	Basel portion-yellowish green,Top portion - reddish. Glands prominent and well distributed on upper and lower surface	Reddish green. Glands dark and prominent
iii) Corolla	Yellowish creamy. Petal claw small. Aestivation of corolla - twisted	Inner surface-dark pink. Outer surface-yellowish patch and veins yellowish. Petal claw small. Aestivation of corolla - twisted	Whitish petal. Reddish border on both side. Petal claw small. Aestivation of corolla - twisted.
iv) Blotch or Petal spot	Absent	Inner surface -prominent red and start from base of petal. Outer surface - prominent red but small on left / right basal corner	Inner surface-red. Outer surface-small on basal right corner
v) Androecium	Loosely and regularly arranged. Anther - yellow. Filament creamy white	Loosely and regularly arranged. Anther colour-Yellow. Filament pigmented (Red)	Loosely and regularly arranged. Anther colour- Yellow. Filament Basal pigmented and remaining creamy.
vi) Gynoecium	Stigma united. Glands: Present on style and ovary. Absent on stigma	Stigma united. Glands: Present on stigma and ovary. Absent on style	Stigma united. Red pigmentation on stigma. Glands on style,stigma and ovary.
Boll	Small semi elongated. Surface-smooth,glands not prominent. Green. Semi pointed at tip	Semi elongated. Surface-rough and glanded. Red. Semi pointed at tip	Semi elongated. Surface-rough and glanded. Red. Semi pointed at tip.

the present study followed this approach for introgression of desirable characters from cultivated *G. arboreum* L. diploid cottons to tetraploid *G. hirsutum* [18].

Materials and methods

The haploid plants used in present investigations were obtained from F_2 of the interspecific cross of *G. hirsutum* × *G. barbadense* [19] which are maintained by vegetative propagation as they were partially fertile. Pollens at anthesis were collected from *G. arboreum* (var. G 27) and dusted on previously bagged flowers of *G. hirsutum haploid*. There was boll and seed set after effecting large number of cross-pollinations.

Parents and F_1 hybrid were raised. Various morphological characters studied in *G. hirsutum haploid*, *G. arboreum* var. G 27 and their F_1 hybrid. For cytological analysis, young flower buds of F_1 hybrid and their respective parents were fixed in Cornoy's fluid (6:3:1) and squashed in 1% aceto-carmine. Analysis of PMCs was made from temporary mounts. Pollen sterility was tested with differential staining method [20]. The pollen germination test was carried out as per Iyengar [21]. Microphotographs were taken on coloured film with the help of Rico 35mm camera mounted on trinocular Leica Microscope.

Results and discussion

In present study, successful production of hybrid have raised hopes for further introgression of resistance to sucking pests and drought from *G. arboreum* cotton on *G. hirsutum* back ground having high spinning value,

bigger boll and greater vigour. Such types if bred will be a boon for rainfed cotton growers to grow them with low input technology within shortest possible time.

The study of quantitative characters (Table 2) indicated that leaf thickness (0.62 mm), bracteole teeth (6.8), anther number (85/anther column), stomata per unit area (17.6) and percent of motes (68.96) and seed length (6.80 mm) in female parent *G. hirsutum* haploid were higher than that of hybrid. Calyx breadth (3.18 cm), petal attachment (0.49), anther size (1.12 mm), locule width (2.08cm) flower per plant (43), boll set per plant (81.40), seeds per locule (7), seed index (6.2) seed length (7.51mm) and fiber diameter (36.58 μ) of male parent (*G. arboreum*) were also higher than hybrid. The hybrid was superior to both parents in plant height, pedicel length, bract length, bract breadth, corolla length, corolla breadth, pollen size and seed breadth indicating exhibition of heterosis for these characters.

In qualitative characters (Table 2) red pigmentation observed in various plant parts and petal spot of parent G 27 were dominant in F_1. Leaf shape and boll attributes were also found intermediate confirming hybridity.

In present study, after thousands of effective pollination of sterile flowers of haploids plants of *G. hirsutum* cotton, it was possible to obtain only three bolls. Of which two were empty and contained motes and undeveloped seed. But from one boll only, four developed seeds were obtained, out of which only one proved hybrid at seedling stage as it expressed pigmented characteristic of male parent, while other three were confirmed as haploid. The parthenocarpic development of seed reported earlier has been confirmed in present case along with crossabilityof haploid plant with other species [22].

The successful hybridization between haploid *G. hirsutum* and diploid *G. arboreum* became possible because of haploidy. Incompatibility appears to be due to lack of harmony between development of zygote and surrounding tissues and imbalance [22]. It is postulated that such balance of zygote and endosperm imbalance is restored in haploid which made it possible for hybrid embryo to develop into normal healthy seed and adult plant.

Hybridity of this plant was confirmed by cytological analysis (Table 3). In haploid, the meiosis was not normal. The range of bivalent formation was 4-9, remaining chromosome being univalents.

In hybrid, numbers of bivalents were seen to vary from 5-9 with the average 6.5. The majority of bivalents had only single chiasmata. In a few PMCs, trivalents were also noticed. At metaphase the bivalents and univalants were seen scattered in cytoplasm and this irregular behaviour is possibly due to the fact that all the chromosomes have not reached the equatorial plate and arranged themselves in the normal compact manner. Although most of the bivalents were arranged at equatorial plate, few of them were seen away from equatorial plate, scattered in the cytoplasm along with univalants. When the paired chromosomes began to separate and an anaphase spindle was formed, the univalants were seen with distribution at random on spindle. Such irregular movement of univalants resulted into various abnormalities at the end of Ist division. Occasionally certain PMCs showed two distinct compact second metaphase plates with unequal number of chromosomes. Abnormal anaphase chromosome movements led to various irregularities of Ist division spindle. Tripolar spindles were also observed. Second metaphase plate, which contained a small number of chromosomes, formed micronuclei and microspores, thus they were without complement any chromosomes. The sporads formed per PMC were 3-9. The resulting pollens formed were with high size variation and sterility. So cytological observations (Table 3) revealed that the parent *G. hirsutum* haploid (Figs. 1-5) and F_1 (Figs 6-10) showed abnormal chromosome behaviour during meiosis. Perfect bivalent formation was observed in male parent *G. arboreum* (G 27) which showed normal separation of bivalent during first meiotic division and chromatid during second meiotic division. Normal sporads were observed which for pollen with equal size well developed exine and 98 % fertility. In haploid plant (Figs. 1-5) 13.0^{I} and 6.5^{II}, 15.2^{I} and 5.4^{II}, were observed at diakinensis and metaphase I, respectively.

However, in hybrid (Figs. 11-15) 13.0^{I} and 6.5^{II}, 15.2^{I}, and 5.4^{II} were observed at diakinensis and metaphase, respectively. The data indicated the possibilities of partial pairing between A and D chromosomes of haploid and *G. arboreum*. Univalents observed might be due to complete nonhomology. Formation of trivalent also indicated the partial homology between A & D chromosomes and providing the chances for exchange of chromatid between chromosomes of the two different species.

The hybrid between haploid *G. hirsutum* and *G. arboreum* (G 27) under report will be a valuable tool to transfer desirable characters e.g. resistance to sucking pests and drought from diploid *G. arboreum* to *G. hirsutum* and a widening of the genetic base.

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Table 2. Comparison of *G. hirsutum* tetraploid (2n = 4x = 52) haploid (2n = 2x = 26), *G. arboreum* (G-27) (2n = 2x = 26) and their hybrid plant on the basis of uantitative traits

Sl. No.	Plant part	Tetraploid *G. hirsutum* (2n = 4x = 52)	Haploid *G. hirsutum* (2n = 2x = 26)	Hybrid (2n = 2x = 26)	*G. arboreum* (G-27) (2n = 2x = 26)
1.	**Plant height (cm)**	128.30	62.00	185.00	148.00
2.	**Leaf observation (cm)**				
a.	Leaf tip to sinus	9.35	6.65	8.98	8.30
b.	I lobe to I lobe	5.25	2.54	3.56	2.86
c.	II lobe to II lobe	3.44	-	7.28	6.26
cl.	III lobe to III lobe	3.56	-	-	-
d.	I lobe to sinus	4.32	2.32	3.22	3.74
e.	Peduncle (cm)	8.40	4.06	5.28	5.12
f.	Leaf thickness (mm)	1.25	0.62	0.47	0.52
3.	**Flower observation**				
a.	Pedicel (cm)	1.89	0.77	2.28	1.60
b.	Epicalyx/Bract				
	i) Teeth (no)	8.80	6.80	4.40	4.00
	ii) Length (cm)	2.26	2.56	4.06	3.18
	iii) Breadth (cm)	2.36	1.51	2.74	2.40
c.	Calyx				
	i) Length (cm)	2.35	0.73	0.75	0.72
	Ii) Breadth (cm)	2.56	2.14	2.82	3.16
d.	Corolla				
	i) Length (cm)	6.30	2.89	3.27	2.39
	ii) Breadth (cm)	5.36	2.37	4.00	2.47
	iii) Petal attachment (cm)	0.89	0.38	0.45	0.49
e.	Biotch/Petal Spot				
	i) Length (cm)	0.00	0.00	1.50	1.21
	ii) Breadth (cm)	0.00	0.00	1.70	1.23
f.	Androecium				
	i) Length (cm)	2.63	1.36	1.43	1.37
	ii) Anther No.	139.25	85.00	57.40	71.80
	iii) Anther size (mm)	0.89	0.58	0.73	1.12
g.	Gynoecium				
	i) Style length (cm)	4.50	1.28	2.00	1.56
	ii) Ovary length (cm)	1.98	0.47	1.00	0.79
	iii) Ovule No.	26.65	22.20	25.20	24.20
h.	Pollen Size (μ)	135.20	82.40	89.38	88.84
i.	Stomata Size (μ)	33.70	32.36	30.50	27.26
j.	Av. Stomata no./Unit area (no.)	11.25	17.60	10.20	14.00
4.	**Boll**				
a.	i) Diameter (cm)	4.25	1.98	2.60	2.27
	ii) Locule width (cm)	2.56	1.51	1.99	2.08
b.	Flower produced/plant (no)	140.25	22.00	35.00	43.00
c.	Boll set/plant (no)	98.00	11.00	12.00	35.00
d.	Boll setting %	69.87	50.00	34.29	81.40
e.	Seed/locule (no)	8.50	3.00	5.40	7.00
f.	Seed index	9.56	2.40	4.60	6.20
g.	Seed length (mm)	10.56	6.80	6.31	7.51
h.	Seed breadth (mm)	7.89	3.96	4.57	4.08
i.	% motes	0.50	68.96	30.00	0.00
j.	Fiber diameter (μ)	32.25	26.60	31.26	36.58

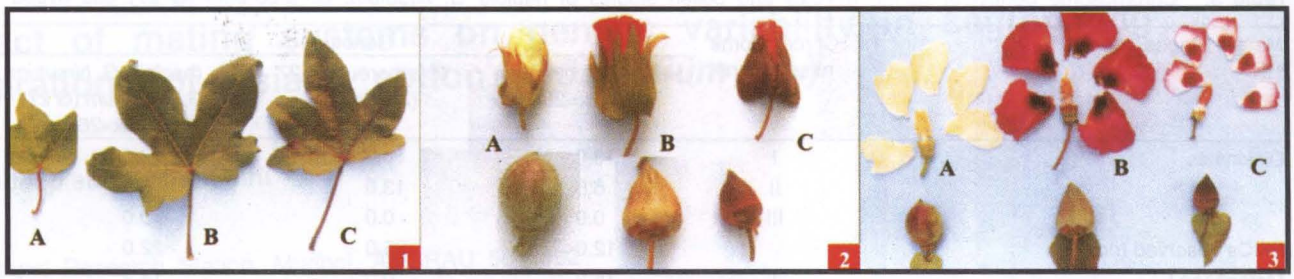

Fig. 1. (1-3) Morphological characters of parents and hybrids. 1. Leaf shape and size; 2. Flower and boll colour, shape and size; 3. Petal and anther shape size and colour (A = *G. hirsutum* L. haploid; B = F_1 hybrid (*G. hirsutum* L. haploid x *G. arboreum* L. var. G 27); and C = *G. arboreum* L. var. G 27)

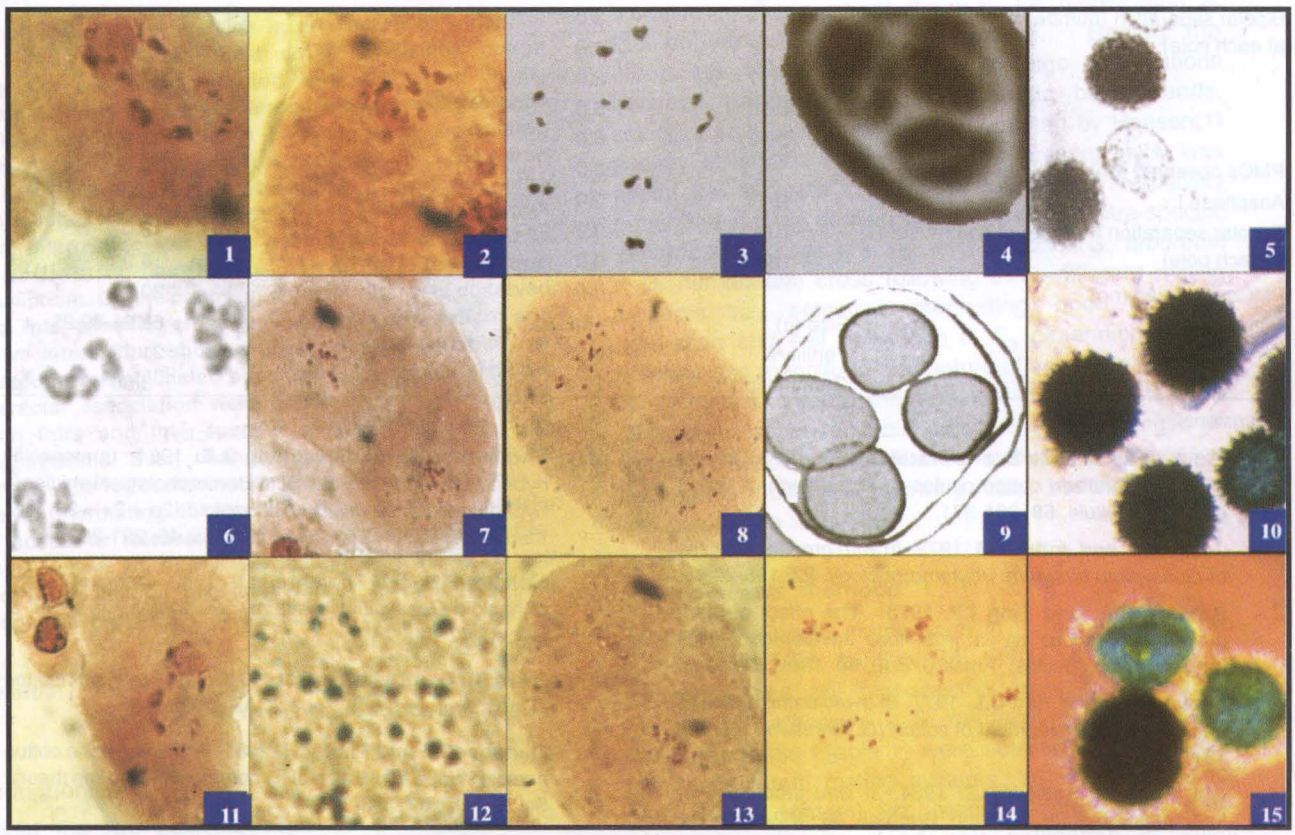

Fig. 2. (1-15): Meiosis in *G. hirsutum* L. haploid, *G. arboreum* L. Var. G 27 and F_1 hybrid. (1-5) Meiosis in *G. hirsutum* L. haploid (2n=2x=26), 1 and 2 Diakinensis (1350 x); 3. Metaphase - I; 4. Tetrads (750 x); 5. Sterile and fertile pollen; (6-10) Meiosis in *G. arboreum* G-27, 6. Metaphase I with 13 II (1250 X); 7 and 8. Normal anaphase I (1150 X); 9. Normal Tetrad (450 X); 10. Normal fertile pollen (450 X); (11-15) Meiosis in F_1 hybrid; 11. Diakinensis with paired and unpaired chromosomes (1050 x); 12. Metaphase with univalent and bivalent chromosomes. (1250x); 13. Metaphase II. (1150x); 14. Anaphase II with laggards. (1050x); 15. Sterile pollen with size variations (650x)

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Table 3. Chromosome behaviour during meiosis and pollen studies of haploid *G. hirsutum*, *G. arboreum* (G 27) and hybrid

Meiotic Stages	Chromosome associations	Genotype		
		Haploid *G. hirsutum* (2n=2x=26)	*G. arboreum* G 27 (2n=2x=26)	F_1 (haploid *G. hirsutum* × *G. arboreum* (G 27) (2n=2x=26))
Diakinesis	I	13.0	0.0	13.0
	II	6.5	13.0	6.5
	III	0.0	0.0	0.0
PMCs observed (no)		12.0	25.0	22.0
Metaphase I	I	15.2	0.0	14.2
	II	5.4	13.0	5.9
	III	0.0	0.0	*
PMCs observed (no)		23.0	20.0	27.0
Anaphase I	17-9	0.0	0.0	1.0
Bipolar separation (number of chromosome at each pole)	16-10	3.0	0.0	2.0
	14-12	4.0	0.0	5.0
	15-11	2.0	0.0	4.0
	16-10	5.0	0.0	3.0
	13-13	6.0	26.0	1.0
PMCs observed (no)		20.0	26.0	16.0
Anaphase I	6-9-11	0.0	0.0	6.0
Tripolar separation (number of chromosome at each pole)	5-10-11	0.0	0.0	16.0
	4-12-10	0.0	0.0	8.0
PMCs observed (no)		0.0	0.0	30.0
Pollen	Size (μ)	78.05-85.5	99.2±0.56	68.25-89.25
	Fertility%	18.50	93.20	21.25
	Germination %	2.60	84.25	3.56

*Occasional

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