Proc. Indian Acad. Sci. (Plant Sci.), Vol. 91, Number 5, October 1982, pp. 409–416.

Anther and pollen development in cotton haploids and their parents

S S MEHETRE

Department of Botany, Mahatma Phule Agricultural University, Rahuri, District, Ahmednagar (MS) 413 722, India

MS received 29 October 1981 ; revised 29 July 1982

Abstract. Development of anther tapetum from premeiotic stages to pollen formation was studied in six x-ray induced haploids of *Gossypium hirsutum*, three interspecific F_2 haploids, and one natural haploid of each of *G. hirsutum* and *G. barbadense*, and the observations were compared with those of their respective parents, a genetic male sterile, a male fertile and a cytoplasmic male sterile line of *G. hirsutum*. Significant differences were recorded for number of anthers per flower, pollen size, pollen viability and number of microspores produced by PMC. Anther development in haploids was normal. Anther dehiscence was also normal in some haploids. Non-dehiscent anthers could be mostly attributed to the formation of immature pollen grains. Normal development of anthers and degeneration of tapetum occurred in the parents and in the genetic fertile line. Contrastingly no degeneration of tapetum was noticed in the cytoplasmic male sterile line.

Keywords. *Gossypium* spp. ; haploids ; anther tapetum ; male sterile.

1. Introduction

Haploids are characterized by significant decrease in size of vegetative plant parts, vigour and fertility (Kostoff 1943) and in diameter of pollen mother cells (Belling and Blakeslee 1923) by half of that in the diploid (Ivanov 1938), and by smaller guard cells (Lamm 1938). Kimber and Riley (1963) indicated a relationship between haploid and diploid guard cells of *G. hirsutum* and *G. barbadense* by a factor of 1·26.

Partial or complete sterility due to halving of chromosome number and several meiotic irregularities in haploids of *G. hirsutum*, *G. barbadense*, their F_2 interspecific crosses and x-ray induced haploids have been reported (Mehetre and Thombre 1981b, c, d). Studies were undertaken to investigate the development of anther tapetum and its role in pollen sterility observed in these haploids. The comparative observations made on haploids, their respective parents, genetic male sterile and fertile lines and one cytoplasmic male sterile line have been reported

Table 1. Data on pollen and anther development in cotton haploids and their parents.

Species	Genotype	Chromosome number ($2n$)	Anthers per flower	Microspores per PMC	Pollen size (μm)		Pollen viability (%)	Average radial width of tapetal cell (μm)				
					Range	Av.		Premeiotic	Prophase-I	Anaphase-I	Tetrad	Pollen
								Stages				
1	2	3	4	5	6	7	8	9	10	11	12	13
G. hirsutum	HG-108	$4x = 52$	88·00± 9·91	4·00	116·30–131·20	117·30	98·2	7·62+ 4·02	8·12± 0·96	6·1± 0·12	4·98± 0·19	3·89± 0·29
	Haploid 1 (Hpl_1)	$2x = 26$	56·30± 14·43	3·77	35·60–86·10	69·16	2·1	6·69± 0·99	6·99± 0·23	5·51± 0·11	5·98± 0·19	4·16± 0·24
	Hpl_2	$2x = 26$	63·68± 14·08	3·90	61·20–117·30	72·30	28·2	6·09± 0·16	6·51± 0·19	6·17± 0·21	5·72± 0·21	4·97± 0·33
	Hpl_3	$2x = 26$	47·10± 17·28	3·74	35·70–112·20	63·14	0·0	5·99± 0·21	6·25± 0·17	5·98± 0·23	5·16± 0·23	4·79± 0·36
	Hpl_4	$2x = 26$	26·30± 10·00	4·24	66·30–112·20	74·19	32·0	6·67± 0·23	6·70± 0·19	6·39± 0·24	6·01± 0·44	5·50± 0·49
	Hpl_5	$2x = 26$	32·30± 8·62	4·15	40·80–107·10	68·16	1·0	6·61± 0·31	6·74± 0·24	5·14± 0·31	5·99± 0·34	5·16± 0·57
	Hpl_6	$2x = 26$	35·70± 10·80	4·28	40·80–105·10	73·25	4·1	6·91± 0·27	7·10± 0·27	6·59± 0·44	6·10± 0·39	5·57± 0·16
	Laxmi (female parent of Varalaxmi)	$4x = 52$	62·10± 9·16	4·00	115·30–137·70	129·15	94·0	6·29± 0·24	7·92± 0·12	6·16± 0·13	4·98± 0·09	4·19± 0·13
G barbadense	S.B. 289-E (male parent of Varalaxmi)	$4x = 52$	70·13± 8·68	4·00	112·20–137·70	131·69	95·2	6·29± 0·77	7·01± 0·19	6·98± 0·10	4·04± 0·12	3·98± 0·39

F_2 of inter-specific cross Varalaxmi	F_2Hpl_1	$2x = 26$	64·00± 25·34	4·67	45·90– 117·30	63·69	3·2	5·78± 0·22	6·28± 0·22	6·01± 0·27	5·28± 0·21	4·78± 0·43
	F_2Hpl_2	$2x = 26$	48·00± 14·83	4·60	45·90– 112·20	72·97	1·2	5·98± 0·39	6·51± 0·17	5·98± 0·20	5·51± 0·39	5·28± 0·48
	F_2Hpl_3	$2x = 26$	36·16± 16·35	4·78	45·90– 117·30	79·17	1·8	6·22± 0·57	6·79± 0·36	6·50± 0·19	5·78± 0·44	5·21± 0·39
G. hirsutum	IAH 468	$4x = 52$	103·12± 9·26	4·00	122·10– 130·50	122·30	94·0	6·12± 0·12	7·76± 0·21	6·29± 0·13	4·98± 0·11	4·12± 0·21
	Haploid	$2x = 26$	63·19± 19·80	4·53	25·50– 112·10	66·05	2·4	5·19± 0·29	5·75± 0·34	5·25± 0·29	5·05± 0·17	4·79± 0·29
G. barbadense	Giza–45	$4x = 52$	86·00± 7·32	4·00	122·20– 130·00	120·26	95·2	6·98± 0·29	7·59± 0·13	6·11± 29	4·59± 0·12	3·29± 0·19
	Haploid	$2x = 26$	53·00± 18·17	4·13	30·60– 81·60	51·31	1·8	5·17± 0·31	5·79± 0·39	5·30± 0·20	5·10± 0·27	4·88± 0·29
G. hirsutum	Gregg genetic male sterile	$4x = 52$	86·13± 6·16	4·00	116·17 129·24	119·16 **	0·0	6·98± 0·17	7·25± 0·25	7·11± 0·16	6·98± 0·39	6·28± 0·21
	Gregg male fertile	$4x = 52$	89·16± 5·29	4·00	86·29**– 112·12 Non-dehiscent anthers	96·15	96·11	7·01± 0·21	7·51± 0·17	6·27± 0·21	5·00± 0·41	4·13± 0·31
	Cytoplasmic male sterile GH : 572	*	82·00± 9·17	*	*	*	*	6·21± 0·17	17·25± 0·21	27·45± 1·39	25·25± 2·17	4·13± 0·29

* PMCs degenerated before premeiotic stages ; therefore chromosome number, microspores/PMC, pollen size and pollen viability could not be studied.

** Significant at 1% (between Gregg male sterile and fertile).

2. Material and methods

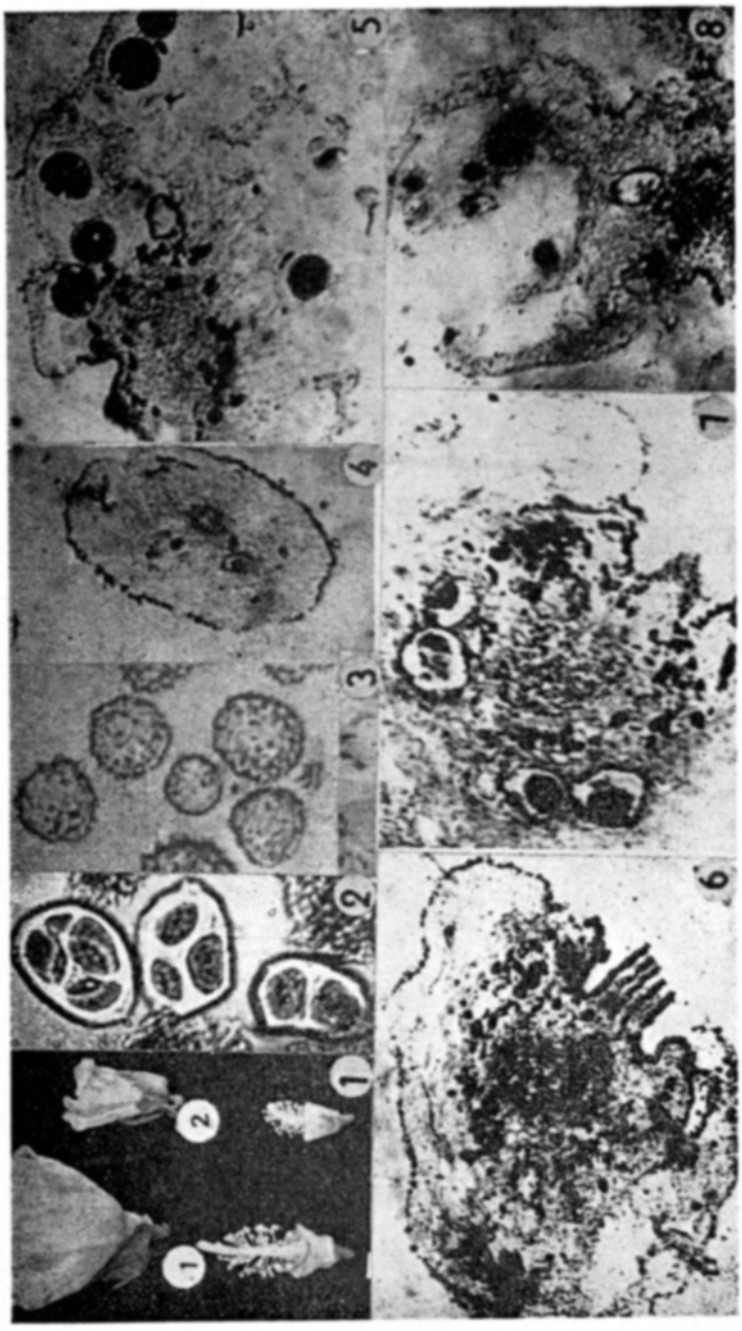
The length of flower bud at various meiotic stages was determined by studying meiosis in the fertile counterparts and haploids. Flower buds of different haploids, their respective parents, genetic male sterile and fertile lines and cytoplasmic male sterile line (table 1) were collected from premeiotic to pollen formation stages and fixed in Randolph's Craf ; after dehydration, the anthers were embedded in paraffin. Sections of 12 μm were cut and stained with iron alum hematoxylin (Johansen 1940). Pollen viability (fertility/sterility) was tested by differential staining with a solution comprising among other organic components malachite green, acid fuschin, and orange G. (Alexander 1969). The data collected from 25 observations for each of the parameters mentioned in table 1 were analysed statistically (Panse and Sukhatme 1953) and standard deviations and significance of differences between means were calculated.

3. Results and discussion

Significant differences were noticed between haploid and diploid plants in size of flowers and androecia, in number of anthers per flower (figure 1), tetrads per microspore (figure 2) and in pollen size and pollen sterility (figure 3). In haploids the average number of microspores resulting from a PMC ranged from 3·77 to 4·78. The large variation observed in pollen size (figure 3) in all the haploids indicated that the pollen grains contained varying number of chromosomes. Although well-developed exine and spines were noticed on some pollen grains, probably microspore mitosis and starch formation had not occurred in them, thus resulting in pollen sterility.

In the cytoplasmic male sterile line the tapetum was well developed and its cells were enlarged. There was normal differentiation of anther wall, but the sporogenous tissue collapsed early during meiosis ; meiosis did not proceed beyond prophase and hence the tapetum remained intact and enlarged (figure 6). Similar observations were recorded by Murthi and Weaver (1974) and Mehetre and Thombre (1981a) for the cytoplasmic and genetic male sterile (MS_5 and MS_6) stocks of *G. hirsutum*. In the present study on the genetic male sterile stock normal development of anther tapetum was noticed, but the microspores aborted due to development of vacuoles in them (figure 7). In the male fertile counterparts (figure 8), in all the tetraploid parents and in all the haploids (figures 4, 5) the tapetal cells begin to digenerate at the time of separation of microspores from the tetrads.

In the fertile lines the microspores develop a thick exine and a thin intine. Spines develop on the exine and the germ pores become distinct. The microspore nucleus divides to produce the generative nucleus and the vegetative nucleus ; at this stage the pollen grain is considered to be mature and ready for shedding. Similar observations were reported by Murthi and Weaver (1974) and Mehetre and Thombre (1981a) for male fertile anthers and Mehetre (1981) for triploid (3x = 39) and tetraploid (4x = 52) anthers. In the haploids, however, after separation of the microspores from tetrads the exine may become well developed but the



Figures 1–8. **1.** Flowers and androecia of (1) Parent H G 108 and (2) Haploid No. 1 (1/2x). **2.** Abnormal "tetrads" of x-ray induced haploid Hpl_3 showing different number of microspores (550 ×). **3.** Sterile pollen grains of x-ray induced haploid Hpl_3 showing variation in size and underdeveloped spines (450 ×). **4.** Trans-section of anther of haploid F_2 Hpl_1 prior to initiation of meiosis (20 ×). **5.** LS non-dehiscent anthers of haploid F_2 Hpl_1 one day before anthesis showing uneven degeneration of tapetum (60 ×). **6.** LS anther of cytoplasmic male sterile line one day before anthesis showing complete intact tapetum (65 ×). **7.** LS anther of genetic male sterile line one day before anthesis showing vacuolated pollen grains (65 ×). **8.** Complete degenerated tapetum of Gregg male fertile line at anthesis (45 ×).

spines are not of uniform size, the germpores are not distinct and the pollen grains do not mature because the mircospore nucleus does not undergo division, as was also observed in the parents of the haploids, such underdeveloped immature pollen does not contain sufficient starch grains.

Decrease in the radial dimensions of tapetal cells occurred in the fertile materials between anaphase I and pollen formation, while in the haploid plants the decrease was observed between anaphase I and tetrad stage only. The extent of decrease was variable from anther to anther, flower to flower and plant to plant.

The data on measurements of radial width of anther tapetum in parents of haploids and in the genetic fertile line indicated that in parents of haploids the degeneration of anther tapetum continues progressively from meiotic anaphase to pollen stages and it ranged from 3 to 6 microns at pollen stage, while in the genetic male sterile line the tapetum remained intact even after microspore tetrad stage. Pollen abortion occurred due to vacuolation of pollen caused presumably by nutritional differences, while in the cytoplasmic male sterile line meiosis did not proceed and hence the tapetum remained intact. A similar behaviour of anther tapetum was also reported by Brooks *et al* (1966) in anthers of different genetic and cytoplasmic male sterile, make fertile and fertility restorer lines of sorghum by Murthi and Weaver (1974) and Mehetre and Thombre (1981a) in cotton.

In all the three groups of haploids a marked variation in the width of tapetal cells from pre-prophase to pollen stage was observed. In some individuals, the degeneration of tapetum was rapid while in some individuals it was slow. Although significant differences in tapetal cell width were noticed in tetraploid and diploid plants, it was not in a 1 : 2 ratio. The pollen abortion and sterility was mainly due to microspores containing variable number of chromosomes and pollen with high variation in size and probably not due to the abnormal development of tapetum.

References

Alexander M P 1969 Differential staining of aborted and non-aborted pollens ; *Stain Technol.* **44** 117–122

Belling J and Balakeslee A F 1923 The reduction division in haploid, diploid and tetraploid Daturas ; *Proc. Natl. Acad. Sci.* **60** 106–111

Brooks M H, Brooks J S and Chien I 1966 The anther tapetum in cytoplasmic genetic male sterile sorghum ; *Am. J. Bot.* **53** 902–908

Ivanov M A 1938 Experimental production of haploids in *Nicotiana rustica* L. ; *Genetica* **20** 295–397

Johansen D A 1940 *Plant Microtechnique*, Tata McGraw-Hill Co. 2nd ed.

Kimber G and Riley R 1963 Haploid angiosperms ; *Bot. Rev.* **29** 480–531

Kostoff D 1943 Haploide *Triticum vulgare* and die Variabiliabat inrer diploiden Nachkommens schaften ; *Zuchter.* **15** 121–125

Lamm R 1938 Note on haploid potato hybrid ; *Herediatas*, **24** 39

Mehetre S S 1981 Anther and pollen development in traploid ($3x = 39$) and tetraploid ($4x = 52$) plants in cotton (*Gossypium* spp.) *Phytomorphology* (in press)

Mehetre S S and Thombre M V 1981a Stages of pollen abortion in male sterile stocks of *Gossypium hirsutum* L.; *J. Maharashtra Agril. Universities* **6** 159–161

Mehetre S S and Thombre M V 1981b Cytomorphological studies in x-ray induced glandless haploids in *Gossypium hirsutum* L. cotton ; *Proc. Indian Acad. Sci.* (*Plant Sci.*) **90** 313–322

Mehetre S S and Thombre M V 1981c Meiotic studies in the haploids ($2n = 2x = 26$) of tetraploid cottons ($2n = 4x = 52$) ; *Proc. Natl. Sci. Acad.* **B47** 516–518

Mehetre S S and Thombre M V 1981d Microsporogenesis in interspecific F_2 haploids of cotton ; *Phytomorphology* (in press)

Murthi A N and Weaver J B 1974 Histological studies on the five male sterile strains of upland cotton ; *Crop Sci.* **14** 658–662

Panse V G and Sukhatme P V 1953 *Statistical methods for agricultural workers* ICAR, New Delhi