Morphological and cytological studies on induced polyploidy

in *Sesamum indicum* L.*

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Sesamum indicum L. are cultivated in Japan as the oil crop. Obtained from the sesame seed the oil is nearly all consumed in foods. This investigation was first undertaken in an attempt to increase the oil yield by colchicine polyploidy.

Although the practical value of the experimental results proved unsatisfactory, several of the noticeable changes in the polyploid plants are of scientific interest.

In induced polyploidy many morphological changes such as vigorous branching and a marked increase in the number of capsules, seem to have been due to changes of relationship between a number of physiological factors.

In the following articles the writers compare the morphological changes observed in these polyploid plants with the diploid plants.

The colchicine polyploids which were successfully induced in 1945 have been cultivated and carefully observed every following summer for seven years.

In the cytological studies in *Sesamum* hitherto reported the somatic chromosome number has been reported as $2n=26$ by Morinaga *et al* (1929), Sugiura (1936), Kumar and Abraham (1941) and Kobayashi (1949a). The meiosis in this genus, on the other hand has been described and reported by Nohara (1934), Kumar and Abraham (1941) and Kobayashi (1949b) as $n=13$.

The phenomenon of secondary pairing in the meiosis of the pollen mother cell is very often observed in diploid plants. In induced tetraploid this secondary pairing appears more conspicuously in the doubling of the number of chromosomes.

The peculiar type of this phenomenon in *Sesamum* is exceedingly interesting from the cytogenetical point of view. The following paper deals briefly with the cytological observations on this problem as a preliminary report. The junior author (Kobayashi) will discuss this subject in further detail in a future paper.

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Materials and Methods

Three of the many forms of *Sesamum indicum* L. ($2n=26$) quite commonly cultivated in Japan were used as the basis for this study. The most distinctive character of these forms is the colour of the seed-coat, and they are popularly called, accordingly

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Table 1. Effect of colchicine treatment (Immersion method).

	Percentage of colchicine	No. of seeds treated	No. of ungerminated seeds or dead seedlings	No. of plants surviving treatment			Percentage of tetraploids to treated seeds
				No. of tetraploid plants	No. of mixoploid plants	No. of unchanged plants	
B ₁ D	0.8	30	23	3	4	0	10.0
B ₂ D		"	26	1	3	0	3.3
YD		"	24	2	4	0	6.6
WD		"	26	2	2	0	6.6
B ₁ D	0.5	"	20	9	1	0	30.0
B ₂ D		"	20	7	2	1	23.3
YD		"	19	8	3	0	26.6
WD		"	22	6	2	1	20.0
B ₁ D	0.2	"	20	5	2	3	16.6
B ₂ D		"	19	4	2	5	13.3
YD		"	16	6	2	6	20.0
WD		"	17	4	3	6	13.3
B ₁ D	0.1	"	16	1	2	11	3.3
B ₂ D		"	19	2	0	9	6.6
YD		"	16	0	1	13	0.0
WD		"	18	1	1	10	3.3
B ₁ D	0.05	"	11	1	4	14	3.3
B ₂ D		"	14	0	1	15	0.0
YD		"	9	0	0	21	0.0
WD		"	13	0	0	17	0.0
B ₁ D	Control	30	0	0	0	30	0.0
B ₂ D		"	1	0	0	29	0.0
YD		"	0	0	0	30	0.0
WD		"	0	0	0	30	0.0

the black sesame, the yellow (or brown) sesame, and the white sesame. These materials were supplied through the Prefectural Agricultural Experiment Station at Kiyosu in Aichi Prefecture.

For the sake of convenience throughout the present investigation the diploid plant of the black-seeded form is designated as BD, of the yellow-seeded form as YD, and of the white-seeded form as WD. Further, BD is divided into two types, which we have designated as B₁D and B₂D respectively. In fact, both B₁D and B₂D are very much alike in plant form, but B₁D has short hairs on the stem and the leaves are broader, the branching more numerous, the seeds larger, and the surface of seeds coarser than those of B₂D.

In May, 1945, we tried to induce the tetraploids of these diploid forms by the colchicine method. Two methods were employed:

- (1) Seeds were immersed in different concentrations, from 0.05 to 0.8% aqueous solution of colchicine for five days (Immersion method).
- (2) Vegetative tips of five- or six-day seedlings were treated with drops of different concentrations of colchicine (0.05 to 0.8%) for five days (Dropping method).

Table 2. Effect of colchicine treatment (Dropping method).

	Percentage of colchicine	No. of seedlings treated	No. of dead seedlings	No. of plants surviving treatment			Percentage of tetraploids to treated seedlings
				No. of tetraploid plants	No. of mixoploid plants	No. of unchanged plants	
B ₁ D	0.8	20	11	2	7	0	10.0
B ₂ D		"	14	1	5	0	5.0
YD		"	10	4	6	0	20.0
WD		"	12	3	5	0	15.0
B ₁ D	0.5	"	6	9	5	0	45.0
B ₂ D		"	4	7	9	0	35.0
YD		"	5	10	4	1	50.0
WD		"	5	6	9	0	30.0
B ₁ D	0.2	"	2	18	0	0	90.0
B ₂ D		"	1	18	1	0	90.0
YD		"	3	15	1	1	75.0
WD		"	1	16	2	1	80.0
B ₁ D	0.1	"	2	16	0	2	80.0
B ₂ D		"	2	14	1	3	70.0
YD		"	1	7	4	8	35.0
WD		"	1	6	6	7	30.0
B ₁ D	0.05	"	1	5	2	12	25.0
B ₂ D		"	0	4	3	13	20.0
YD		"	0	10	1	9	50.0
WD		"	1	4	0	15	20.0
B ₁ D	Control	20	0	0	0	20	0.0
B ₂ D		"	0	0	0	20	0.0
YD		"	1	0	0	19	0.0
WD		"	0	0	0	20	0.0

The most suitable concentration of colchicine for induction of polyploidy in sesame, irrespective of method, was found to lie between 0.2 and 0.5% (Tables 1 and 2). In the immersion method, after the treatment each seedling which survived was washed and carefully transplanted in the seedling bed, along with untreated young plants, in a green house and grown under similar conditions. Generally the treated plants had peculiar external features which remained even in the adult plants, but in some plants they gradually disappeared and at the adult stage it was nearly impossible to distinguish between the treated and untreated plants. The final determination of tetraploid plants, therefore, was done by the examination of pollen grains and by chromosome counts.

Of the treated seedlings, besides the tetraploid, some mixoploid plants which had an admixture of diploid and tetraploid tissue were often obtained (Tables 1 and 2).

Some of the mixoploids were systematically observed by way of comparative study.

Both root tips and flower buds from treated and untreated individuals, were fixed with Navashin's solution. Sections were cut from 10-12 μ by the paraffin method

and stained either with Heidenhain's iron-alum hematoxylin, sometimes with Newton's gentian violet method. Some of the materials were prepared for microscopic observations by acetocarmine smear method.

Experimental results

I. Morphological change

a) **Seedlings.** In both methods, the immediate effect of the treatment was a general retardation of development evinced in the slow germination or growth of the seedlings. Such inhibition was especially evident after treatments with a concentrated solution or after excess in applications, often leading to the death of the seedlings (Tables 1 and 2).

For several days after the treatment by the dropping method, the plants would stop growing completely; then they would resume growth. In both methods during the early cotyledonary stage differences between the treated and untreated seedlings were marked in that the treated plants were shorter, darker green, thicker, and their leaves stouter (Figs. 1, 2, 3, and 4).

Fig. 1. Plumping radicles of *S. indicum* by colchicine treatment (Immersion method). Upper row: Yellow; Lower row: Black₁. From left to right, control, 0.05, 0.1, 0.5, and 0.8% respectively (photographed five days after treatment). **Figs. 2-3.** Young seedlings of *S. indicum*, 12th day after the sowing. Fig. 2; control, Fig. 3; seedlings treated with colchicine (Dropping method). **Fig. 4.** In 35th day later, growth of two seedlings of Black₂. Left: seedling came from seed treated with 0.2% colchicine. Right: control. **Figs. 5-6.** Stomata and pollen grains from a mixoploid respectively. Fig. 5 $\times 140$, Fig. 6 $\times 50$.

b) Growth habits. The induced tetraploid plants, which were extremely robust, had thicker and broader leaves with shorter petioles than the original diploid plants (Pl. VI, Figs. 15-18). But the growth rate at the beginning of the growing stage of tetraploids was very much slower than that of diploids, then it quickened gradually, so that the tetraploids came into full blooming about one week later than the diploids, and the harvest of tetraploids, further, was about 10–20 days later in comparison with the diploids. The growth rate of diploids and tetraploids is indicated in Fig. 7.

Fig. 7. Growth rate of diploids and tetraploids in *S. indicum*.

c) Leaves. The dimensions of tetraploid leaves at the harvest were larger than those of diploid and showed somewhat shrunk forms, especially the tetraploid Black₁ (Table 3), but the length of petioles was rather shorter than in the diploids. The increase of leaves in thickness is shown in Table 4.

Table 3. Size (length×breadth in cm.) of the largest leaf in diploid, tetraploid and mixoploid.

	Black ₁	Black ₂	Yellow	White
Diploid	14.0 × 4.2	12.0 × 4.3	14.0 × 6.0	17.0 × 5.5
Tetraploid	21.0 × 10.0	13.5 × 7.0	18.0 × 13.0	23.0 × 6.3
Mixoploid	14.0 × 6.0	12.8 × 5.0	14.2 × 11.0	17.0 × 6.3

Table 4. Average thickness of leaves (μ).

	Black ₁	Black ₂	Yellow	White
Diploid	37.2±0.172	36.0±0.201	38.0±0.102	37.0±0.089
Tetraploid	43.4±0.095	40.8±0.322	42.9±0.116	41.4±0.136
Mixoploid	39.5±0.121	36.2±0.091	40.0±0.157	38.8±0.108

d) **Stomata.** To see whether or not the epidermis of the plants had become polyploid, stomata were examined. The normal diploids have smaller stomata and about twice as many per given area as tetraploids (Table 5, Pl. IV, Figs. 1 and 2). On the mixoploid leaves were mixed diploid- and tetraploid-like stomata (Fig. 5).

Table 5. Number and size (length $\times$ width in μ) of stomata.

	Numbers of stomata*				Size of stomata (μ)			
	Black ₁	Black ₂	Yellow	White	Black ₁	Black ₂	Yellow	White
Diploid	24.1	22.3	23.5	23.4	27.9 $\times$ 18.0	26.5 $\times$ 17.7	24.8 $\times$ 17.0	26.9 $\times$ 18.7
Tetraploid	11.8	10.2	12.1	11.9	31.0 $\times$ 24.8	30.0 $\times$ 25.1	27.9 $\times$ 23.2	31.5 $\times$ 23.8

* The average of twenty microscopic fields.

e) **Flowers.** The tetraploid flowers tend to be larger and have broader petals and thicker corolla tubes (Table 6, Pl. IV, Fig. 5). The stigma is larger in diameter but the style becomes shorter in length (Pl. IV, Fig. 6). The filaments are thicker and shorter, and the anthers are heavily laden with comparatively good pollen grains.

Further, the flower pigments of the tetraploids generally increased as compared with the diploids. The mixoploid flowers show an asymmetric corolla without exception.

Table 6. Average size (corolla diameter $\times$ flower length in cm.) of flowers.

	Black ₁	Black ₂	Yellow	White
Diploid	2.7 $\times$ 3.9	2.6 $\times$ 3.7	2.2 $\times$ 3.5	2.0 $\times$ 3.5
Tetraploid	3.3 $\times$ 4.7	3.0 $\times$ 4.5	3.1 $\times$ 4.2	2.2 $\times$ 3.9
Mixoploid	2.8 $\times$ 4.1	2.8 $\times$ 3.9	2.5 $\times$ 3.9	2.0 $\times$ 3.6

f) **Pollen grains.** The pollen grains of the diploids can easily be distinguished from those of the tetraploids. So far we have found no exception to the rule that doubling the chromosome number increases notably the size of the pollen grains. The tetraploid grains are about 1.25 times greater in diameter through each of three forms than the diploid grains (Table 7, Pl. IV, Figs. 3 and 4). The tetraploid plants produced many good pollens.

In consequence, its fertility is comparatively high (Table 8). In the mixoploid grains examined, mixed diploid- and tetraploid-like grains in the microscopic fields were very frequent (Fig. 6).

Table 7. Size of pollen grains (μ).

	Black ₁	Black ₂	Yellow	White
Diploid	73.7 $\pm$ 0.335	42.0 $\pm$ 0.292	70.3 $\pm$ 0.308	82.6 $\pm$ 0.421
Tetraploid	98.4 $\pm$ 0.297	95.2 $\pm$ 0.313	100.2 $\pm$ 0.411	100.0 $\pm$ 0.508

Table 8. Fertilities of diploid and tetraploid pollen grains.

		No. of sterile pollen grains	No. of fertile pollen grains	Total	Fertility(%)
Black ₁	Diploid	17	483	500	96.60
	Tetraploid	141	359	"	71.80
Black ₂	Diploid	13	487	"	97.40
	Tetraploid	132	368	"	73.60
Yellow	Diploid	8	492	"	98.40
	Tetraploid	124	376	"	75.20
White	Diploid	10	490	"	98.00
	Tetraploid	122	378	"	75.80
Av. {		Diploid			97.60
		Tetraploid			74.10

g) Branching and node numbers. The branching in the Black₁ and Black₂ group was numerous, while in the Yellow and White it was entirely missing. This can be recognized as one of the chief differences between the Black and the other two groups.

The induced tetraploid of Black₁ and Black₂ grow densely with many branches at the adult stage (Fig. 8). In Black₁, the node numbers of the tetraploids, especially, increase in proportion to the increase of their growth (Table 9), and the length of the internodes is also longer than in the diploids.

Table 9. Number of branches and nodes.

	Black ₁		Black ₂		Yellow	White
	No. of branch	No. of node	No. of branch	No. of node	No. of node	No. of node
Diploid	3.4±0.262	74.6±0.492	3.2±0.099	31.0±0.316	35.1±0.229	40.0±0.211
Tetraploid	7.1±0.335	132.2±0.635	5.8±0.134	45.5±0.411	47.8±0.364	49.4±0.435

h) Capsules. In Black₁ and Black₂, the capsules are solitary in the axils of the leaves (Pl. IV, Fig. 7). On either side of each flower a yellow nectary gland which secretes a mucilagenous substance is to be found. Arrangements of the capsules on the stem are comparatively sparse on account of their having alternate phyllotaxis.

In the capsules of the Yellow and White, however, two nectary glands of one flower develop into two functional flowers, giving three flowers per leaf axil instead of the usual one. All three flowers produce three capsules as a general rule (Pl. IV, Fig. 8). Arrangements of the capsules on the stem are very dense as if they have opposite phyllotaxis and, further, the internodes are shorter than in Black₁ and Black₂.

Table 10. Number of capsules per one plant.

	Black ₁		Black ₂		Yellow		White	
	No. of cap.	Ratio	No. of cap.	Ratio	No. of cap.	Ratio	No. of cap.	Ratio
Diploid	117.65±0.306	100	93.63±0.021	100	148.07±0.324	100	155.22±0.097	100
Tetraploid	165.22±0.431	140	61.05±0.421	65	111.23±0.403	75	101.13±0.321	65

The number of capsules per plant in the tetraploids is generally smaller than in the diploids except for Black₁ (Table 10). The larger number in the Black₁ tetraploids results chiefly from the more numerous branchings than in the diploids. In a few tetraploids of Yellow and White, we often found, the number of capsules per one opposite phyllotaxis had increased though the total number of capsules per plant had decreased. The maximum number per one opposite phyllotaxis we found to be nine (Fig. 8). This flourishing physiological condition may be due to the metamorphosis of nectary glands in axis, as a result of the diploid's being transformed into a tetraploid.

Fig. 8. Diagram to show the branchings and the number of capsules of the highest plant in diploids and tetraploids in the harvest.

Table 11. Size (length×breadth in mm.) and weight (mg.) of seeds of diploid and tetraploid.

		Size	Ratio	Weight	Ratio
Black ₁	Diploid	3.2±0.012×2.0±0.021	100×100	2.4±0.004	100
	Tetraploid	4.0±0.005×2.5±0.034	125×125	4.3±0.019	179
Black ₂	Diploid	3.0±0.003×1.9±0.022	100×100	2.0±0.021	100
	Tetraploid	3.0±0.014×2.5±0.037	100×132	3.2±0.035	155
Yellow	Diploid	3.0±0.028×2.0±0.008	100×100	2.3±0.014	100
	Tetraploid	3.0±0.017×2.5±0.006	100×125	4.0±0.121	174
White	Diploid	3.0±0.006×2.0±0.007	100×100	2.3±0.007	100
	Tetraploid	3.0±0.009×2.3±0.008	100×115	3.7±0.012	161

Though length of tetraploid capsules is shorter, the width is, in general, much increased (Pl. IV, Figs. 9 and 10, Pl. V, Figs. 11, 12, 13, and 14). In such capsules the placenta and the ovules have extremely enlarged in width and consequently they contain a smaller number of seed.

i) **Seeds and fertilities.** Plate VII, Figs. 24—27 show seeds harvested from diploid and tetraploid capsules respectively. Increased seed size has proved an unfailing criterion of tetraploidy (Table 11). And the seeds from one tetraploid capsule are fewer in number than from that of diploid (Table 12). It can be seen from Table 12 that the seed fertility of the tetraploids is comparatively high through each form.

Table 12. Comparison of the fertility per capsule of diploids and tetraploids.

		Fertile seeds	Sterile seeds	Total	Fertility(%)
Black ₁	Diploid	74.4	3.5	77.9	95.51
	Tetraploid	40.0	14.0	54.0	74.07
Black ₂	Diploid	69.2	2.0	71.2	96.91
	Tetraploid	39.5	10.3	49.8	79.32
Yellow	Diploid	81.6	2.8	84.4	96.68
	Tetraploid	50.4	13.9	64.3	78.38
White	Diploid	78.8	2.6	81.4	96.81
	Tetraploid	57.5	15.1	72.6	79.20
Av. { Diploid					96.48
{ Tetraploid					77.74

The yield of seeds per plant of the tetraploid is generally lower than in the diploid except in Black₁.

In Black₁ the number of capsules increased because of the vigorous branching resulting in a total overall increase in the yield of seeds per plant. However the weight of seeds per one capsule was reduced. Thus, the forms showing a sparse arrangement of capsules on a stem, but having many branches, are more productive than forms with a dense arrangement on a single stem without branching.

Table 13. The yield of seeds per plant.

		Average number of capsules per plant	Average weight of fertile seeds per capsule (gm.)	Average weight of fertile seeds per plant (gm.)	Ratio
Black ₁	Diploid	117.25±0.325	0.178±0.003	20.827±0.105	100
	Tetraploid	165.36±0.627	0.172±0.125	28.380±0.420	136
Black ₂	Diploid	93.13±0.220	0.138±0.011	12.834±0.311	100
	Tetraploid	61.41±0.761	0.126±0.321	7.686±0.394	59
Yellow	Diploid	169.09±0.032	0.187±0.005	31.434±0.207	100
	Tetraploid	111.91±0.441	0.202±0.230	22.222±0.391	71
White	Diploid	155.25±0.266	0.181±0.012	28.055±0.022	100
	Tetraploid	101.03±0.374	0.212±0.039	21.412±0.410	76

j) **Aphis resistance.** All diploid forms generally receive a severe attack of aphides, and they sometimes are completely destroyed. But the tetraploid forms showed

extreme resistance to the insects. Their resistance may be due to a physiological difference or to a difference in chemical content in the tetraploid itself. The fact itself, however, is worthy of note not only in further experimental planting but also in practical planting in the field.

I. Cytological observation

At the somatic metaphase of the diploids 26 chromosomes were usually observed (Pl. VI, Figs. 16 and 18). But at that of the tetraploids 52 chromosomes were distinctly counted in all the examined preparations (Pl. VI, Figs. 15 and 17). The karyotypes of the diploid and tetraploid forms were determined as

$$B_1D \text{ (or } B_2D) = K(2n) = 26(2b) = 2A^{st} + 18B^m + 6C$$

$$B_1T \text{ (or } B_2T) = K(4n) = 52(4b) = 4A^{st} + 36B^m + 12C$$

$$YD \text{ (or } WD) = K(2n) = 26(2b) = 2A^{st} + 2B^{st} + 22B^m$$

$$YT \text{ (or } WT) = K(4n) = 52(4b) = 4A^{st} + 4B^{st} + 44B^m$$

The mean of length of A, B, and C was 1.85, 1.43, and 1.21 micron respectively.

The lengths measured on the chromosomes agree with those of Sugiura's data (1936) approximately. It is noteworthy that some of the homologous chromosomes showed a tendency to somatic pairing.

The meiosis of the diploids goes quite normally, producing good pollens. Thirteen bivalents were easily recognized in polar view of metaphase I (Pl. VII, Fig. 28). A characteristic feature at this stage was secondary pairing of chromosomes without exception in all examined preparations. The characteristic secondary pairing type was $4_{II-II} + 5_{II}$ with the maximum frequency. This formula shows four groups of two bivalents each and five ungrouped bivalents (Table 14, Pl. VII, Fig. 28).

Table 14. Frequencies of secondary pairing types at metaphase I in pollen mother cells in diploid *S. indicum*.

Type of pairing		Configurations ¹⁾	Cells observed	%
13 _{II} {	1 _{II} -II + 11 _{II}	13 (12)	106	6.75
	2 _{II} -II + 9 _{II}	13 (11)	211	13.44
	3 _{II} -II + 7 _{II}	13 (10)	306	19.49
	4 _{II} -II + 5 _{II}	13 (9)	869	55.35
	1 _{IV} + 11 _{II} *	12	21	1.34
	1 _{IV} + 1 _{II} -II + 9 _{II}	12 (11)	8	0.51
	1 _{IV} + 2 _{II} -II + 7 _{II}	12 (10)	10	0.64
	1 _{IV} + 3 _{II} -II + 5 _{II}	12 (9)	7	0.45
	2 _{IV} + 9 _{II} *	11	5	0.32
	2 _{IV} + 7 _{II} -II + 7 _{II}	11 (10)	4	0.25
	2 _{IV} + 2 _{II} -II + 5 _{II}	11 (9)	7	0.45
	3 _{IV} + 7 _{II} *	10	3	0.19
	3 _{IV} + 1 _{II} -II + 5 _{II}	10 (9)	4	0.25
	4 _{IV} + 5 _{II} *	9	9	0.57
Total			1570	100.00

1) The numbers in brackets are the number that one group of two bivalents exhibiting secondary pairing was counted as one chromosome configuration.

* No indicate any secondary pairing in these formulas, but we applied them in the present table because these types are concerned with secondary pairing.

In the diploids, occasionally one or more quadrivalents, of the ring and chain type, were noticed (Pl. VII, Fig. 29). The maximum number of the quadrivalents was four : $4_{IV} + 5_{II}$ (Table 14). Secondary pairings, also, were recognized on side views at metaphase I (Pl. VII, Fig. 30). These phenomena were very often remarked at the second metaphase.

Beyond expectation, the occurrence of the quadrivalents in the tetraploids was more frequent than those of the bivalents, and the occurrence of univalents, trivalents, and octavalents was very rare (Table 15). With regard to the type of secondary pairing in the tetraploids, we have designated the chromosome configurations from 9 to 13 as class A and the others as class B. As indicated in Table 15, the configurations belonging to class A were more frequent (70.95%) than those of class B (29.05%).

Table 15. Chromosome configurations and associations at metaphase I of pollen mother cells in tetraploid *S. indicum*.

Class	Configurations	No. of PMC	Association of chromosomes				
			I	II	III	IV	VIII
A	9	7				35	28
	10	11				77	33
	11	18				162	36
	12	115				1265	115
	13	434 + (6)*		(48)*		5642 + (6)*	(24)*
B	14	26	2	44	2	318	6
	15	11	2	47		113	3
	16	19	2	107	6	185	1
	17	13		104		117	
	18	12	1	132	1	98	2
	19	17		204		119	
	20	17		237	2	101	
	21	22		360		100	2
	22	24	2	420	2	93	1
	23	17		340		51	
	24	51		330		30	
	25	21		504		21	
	26	18	2	491		1	
	27	3	26	161		1	
	28	7	12	74			
Total	A	591 (70.95 %)	0	48	0	7187	236
	B	242 (29.05 %)	49	3555	13	1348	15
		833	49	3603	13	8535	251
Gemini	A	15366	0	48	0	14374	944
	B	6355	24.5	3555	19.5	2696	60
		21721	24.5	3603	19.5	17070	1004
Frequency(%)	A	100	0	0.31	0	93.54	6.15
	B	100	0.39	55.94	0.31	42.42	0.94
Frequency to A + B		100	0.11	16.59	0.08	78.59	4.63

* These are the number that did not exhibit any secondary pairing.

In the numbers observed as having 13 configurations, 6 cases (0.72 %) that did not have any secondary pairing are included, but this is a negligibly small number.

It was commonly observed in the class A that some of the quadrivalents happened to lie close together : therefore, they showed different numbers of secondary pairing.

On the contrary, in the class B, several chromosome associations were seen, and in those associations bivalents were most frequent, quadrivalents were next, and univalents, trivalents, and octavalents were very small in number. Furthermore, in this class secondary pairing was almost never observed.

Table 16. Frequencies of secondary pairing types of class A in pollen mother cells at metaphase I in tetraploid *S. indicum*.

Type of pairing		Configurations ¹⁾	Cells observed	%
13 _{IV}	1 _{IV} -1 _{IV} +11 _{IV}	13 (12)	32	5.47
	2 _{IV} -1 _{IV} + 9 _{IV}	13 (11)	41	7.01
	3 _{IV} -1 _{IV} + 7 _{IV}	13 (10)	58	9.91
	4 _{IV} -1 _{IV} + 5 _{IV}	13 (9)	303	51.79
	1 _{VIII} + 11 _{IV} *	12	46	7.86
	1 _{VIII} + 1 _{IV} -1 _{IV} + 9 _{IV}	12 (11)	39	6.67
	1 _{VIII} + 2 _{IV} -1 _{IV} + 7 _{IV}	12 (10)	18	3.08
	1 _{VIII} + 3 _{IV} -1 _{IV} + 5 _{IV}	12 (9)	12	2.05
	2 _{VIII} + 9 _{IV} *	11	8	1.37
	2 _{VIII} + 1 _{IV} -1 _{IV} + 7 _{IV}	11 (10)	6	1.03
	2 _{VIII} + 2 _{IV} -1 _{IV} + 5 _{IV}	11 (9)	4	0.68
	3 _{VIII} + 7 _{IV} *	10	6	1.03
	3 _{VIII} + 1 _{IV} -1 _{IV} + 5 _{IV}	10 (9)	5	0.85
	4 _{VIII} + 5 _{IV} *	9	7	1.20
Total			585	100.00

1), * See Table 14.

Table 16 gives a summary of the various pairings met with in the class A. The maximum secondary pairing was 4_{IV}-1_{IV}+ 5_{IV} (Pl. VII, Fig. 31). It is of further interest that four octavalents were observed as the maximum chromosome pairing in the tetraploids : 4_{IV}-1_{IV}+ 5_{IV} (Pl. VII, Fig. 32).

Chromosome separation of the quadrivalents and the octavalents at anaphase I is comparatively normal (Pl. VII, Fig. 33) and secondary pairing is maintained : the side views at this stage show that some groups with secondarily paired chromosomes were still visible. But a few irregularities in the division, viz. lagging chromosomes, chromosome bridges and others, were seen through the first and second metaphase.

Consequently, the tetraploid pollen grains yielded were comparatively fertile ones.

Discussion

In our experiments the use of colchicine proved to be highly efficacious in inducing an abundance of tetraploids in *S. indicum*. Of our two methods of application, immersion and dropping, to induce the tetraploids plants, the latter was more effective than the former : five or six- days treatment of seedlings with 0.2% colchicine solution resulted in a 75 ~ 90 % yield through several forms. The former, on the contrary, resulted in the comparatively low occurrence of the tetraploids of only 20 ~ 30 %, with

a maximum 0.5% colchicine solution. This lower occurrence of tetraploids by the immersion method was due to the relatively large number of seeds which did not germinate and of seedlings which died. In fact, the hypocotyle plumped abnormally and died away soon after the germination. These phenomena, especially, were more remarked when high concentrations of colchicine were used.

For a few days after the treatment with colchicine, the plants stopped growing and seemed to remain in this state for some time, and then they resumed growth gradually. So that the life period of the tetraploids was longer than that of the diploids.

The growth rate of the tetraploids outstripped that of the diploids about 40 days after the treatment, furthermore the difference in growth habits between them became more obvious. Tetraploid Black₁ particularly, with its branching habit, was the most conspicuous.

It has been noted by previous investigators that the autopolyploid plants are very often more robust, with thicker stems and larger leaves, flowers, and seeds than the diploids. In our materials these facts were well substantiated.

It is of interest that the fertility of the tetraploids in *Sesamum* produced by the colchicine method is comparatively high in comparison with that other induced polyploids. This has been already pointed out by Langham (1942).

The fact that autotetraploids of *S. indicum* show comparatively high fertility can be partially explained by our cytological observation that thirteen quadrivalents has a prevalent frequency as the chromosome pairing at metaphase I while univalents and trivalents appear infrequently.

The cause of fertility depends, of course, upon various factors and is not so simple that it can be attributed to one, but an important possibility lies in the assumption that every quadrivalent chromosome is equally divided into two and that the disjunction takes place regularly and since no irregularity in poleward movement occurs, good pollens are produced and normal seed setting takes place.

In cytological studies of the diploids, one notable feature is that there are secondary pairing of chromosomes in many preparations. Kumar and Abraham (1941) observed in diploid *S. orientale* that on the secondary association the most frequent type of association was into groups of seven : $2_3 \ 2_2 \ 3_1$ and also $1_3 \ 4_2 \ 2_1$. As a result they have suggested that the original basic number of the genus is probably seven.

However, as already indicated, in our observations we never saw such a type. In our studies the type of secondary pairing, $4_{II-II} + 5_{II}$, was the maximum frequency. This type means four groups of two bivalents each and five ungrouped bivalents.

A further characteristic feature to be noted is that at metaphase I of the diploids some quadrivalents were seen though their appearance was very rare. The maximum number of the quadrivalents was four : $4_{IV} + 5_{II}$. This fact reveals *S. indicum* as one of the secondary polyploids. Our consideration tends to suggest that it is highly probable that the original ancestor of *S. indicum* had nine as the basic number from which the cardinal number thirteen has been derived.

Summary

1) Induced polyploidy in *Sesamum indicum* L. by the colchicine method is described; two different methods, namely the immersion and dropping, were used, the latter proving more effective than the former.

2) As the characteristics of autopolyploidy, the tetraploid plants were extremely robust, with much thicker stems, larger leaves, stomata, flowers, and seeds than the diploids.

3) The fertilities of pollen grains and seeds per capsule from the tetraploids were comparatively high with averages of 74.10% and 77.74% respectively. But the yield of seeds per plant of the tetraploids was unfavorable.

4) Details of meiosis in diploid and tetraploid *S. indicum*, especially on secondary pairing, have been recorded.

On the nature of secondary polyploidy in *S. indicum* was discussed.

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Explanation of plates

Plate IV.

- Figs. 1-2. Stomata from leaves of diploid (Fig. 1) and tetraploid (Fig. 2). $\times 140$.
 Figs. 3-4. Pollen grains from diploid (Fig. 3) and tetraploid (Fig. 4). $\times 50$.
 Fig. 5. Flowers from diploid and tetraploid. Upper row : tetraploid; lower row : diploid. From left to right, Black₁, Black₂, Yellow and White. ca. $\times 2/5$
 Fig. 6. Ovary and style from diploid (left) and tetraploid (right). Actual size
 Figs. 7-8. Showing two ways of arrangements of the capsules in the axils of the leaves in the diploids. Fig. 7. Black₁ with alternate phyllotaxis. Fig. 8. Yellow with opposite phyllotaxis.
 Figs. 9-10. Comparison of three-capsules from diploid (above) and tetraploid (below). Fig. 9. Yellow, Fig. 10. White. Actual size.

Plate V.

- Figs. 11-14. Comparison of mature capsules from diploid (left) and tetraploid (right). Fig. 11. Black₁, Fig. 12. Black₂, Fig. 13. Yellow and Fig. 14. White respectively. Actual size.

Plate IV.

- Figs. 15-18. Diploid and tetraploid plants which were grown under similar conditions in the green house, and their somatic chromosomes. Fig. 15. Tetraploid Black₁ : $2n = 52$, Fig. 16. Diploid Black₁ : $2n = 26$, Fig. 17. Tetraploid Yellow : $2n = 52$ and Fig. 18. Diploid Yellow : $2n = 26$.
 Figs. 19-23. Capsules on stem of diploid and tetraploid in the harvest. Fig. 19. Diploid Black₁, Fig. 20. Tetraploid Black₁, Fig. 21. Diploid Yellow, Fig. 22. Tetraploid Yellow and Fig. 23. Tetraploid White.

Plate VII.

- Figs. 24-27. Seeds from diploid (below) and tetraploid capsules (above). Fig. 24. Black₁. Fig. 25. Black₂, Fig. 26. Yellow and Fig. 27. White. Actual size.
 Figs. 28-30. Meiotic chromosomes in dioloid. Fig. 28. Metaphase I, 13_{II}, showing the maximum secondary pairing : $4_{II-III} + 5_{II}$. Fig. 29. Shows the maximum number of the quadrivalents : $4_{IV} + 5_{II}$. Fig. 30. A side view at metaphase I, showing one group exhibiting secodary pairing on the middle. $\times 3000$.
 Figs. 31-33. Meiotic chromosomes in tetraploid. Fig. 31. Metaphase I, 13_{IV}, showing the maximum secondary pairing : $4_{IV-IV} + 5_{IV}$. Fig. 32. Shows the maximum number of the octavalents : $4_{VIII} + 5_{IV}$. Fig. 33. A side view at early anaphase I, showing a normal separation of one octavalent. $\times 3000$.