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Short Communication

Cytomorphology of dihaploid of a interspecific cross between *Gossypium hirsutum* L. × *Gossypium barbadense* L.

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Haploids have been reported in cotton by semigamy; interspecific hybridization and x-ray treatment, polyembryony, and twinning [1], use of cotton haploids in cotton breeding is discussed [2]. Doubled haploids [3] of cotton have been found to be similar to their parent varieties in yield, yield stability, productivity of their crosses, interactions with locations and type of gene action [4]. Further reports [5] indicate that completely homozygosis will not be a disadvantage due to developmental homeostasis associated with heterozygosity i.e. developmental homeostasis has not been found in the allopolyploids. Authors recorded one spontaneous haploid plant in the field population of a interspecific (*G. hirsutum* × *G. barbadense*) cotton hybrid 'Phule-388'. The detailed morphological and cytological observations on meiotic chromosome behavior of this haploid in comparison with hybrid and its parents are reported in the present paper.

The various morphological characters of haploid, and its tetraploid parents *G. hirsutum* var. RHC 006, *G. barbadense* RHCb-001 and hybrid RHB-0388 were studied (Table 1). Data analysis, cytological studies, pollen viability and germination tests were conducted as per our earlier report [6]. For studying meiosis in parents, hybrids and haploid, the flower buds of various size were fixed in Cornoy's fluid (6:3:1) and squashed in 1% aceto-carmine. The analysis of PMCs was made from temporary mounts. Pollen sterility was tested with differential stain [7] and cytological analysis was made from temporary preparations. The pollen germination tests were carried out as per Iyengar [8].

Comparison of morphological characters of haploid, F_1 hybrid, and its parents (Table 1) revealed that, though reduction in morphological characters was significant over F_1 hybrid the differences were non significant between haploid and their either of the parents (Plate I: Figs. 1-3). Meiotic studies in F_1 indicated PMCs had n=26 chromosomes and normal meiosis and pollen development (Plate II: Figs. 4-6) while haploid PMCs contained n = 13 chromosomes with on an average 9.2^{II} + 8.4^{I} chromosome associations (Figs. 7&8). Irregular and unequal separation of chromosomes and chromatids at anaphase-I and II respectively, as reported earlier lead to formation of abnormal sporads containing 1-7 microspores (Fig. 9) and sterile pollens with high size variations (Fig. 10).

Marked and significant reduction was observed in all morphological characters (Table 1). The reduction was approximately in 1:2 ratios. Similar trend of variation were observed at both ploidy levels except for characters like stomata size, stomata/unit area, chloroplast/stoma. For bracteole teeth non-significant differences were observed. The results reported here corroborate with those obtained by earlier workers [2, 9]. Further, differences observed between haploid and its tetraploid parent for size of stomata and chloroplasts/stomata [10] were also confirmed during present investigations.

Cytological studies : Varying degrees of bivalents and chiasma per bivalent were observed in the first meiotic division in these haploids. The term bivalent other than associations used here as chiasma formation appeared in the synapsed chromosomes (Table 2). Observations of chromosome behavior reported here are confirmatory to earlier one i.e., chromosome associations ranging from 7-9 without any chiasma between A and D chromosomes during pachytene of cotton haploids [11].

The observations of chromosome behavior were confirmatory to the previous workers [9]. Further, Brown [11] reported 7-9 associations without any chiasma formation between 'A' and 'D' chromosomes during pachytene of *G. hirsutum* haploid might be due to inter-genomic homologies [1] and perfect bivalent formation observed might be due to the gene controlled phenomenon. Accordingly small amount of pairing observed might be due to polyhaploid and the affinities between 'A' and 'D' genomes leading to reduced possibility of multivalent formation and thus only occasional bivalents are formed.

Formation of bi or/ tripolar spindles univalents outside the spindles resulting in appearance like "meta-anaphase" were also observed. Unequal separation of chromosomes at metaphase-I, formation of more than four microspores, pollen grains with high size variations and without spines on their exines and

Table 1. Comparison of different morphological characters of haploid and its tetraploid parents (*G. hirsutum*, RHC-006, *G. barbadense* RHCb-001) and hybrid RHB-0388)

Sr. No.	Characters	Haploid		Hybrid RHB-0388		RHCb-001 (male)		RHC-006 (female)	
		Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	Plant height (cm)	87.0	0.0	120.0	9.6	130.0	16.0	100.0	15.0
2	Number of sympodia/plant	16.0	0.0	14.0	8.2	18.0	8.2	14.0	8.9
3	Number of monopodia/plant	4.0	0.0	3.0	10.1	4.0	3.0	5.0	4.8
4	Internodal length (cm)	5.0	0.0	4.0	9.8	5.0	8.2	4.0	4.9
5	Leaf area (cm2)	67.60	16.0	169.6	20.6	220.2	16.0	69.39	14.6
6	Petiole length (cm)	10.10	5.1	25.5	19.6	14.0	9.8	12.0	9.1
7	Stomata size (microns)	24.87	21.9	46.0	20.1	45.3	10.0	35.3	10.9
8	Number of chloroplast/stoma	11.50	15.7	24.1	17.1	22.1	7.1	22.0	9.6
9	Number of anthers/flower	36.0	17.6	115.6	17.9	105.6	9.8	103.0	9.1
10	Length of style(cm)/flower	3.0	8.5	9.1	11.2	5.2	2.7	4.4	1.8
11	Petal length (cm)/petiole	4.12	6.6	7.5	9.6	5.5	7.2	5.0	9.7
12	No. of bracteoles/teeth	9.8	16.9	11.2	15.6	11.2	11.0	10.0	19.6
13	Boil diameter (cm)	1.2	0.0	4.2	16.8	3.2	9.9	4.0	5.2
14	Number of bolls/plant	9.0	0.0	65.8	20.2	52.0	20.0	40.0	12.2
15	Boll weight (g/boll)	1.98	0.0	3.5	20.1	3.2	13.0	3.7	9.2
16	Lint weight (g/boll)	0.59	0.0	1.02	4.5	1.2	10.0	1.4	4.2
17	Seed weight (g/boll)	1.39	0.0	2.5	9.2	1.9	9.9	2.2	7.8
18	Number of seeds/boll	17.2	0.0	25.3	8.2	20.2	5.1	26.4	4.3
19	Number of motes/boll	2.0	0.0	5.0	7.2	2.1	3.2	0.0	0.0
20	Yield (g/plant)	9.80	0.0	70.3	12.2	38.7	11.0	40.3	9.8

Table 2. The observations on chromosome behaviour during meiosis studied in haploid (2n=2x=26) and its tetraploid (2n=4x=52) parents (*G. hirsutum*, RHC-006, *G. barbadense* RHCb-001 and hybrid RHB-0388)

Ploidy level	Meiotic stages	Mean chromosome associations		PMCs observed (no)
		I	II	
Haploid (2n=2x=26)	Pachytene	25.2	0.4	26
	Diakiness	6.4	9.8	23
	Metaphase-I	7.6	9.2	25
Tetraploid Hybrid	Pachytene	26.0	0.0	12
RHB-0388	Diakinesis	26.0	0.0	10
(2n=4x=52)	Metaphase-I	26.0	0.0	12
Tetraploid	Pachytene	26.0	0.0	12
RHCb-001 (male)	Diakinesis	26.0	0.0	10
(2n=4x=52)	Metaphase-I	26.0	0.0	12
Tetraploid	Pachytene	26.0	0.0	12
RHC-006(Female)	Diakinesis	26.0	0.0	10
(2n=4x=52)	Metaphase-I	26.0	0.0	12

Table 3. Chromosome separation observed at anaphase-I of meiosis of the haploid and its tetraploid parents in *Gossypium* sp.

Ploidy level	Chromosome distribution at each pole						Pollen studies		
	21-5	20-6	19-7	18-8	17-9	16-10	Size (μ)	Fertility %	Germination %
Haploid	5	12	22	25	24	5	83.2	2.35	-
Tetraploid	-	-	-	-	-	-	112.2	89.25	85.2

rarely fertile pollens were observed in present studies.

Fixation of F_1 hybrid vigour by "One Line Theory" i.e., production of doubled haploid (DH) of commercial hybrids or by inducing apomictic or parthenocarpic seed development has a tremendous practical significance in hybrid cotton cultivation. Once such lines are produced, the commercial heterosis will be "locked" in them. Their seed production on large scale at cheaper rate will become possible only by simply selfing/ growing them strictly under isolation. Hence, further, exploitation of this material in the above direction is in progress.

References

1. **Kimber G. and Riley R.** 1963. Haploid angiosperms. Bot. Rev., **29**: 480-431.
2. **Harland S.C.** 1955. The use of haploids in cotton breeding. Indian J. Genet., **15**: 15-17.
3. **Feaster C.V. and Turcotte E.L.** 1973. Yield stability in doubled haploids of American Pima cotton. Crop Sci., 13: 232-233.
4. **Meredith W.R. Jr., Bridge R.R. and Chism J.F.** 1970. Relative performance of F_1 and F_2 hybrids from doubled haploids and their parent varieties in upland cotton, *Gossypium hirsutum* L. Crop Sci., 10: 295-298.
5. **Kohel R.J. and White T.G.** 1963. An analysis of the variability of five parents and their F_1 progenies in *Gossypium hirsutum* L. Crop Sci., **3**: 359-361.
6. **Mehetre S.S., Patil V.R. and Aher A.R.** 2002. *Gossypium raimondii* source of fertility restorer for cytoplasmic male sterility of *Gossypium hirsutum* background J. *Caryologia*, **55**: 227-232.
7. **Alexander M.P.** 1969. Differential staining of aborted and non aborted pollen. Stain Tech., **44**: 117-122.
8. **Iyengar N.K.** 1939. Pollen tube studies in *Gossypium*, spp. J. Genet., **37**: 69-106.
9. **Mehetre S.S. and Thombre M.V.** 1982. Comparative studies of morphological characters of haploid and diploid plants of cotton. Indian J. Agric. Res., **16**: 215-218.
10. **Mehetre S.S.** 1982. Observation on the stomata and chloroplasts in polyhaploids and tetraploids of cotton (*Gossypium*, spp.). Sci. & Cul., **48**: 75-77.
11. **Brown M.S.** 1961. Chromosome differentiation in *Gossypium*. Amer. J. Bot., **48**: 532.

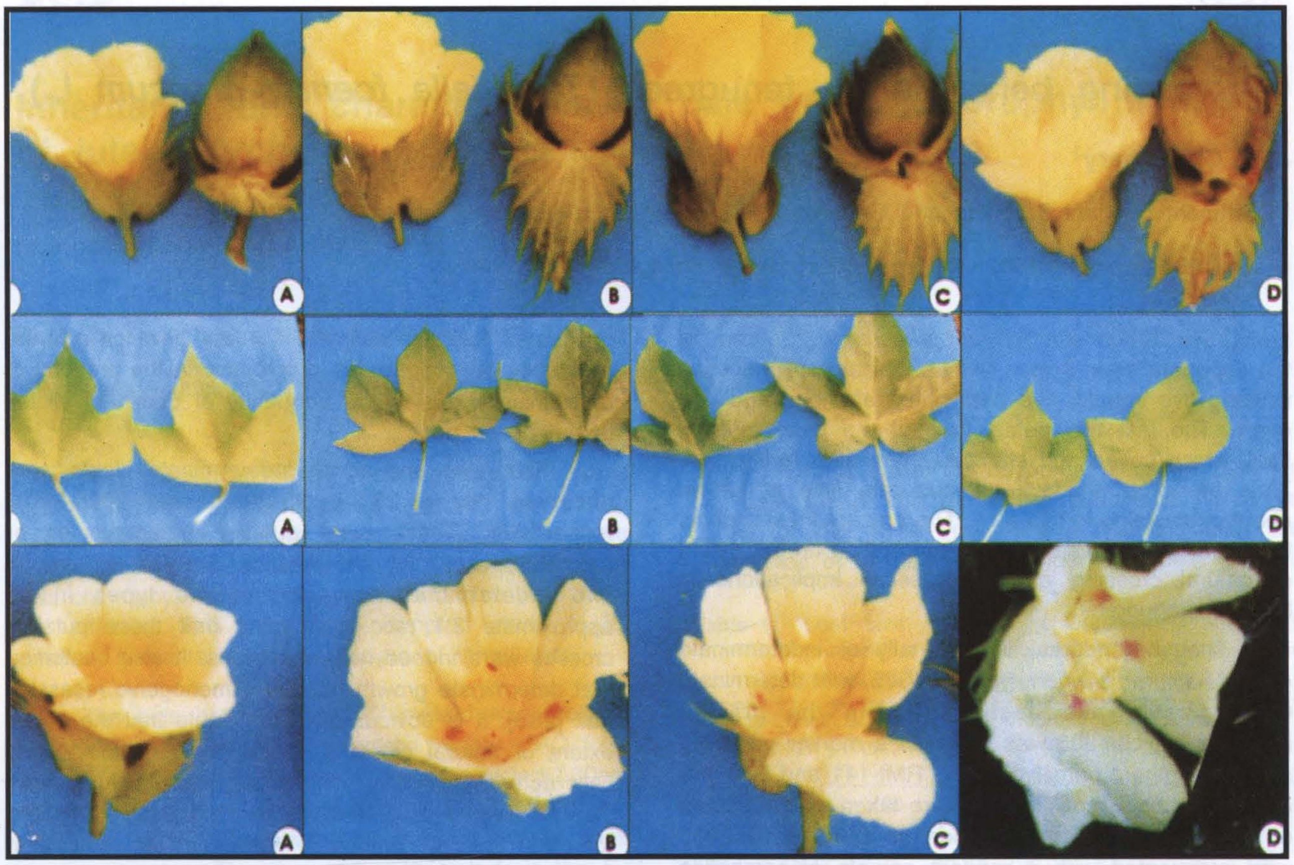

Plate I: Figs. 1-3. (1) Flower and boll size and shape; (2) Leaf size and shape and (3) presence of petal spot and colour of petal in A) Tetraploid *G. hirsutum* RHC-006 (female) (2n=2x=52); B) F_1 Hybrid RHB-388; C) Tetraploid), *G. barbadense* RHC-001 (Male) (2n=2x=52) and D) *G. hirsutum* haploid (n=2x=26)

Plate II: Figs. 1-10. (1) Comparative size and shapes of flowers and leaves; (2&3) Expression of petal spot, petal size and shape, A. Female *G. hirsutum* var. RHC-006; B. *Male G. barbadense* var. RHCb-001; C. Hybrid-Phule-388; D. Haploid; (4-6) Meiosis and pollen development in F_1 hybrid, 4. Normal bivalent formation (950x); 5. Normal tetrad formation (550x); 6. Normal fertile pollen (450x), (7-10) Meiosis and pollen development in haploid, 7. Metaphase I in $6^{II} + 14^{I}$ (950x); 8. Metaphase with 26I (950x); 9. Abnormal tetrads (780x); 10. Sterile pollen with size variation (550x)