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CYTOMORPHOLOGICAL STUDIES IN INDUCED TETRAPLOID *G. ARBOREUM* AND ITS INTERSPECIFIC HYBRID WITH TETRAPLOID *G. HIRSUTUM* L.

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ABSTRACT

Polyploidy was induced in the diploid cotton species *G. arboreum* (2n = 26), and the tetraploid was crossed with *G. hirsutum* (2n = 52), giving rise to a new interspecific hybrid (2n = 52). The new hybrid produced 27.6% fertile pollen. The leaf shape, plant habit, petal spot, petal colour and pollen colour of F_1 plants were similar to those of *G. arboreum*, while bud and flower size resembled *G. hirsutum*. The F_1 hybrid was tolerant to the sucking pest complex. The F_1 males with 2n = 52, crossed with male sterile *G. hirsutum* lines, gave some seed set. With the available materials it may be possible to undertake introgression of favourable genes of the diploid and tetraploid *Gossypium* species in one species after wide crossing.

Key words: Polyploidy, cotton, introgression.

Of the four cultivated *Gossypium* species, the diploid species *G. arboreum* (2n = 26 A2) is predominantly cultivated in India, particularly in Maharashtra, as it has wider adaptability and resistance to pests and diseases. The species, however, has inferior fibre qualities and lower yield potential because of smaller bolls than the allotetraploid species *G. hirsutum* (2n = 4x = 52, 2AD1). So far interspecific hybridization has been attempted between allotetraploid 2n = 4x = 52 (2A D1) *G. hirsutum* and diploid 2n = 2x = 26 (A2A2) *G. arboreum*. The F_1 being triploid 2n = 39 (A2D1) was sterile. Fertility had to be restored in such hybrids by chromosome doubling followed by back crossing with *G. hirsutum* [1].

Considering the limitations in interspecific transfer of *G. hirsutum* characters like bigger boll size and superior fibre quality, attempts were made to introduce polyploidy in *G. arboreum* to obtain 4n = 52 (4A2) type and then to cross it with *G. hirsutum* (2AD1) to produce hybrid with 2n = 4x = 52, 2A2 AD1 genome.

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MATERIALS AND METHODS

Polyploidy was induced in *G. arboreum* variety PA-85/85 by immersing the seedlings in 0.5% colchicine solution for 8, 12 and 24 h.

Four plants having 4n = 52 were detected and cytologically confirmed. The 4n *arboreum* so obtained was crossed with *G. hirsutum* variety Purnima. From 250 crosses attempted, three bolls were obtained. Thirty seeds obtained from these three bolls were sown in separate rows and 11 germinated and produced vigorous F_1 hybrids.

For meiotic observations, young flower buds of both 4n *arboreum* and interspecific hybrid were fixed in Carnoy's fluid and squashed in 1% acetocarmine. Pollen fertility was tested with acetocarmine stain.

RESULTS AND DISCUSSION

MORPHOLOGICAL CHARACTERS

The distinguishing morphological characters of parents, 4n *arboreum*, and interspecific F_1 hybrid are presented in Table 1.

Induced tetraploid plants (Fig. 2) were taller than diploid (Fig. 1) and had more number of monopodia. The leaves were bigger, thicker and slightly more ovate than in the diploid. The size of the bracteole, bud and flower was larger than in diploid.

The pubescence on stem and leaves was intense in tetraploid than in the diploid. The petal size was longer and petal spot darker in tetraploid than in diploid. Expression of gigas characters for hairiness, leaf size and petal size in tetraploids may be due to higher gene doses as a result of chromosome doubling. The pollen size increased and pollen grains were of varying sizes and shape as compared to small and uniform size of diploids. Pollen fertility up to 65.8% was observed in the tetraploid. However, the higher sterility was due to irregular meiosis, particularly during anaphase II. Induced polyploidy in *G. arboreum* has been reported earlier [2, 3].

The F_1 (Fig. 4) was taller than induced tetraploid of *G. arboreum* and *G. hirsutum* (Fig. 3). In F_1, the bract shape and leaf size of *G. hirsutum* was dominant while petal colour, petal spot, anther colour of 4n *G. arboreum* were dominant. The pollen grains were slightly bigger and of varying size. Such interspecific transfer of characters has been reported earlier in cotton [4–6].

Table 1. Comparative morphological characters of 2n *G. arboreum*, 4n *G. arboreum*, interspecific 4n F_1 (*G. arboreum* x *G. hirsutum*), and 4x *G. hirsutum*

Character	Diploid *G. arboreum* (2n = 26)	Autotetraploid *G. arboreum* (4n = 52)	4n *G. arboreum* x *G. hirsutum* F_1 (2n = 4x = 52)	Tetraploid *G. hirsutum* (2n = 4x = 52)
Plant height (cm)	115	140	192	75
No. of monopodia	0–1	4–5	5–7	1–2
First sympodia (node No.)	3–4	5–6	7–8	3–4
Leaf size:	5-lobed deeply serrated	5-lobed	3–5 lobed	3–1 lobed
a. Leaf length (cm)	5.5	6.5	4.5	4.5
b. Lobe length (cm)	4.0	4.0	2.5	2.5
c. Lobe breadth (cm)	1.6	3.0	2.0	2.5
d. Sinus length (cm)	1.5	2.0	2.0	2.4
Bracteoles:	3, 5–7 teeth	3, united base	3, with broad teeth 3–5 teeth	3 5–7 longer teeth
a. Length (cm)	2.6	3.6	3.2	2.4
b. Breadth (cm)	2.5	4.0	3.4	2.0
Calyx tube length (cm)	0.9	1.2	1.1	0.8
Corolla	Yellow petal, dark red eye spot	Yellow petal, red eye spot	Yellow, light red eye spot	Cream, without eye spot
Pollen colour	Dark yellow	Dark yellow	Dark yellow	Cream
Pollen fertility (%)	96.7	65.7	27.7	92.6
Tetrad %	95.0	80.0	72.1	92.0
Diameter of pollen grain (μ)	73.1	109.2	99.6	91.2
Ovary	4 locules	4 locules	Abortive	4 locules
Pubescence	Medium	Dense	Medium	Less

CYTOLOGICAL OBSERVATIONS

G. arboreum (2n = 26, 2A2). Meiosis in *G. arboreum* was normal. The metaphase had 13 bivalents. The anaphase had equal separation of bivalents in the first division and of chromatids in the second. The formation of tetrads was normal, giving 97% pollen fertility.

Induced tetraploid G. arboreum (4n = 52, 4A2). Meiotic studies in induced tetraploids (2n = 52) confirmed their tetraploid nature. Multivalent formation was observed at metaphase I. Chromosome association at metaphase I was studied in 38 PMCs (Table 2). Analysis of metaphase I showed that about 55.3% PMCs had 1 to 4 univalents. The number of bivalents ranged from 6 to 24, and 21.0% cells had 20 bivalents, 18.2% cells had 15 bivalents, and 13.2% PMCs had 24 bivalents. Number of trivalents ranged from 0 to 4 and 34.2% PMCs had one trivalent while 47.4% PMCs carried no trivalents. The range of quadrivalents was 0–7 and

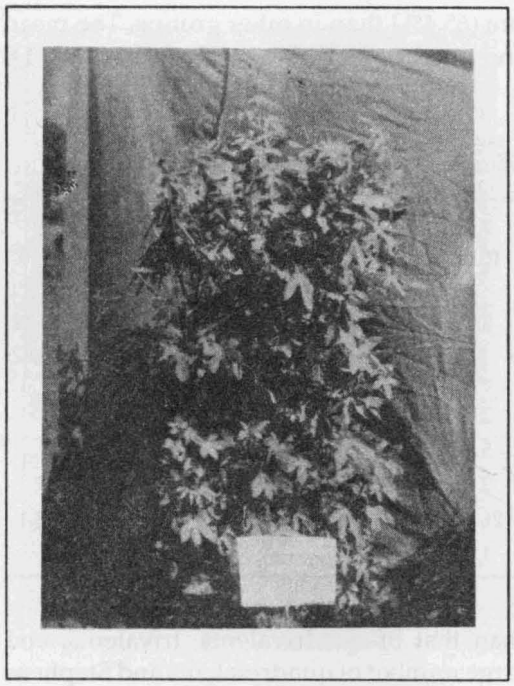

Fig 1. Diploid *G. arboreum.*

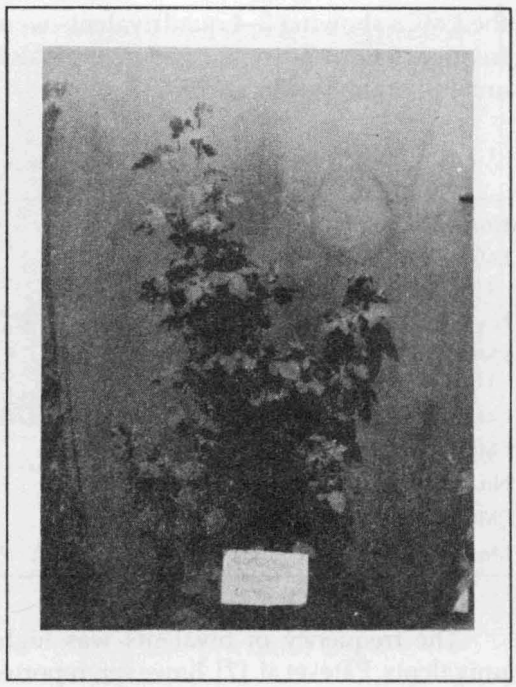

Fig 2. Induced tetraploid 4n *arboreum* (4n=52).

Fig 3. G. *hirsutum.*

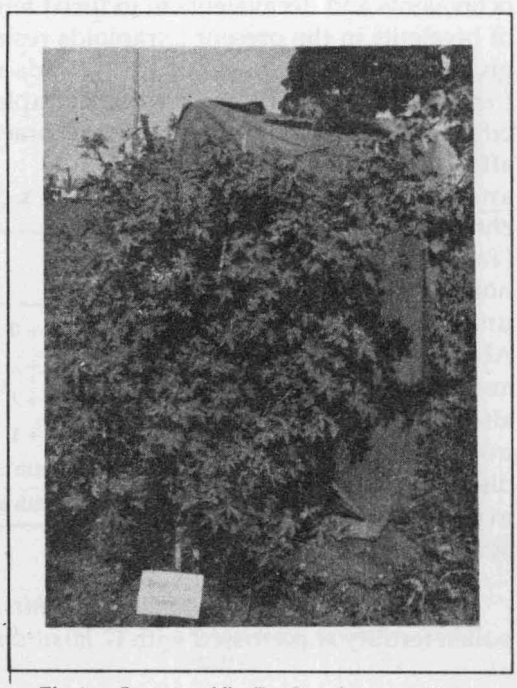

Fig 4. Interspecific F_1 *G. arboreum* (4n) x *G. hirsutum.*

the PMCs showing 2–4 quadrivalents were more (65.4%) than in other groups. The mean frequency of univalents, bivalents, trivalents and quadrivalents per cell was 1.5, 17.0, 1.0 and 3.4, repsectively (Table 2).

Table 2. Univalents, bivalents, trivalents and quadrivalents at metaphase I in 4n *G. arboreum*

No. of univalents	0				1				2			3			4					
PMC at metaphase I (%)	44.7				10.5				10.5			21.0			13.2					
No. of bivalents	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
PMC at metaphase I (%)	13.1	0	0	0	0	0	5.3	2.6	0	18.4	2.6	0	7.9	7.9	21.0	0	7.9	0	13.2	
No. of trivalents	0				1				2			3			4					
PMC at metaphase I (%)	47.4				34.2				5.3			0			3.1					
No. of quadrivalents	0				1				2			3			4		5		6	7
PMC at metaphase I (%)	2.6				10.5				26.3			15.4			26.3		5.3		0	13.1
Mean per PMC	1.5^{I},				17.0^{II},				1.0^{III},			3.4^{IV}								

The frequency of bivalents was higher than that of quadrivalents, trivalents, and univalents. Patel et al. [7], however, reported a large number of quadrivalents and Stephens [8] indicated even higher order of multivalent formation in the form of hexavalents, octavalents and decavalents in induced tetraploids of *G. arboreum*. The higher frequencies of bivalents in the present tetraploids resulted in less complicated anaphase disjunction, giving higher frequency of normal tetrads and higher pollen fertility (up to 65.7%). A large frequency of bivalents in individual tetraploids may be due to multivalent suppressor gene effects. Such tetraploids can be of great practical value for introgression of favourable genes after wide hybridization. The analysis of separation of chromosomes at anaphase I (Table 3) revealed that 82.3% normal separation and 17.6% unequal separation. Abnormalities like laggards and tripolar separation were also noticed in a few pollen mother cells. Abnormal distribution of chromosomes in majority of the PMCs was seen at anaphase II.

Table 3. Chromosome separation at anaphase I in 4n *G. arboreum*

Group	PMC in different groups total	%
26 + 26 + 0	28	82.3
25 + 25 + 2	2	5.9
27 + 23 + 2	2	5.9
26 + 25 + 1	2	5.9
PMC with normal distribution, %		82.35
PMC with abnormal distribution, %		17.64

Interspecific F_1 4n G. arboreum x *G. hirsutum*. The 4n *G. arboreum* having considerable pollen fertility was crossed with *G. hirsutum* variety Purnima. Metaphase I and anaphase I

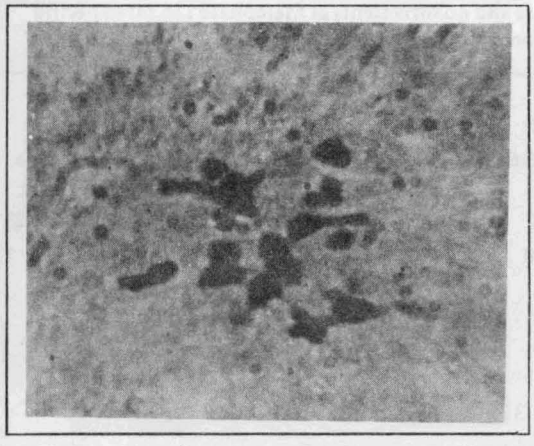

Fig. 5. Metaphase I showing chromosome conjugation 12 I, 16 II and 2 IV.

Fig. 6. Metaphase I showing maximum bivalent formation (2n=52).

Fig. 7. Anaphase I showing equal chromosome separation 26:26.

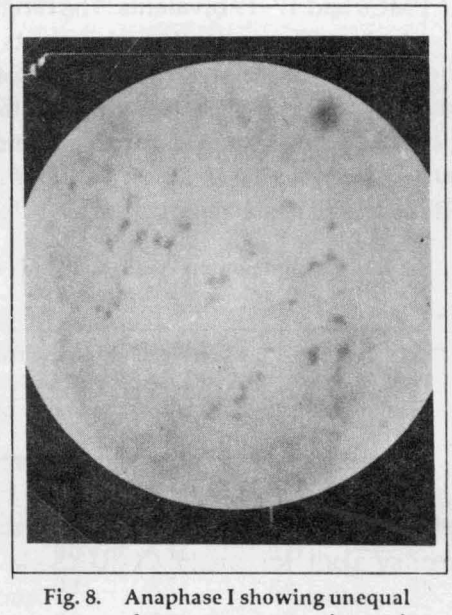

Fig. 8. Anaphase I showing unequal chromosome separation and laggards.

Table 4. PMC with univalents, bivalents, trivalents and quadrivalents at Metaphase I in interspecific F_1 (4n *G. arboreum* x *G. hirsutum*)

Frequency of univalents	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
PMC at M I (%)	3.8	0	7.5	3.8	1.9	17.0	5.6	17.0	5.6	18.8	1.9	7.5	0	1.9	0	3.8	3.8	
Frequency of bivalents	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
PMC at M I (%)	1.9	0	0	0	0	0	0	1.9	0	7.5	9.4	13.2	24.5	15.1	9.4	7.5	7.5	1.9
Frequency of trivalents	0		1															
PMC at M I (%)	67.9		32.1															
Frequency of quadrivalents	0		1		2		3											
PMC at M I (%)	17.0		60.4		20.7		1.9											

stages were studied in the interspecific hybrids thus obtained (Tables, 4, 5 and 6). The 52 chromosomes at metaphase I of F_1 were in the form of univalents, bivalents, trivalents and quadrivalents (Figs. 5 and 6). Analysis of metaphase I (Table 4) showed that the range of univalents was from 3–19 and about 17.0% PMCs had 3–7 univalents, 64.1% PMCs had 8–12 univalents, and 18.5% PMCs were with 13–19 univalents. The number of bivalents ranged from 6 to 23, and 88.6% PMCs had 16–23 bivalents. The study further indicated that 52.5% PMCs had 17–19 bivalents. The range of 0–1 trivalent was observed and 32.1% PMCs had 1 trivalent. The range of quadrivalents was from 0 to 3, and 81.1% PMC had 1–2 quadrivalents. Further, it was noted that chromosome conjugation in the form of multivalents was less in interspecific hybrid (F_1) than in the induced *G. arboreum* tetraploid. Mean number of univalents, bivalents, trivalents and quadrivalents per PMC was 8.9, 19.5, 0.5 and 0.7, respectively (Table 5).

Table 5. Chromosome conjugation in F_1 of 4n *G. arboreum* x *G. hirsutum*

Total PMC examined	Mean No. of chromosomal conjugations per PMC			
	univalents	bivalents	trivalents	quadrivalents
7	10.0	21.0	0.0	0.0
2	5.0	22.0	1.0	0.0
29	11.2	17.7	0.0	1.3
15	9.3	17.7	1.0	1.3
Mean	8.9	19.5	0.5	0.7

Table 6. Chromosome separation at anaphase I in F_1 of 4n *G. arboreum* x *G. hirsutum*

Group	Distribution	
	No.	%
26 + 26 + 0	14	46.7
28 + 24 + 0	6	20.0
30 + 22 + 0	2	6.6
26 + 25 + 1	2	6.6
28 + 22 + 2	6	20.0
Total	30	99.9
PMC % with:		
normal distribution		46.7
abnormal distribtuion		53.3

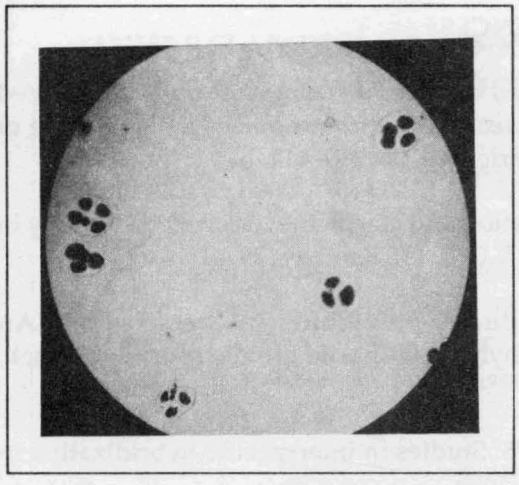

Fig. 9. Formation of triads, tetrads and pentads.

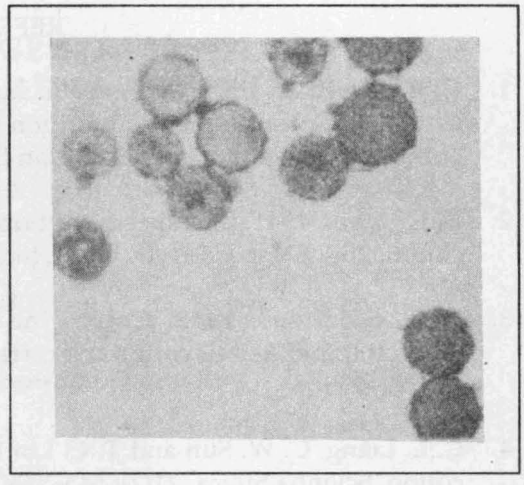

Fig. 10. Sterile and fertile pollen grains with varying size.

The analysis of chromosome separation at anaphase I (Table 6) indicated that normal chromosome separation occurred in 46.7% PMCs (Fig. 7). Laggards were observed at anaphase I and II (Fig. 8). Meiotic irregularities at anaphase, particularly anaphase II, resulted in the formation of diads, triads, pentads, and hexads (Fig. 9) to the extent of 26.0%. Formation of normal tetrads was 74%. Because of the occurance of 1 or 2 micronuclei in most of the tetrads, the fertility of pollen grain was 27.7% (Fig 10).

The cytology of interspecific hybrid between diploid and allotetraploid new world cotton has been studied by several workers [2, 9]. These authors reported 13–15 univalents and 12–13 bivalents with 2n = 39. Both male and female sides were sterile in triploid F_1s so obtained. The 4n F_1 with 2n = 52 had up to 27.6% pollen fertility and complete female sterility. The pollens of these plants when used to pollinate the cytoplasmic male sterile lines of *G. hirsutum* produced some seeds.

Meiosis in G. hirsutum (2n = 4x = 52, 2 AD1). Regular meiosis with 2n = 52 was observed in *G. hirsutum,* 26 bivalents being regularly observed at metaphase I. The separation was normal at anaphase I and anaphase II, giving normal tetrads and fertile pollen.

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