Cyto-Morphological Studies on C_2, C_3 Progeny of the Cross between C_2 Tetraploid and Diploid of Sesame

(Sesamum indicum L.)*

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The realisation that many of the valuable crop plants, such as wheat, cotton, tobacco sugarcane etc. are natural polyploids, and that they possessed superior economic properties over the diploids led to a more exhaustive exploration of the scope of polyploidy being introduced artificially in the field of plant breeding, with a view to evolving promising crop varieties.

Real advancement in the line of induced polyploidy in the field of plant breedidg has been recorded only since the adoption of Colchicine technique of induction of polyploidy proposed by Blakeslee and Avery (1937). Colchicine-induced polyploids of many crop plants, such as sugarbeet, rye, red clover, water-melon etc. have become established themselves as promising crop varieties.

Induction of polyploidy in sesame *(Sesamum indicum* L.) by Colchicine treatment has been tried by Langham (1940), Richharia and Persai (1940), Kobayasbi and Shimamura (1948), Shrivastava (1956) and Nair (1965).

Sesame *(Sesamum indicum* L.) is an important oil yielding annual crop. The chromosome number of the species is 2n : 26 (Moringa *et al.,* 1929).

The present investigation was taken up in continuation of the work done by Nair (1965), with a view to studying the C2 and C3 generations, as well as the progeny of the cross between C2 tetraploid and diploid.

Materials and Methods

A. Materials

Fourteen seed types of *Sesamum indicum* L. (Strain TMV. 2) obtained by Nair (1965) from the C_1 generation formed the seed material for the study of generation. The seeds were collected from suspected 'polyploids' under different treatments of C_1 generation. Seeds of normal diploid were used as control.

The C_2 tetraploid was crossed with diploid reciprocally. The crossed seeds were tried along with the selfed seeds of the parents in the C_3 generation,

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B. Methods

For correct statistical comparison of the different suspected 'polyploid' types of C_1 generation, a randomised block design with 15 types and 4 replications was laid out for the study of C_2 generation.

For C_3 generation a randomised block design with 6 types and 8 replications was laid out. The first 4 types were selfed seeds of C_2 tetraploid. Type 5 was selfed seeds of diploid parent, and type 6 was crossed seeds of C_2 tetraploid and diploid.

The following characters were studied: –

1. Germination percentage of C_1, C_2 and 4x X 2x seeds.
2. Height of plants—at weekly intervals from 3rd week after sowing till harvest.
3. Leaf characters
   a) Mean area of leaves:– By Darrow's method
   b) Mean thickness of leaves:– From 100 hand sections from each type measured in-micron
4. Size and distribution of stomata:-Lower epidermis of 10 randomly collected leaves of each type was stained in 0.5% safranin. Frequency of stomata in unit area was determined by counting 100 randomly selected microscopic fields for each type. Length and width of 100 randomly selected stomata were measured in micron (μ) for each type using a standardised ocular micrometer
5. Number of flowers:- Flower counts were taken daily.
6. Size and sterility of pollen grains:- Pollen grains of each type were stained in glycerine-aceto-carmine. Ten fields for each treatment were scored for sterile and fertile grains. Diameter of 100 randomly selected pollen grains was measured in micron (μ) using a standardised ocular micrometer.
7. Cytological observations

Suspected polyploids based on their gigas characters were marked, and flower buds of appropriate size from these plants, as well as from other plants, were fixed separately in a 3:4: 1 mixture of absolute alcohol, chloroform and propionic acid with traces of ferric chloride. Fixation was done between 11.15 A.M and 11.45 A.M. After keeping 24 hours in the fixative the buds were stored in 70% ethyl alcohol. Anthers were squashed in 1% propiono-carmine. Gentle warming favoured excellent spreading and differential staining of chromosome and cytoplasm.

8. Crossing tetraploid and diploid.

The single plant of generation as found to be at tetraploid was crossed reciprocally with diploid.

Emasculation was done on the previous evening of anthesis and pollination on the next day at 7 A. M.

9. Selfing

Selfed seeds of tetraploid and diploid parents were collected by covering mature flower buds with a paper cover.

10. Number and size of capsule

Length and girth of capsules were measured for each type from 10 randomly selected capsules at the time of harvest.

11. Yield of seed

10 capsules from each type were collected at random and the seeds were counted.

12. Weight of seeds

Weight of 1000 seeds from each type was recorded in grams.

13. Study of triploids

The progeny of the 4x X 2x cross was studied along with the C_3 generation as suspected triploids, for all the characters mentioned.

The recorded data were statistically analysed. Analysis of variance was worked out for 7 characters, viz., height of plants, number of branches, flowers and stomata, pollen size, leaf thickness and number of pods. Comparison of other characters under different types was made by calculating the mean and standard error.

Results and Discussion.

1. *Percentage of germination*

The 14 seed types of the C_1 suspected polyploids showed only a slight reduction in germination compared to the normal diploid. (Table. 1)

In the C_3 generation the seeds of C_2 tetraploid showed minimum germination andthe 4x X 2x crossed seeds showed maximum germination. Such a high germinability of seeds from tetraploid seeds as seen in generation was recorded by Kibayashi and Shimamura (1949, 1952) and Srivastava (1956) in the same crop. The comparatively higher percentage of germination in C may be due to the smaller number of diploid gametes fertilized. Further, such tetraploid seeds might not have germinated. Only seeds developed from normal haploid gametes were seen germinated, as only diploids were seen in the progeny. The same reason can be attributed to the progeny of the tetraploid. The higher germination percentage of the crossed seeds may be due to the extra vigour obtained by cross pollination.

2. *Growth of plants*

The C_2 types did not show any significant difference in growth rate, compared to normal diploid. In appearance they were more vigorous in growth than normal diploid and 4x x 2x progeny. Progeny of the cross was intermediate between the parents. Similar results were observed by Nair (1965) and Srivastava (1956) in the same crop.

3. *Height ofplants and number of branches*

The suspected C_2 polyploid types did not show any significant difference in height compared to normal diploid. Types 1 and 5 showed significant increase in number of branches. (Table I)

In C_3 generation the progeny of tetraploid showed significant increase in height as well as number of branches. The progeny of the cross (4x x 2x) was intermediate between the parents.

Profuse branching nature of tetraploids has been recorded by Tandon (1961) in *Brassica oleraceae*

Even though the progeny of the suspected C_1 tetraploids and the progeny of the 4x x 2x cross were all diploids (except one sectorial polyploid in C generation) the vigorous growth and profuse branching may be due to the inheritance of these characters from C_1 generation. Due to cross pollination the X 2x progeny might have obtained some excess vigour. The intermediate nature of such progeny in characters like height etc. may be due to the expression of quantitative characters by the F_1.

Leaf area and thickness.

In the C_2 generation only 5 types have shown an increase in leaf area compared to normal diploid. Only 4 types exceeded diploid in leaf thickness. (Table I.)

In C_3 generation, the difference in leaf area was negligible. But the 3 types differed significantly in thickness. Progeny of the C_2 tetraploid possessed maximum leaf area and thickness followed by progeny of 4x x2x cross and then by the normal diploid.

The same reason as given in item 3 can be attributed in this case also.

5. *Size and distribution of stomata.*

There was no significant reduction in the number of stomata per unit area among the C_2 types compared to diploid. Most of them showed slight increase in size of stomata. (Table II.)

In C_3 generation the progeny of C_2 tetraploid had the minimum number of stomata, followed by the progeny of 4x x 2x, and then by diploid The size of the stomata also was in the same order, the progeny of the Cjj tetraploid having bigger sized stomata.

Hertzsch (1951) recorded similar rusults in tetraploids of *Vicia villosa*. But Graner (1941) in *Manihot utilissima*, and Langham (1942) and Srivastava (1956) in sesame, have recorded results contradictory to this.

The diploid nature of the progeny of the C_1 and C_2 (except one sectorial polyploid in C_2), as well as the 4x × 2x progeny, may be the reason for such results.

6. *Number and size of flowers*

The number of flowers produced by the C_2 types, as well as the progeny of the 4x × 2x cross and progeny of C_2 tetraploid, did not show any significant difference. (Table III). In general they produced larger flowers and showed delay in flowering compared to normal diploid. Randolph (1944) in maize, Tandon and Chinoy (1950) in *Amaranthus blitum* and Srivastava (1956) in sesame observed prolonged vegetative growth and delayed flowering in autotetraploids. Production of bigger flowers in larger numbers was recorded in autotetraploids of many crops by Parthasarathy and Kedarnath (1945), Kobayashi and Shimamura (1949, 1952), Srivastava (1956) and Nair (1965) in sesame.

The slight increase in the number and size of flowers produced by C_2 types, as well as the progeny of tetraploid and the progeny of the 4x × 2x cross may be only due to their vigorous growth habit, as no genuine tetraploid was observed.

7. *Cytological observation*

The behaviour of chromosome during meiosis of all the plants of both generations was studied. Only in one plant of C_2 generation tetraploid chromosome number (2n : 52) was noticed. Here also the anaphasic separation was normal, i. e. 26/26. In the same plant pollen mother cells with diploid chromosome number (2n 26) was also seen. All the other plants behaved as normal diploid forming 13 bivalents at metaphase—I. Meiosis was found to be regular in all the plants. Only bivalents were found in all the cases at diakinesis and metaphase—I. Persistence of more than one secondary nucleolus was noticed in pollen mother cells and microspores. The progeny of the C_2 tetraploid x diploid cross were proved to be all diploid instead of triploids. Selfed progeny of this C_2 tetraploid was also found to consist only of diploids, Ramuson and Levan (1959) observed diploid and tetraploid sectors in the same branch or even in the same flower in the case of sugarbeet. Sen and Cheda (1958) observed in black gram that the Colchicine induced polyploids were either complete branch or sectorial polyploids. Normal anaphasic separation in autotetraploids was observed by Kundu and Sarma (1956) in *Corchorus olitorius*, and by Visweswara and Chinnappa (1965) in *Coffeacanephora*.

The apparent reversion of C, tetraploids to diploids in subsequent generation may be due to following reasons :

The C_1 tetraploids may not be genuine tetraploids. Instead some of them might have been periclinal ploid chimeras. Consequently the seeds collected consisted only of diploid seeds. Some of the C tetraploids might be of sectorial polyploid type instead of complete polyploid. From such plants a mixture of tetraploid and diploid seeds might have been obtained of which only diploid seeds germinated.

8. *Cross between tetraploid and diploid*

Only one cross between C_2 tetraploid as female parent and diploid as female parent yielded a few viable seeds. In the reciprocal cross (2x 0 X 4x 0) only shriveiled and non-viable seeds were obtained.

Similar results were recorded by Srivastava (1956) in sesame and Toyao (1960) in tea.

The failure of reciprocal cross (2x 0 x 4x 0) may be due to the slow growth rate of diploid pollen tube of the C_2 tetraploid.

The few seeds obtained in the cross 4x 0 x 2x 0 were proved to be of diploid instead of triploids. This may be due to the sectorial polyploid nature of the C_2 tetraploid used as female parent. Out of the diploid and haploid gametes produced only the hoploid ones might have succeeded in developing into viable seeds. The 3x embryo and its endosperm which resulted from the fusion of haploid and diploid gametes might have collapsed in the early developmental stages. Consequently only a few seeds which were diploid were obtaind in the cross.

9. *Size and sterility of Pollen*

Few C_2 types showed significant increase in pollen size compared to normal diploid But there was no significant difference in sterility. (Table III)

In C_3 generation the progeny of C_2 tetraploid possessed larger pollen followed by 4x X 2x progeny and then by normal diploid. The three types did not show any significant difference in sterility.

Larger pollen size in autotetraploids has been recorded by Amin (1940) in cotton, by Pal, Ramanujam and Joshi (1941) in *Capsicum* and *by* Parthasarathy add Kedarnath,(1945) Srivastava (1956) and Nair(1965) in sesame.

There exists a great deal of diversity regarding pollen sterility inautotetraploids. Fertility comparable to diploids was recorded by Ramuson and Levan (1939) in sugarbeet, and Langham (1942) Kobayashi and Shimamura (1949, 1952) and Srivastava (1956) in sesame. Even an increase in fertility than" diploid was noticed by Kundu and Sarma (1956) in autotetraploids of *Corchorus olitorius.*

TABLE I

Data showing germination percentage, height of plants, number of branches, mean area and thickness of leaf

Type of plant	Germination %	Mean height of plants (Cm)	Number of branches	Mean area of leaf (sq cm)	Mean thickness of leaf
	I	II	III	IV	V
C_2 Generation					
Control (Diploid)	65	88.68	4.37	68.1+or—5.96	246.30+or—2.73
1	43	78.40	6.57	67.5+or—5.18	234.15+or—3.52
2	42	84.25	4.87	67.9+or-4.16	254.55+or—3.91
3	47	97.75	4.55	67.1+or—3.56	253.50+or—2.70
4	47	92.25	4.95	71 2+or—3.25	263.40+or—3.70
5	44	93.62	6.12	67.8+or—5.75	279.00+or—4.65
6	59	94.95	4.30	67.9+or-3.87	242.25+or—3.25
7	57	91.62	4.10	65.7+or—3.87	246.25+or—3.24
8	53	91.00	4.30	71.5+or—5.68	241.80+or—3.13
9	42	89.25	4.02	71.0+or-4.25	245.40+or—3.03
10	55	88.17	3.67	68.5+or-4.71	234.30+or-2.35
11	57	95.95	5.42	66.9+or-4.41	238.80+or—3.28
12	61	97.40	4.55	68.8+or—2.75	244.50+or—3.16
13	57	94.97	3.62	70.9+or—4.87	235.20+or-2.62
14	54	95.55	4.47	66.0+or—3.09	253.35+or-3.06
C. D. (5%)					0.4704

C_3 Generation

	I	II	III	IV	V
4x	21.3	103.68	4.52	7.46+or—4.59	274.20+or—3.45
2x	43.4	94.23	2.77	69·95+or—4.68	250.35+or—2.73
4x x 2x	62.5	98.18	4.06	69.82+or—1.97	253.50+or—3.07
C. D. (5%) for 4x Means		5.85	0.23		0.49
C. D. (5%) for 2x and 4x × 2x Means		7.67	0.36		0.62

TABLE II

Data showing number of stomata per unit area and size of stomata

Types	Number of stomata per unit area	Size of stomata (μ)	
		Mean width	Mean lengtb
	I	II	III
		C_2 Generation	
Control (Diploid)	23.84+or—0 08	15.07+or—0.28	24.21+or—0.16
1	23.49+or-0.08	15.04+or—0.23	23.32+or—0.16
2	23.18+or—0.09	17.52+or—0.14	25.94+or—0.26
3	24.25+or—0.12	14.49+or—0.15	28.25+or—0.28
4	23.21+or-0.17	15.21+or—0.22	26.42+or—0.31
5	22.19+or—0.14	17.83+or—0.18	26.25+or—0.22
6	22 81+or—0.11	1932+or—0.21	26.22+or-0.22
7	23.37+or—0·25	15.97+or—0.25	25.90+or—0.20
S	22.56+or—0.12	17.56+or—0.16	25.87+or—0.24
9	227o+or 0.10	17.18+or—0.21	27.60+or—0.12
10	23.27+or-0.10	15 93+or—0.13	25.08+or—0.03
I I	23.41+or—0 10	15.28+or—0.13	24.25+or—0.24
12	23.18+or—0.09	17.31+or—0.09	26.32+or—0.24
13	22.90+or—0.09	15.69+or—0.22	26.42+or—0.25
14	21.42+or—0.12	18.21+or—0.14	26.42+or—0.23
C. D. (5%)	0.3324		

C_3 Generation

	I	n	III
4x	23.30+or-0. 10	17.59+or—0.17	27.73+or—0.24
2x	24.00+or—0.12	16.76+or—0.26	23.14+or—0.25
4x × 2x	23.48+or—0.15	16.90+or-0.24	24.08+or—0.21

C. D. (5%) for 4x Means	0 294
C. D. (5%) for 2x and 4x × 2x Means	0.352

TABLE III

Data showing number of flowers and size and sterility of pollen grains

Types	Mean number of flowers	Mean diameter of pollen (μ)	Pollen sterility (%)
	I	II	III
		C_2 Generation	
Control (Diploid)	59.95	68.20+or-0.28	6.08
1	55.92	70.72+or–0.26	5.59
2	58.05	68.96+or–0.36	6.85
3	78.57	68 24+or–0.30	5.03
4	69.42	70.72 + or–0.27	3.52
5	89.37	71.07+or–0.34	3.92
6	58.05	67.93 + or–0.28	4.07
7	59.55	70.13+or–0.36	9.37
8	6505	68.58+or–0.31	6.67
9	56.37	68.51+or–0.31	6.19
10	58.10	68.68+or–0.23	4.97
11	69.75	68.55+or–0.36	7.64
12	69.27	70.75 + or-0.32	6.66
13	67.85	68.41+or–0.33	12.34
14	65.35	70.03+or–0.36	7.31
CD.(5%)		0.254	
		C_3 Generation	
	I	II	III
4x	64.70	70.89+or–0.32	8.3
2x	60.86	67.86+or–0.25	6.4
4x × 2x	60.72	68.55 + or-0.33	8.1
C. D. (5%) for 4x Means		0.196	
C. D. (5%) for 2x and 4x × 2x Means.		0.803	

TABLE IV

Data showing number and size of capsules and 1000 seed weight

Types	Mean number of capsules	Size of capsules (cm)		Weight of 1000 seeds (g.)
		Length	Girth	
	I	II	III	IV
		C_2 Generation		
Control (Diploid)	38.80	2.41 + or—0.007	3.71 + or—0.014	2.25
1	41.65	2.61 + or—0.010	3.93 + or-0.010	2.37
2	36.47	2.70 + or—0.011	4 03 + or—0.009	2.45
3	55.95	2.62+or-0.013	3.88 + or—0.013	2.42
4	45.45	2.50 + or—0.008	3.62 + or—0.007	2.40
5	54.12	2.43 + or—0 013	3.90 + or—0.001	2.50
6	36.22	2.54 + or—0.011	3 65 + or—0.011	2.35
7	35.72	2.46+or-0.011	3.91 + or—0.009	2.32
8	38 00	2.46 + or—0.010	3.68 + or—0.011	2.36
9	32.77	2.51 + or—0 012	3.79+or-0.016	2.29
10	31.30	2.46 + or—0.009	3:78 + or -0.014	2.46
11	36.32	2.52 + or—0.015	3.78+or—0 011	2.38
12	39.52	2.50 + or—0.012	3.70 + or—0.014	2.45
13	40.20	2.58 + or—0.007	3.65 + or—0.011	2.38
14	42.67	2.57 + or—0.009	3.79 + or—0.009	2.45
		C_3 Generation		
	1	II	III	IV
4x	30.70	2.69 + or—0.070	3.97 + or—0.030	2.62
2x	28.48	2,57 + or—0.047	3.80 + or—0.017	2.30
4x x 2x	36 67	2.67 + or—0.068	3 83 + or—0.284	2.41

CD. (5%) for 4x Means 3.11
CD. (5%) for 2x and
4x x 2x Means 3.95

On the contrary, considerable reduction in fertility among autotetraploids was recorded by Pal, Ramanujam and Joshi (1941) in *Capsicum* and by Parthasarathy and Kedarnath (1945), and Nair (1965) in sesame. Very low fertility was recorded by Moringa and Fukuzhima (1935) and Ramanujam (1937) in the case of the progeny of tetraploid x diploid cross in *Oryza sativa*.

The high fertility of the progeny studied can be attributed to the orderly meiotic behaviour and diploid nature, except one tetraploid, in C_2 generation. Slight increase in sterility in some types may be due to the presence of more nucleoli in the microspores.

The larger size of pollen grains may be due to the larger pollen mother cells produced by the C_1 progenies, since they possessed gigas characters of tetraploids.

10. Capsule setting and yield

The C_2 types did not differ significantly in capsule production. But the capsules produced were slightly larger in size compared to normal diploid. The number of seeds produced also did not show significant variation. The seeds produced by the different types showed slight increase in weight than the normal diploid. (Table IV)

In C_3 generation there was significant difference in capsule production. The progeny of the cross (4x X 2x) showed significant increase in capsule production than the parents. There was not much difference in size of capsules. The progeny of the C_2 tetraploid produced slightly larger capsules. The yield and weight of seeds did not show much variation.

Langham (1940), Kobayashi and Shimamura (1949, 1952) and Srivastava (1956) observed in sesame that the tetraploids did not differ from diploids in capsule setting and yield, even though they possessed larger capsules and seeds.

The larger size of capsules and seeds of C_2 types and progeny of C_2 tetraploid can be attributed to their morphological similarity with tetraploids, even though they were diploids cytologically.

The increase in capsule setting by the progeny of the cross 4x X 2x, without such a significant increase in flower production, may be due to its lesser susceptibility to the caterpillar ***Antigastra catalaunalis*** which attacks flower buds also. This character may be due to excess vigour of cross pollination.

Nair (1965), after studying the C_1 generation, concluded that colchicine technique in improving sesame crops is considerably limited. After studying the progeny of C] and C_2 generation it is seen that there are some promising types in C_2 having good economic characters, even though they were only diploids. The progeny of the cross between C_2 tetraploid and diploid, though diploids, showed excess vigour than normal diploids and also lesser susceptibility to the leaf caterpillar.

Summary and Conclusions

The present investigation was undertaken in the Agricultural Botany Division of the Agricultural College and Research Institute, Vellayani, to study the nature of polyploids in C_2 and C_3 generations of ***Seasamum*** *indicum* L , as well as to study the progeny of the cross between C_2 tetraploid and diploid.

Fourteen selfed seed types of C_1 studied by Nair (1965) were carried forward to study the C_2 generation. Reciprocal crosses were made with C_2 tetraploid and diploid and the progeny studied along with C_3. Morphological and cytological behaviours of these generations were studied.

The suspected C_1 tetraploid types gave rise to only diploids except one sectorial polyploid in C_0 generation. This was confirmed cytologically. The C_2 plants, though diploids, were having morphological characters of tetraploids. The C_2 sectorial tetraploid, when crossed with diploid reciprocally, produced a few viable seeds in only one cross, i. e., 4x ♀ X 2x ♂. The progenies of the cross were also diploids only. The selfed progenies the C_2 tetraploid were also diploids.

The absence of tetraploids in the progeny of C_1 and C_2 tetraploids may be due to the failure of diploid gametes to function normally or due to the failure of tertaploid seeds to germinate. The absence of triploids in the cross between C_2 tetraploid and diploid may be due to the collapse of 3x embryo and its endosperm in its early developmental stage. The failure of reciprocal cross (2x ♀ x 4x ♂) to set seeds may be due to the failure of diploid pollen tube to grow through the normal stigma.

Except in one sectorial polyploid plant in C_2, all the other plants studied were diploid having 2n : 26. Though they were diploids many of the C_2 plants were having gigas nature and morphological similarity of tetraploids and possessed economic qualities like branching, flowering, capsule production and yield of seeds. The progenies of the cross between C_2 tetraploid and diploid were found to be superior in capsule setting and showed lesser susceptibility to caterpillar *Antigastra catanaunalis*, compared to the parents.

It was concluded that the effect of colchicine in inducing polyploidy in *Sesamum indicum* was not substantiated by the observations made in the present investigations. However, there were some promising types in C_2 generation having morphological similarities with tetraploids and possessing good economic qualities like capsule production and yield. The progeny of the cross between C_2 tetraploid and diploid also showed excess vigour of cross pollination in capsule setting and lesser susceptibility to leaf caterpillar. These promising tyes can be utilised for further selection.

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