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Cytomorphology of interspecific hybrids between *Gossypium hirsutum* L., its haploid and *Gossypium raimondii*

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Abstract

Interspecific hybrids between *G. hirsutum*, its haploid and *G. raimondii* were obtained. Morphological studies of different characters indicated that flower colour, petal spot, filament colour, pollen colour, stems, leaf hairiness etc. of *G. raimondii* were found to be dominant in both the hybrids while other growth characters and habit were intermediate. Meiosis in *G. hirsutum* and *G. raimondii* was normal leading to formation of viable pollen, however, in haploid and both the hybrids it was abnormal. On an average chromosome associations at diakinesis and metaphase-I were $4.11^{I} + 4.69^{II} + 4.17^{III}$ and $5.30^{I} + 4.20^{II} + 4.10^{III}$ and $10.55^{II} + 10.27^{II} + 1.13^{III} + 1.13^{IV}$ and $10.29^{I} + 10.99^{II} + 1.39^{III} + 0.64^{IV}$, in diploid (*G. hirsutum* haploid × *G. raimondii*) and triploid (*G. hirsutum* × *G. raimondii*), hybrids, receptively. Further, 4 to 6 bivalents observed in hybrid between *G. hirsutum* haploid × *G. raimondii* were due to pairing between. A_h D_5 chromosome of *G. hirsutum* and *G. raimondii*, respectively and also Ah with D_h and D_5 chromosomes. In addition, bivalents formed in diploid hybrid and trivalent and quadrivalents in triploid hybrid due to pairing between non-homologous chromosomes. Pattern of chromosome pairing indicated that chromosomes from D_5 genome of the *G. raimondii* have closer homology with A_h D_h, chromosomes of *G. hirsutum*. Formnation of multivalents, univalents and unequal separation of chromosome has resulted in formation of sporads containing 1-7 spores leading to formation of sterile pollens with high size variation.

Key words: Cytomorphology, *Gossypium hirsutum*, *Gossypium raimondii*, haploid, interspecific hybrids

Introduction

More than fifty wild species of genomic constitution A to G and K have been reported. In addition to this, a number of primitive perennial races having economically useful attributes are also available in the wealth of genus *Gossypium* [1]. Many inter-specific hybrids have been made and studied in this genus. These have provided (i) useful information for understanding species relationship in the genus and (ii) new source of germplasm to be incorporated in to breeding programme [2].

In inter specific hybridization resorted for transplanting gene/genes which are not available within the limits of cultivated species. From the literature reviewed so far, it appeared that majority of the work has been done between cultivated tetraploid and wild diploid. Since the cultivated American cottons are allotetraploid (2n = 4x = 52) and the majority of the wild species are diploids (2n = 2x = 26), hybrids produced between these two chromosomal types are sterile triploids. Doubling of the chromosomes of sterile triploid produces an allohexaploid, which usually have sufficient fertility for self pollination and for backcrossing to the cultivated tetraploid cotton. This is a long and time consuming program as it involves chromosome doubling of sterile triploid hybrid and backcrossing this hexaploid with cultivated tetraploid [3]. In the utilization of derived allohexaploids in a breeding programme [4] pointed out that the breeding technique to be used by cotton breeders depends upon the extent of segregation in the allopolyploid and its immediate progeny generation.

Utilization of the haploids of tetraploid cotton for direct crossing with wild diploid is reported to the shortcut method for such gene transfer [5, 6]. Such interspecific F_1 hybrids between *G. hirsutum* (L.) haploid and *G. thurberi* [5] and *G. anomalum* [6] were also reported.

G. raimondii, has a 13 haploid (n) chromosomes [7] closely related to *G. klotzschianum* of the Galapagos Island, mainly confined to the valleys of Jequetepeque and Chicama North Peru where it harbor the insect pest *Dysdercus ruficollis*, *G. raimondii*, is resistant to jassids, thrips, leaf roller, bollworms - *Heliothis*, rust, bacterial blight and drought [1]. Further, it is known to contribute other desirable characters for high fiber strength, fineness, lint index, and strength, mechanical resistance of fibers, high ginning, boll size and hairiness [1, 3]. In addition to this, it has a unique terpenoids missing species [8]. Hence present investigation was attempted for (i) introgression of desirable genes from *G. raimondii* into *G. hirsutum* cotton within shortest

possible time utilizing haploids of *G. hirsutum* cotton and (ii) to develop new synthetic lines with in-built inherent resistance to biotic and abiotic stresses coupled with desirable fiber quality traits in addition to desired economic characters.

Materials and methods

Pollen at anthesis from *G. raimondii* plants were collected and were dusted on previously emasculated flowers of *G. hirsutum* and its haploid plants. There was seed set after pollination and twenty-five F_1 hybrids were raised and studied with reference to characters of genetical importance.

The various morphological characters were studied in these *G. hirsutum*, its haploid, *G. raimondii* and their F_1 hybrid (Table 1). For analysis, young flower buds of all these plants and their respective parents were fixed in cornoy's fluid (6:3:1) and squashed in 1% aceto-carmine. The cytological analysis of chromosome behaviour in PMCs was made from temporary mounts [5, 6]. Pollen sterility was tested with differential stain [9]. The pollen germination was tested [10]. Microphotographs were taken on coloured film with the help of Ricoh XR-X-3000 35mm Camera mounted on Leica, DMLS Microscope.

Result and discussion

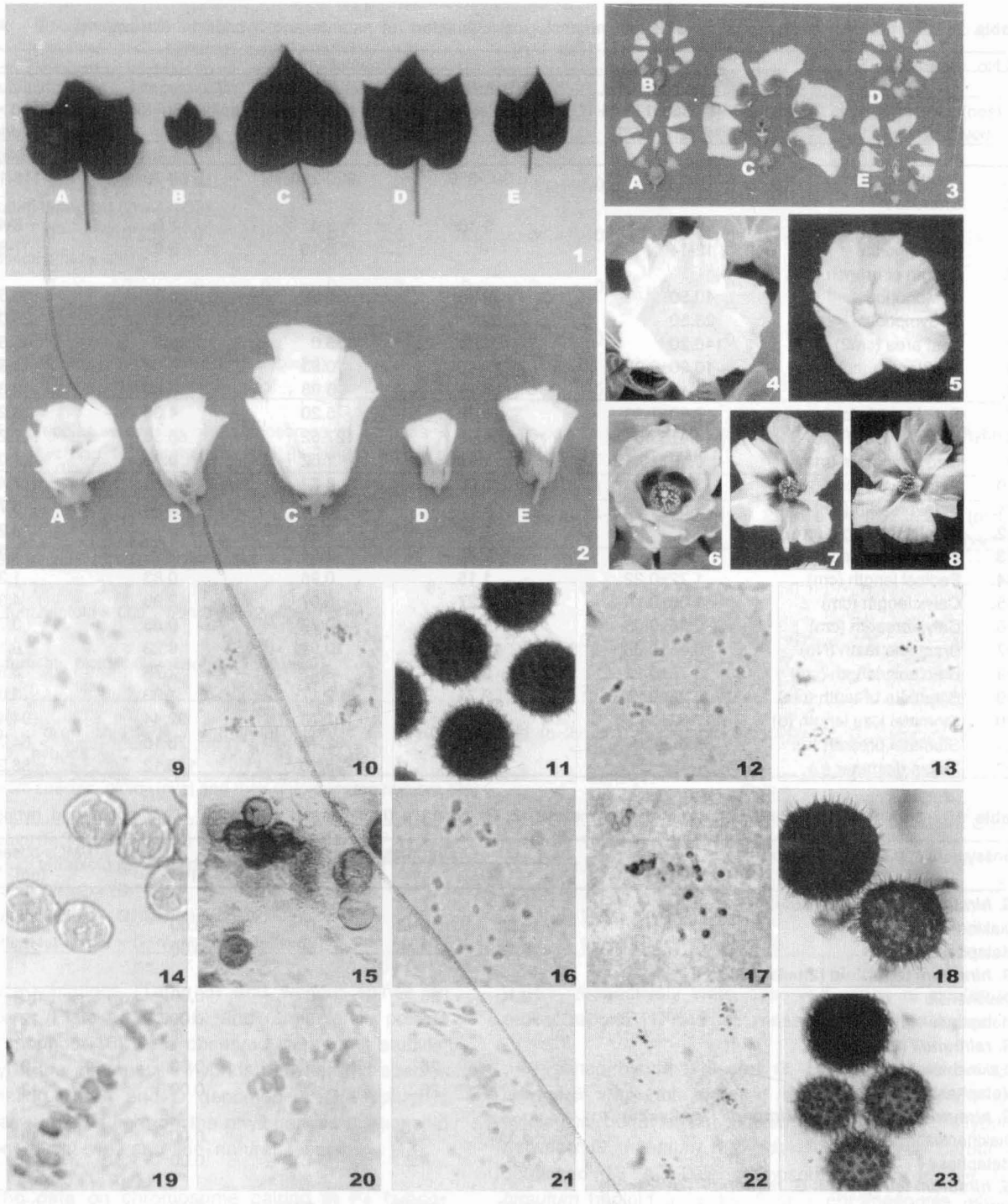
Morphological characters: Comparison of distinguishing morphological characters of parents and F_1,s are given in Tables 1 and 2. F_1 hybrids were found intermediate between both the parents in plant height and growth habit. The leaf shape (Plate 1; Fig. 1 A-E) flower shape, size (Plate 1; Fig. 2 A-E) and colour (Plate 1; Fig. 4-8), petal spot, filament colour (Plate 1; Fig. 3 A-E), pollen colour, stem and leaf hairiness etc., of *G. raimondii* was found to be dominant in both the hybrids.

Cytological studies: Meiosis in wild *G. raimondii* was found normal (Plate 1; Fig. 9-11). Varying degrees of bivalents, their shape and size were observed in the first meiotic division in the haploids. The term bivalent other than associations used here as chiasma formation appeared in the synapsed chromosomes (Table 3). Further, unequal separation of chromosomes (Plate 1; Fig. 12) and chromatids (Plate 1; Fig. 13) at anaphase I and II, respectively (Plate 1; Fig. 14) was observed. Such unequal separation lead to formation of sporads with varying number of chromosomes. Pollen grains formed were sterile with size variations (Plate 1; Fig. 15).

The observations of chromosome behaviour were confirmatory to earlier workers [11, 12]. Associations ranging from 7 to 9 without any chiasma between A and D genomic chromosomes during pachytene were reported in *G. hirsutum* haploids [12,13]. Perfect bivalent formation observed might be due to the gene

Table 1. Various morphological characters of *G. hirsutum haploid*, *G. raimondii* and their F_1 hybrid

Characters	*G. hirsutum* (haploid) n = 26	*G. raimondii* n = 13	F_1 hybrid
Plant height (cm)	69	250-300	Av. 118.6
No. of monopodia	1 (25.25 cm)	2.7 (95.23 cm)	5.2 (69.63 cm)
Leaf	2-5 lobed, moderately deeply lobed, 6.06 × 6.5 cm, Petiole length 5.2 cm	Entire,cordate,acuminate densely hairy. Large those on the main stem usually 15 cm or more long, av. 17.16 × 17.14 cm, Petiole length 10.08 cm	2.5 lobed,with shallowly lacinated lower surface hairy, 12.66 × 12.84 cm., Petiole length 9.28 cm
Nectary	Leaf-1 floral-3 outer	Leaf-1 floral 3	Leaf-3 floral 3
Gland color	Black to dull black	Brown	Black
Bracteoles	Broad at the base, cut into 8-9 very long threadlike, 3.47 × 2.17 cm	Small, broad at the base,but cordate cut into 15 or more very long almost thread like, tails along the upper margin	Small broad at the base densely hairy, cordate cut in to 13 very long
Calyx tube (cm)	2.93 × 0.68 cm	4.27 × 3.3. cm	4.35 × 0.72 cm
Corolla	Light yellow, (3.68 × 3.02), spot absent	(7.0 × 6.1 cm) Cream colour, spot dark very large covering almost lower half of petal	Cream colour, (7.49 × 6.77 cm) spot light red (5.26 × 1.91 cm) cream
Pollen (color and size)	Yellow, 96.25 microns	Cream, 105.20 microns	Yellow, 79.60 microns
Staminal column anthers/flower (no)	Arranged regularly, compact	Arranged regularly but not in ranks 145-160	Arranged regularly loosely arranged 147.25
Average anther size (mm)	53-60 1.62	1.96	1.03
Stigmatic exertions	Style strength, medium 2.30 cm stigma ununited to the top	Style straight, long 3.12 cm, stigma united to the top	Style straight and long –3.52 cm, stigma un-united to the top
Ovary	Ovary 3-4 loculed, ovules 6-7/locule	Ovary 3-4 loculed, ovules 6-7 locule	Ovary 3-4 loculed, ovules 5-6/locules
Pubescence	Mildly pubescent to slightly hair	Pubescent	Mildly pubescent



Figs. 1-23. 1. Expression of leaf shape in (a) *G. hirsutum* L A line, (b) *G. hirsutum* haploid, (c) *G. raimondii*, (d) *G. hirsutum* L. A line × *G. raimondii*, (e) F_1 hybrid (*G. hirsutum* haploid × *G. rainmondii*); 2. Expression of flower shape and size in (a) *G. hirsutum* L A line, (b) *G. hirsutum* haploid, (c) *G. raimondii*, (d) *G. hirsutum* L. × *G. raimondii*, (e) F_1 hybrid *G. hirsutum* haploid × *G. raimondii*); 3. Expression of flower, anther and petal colour and petal spot in: (a) *G. hirsutum* L. A line, (b) *G. hirsutum* haploid, (c) *G. raimondii*, (d) *G. hirsutum* L. A line × *G. raimondii*, (e) F_1 hybrid (*G. hirsutum* haploid × *G. raimondii*); 4-8. Expression of flower colour, size and shape in: (4) *G. hirsutum* L A line, (5) *G. hirsutum* haploid, (6) *G. raimondii*, (7) *G. hirsutum* L. × *G. raimondii*, (8) F_1 hybrid *G. hirsutum* haploid × *G. raimondii*); 9-11. Meiosis in *G. raimondii*: (9) Metaphase-I with 13^{II}, (10) Anaphase-II with normal separation chromatids, (11) Normal fertile pollens; 12-15. Meiosis in *G. hirsutum* haploid, (12) Aanaphae-I with laggards, (13) Mataphase-II, (14) Abnormal sporads with 2-5 spores and micronuclei, (15) Abnormal pollens without spines on exine 3 days before flower opening; 16-17. hybrid (*G. hirsutum* haploid × *G. raimondii*): (16) Early metaphase-I with $9^{I}+4^{II}+3^{III}$, (17) Metaphase-I showing one cluster of chromosomes; 18-23. *G. hirsutum* L. × *G. raimondii*: (18) Fertile and sterile pollen; (19) Metaphase-I with $3^{I}+17^{II}+4^{III}$, (20) Metaphase-I different chromosome associations, (21) Early anaphase with chromosome bridges, (22) Anaphase-II with four groups of unequal chromosomes and few chromosomes lying outside the groups, (23) Fertile (red and larger) and sterile (green and smaller) poliens

Table 2. Biometrical observations on different morphological characters of parents and hybrids in *Gossypium*

S.No.	Characters	Paents/hybrids				
		G. hirsutum L. CMS176 IH 2n=4x=52	*G. raimondii* 2n=2x=26	G. hirsutum L. × *G. raimondii* 2n=3x=39	*G. hirsutum* L. haploid 2n=2x=26	*G. hirsutum* L. haploid × *G. raimondii* 2n=2x=26
1.	Plant height (cm)	125-130	300-350	220-230	60-70	115-120
2.	Branches					
	a) Monopodia	2-3	9-10	3-4	1.0	3-4
	b) Sympodia	12-14	-	8-10	3.0	7-8
3.	Length of branch (cm)					
	a) Monopodia	40.50	90.95	80.56	20.0	30.50
	b) Sympodia	25.50	150.0	110.50	20.50	25.00
4.	Leaf area (cm2)	146.20	195.50	135.0	90.3	90.50
5.	Leaf length (cm)	10.90±0.962	17.16	10.93	6.06	12.66
6.	Leaf breadth (cm)	13.10±0.54	17.14	6.98	6.50	12.84
7.	Petiol length (cm)	8.60±1.38	10.08	5.20	4.30	9.28
8.	Anthers/flower (No)	114.4±6.46	145.00	127.62	86.50	147.25
9.	Size of anthers (mm)	0. 97±0.13	1.92	1.62	0.78	1.03
10.	Petal length (cm)	3.70±0.33	7.00	6.72	3.68	7.49
11.	Petal breadth (cm)	3.58±0.08	6.10	5.84	3.02	6.77
12.	Petal spot length (cm)	-	2.48	3.50	-	3.26
13.	Petal spot breadth (cm)	-	2.98	2.54	-	1.91
14.	Pedicel length (cm)	1.72±0.22	1.15	0.96	0.83	1.34
15.	Calyx length (cm)	1.08±0.19	4.27	3.07	2.93	4.35
16.	Calyx breadth (cm)	0.55±0.05	3.30	0.62	0.68	0.72
17.	Bracteole teeth (No)	10.45±0.68	13.35	10.98	9.23	8.13
18.	Bracteole length (cm)	3.78±0.22	2.85	3.47	2.73	3.83
19.	Bracteole breadth (cm)	2.92±0.24	2.74	2.17	1.83	3.09
20.	Stomatal size length (u)	117.32±14.84	85.12	107.73	92.44	94.43
21.	Stomatal breadth (u)	65.06±4.04	64.76	82.46	73.15	64.77
22.	Pollen diameter (u)	-	111.72	93.10	110.12	85.29

Table 3. Chromosome pairing behaviour during meiosis of *G. hirsutum* (haploid and tetraploid) *G. raimondii* and hybrids

Gossypium spp./hybrids Meiotic stages	Chromosome associations				PMCs observed
	I	II	III	IV	(no)
***G. hirsutum* haploid (2n=2x=26)**					
Diakinensis	19.02	3.49	0	0.00	23
Metaphase-I	19.10	3.45	0	0.00	25
***G. hirsutum* tetraploid (2n=4x=52)**					
Diakinensis	0	26	0	0,00	10
Metaphase-I	0	26	0	0.00	12
***G. raimondii* (2n=2x=26)**					
Diakinensis	0	26	0	0.00	10
Metaphase-I	0	26	0	0.00	12
***G. hirsutum* haploid × *G. raimondii* (2n=2x=26)**					
Diakinensis	4.11	4.69	4.17	0.00	19
Metaphase-I	5.3	4.2	4.1	0.00	22
***G. hirsutum* tetraploid × *G. raimondii* (2n=2x=39)**					
Diakinensis	10.65	10.27	1.13	1.13	27
Metaphase-I	10.29	10.99	1.39	0.64	29

controlled phenomenon [14]. Due to which, 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. Observations made in the present studies reflect a similar mechanism operative in the poly haploid under present study. Formation of bi or/ tripolar (Tables 3 and 4) spindles univalents outside the spindles resulting in appearance (Plate 1; Fig. 13) like "meta-anaphase" [15] was observed in present studies in both the hybrids.

Unequal separation of chromosomes at metaphase-1, (Table 4 and 5) formation of more than four microspores (Plate 1; Fig. 14), pollen grains with

Table 4. Bipolar separation of chromosomes at anaphase-I during meiosis in the *G. hirsutum* haploid (n=2x=26), its tetraploid (2n=2x=52) *G. raimondii* and F_1 hybrids

Chroinosome distribution at each pole														
21-5	20-6	19-7	18-8	17-9	16-10	13-26	15-24	16-23	17-22	18-21	19-20	26-26	13-13	PMCs (nos) observed
G. hirsutum haploid (2n=2x=26)														
4	9	7	5	4	13	0	0	0	0	0	0	0	0	42
G. hirsutum tetraploid (2n=4x=52)														
0	0	0	0	0	0	0	0	0	0	0	0	40	0	40
G. raimondii (2n=2x=26)														
0	0	0	0	0	0	0	0	0	0	0	0	0	25	25
G. hirsutum haploid × *G. raimondii* (2n=2x=26)														
2	3	1	2	3	4	0	0	0	0	0	0	0	0	15
G. hirsutum tetraploid × *G. raimondii* (2n=2x=39)														
0	0	0	0	0	0	1	4	3	5	2	1	0	0	16

Table 5. Tripolar separation of chromosomes at anaphase-I during meiosis in the *G. hirsutum* haploid (n=2x=26) and F_1 hybrids involving *G. hirsutum* haploid (n=2x=26), its tetraploid (2n=2x=52) × *G. raimondii*

Chromosome distribution at each pole during anaphase-I of meiosis														
18-5-3	17-6-3	16-7-3	15-7-4	14-7-5	13-7-6	12-7-7	13-13-13	14-15-10	16-14-9	18-9-9	20-10-9	22-12-5	24-13-2	PMCs (nos) observed
G. hirsutum haploid (2n=2x=26)														
2	1	3	2	1	2	1	0	0	0	0	0	0	0	12
G. hirsutum haploid × *G. raimondii* (2n=2x=26)														
1	3	1	2	2	2	1	0	0	0	0	0	0	0	12
G. hirsutum tetraploid × *G. raimondii* (2n=2x=39)														
0	0	0	0	0	0	0	1	4	7	2	1	2	1	18

Table 6. Pollen, size fertility and germination in *G. hirsutum* haploid (n=2x=26), its tetraploid (2n=4x=52) *G. raimondii* and F_1 hybrids

Gossypium species, ploidy level and their chromosome number (2n)	Pollen		
	Size (micros)	Fertility %	Germination %
G. hirsutum haploid (2n=2x=26)	96.25	2.50	0.0
G. hirsutum tetraploid (2n=4x=52)	138.50	26.25	89.50
G. raimondii (2n=2x=26)	105.20	86.25	69.36
G. hirsutum haploid × *G. raimondii* (2n=2x=26)	79.60	0.0	0.0
G. hirsutum tetraploid × *G. raimondii* (2n=3x=39)	95.25	40.0	28.78

high size variations (Table 5) and without spines on their exine (Plate 1; Fig. 15) and rarely fertile pollens formed monads [16] were observed in present studies. A study of the interspecific hybrids between the species representing the A and D genomes in *Gossypium* is useful as it throws light on the phylogenetic relationship between these two, and the natural tetraploids [17].

The data on chromosome pairing in F_1 hybrids are presented in Table 3 (Plate 1; Fig. 19-22). In F_1 hybrid involving *G. hirsutum* CMS × *G. raimondii* on an average $12.78^{I} + 11.13^{II} + 1.32^{III}$ were noticed at metaphase-1 [23]. Earlier workers [18] reported 12.57^{I} and 0.125^{IV}. In the present studies in hybrid between *G. hirsutum* haploid × *G. raimondii* and *G. hirsutum* × *G. raimondii* on average chromosome associations at diakinesis and metaphase-1 were $4.11^{I} + 4.69^{II} + 4.17^{III}$ and $5.3^{I} + 4.20^{II} + 4.1^{III}$ and $10.55^{I} + 10.27^{II} + 1.13^{III} + 1.13^{IV}$ and $10.29^{I} + 10.99^{II} + 1.39^{III} + 0.64^{IV}$, respectively were observed which is similar to earlier reports [17, 18] in respect of triploid hybrid.

Since hybrid between *G. hirsutum* haploid × *G. raimondii* was not reported earlier, the data on its cytological behavior not availed for comparison. Hybrid between *G. hirsutum* haploid × *G. raimondii* certainly contained 13 A_h D_h chromosomes from female *G. hirsutum* haploid (n = 13: 6 or 7 chromosomes either from both A and D genomes) and remaining 13 D_5 from male parent *G. raimondii*. Thus $4\text{-}6^{II}$ observed might have resulted not only due to the pairing between D_h and D_5 chromosomes but may also be due to inter genomic pairing between Ah chromosomes. It was possible to obtain and study hybrids between *G. hirsutum* haploid × *G. thurberi* [5] and *G. anomalum* [6]. Specific chromosome pairing instead of random and closer homology of D genomes of chromosomes of A than D was observed in these hybrids [19]. As reported

earlier, 7-9^{II} / PMC were observed in haploids. Thus chromosome pairing in earlier work indicated the chromosome pairing of non-homologues as well as due to the residual chromosomal homologies [19]. Thus, bivalents and trivalent formed in this hybrid was due to both inter and intra genomic pairing. Further, quadrivalents observed in triploid hybrid was certainly due to the similar phenomenon. Pattern of chromosome pairing observed in present studies indicated the closer homology of *G raimondii* D chromosomes to *G. hirsutum* AD chromosomes.

However, several theories have been put forward on the time and place of the origin of the tetraploid species, which is discussed [20]. *G. barbadense* has evolved in South America as a hybrid between *G. herbaceum* and *G. raimondii* [21]. It is also further confirmed by comparison of 2C nuclear DNA content of *G. herhaceum, G. raimondii* and *G. barbadense* [22].

Formation of multivalent, (Plate 1; Figs. 19-22) and univalent rather than bivalents as well as unequal separation of chromosomes and chromatids at anaphase -I and -II (Plate 1; Fig. 21-22) in these hybrids, respectively led to formation of sporads containing 1-7 sporads with unequal number of chromosomes which resulted into formation of sterile pollen grains with (Plate 1; Fig. 23) size variations (Table 5).

From the attempts reported here, it has been possible to obtain hybrid between both *G. hirsutum* L. and its haploid with *G. raimondii*. Further attempts are in progress to induce chromosome doubling and fertility it will certainly lead to achieve desired segregates combined with tolerant to sucking pest and improved fibre properties of *G. hirsutum* cotton.

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