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Cytomorphological studies in x-ray induced glandless haploids in *Gossypium hirsutum* L. (cotton)

S S MEHETRE* and M V THOMBRE
All India Co-ordinated Cotton Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar 413 722, India
* Present address: Department of Botany, Mahatma Phule Agricultural University, Rahuri, India.

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Abstract. Six haploid plants were obtained in M_2 generation of the 25 kr. x-ray irradiated *Gossypium hirsutum* L. cotton variety H.G. 108. The cytomorphological studies on these plants indicated highly irregular meiosis, giving on an average six bivalents, the range being 0–9. Unequal separation of chromosomes and chromatids at anaphase-I and II respectively led to formation of abnormal tetrads and pollens with high size variations leading to high pollen sterility. These plants were characterized by miniature stature, shorter stem and internodes, smaller leaves, flowers and stomata with fewer chloroplasts, male and female sterility and halving of chromosomes. The reduction in morphological characters was nearly in the proportion of 1 : 2 as compared to their diploid counterparts.

Keywords. Induced haploidy; cytomorphological studies; *Gossypium hirsutum* L. cotton.

1. Introduction

Haploids with gametic chromosome complements helped in understanding the cytogenetic structure of various crop plants over the past five decades. Kimber and Riley (1963), Magoon and Khanna (1963), Kirilova (1966) and Chase (1969) have reviewed the various aspects of haploids and discussed their possible uses in genetical and cytogenetical research. Natural or spontaneous monoembryonic haploids have been reported in many plant species. Spontaneous haploids have been reported in 36 species belonging to 26 genera and 10 families including the genus *Gossypium* (Kimber and Riley 1963). Haploids have been reported in cotton by semigamy (Turecotte and Feaster 1974), interspecific hybridization (Lee 1970) and x-ray treatment (Mehetre and Thombre 1977). The detailed cytological studies and morphological observations in x-ray induced haploids are reported in the present paper.

2. Materials and methods

Six conspicuous plants with small leaves with profuse but sterile flowers were detected in M_2 generation of the x-ray irradiated *G. hirsutum* cotton variety H.G. 108. The plants though self-sterile were slightly cross-fertile and set few bolls. The various morphological characters were studied in detail in these haploids and compared with their parent. Young flower buds were fixed in Cornoy'f Fluid (6 : 3 : 1) and anthers were squashed in 1% propionocarmine.

3. Results

Marked and significant reduction was observed in all morphological characters (table 1). The reduction was approximately in 1 : 2 ratio. Similar trends of variation were observed at both the ploidy levels except for characters like stomata size, number of stomata/unit area and chloroplasts/stoma. For bracteole teeth non-significant difference was observed.

Few bolls were obtained on haploid, plants, the boll number, size, number of seeds, seed (figures 1–6) and lint weight per boll and yield per plant were found to be significantly reduced while the number of motes was found to be significantly increased in haploids than the checks.

4. Qualitative characters

The variety H. G. 108 (*G. hirsutum* cotton) is highly glanded and it had leaf and floral nectaries, dark green leaves, cream pollens, cream petals and light green leaves. However, the mutant plant in X_1 H.G. 108–25–1 was completely glandless, with floral and extrafloral nectaries while petals and pollens were slightly yellowish and with wrinkled leaves. The haploids plants (1–6) were glandless but similar to that of their mother plant H. G. 108–25–1 in respect of other characters.

4.1. *Cytological observations*

The observations on the chromosome behaviour during meiosis and pollen development were made (figures 7–12). The analysis of chromosome associations observed during different stages of meiosis presented in table 2 indicates that the range of bivalent and univalent formation was from 1·9 and 22·2 (Hpl_1 and Hpl_2) to 7·0 and 14·0 (Hpl_4) and chaisma/II 0·0–0·7 (Hpl_4) to 0·88 (Hpl_4).

At second meiotic division the chromosome separation was highly irregular. The analysis of 100 PMCs at anaphases-I is presented in table 3.

In addition to abnormal distribution, tripolar separation was also observed in these PMCs due to groups of chromosomes lying outside the spindle. The division of univalents was also observed in a few cases at anaphase-I. The tripolar separation also resulted in formation of triads and other higher polyads (table 4). The range of polyads observed was 1 to 6. Monads were observed particularly in haploids 2 and 4 while dyads and other higher polyads were common in all haploids. In general, triads, tetrads and pentads were commonly observed in all haploids while monads, dyads and hexads were comparatively low. The size

Table 1. Means and coefficients of variations for the morphological characters studied in x-ray induced haploids (Hpl_1 to Hpl_6) in M_2 generation and mutant H.G. 108-21-1.

Sl. No.		Mutant (M_2) H.G. 108-25-1	Haploids					
			Hpl_1	Hpl_2	Hpl_3	Hpl_4	Hpl_5	Hpl_6
1.	Plant height (cm)	99·56	64·50	67·50	48·00	63·00	45·50	47·50
2.	No. of sympodis	2·00	3·00	3·00	3·00	6·00	2·00	..
3.	No. of monopodis	6·00	4·00	6·00	3·00	1·00	6·00	8·00
4.	Internodal length (cm)	6·55 (17·21)	6·00 (12·20)	2·70 (31·11)	2·94 (22·94)	3·40 (24·11)	3·10 (13·54)	2·90 (14·48)
5.	Leaf area (cm^2)	133·50 (5·12)	57·94 (16·66)	97·83 (21·44)	42·71 (7·93)	64·04 (26·79)	61·23 (30·05)	55·81 (8·45)
6.	Petiole length (cm)	8·54 (6·83)	6·51 (19·81)	9·97 (15·24)	7·61 (10·11)	8·91 (16·29)	6·78 (13·22)	7·22 (18·03)
7.	Stomata number (L./mm^2)	26·39 (10·91)	23·43 (20·69)	26·48 (25·01)	27·78 (22·11)	23·94 (19·23)	19·38 (24·16)	20·48 (26·17)
8.	Chloroplast number/ stoma	18·70 (5·98)	9·80 (19·28)	10·40 (19·26)	8·40 (13·17)	7·30 (16·60)	6·80 (17·61)	8·15 (17·57)
9.	No. of anthers/ flower	88·00 (9·91)	56·50 (14·43)	68·80 (14·08)	47·10 (17·28)	26·30 (10·00)	32·20 (8·62)	35·70 (10·80)
10.	Length of style (cm)	3·10 (1·91)	2·65 (7·12)	2·56 (5·07)	2·70 (10·75)	2·98 (8·12)	2·66 (1·95)	2·79 (5·01)
11.	Petal length (cm)	4·00 (19·62)	3·43 (9·43)	3·72 (6·56)	3·00 (13·00)	3·26 (12·67)	3·46 (4·44)	3·41 (7·40)
12.	No. of bracteole teeth/bracteole	10·10 (1·69)	8·40 (16·44)	10·00 (15·60)	6·50 (16·61)	7·90 (18·35)	8·20 (20·60)	9·20 (22·17)
13.	Boll diameter (cm)	2·00 (3·96)	1·62 (11·89)	1·67 (11·45)	1·63 (12·90)	1·67 (3·03)	1·69 (2·33)	1·37 (15·18)
14.	No. of bolls/ plant	49·00 (12·69)	8·00 ..	20·00 ..	8·00 ..	10·00 ..	9·00 ..	11·00 ..
15.	Boll wt. g/ boll	3·59 (10·87)	2·11 (20·45)	2·25 (22·45)	2·50 (15·45)	2·75 (16·20)	2·45 (13·40)	2·45 (17·10)
16.	Lint wt./boll	1·47	0·85	1·15	0·95	1·05	1·15	0·88
17.	Seed wt. (g/boll)	2·32 (11·19)	1·26 (18·38)	1·10 (52·54)	1·55 (6·34)	1·70 (8·87)	1·30 (8·09)	1·57 (9·98)
18.	No. of seeds/ boll	24·20 (8·67)	8·80 (27·50)	6·57 (26·44)	3·90 (38·97)	6·55 (20·30)	4·80 (21·45)	5·20 (23·65)
19.	No. of motes/ boll	0·80 (21·00)	8·20 (24·26)	8·23 (28·65)	7·30 (34·01)	7·00 (33·71)	6·50 (54·92)	6·22 (43·08)
20.	Seed cotton yield/plant (g)	176·0 (11·70)	24·80 ..	45·00 ..	12·00 ..	27·50 ..	22·05 ..	25·95 ..

Note : Figures in the bracket are of coefficients of variation.

Table 2. Chromosome pairing observed in x-ray induced *G. hirsutum* var. H.G. 108 (Hpl_1 to Hpl_6).

Haploids	Meiotic stages	No. of PMCs examined	Chromosome associations average per PMC		X' ta/II
			I	II	
Hpl_1	Pachytene	16	22·2	1·9	0·21
	Diakinesis	30	22·0	2·0	0·21
	Metaphase-I	30	20·0	3·9	0·22
Hp_2	Pachytene	10	21·2	2·4	0·18
	Diakinesis	40	14·0	6·0	0·22
	Metaphase-I	45	14·0	6·0	0·22
Hpl_3	Pachytene	7	22·0	2·0	0·09
	Diakinesis	27	22·2	1·9	0·08
	Metaphase-I	29	14·0	6·0	0·07
Hpl_4	Pachytene I	10	16·0	3·0	0·11
	Diakinesis	15	12·0	7·0	0·09
	Metaphase-I	22	14·0	6·0	0·88
Hpl_5	Pachytene	8	..	..	..
	Diakinesis	18	18·4	3·8	0·22
	Metaphase-I	21	15·2	5·4	0·18
Hpl_6	Pachytene	6	..	..	..
	Diakinesis	25	20·2	2·9	0·21
	Metaphase-I	32	15·2	5·4	0·10

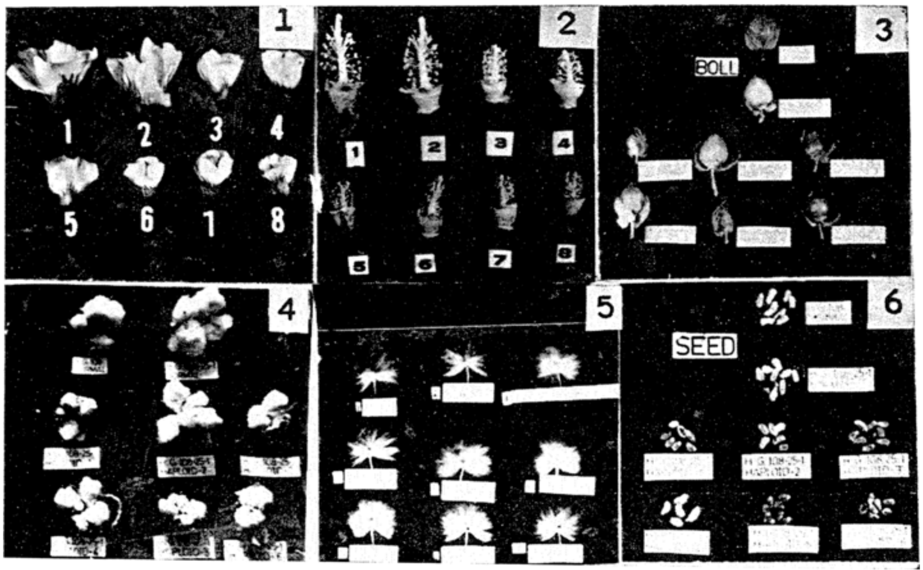
of pollen in each haploid varied from 35·70 to 117·30μ as against 112·80 to 142·80μ in their normal diploid counterparts (table 5).

Pollen fertility in diploid is 92% while 2, 28, 0, 32, 1 and 4 per cent in haploids 1–6 respectively.

5. Discussion

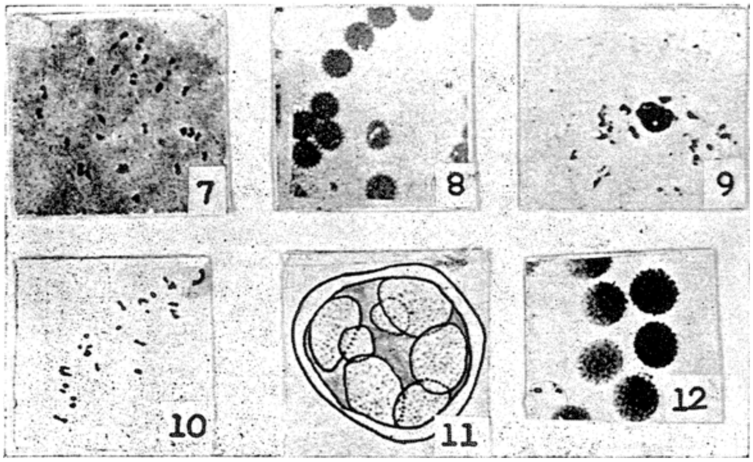
The results of the different morphological characters indicated significant reduction closer to 1 : 2 ratio. The values of coefficients of variation indicated that there were similar trends of variation at both the ploidy levels. The slightly higher variation observed in some characters might be due to sampling error.

The results reported here coroborate with those obtained by Dergach (1970), Harland (1955), Lee (1970), Owing *et al* (1964), Roux (1958), Silow and Stephens (1944), Tiranti (1965) and Mehetre and Thombre (1977). The miniature stature of haploid, the shorter stem and internodes the smaller leaves and flowers, the smaller stomata with fewer chloroplast, and the non dehiscent reported by Barrow



Figures 1–6. Morphological characters of H.G. 108 (parent), its mutant (H.G. 108–25–1) and haploids 1–6 obtained from the mutant. Note : 1. H.G. 108–parent ($2n = 4x = 52$). 2. H.G. 108–25–1—Mutant ($2n = 4x = 52$). 3–8. Glandless haploids 1–6 ($n = 2x = 26$).

1. Flowers of parent, (1) mutant (2) and haploids (1–6). **2.** Dehiscent (1 and 2) and non-dehiscent (3–8) anthers in parent, mutant and haploids. **3.** Glanded (1) and glandless (2), (3–8) bolls in parents and haploids. **4.** Open bolls in parent (1), mutant (2) and haploids (1–6). **5.** Fibre length in H.G. 108 parent (1), mutant (2 and 3) and haploids (4–9). **6.** Seed size in parent (1), mutant (2) and haploids (1–6).



Figures 7–12. Meiosis and pollen development in glandless mutant H.G. 108-25-1 ($2n = 4x = 52$) and its haploids ($2n = 2x = 26$).

Meiosis in glandless mutant H.G. 103-25-1 ($2n = 4x = 52$). **7.** Metaphase-I with 26^{II}. **8.** Normal fertile pollen grains with well developed exine.

Meiosis in glandless haploid-2 ($n = 2x = 26$).

9. Pachytene showing unpaired univalent and bivalents. **10.** Metaphase-I with $4^{II} + 18^{I}$. **11.** Camera lucida drawing showing abnormal sporads. **12.** Unstained sterile and stained normal fertile pollen grains.

(All chromosomes magnified at 750 × and pollen grains and tetrads at 350 ×).

Table 3. The distribution of chromosomes at anaphase-I in x-ray induced haploids in *G. hirsutum* cotton

Haploids	Distribution of chromosomes at anaphase-I						
	13–13	14–12	15–11	16–10	17–9	18–8	19–7
Hpl_1	6	20	19	20	15	12	8
Hpl_2	20	25	15	15	16	8	7
Hpl_3	2	20	25	20	23	5	5
Hpl_4	22	26	22	8	7	7	8
Hpl_5	2	30	29	10	9	10	10
Hpl_6	8	22	22	29	9	5	5

Table 4. Frequency of microspores produced in PMCs at sporad stage in x-ray induced haploids of *G. hirsutum* cotton.

Haploids	Number of microspores produced per PMC							Av./PMC
	1	2	3	4	5	6	Total	
Hpl_1	0	7	39	29	14	10	100	3·77
Hpl_2	1	6	25	40	26	2	100	3·90
Hpl_3	..	7	29	36	21	5	100	3·74
Hpl_4	3	10	26	42	19	10	100	4·24
Hpl_5	1	2	30	30	22	15	100	4·15
Hpl_6	..	9	20	25	26	20	100	4·28

(1971), Chaudhari and Barrow (1975), Meyer and Justus (1961), Sastry and Swaminathan (1960) and Turecotte and Faster (1974) are in agreement with the present results.

4.2. *Meiotic studies*

The varying degrees of bivalents and chiasma per bivalent were observed in the first meiotic division of these haploids. The term bivalent is used to distinguish these from associations on the basis of chiasma observed in synapsed or paired chromosomes.

These results are similar to those reported by Beasley (1942), Endrizzi (1959) and Brown (1961) who reported average of 5, 0·16 and 7–9 bivalents per PMC in haploids respectively. Brown (1961) reported 7–9 associations without any chiasmata between A and D chromosomes at pachytene in *G. hirsutum* haploid. However, such details are not available for comparison on meiotic behaviour of *G. hirsutum* cotton haploids reported by Beasley (1940 and 1941), Harland (1936), Silow and Stephens (1944) and Meyer and Justus (1960).

Table 5. Pollen grain sizes in x-ray induced haploids (Hpl_1 to Hpl_6) of *G. hirsutum* cotton Var. H.G. 108.

Haploids	Pollen grain size in cocular units (1 unit = 5·10 microns)																						Total
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Hpl_1	1	2	..	..	..	9	10	10	9	21	20	10	2	2	4	..	..	..	..	..	..	..	100
Hpl_2	..	..	..	..	..	5	10	9	13	12	20	10	55	5	4	5	1	..	..	..	..	..	100
Hpl_3	1	2	1	2	1	9	20	11	10	19	10	4	4	4	1	1	..	..	..	..	..	..	100
Hpl_4	..	..	..	..	..	..	10	21	19	10	9	11	11	4	3	2	..	..	..	..	..	..	100
Hpl_5	..	3	1	3	2	7	9	10	11	11	11	9	10	5	5	..	..	..	..	..	..	..	100
Hpl_6	..	2	3	3	1	10	7	9	9	11	14	11	9	6	5	..	..	..	..	..	..	..	100
Diploids	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	25	40	20	5	4	3	3	120

According to Burnham (1962) occasional rod-shaped or ring bivalents are observed at meiosis in haploids where normally univalents are expected. Catcheside (1937) considered that occurrence of chromosome associations in haploid as evidence for duplicated segments.

Exceptional division of univalents at anaphase-I has been observed in these haploids is also confirmatory to the observations reported by Brown (1958) and Barrow (1971) who reported it in *Gossypium* spp. and haploids respectively. Beasley and Richmond (1939) reported x-ray-induced haploids with 7–9 bivalents/ PMC. These workers have also reported chiasma from pachytene to metaphase-I and explained it on the basis of differential rate of coiling of A and D chromosomes.

The data on bivalents observed on allopolyhaploids indicated bivalent formation which was explained on the basis of pairing between homologous chromosomes from component genomes (Kimber and Riley 1963). The normal bivalents formation in allotetraploid cotton is attributed to the critical differentiation which occurred between the constituent genomes (AD) due to structural modifications and divergence of chromosome sets. The possibility of perfect bivalents formation observed might be due to gene-controlled phenomenon (Kimber 1961). Accordingly the small amount of pairing observed might be due to polyhaploid and the affinities between A and D genomes reduced possibility of multivalent formation and thus, only occasional bivalents are formed. Such genetic isolation of homologous chromosomes is reported from the behaviour of allopolyhaploid *Nicotiana tabacum* (Lammerts 1934).

References

Barrow J R 1971 Meiosis and pollen development in haploid cotton plants; *J. Hered.* **66** 277–280

Beasley J O 1940 The production of polyploids in *Gossypium*; *J. Hered.* **31** 39–48

Beasley J O 1941 Hybridization, cytology and polyploids in *Gossypium*; *Chron. Bot.* **6** 394–395

Beasley J O 1942 Meiotic chromosome behaviour in species, species hybrids, haploids and induced polyploids of *Gossypium*; *Genetics* **27** 25–54

Beasley J O and Richmond T R 1939 Cytogenetics and improvement in cotton; *52nd Rep. Tex. Agric. Expt. Sta.* p. 14

Brown M S 1958 The division of univalent chromosomes in *Gossypium*; *Am. J. Bot.* **45** 24–52

Brown M S 1961 Chromosome differentiation in *Gossypium*; *Am. J. Bot.* **48** 532

Burnham C R 1962 *Discussions in cytogenetics*; Burgess Publ. Co. Minneosota (USA)

Catcheside D G 1937 Secondary pairing in *Brassica oleraceae*; *Cytologia* Fujii Jap. 336

Chase S S 1969 Monoploid and monoploid derivatives of maize (*Zea mays* L.); *Bot. Rev.* **35** 117–167

Chaudhari H K 1975 Morphology and breeding behaviour of cotton haploids; *Canad. J. Genet Cytol.* **18** 673–678

Chaudhari H K and Barrow J R 1975 Identification of cotton haploids by stomatal chloroplast count technique; *Crop. Sci.* **15** 760–763

Dergach G V 1970 Morphological and anotomical characteristics of haploid plants of *Gossypium barbadenase*; *Plant Breed. Abstr. No.* 5560 (1972)

Endrizzi J E 1959 Cytogenetics of four sets of twin in cotton; *J. Hered.* **50** 222–226

Harland S C 1936 Haploids in the polyembryonic seeds of Sea Island cotton; *J. Hered.* **27** 229–231

Harland S C 1955 The use of haploids in cotton breeding; *Indian J. Genet.* **15** 15–17

Kimber G 1961 The basis for diploid like cytological behaviour of polyploid cotton; *Nature* (*London*) **191** 98–100

Kimber G and Riley R 1963 Haploid angiosperms; *Bot. Rev.* **29** 480–451

Kirilova G A 1966 The phenomenon on haploids in angiosperms; *Genetica* **2** 137–147

Lammerts W E 1934 On the nature of chromosome association in *Nicotiana tabacum* haploid; *Cytologia* **6** 38–50

Lee J A 1970 On the origin of haploid/diploid twinning in cotton; *Crop Sci.* **10** 453–454

Magoon M L and Khanna K R 1963 Haploids; *Caryologia* **16** 191–235

Mehetre S S and Thombre M V 1977 Glandless haploids $n = 2x = 26$ in *Gossypium hirsutum* L. cotton; *Curr. Sci.* **46** 350–351

Meyer J R ad Justus N 1960 Potential value of doubled haploids in cotton; *Genetics* **45** 999

Meyer J R and Justus N 1961 Properties of doubled haploids in cotton; *Crop. Sci.* **1** 462–464

Owing A D, Sarvella P and Meyer J R 1964 Twinning and haploidy in a strain of *Gossypium barbadense* L. cotton; *Crop Sci.* **4** 652–653

Roux J B 1958 L haploidie chez le cotonnier ; *Cotton Fibres Trop.* **13** 289–292

Sastry G P K and Swaminathan M S 1960 Chromosome association in haploid *Gossypium barbadense*; *Curr. Sci.* **29** 398–400

Silow R A and Stephens S G 1944 "Twinning" in cotton; *J. Hered.* **35** 76–78

Tiranti I N 1965 Chromosome association and some characters of haploid plant in *Gossypium hirsutum* L.; *Bio. Genet. Inst. Fititec. Caster.* No. **1** 21–26

Turecotte C V and Feaster C V 1974 Semigamatic production of cotton haploids in *Haploids in higher plants-advances and potential.* (ed.) K J Khasha, Univ. Guelph, Canada pp. 63–64.