CYTO-MORPHOLOGICAL STUDIES ON C_2, C_3 AND THE PROGENY OF THE CROSS BETWEEN C_2 TETRAPLOID AND DIPLOID OF SESAME (*Sesamum indicum* L.)

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THESIS

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C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bona fide research work carried out by Shri P. Balachandran, under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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INTRODUCTION

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INTRODUCTION

The phenomenon of polyploidy was one of the objects in early cytogenetical studies. Commencing with the discovery of polyploidy in Oenothera by Lutz (1907), this was subsequently found to be one of the most widespread and distinctive features of fairly large number of plant species. The realisation that most of the valuable crop plants such as wheat, oats, cotton, tobacco, potato, banana, coffee, sugarcane etc. are natural polyploids and that many of the polyploids possessed superior economic properties over the diploids, led to a more exhaustive exploration into the scope of its being introduced artificially into the field of plant breeding, with a view to evolve promising crop varieties.

The foremost successful attempt on the artificial production of polyploid was made by Winkler (1916) in Solanum nigrum, by decapitation method. He was able to obtain a tetraploid form of Solanum nigrum by this method. Later many workers have tried to induce polyploidy in plants artificially by different treatments such as temperature, and by chemicals like acenaphthene, chloral hydrate, ethyl mercuric chloride, etc. None of these processes however was very rewarding from an experimental point of view. It was with the practical application of colchicine techni-

que, proposed by Blakeslee and Avery (1937), that the way was paved for the production of polyploids in virtually unlimited numbers.

The alkaloid colchicine is extracted from the corms and seeds of the autumn crocus (Colchicum autumnale) belonging to the family Liliaceae.

Induced polyploidy has a distinct area of usefulness in plant breeding. As one of the recent techniques, it is found to be readily applicable as a valuable tool in crop improvement. Colchicine induced polyploids of many crop plants have become established themselves as promising varieties. Eg:- Red Clover (Levan, 1948); Turnip (Levan, 1948); Water Melon (Kihara, 1951); Rye (Muntzing, 1951); Raddish (Nichyamo, 1952); Grapes (Olmo, 1952); Sugarbeet (Matsumura, 1953); Berseem (Mehta and Swaminathan, 1965).

It may be worthwhile to have in mind, the remarks of Bogyo (1941) in his study on the role of polyploidy in the origin and propagation of species. He concludes that the plant breeder need not expect any miracles from the application of polyploidy in plant breeding. Its most useful application, as he considers, is the production of fertile hybrids (Allopolyploids).

Sesamum (Sesamum indicum, L.) is one of the popular oil yielding crops of India. It belongs to the family

Pedaliaceae and known by various names viz. sesame, til, gingelly, simson etc. It is cultivated mainly in India, Burma, Vietnam, Manchuria, Japan and Africa, for its high quality oil. Sesamum indicum and S. orientale were used as synonyms, but now the name S. indicum is preferred to the other name, as this indicated the country to which the species belonged (Bruce, 1953 a). The chromosome number of the species is 2n = 26 (Moringa et al, 1929). The scope of polyploidy breeding in this crop has been studied by Richharia and Persai (1940), Kobayashi and Shimamura (1948) and Shrivastava (1956).

To throw more light on the scope of induced polyploidy in this crop Nair (1965) has taken up the study of induction of polyploidy in Sesamum by colchicine technique, with a view to evaluate the economic aspects on its practical utilization, and to study the morphological and cytological abnormalities associated with colchicine technique.

He has isolated some suspected tetraploids from the C_1 generation, and the present investigation is the continuation of his work using the seeds from the suspected polyploids.

The objects of the present study are:-

1. To study the C_2 generation of the suspected polyploids in comparison with the diploids, and to study the extent of polyploids in C_2 generation.

2. To cross tetraploids and diploids reciprocally.

3. To study the C_3 generation of the tetraploid, along with plants from the crossed seeds and diploids, to understand the extent of triploids obtained from the crossed seeds, and to have a comparative idea of the morphological and cytological behaviour of tetraploids, triploids and diploids.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Polyploidy was first discovered by Lutz and Gates (1909) in the giant plants of Oenothera Lamarckiana.

Later, Muntzing (1936) and Stebbins (1950) have made an estimate of polyploidy in angiosperms, and found that more than 50% of the angiosperms are polyploids. This showed that it is a feature of wide occurrence in nature.

Stebbins (1938) has estimated that the highest percentage of polyploids are found in perennial herbs, smaller frequency in annuals and lowest frequency in woody plants.

Some of the polyploid crop plants are rice, wheat, maize, cotton, potato, groundnut, sweet potato, banana, sugarcane, coffee, brassicas etc.

Induction of Polyploidy in plants.

Winkler (1916) tried the decapitation and callus method for inducing polyploidy in Solanum nigrum.

Later, Lindstrom and Koos (1931) tried this method successfully in haploid tomatoes.

Greenleaf (1938) treated the cut surface of Nicotiana with 1% Indol Acetic acid in anhydrous lanoline to

promote the formation of callus tissue and adventitious buds. 13.7% of the adventitious buds formed from the callus tissue were polyploids.

Randolph (1932) has succeeded in inducing polyploidy in Maize, by treating the ear at high temperature at the time of zygote formation.

Muntzing (1938) was able to isolate polyploids from the twin seedlings of Rye.

Colchicine technique for induction of polyploidy.

Colchicine is a poisonous alkaloid, generally extracted from the seeds and corms of autum crocus (Colchicum antumnale, L.) belonging to the family Liliaceae.

Colchicine in its present role as a valuable tool in biological research was first introduced by Dustin (1934) in Belgium. He recognised it as a mitotic poison.

Nebel and Ruttle (1937) clearly demonstrated that Colchicine acted upon mitosis. For the first time they brought to light its importance as a tool in inducing polyploidy in plants. Further the efficiency of colchicine was made clear by Blakeslee and Avery (1937) in their experiments on Datura stramonium and other plant species.

The horizon of colchicine research widened quickly when many other botanists learned how effectively the drug could be used. Its high solubility in water and its non-toxic nature to plants at concentration that are effective in inducing polyploidy, are the major advantages attributed to colchicine, in its successful application in botanical research.

Many of the valuable crop plants such as wheat, oats, cotton, tobacco, potato, banana, coffee, sugarcane are found to be natural polyploids and these polyploids possessed superior economic qualities over their diploids. This, together with the more recent invention of the well reputed colchicine technique has been responsible for the beginning of a new trend in agricultural research. The colchicine technique subsequently has found unbounded applicability in crop improvement.

Colchicine induced polyploids.

Neblé et al and Ruttle (1938) treated cuttings of Tradescantia reflexa with 0.2% colchicine for 4 hours and induced tetraploidy.

Pal et al (1938) induced polyploidy in chillies by treating roots of seedlings with 0.05% - 0.4% colchicine for 4 - 6 hours at time of transplanting.

Muntzing and Runquist (1939) treated seeds of Pinus ponderosa with 0.02% aqueous solution of colchicine

for 5 days and obtained polyploid varieties.

Levan (1939) has induced tetraploidy and octoploidy by colchicine in diploid Petunia, by treating seedlings with 1% colchicine.

Ramuson and Levan (1939) have proudced tetraploid sugar beet by colchicine treatment.

Sando W.J. (1939) has induced tetraploid in Buck wheat (Fagopyron tataricum) by colchicine.

Shimamura (1939) induced tetraploidy in tomato by treating seedling with 0.2% colchicine in lanolin paste 2 or 3 times a week. He has also induced chromosome doubling by immersing roots of Alium cepa 1.0 - 1.5 cm. in length in 0.4% aqueous colchicine for 2 hours.

Riebharia and Persai (1940) could get tetraploid sesame by seed treatment with 0.06% colchicine for 2 hours, but the percentage was very low.

Beasly (1940) produced polyploids from 11 type of Gossypium by colchicine treatment of seedlings.

Straub (1940) found application of 0.025% colchicine in cotton wads for 2 successive mornings to the growing tips of shoots in young seedlings, an effective method to induce tetraploidy in Pisum sativum.

Emsweller et al (1940) could get tetraploid bulblets of Lilium formosanum by immersing the growing points in solution of colchicine of 0.2% to 1.0% concentrations for 2 hours.

Langham (1940) treated the axillary buds of sesame with 0.5% colchicine, and with 0.4% colchicine emulsion. In both treatments severe burning and dying back of the leaves occurred, followed by the formation of callus like tissues and new buds, which developed into tetraploid branches.

Ramanujam and Joshi (1941) produced tetraploid gram (Cicer arietinum) by treating seeds with aqueous solution of colchicine. 0.25% solution for ½ hour was found to be most successful.

Pal, Ramanujam and Joshi (1941) have succeeded in getting tetraploid chillies by treating seeds in 0.05%, 0.1%, 0.2% and 0.4% aqueous colchicine for 1-8 days.

Kumar and Abraham (1942) and Kumar (1945) found 0.4% colchicine agar applied to the apical bud of seedlings was most effective for inducing tetraploidy in Phaseolus radiatus.

Shifriss (1942) recommended an efficient method for mass production of polyploids in Cucumis sativus by treating the short apex at the cotyledonary stage of growth with 0.3% to 0.5% colchicine emulsion.

Rao et al (1944) produced tetraploid strains of Corchorus olitorius by treating dry seeds with colchicine solutions of 0.05% - 1% concentrations for 12 - 24 hours.

Beachel and Jones (1945) produced tetraploid rice by temperature and colchicine treatments.

Hofmeyr (1945) could induce tetraploidy in Carica papya by treating seedlings with 0.1% colchicine applied in drops 6 times a day for 4 or 5 days.

Kobayashi and Shimamura (1945) reported that drop method using 0.2% - 0.5% colchicine to wet the growing stem tips to be the most effective method in inducing polyploidy in sesame.

Ramanujam and Deshmukh (1945) induced autotetraploidy in self sterile species of Brassica campestris var. toria, Brassica nigra and in self sterile species of Brassica campestris var. sarson, Brassica tournefortii and Brassica juncea by treating dry seeds in aqueous colchicine of 0.1% to 0.4% strength for 24 - 48 hours; and germinated seeds in 0.025% to 0.1% for 8 - 24 hours.

Toole and Bamford (1945) successfully doubled eight haploid peppers to their diploid forms; by smearing 0.1% to 1.0% colchicine emulsion on the growing tips, once or twice a week.

Warmke (1945) could induce polyploidy in Taraxacum kok-saghyz by immersing seeds in 0.05% - 0.8% colchicine for 1, 2 and 4 days in covered dishes at room temperature.

Tandon and Chinoy (1949) have succeeded in inducing polyploidy in Amaranthus blitum, by treating the growing points with 0.1% solution of colchicine for 13 hours.

Derman (1954) induced tetraploidy in grapes by applying 0.5% colchicine in 10% glycerine in water, on buds once in two days for 3 times.

Hertzsch (1954) obtained tetraploid forms of Trifolium hybridum by immersing shoots in 0.05% colchicine for 24 hours.

Mazzani (1954) produced autotetraploidy in Sesamum by colchicine treatment.

Zulunga and Garginlo (1954) obtained a tetraploid variety of Vitis vinifera by treating newly formed buds with injections of 0.1% to 0.3% colchicine in 5% pure glycerine.

Bragdo (1955) has reported that immersion of young seedlings, when the coleoptiles are about 5 mm. long, with 0.2% colchicine for 20 minutes has given good results in winter rye.

Srivastava (1955) studied varietal differences among polyploids obtained from different strains of Cicer arietinum. He found that seed treatment with 0.25% colchicine for 30 minutes was superior to seedling treatment in inducing tetraploids.

Choudhury et al (1956) produced tetraploids of Corchorus capsularis and Corchorus olitorius by treating the seedlings with 0.1% to 0.2% colchicine for 24 hours.

Dereskevicus (1956) obtained polyploid tomatoes by applying 0.01% to 1.0% colchicine at the growing point of the seedlings.

Kundu and Sarma (1956, 1957) observed that 0.0125% to 0.10% solution of colchicine is effective in inducing tetraploidy in Corchorus olitorius, when treated for 6 - 24 hours.

Srivastava (1956) produced fertile autotetraploids in Sesamum and studied their breeding behaviour. The seeds were soaked in water for 12 hours and treated with 0.04%, 0.06%, 0.08% and 1.0% aqueous solutions of colchicine for various durations. Only a few treated seeds attained maturity, of which 3 were identified as tetraploids. 25 seeds treated with 0.04% and 0.06% for 2 and 6 hours period produced 1 triploid each, and 25 seeds treated with 0.10% for 4 hours produced one tetraploid.

Bali and Tandon (1957) found that in Linaria vulgaris the most successful treatment for the induction of polyploidy to be colchicine of 0.10% to 0.20% concentrations applied to the growing points of seedlings for 6 hours.

Knight (1957) reported the optimum concentration-time combination in inducing tetraploidy in Theobrama cocoa to be 0.6% colchicine in agar applied to apical buds for 24 hours.

Kumar et al (1957) studied the cause of sterility in the interspecific hybrids of the genus Arachis. The triploid hybrid obtained in cross between tetraploid and diploid Arachis hypogaea was found to be sterile, and with a view to overcome the sterility, a fertile allohexaploid plant was produced by doubling the chromosome number by applying 0.2% aqueous colchicine to the buds of the sterile hybrids.

Storbakov (1957) succeeded in getting 100% polyploidy in many ornamental plants by colchicine treatment of concentrations 0.1% - 0.2% for 4 - 6 hours.

Yokayama and Matzui (1957) noted that in Tea 0.3% colchicine is effective in inducing polyploidy when the seeds were treated for 180 - 240 hours.

Kloon (1957) induced polyploidy in 4 varieties of sugar beet and in 3 varieties of mangel by treating germinated seeds with 0.1% - 0.2% colchicine solution in water at

28°C for different durations.

Hull and Britton (1958) obtained a number of polyploid black berries and raspberries by treating germinating seeds with 0.2% colchicine at 86°C for 8 hours.

Sikka et al (1958) studied the effectiveness of different concentrations of colchicine and durations of different treatments, in inducing polyploidy in Trifolium alexandrinum and Melilotus indica. Most effective treatments in these two crops comprised the immersion of shoots of one week old seedlings in 0.10% colchicine for 8 hours, and the immersion of whole seedlings of similar age in 0.05% for 4 to 8 hours respectively.

Sen and Chedda (1958) observed that treatment of seedlings of Phaseolus mungo with 0.25% to 0.5% colchicine for 6 to 9 hours for one to two days could give about 50% polyploid sectors.

Saharov et al (1959) studied the concentration - duration effect for the induction of polyploidy in sugar beet. A higher concentration for shorter duration of treatment enhanced the production of polyploids considerably.

Thombre and Desai (1960) treated the germinating seeds of Agave cantala with 0.4% colchicine for 6 hours and produced tetraploid plants with 2n = 90.

Aleksic (1960) produced tetraploid chillies by treating seeds with 0.8% colchicine solution.

Chopra and Swaminathan (1960) induced polyploidy in water melon by treating the terminal buds in the cotyledonary stage with an emulsion of 0.2% colchicine, stearic acid, morpholine, and lanoline.

Kumar (1960) succeeded in producing tetraploids of Delphinum ajacus with 0.1% colchicine solution for 12 hours.

Sen and Bhowal (1960) induced tetraploidy in 6 varieties of Vigna sinensis. Treatment of seedlings was much more effective than that of seeds in inducing 4x sectors.

Sen and Vaidyabhushan (1960) obtained tetraploid Horse gram by treating the seedlings with 0.1%, 0.25% and 0.5% colchicine for 3 and 9 hours.

Moffet and Nixon (1961) opined that soaking the seeds of Black wattle, in 0.01% - 0.02% or 0.02% - 0.03% colchicine for 4, 8, and 12 hours. Treatment of 0.1% for 4 hours and 0.05% for 8 hours gave best results.

Bouharmont (1961) induced polyploidy in rice, by treating 20 days old seedlings with 0.1% colchicine solution.

Schank and Knowles (1961) applied 0.1% colchicine to the cotyledon of Carthamus tinctorius L., 4 times daily, consecutively for 3 days, which resulted in the production of highest number of polyploids.

Galcenko (1961) found that the optimum concentration for polyploid induction in cucumber consisted of a 0.05% solution applied either at 18°C for 48 - 72 hours or at 30° - 35°C for 24 - 27 hours.

Janaki Ammal (1962) could get tetraploid Rauvolfia serpentina by subjecting cuttings of the plant to colchicine treatment.

Raman and Kesavan (1963) reported an instance of doubling the chromosome number of germinating seedlings of Arachis duranensis by treating apical buds with 0.5% colchicine in water, for a period of 90 minutes each day, in the morning for 3 consecutive days.

Sobti (1963) obtained 4x strains of Menta piperata (2n = 144) by treating suckers of diploid plants with 0.1% colchicine for 24 hours.

Vig (1963) induced autotetraploids in Pb IV type of Cyamopsis psoralioides D.C. by treating young seedlings with 0.2% colchicine in lanolin paste.

Srivastava and Bajpai (1964) treated 25 healthy branches of loquat (Eriobotrya japonica, L.) with 0.12% to 1.0% colchicine for six to 24 hours and induced polyploidy to study the effect of colchicine on fruit set and pulp - seed ratio.

Sankaran (1964) produced autotetraploids in Sorghum vulgare and studied its hybrids with S. halepense.

Amritha Deva Ratnam (1964) studied the inter-specific hybrid of Sesamum indicum x S. laciniatum, and its amphidiploid by doubling the chromosome number of the hybrid by colchicine.

Visweshwara and Chinnappa (1965) succeeded in inducing tetraploidy in Coffea canephora (2x = 22) by treating growing shoot tips of 4 month old seedlings, with 0.3% colchicine at room temperature for 12 hours.

Mehta and Swaminathan (1965) were able to release the Pusa Giant Berseem which is the first colchicine induced tetraploid released for cultivation in India.

Nair (1965) has produced tetraploids of Sesamum by treating seeds and seedlings at different concentrations and durations.

Mehta et al (1966) was able to induce polyploidy in Methra (Trifolium foenum-graecum, L.) (2n = 16 - diploid)

by applying 0.2% of colchicine to apical buds of seedlings for 6 and 9 hours for 2 consecutive days which gave highest percentage of tetraploids.

Behaviour of autotetraploids and effect of colchicine.

Smith (1939) while inducing polyploidy in Nicotiana species and species hybrids by colchicine, found that the autotetraploids of Nicotiana rustica, N. tabacum and N. glauca were characterised by smaller plant habit, and the presence of smaller and thicker leaves.

Warmke and Blakeslee (1939) observed that the tetraploid Nicotiana showed larger stigma, thicker and longer anthers, thicker corolla and flower stalks.

Müntzing and Rungquist (1939) treated 16 species of plants with colchicine. But in only 3 species chromosome doubling was observed (0.05% or 0.025% solution - seed treatment for 3 - 6 days). They found chromosomal variation in the offspring of the autotetraploid and also among the primary autotetraploids. Besides the production of polyploids in some species, another effect of colchicine was found to be of a growth stimulating nature. This was noticed in Festula pratensis and Lolium perenne. The plants treated with weak colchicine solution showed vigorous characters of polyploids. But the chromosome counts, however, showed that there is no

doubling in their number. Such an action of colchicine on the growth hormone of plants was noticed by Havas (1938) also.

Ramuson and Levan (1939) induced tetraploidy in sugar beet and observed the following features:

Seed treatment and application of colchicine agar to seedling and first year plants had an immediate effect and showed polyploid characters, but the effect disappeared gradually and the growing points, exhibited an inclination to revert to diploidy, if partially changed into tetraploidy.

The treatment of shooting flower buds gave best results. Both tetraploid and diploid sectors were found in the same branch, or even in the same flower. During meiosis 0 - 4 quadrivalents were formed, but pollen fertility was not reduced. From the tetraploid branches 84 seedlings were grown. Out of this 3 plants were tetraploid, 13 triploid and 68 diploid. The tetraploids were as fertile as the diploids.

Even after removing all the diploid branches of the plant, the majority of seeds produced by the tetraploid branches were normal diploid.

Amin (1940) reported that the autopolyploids of cotton showed retardation of initial growth, and possessed

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broader leaf lobes, prominent leaf veins, broader bracts, bigger glands, bigger flowers, large pollen and bigger seeds.

Richharia and Persai (1940) reported that the tetraploid seedlings of Sesamum were recognisable by their smaller hypocotyl, thick cotyledons and short stunted roots. The plants were shorter, stiffer and with thicker stem and leaves were coarse, dark green, broad and thick, somatic chromosome number was found to be 52. 0-5 quadrivalents were noticed in autotetraploids.

Pal, Ramanujam and Joshi (1941) studied the colchicine induced polyploids of chillies (Capsicum annum, L.) Out of 244 plants grown from the treated seeds only 54 were found to be tetraploids. They possessed thicker stem, bigger and thicker leaves, larger stomata, bigger pollen grains with 30 - 90% pollen sterility.

Out of the 244 plants of C_1 generation, 54 were tetraploids, 4 were periclinal ploid chimeras, with 4n epidermis and 2n pollen; and 186 were diploids. In the C_2 generation some progenies of the tetraploids were found to consist of only tetraploids, but others of diploid and tetraploid, showing that the parent plants of the latter was really mixoploids. The periclinal ploid chimera gave rise to normal diploid progenies as expected.

In the C_3 generation the tetraploids again gave rise to tetraploids. The fruit formation and seed setting were appreciably more in tetraploids of the 3rd generation.

Tetraploids showed 0 - 7 quadrivalents during meiosis at diakinesis and metaphase I.

They have pointed out that colchicine treatment may produce numerical and structural changes in plants apart from polyploidy.

Langham (1942) has produced fertile tetraploids of sesame (Sesamum indicum, L.) by treating the axillary buds of the plants with 0.5% colchicine in lanoline and 0.4% in emulsion. Some of the branches possessed large mucilage glands and larger but fewer number of stomata. Chromosome counts of pollen mother cells from such branches showed that few of them had the tetraploid number of 2n = 52. While others had between 26 and 52. Some of the tetraploid branches were fertile. There was no difference in the number of pods or number of seeds per pods between diploid and tetraploid branches. The 1,000 seed weight of diploid branch was 3.41 gm. whereas that of tetraploid branch has 5.04 gm. By subsequent colchicine treatment hybridization and selection, haploids, diploids, triploids, tetraploids, hexaploids, octoploids etc. were obtained.

Ramanujam (1942, 1944) has obtained the amphidiploid (2n = 58) Sesamum indicatum by doubling the F_1 hybrid, of a cross between S. orientale (2n = 26) x S. prostratum (2n = 32); with 0.4% solution of colchicine when the plants were 6 - 8 leaves stage. In the A_2 generation 31 out of 35 plants showed 58 as their somatic number, which is equivalent to the somatic number of the 2 parental species. At meiosis they formed 29 bivalents. The anaphasic separation was normal with 29 + 29, although in some cases 30 + 28 seaparation was also noticed. In spite of regular meiosis 60 - 90% pollen sterility was noticed. Four plants of the A_2 generation resembled the S. prostatum parent with 2n = 45 and showed a typical Drosera type of pairing at meiosis, with 16 bivalents and 13 univalents. These plants had resulted from natural crossing between doubled hybrid and S. prostatum. He also records the production of 3 primary trisomics with 2n = 27 and an aneuploid with 2n = 28.

Badenhuizen (1941) treated Hibiscus cannabinus with colchicine and observed that the 4n plants were less branched and the difference in the rate of growth of 4n and 2n plants was less marked.

Baker (1943) obtained periclinal chimera in colchicine treated potato, with 8n epidermis and 4n chore.

Randolph et al (1944) reported that the autotetraploids of maize possessed thicker leaves, and showed delayed

flowering and prolonged vegetative growth.

Bhaduri et al (1948) stated that in Corchorus olitorius, periclinal chimera with 2n and 4n branches were found among the colchicine treated plants.

Parthasarathy and Kedarnath (1945) have produced a number of autotetraploids, showing gigas characters; but the tetraploids were far behind their diploids in fertility. The seed weight of the tetraploid was 50% more than the diploid. The tetraploids possessed a longer growth period; more number of branches and produced bigger sized flowers in large numbers. The pollen size was bigger. The capsules were lesser in number and shorter, broader, and thicker. Seven diploids were noticed in a tetraploid population comprising of 1029 plants.

Kobayashi and Shimamura (1949, 1952) studied the cytology of tetraploid Sesamum after 7 years of induction. The tetraploids were extremely robust, with thicker stem, larger leaves, bigger stomata, flowers and seeds. The pollen fertility was 74.10% and seed fertility 74.74%. They noticed frequent occurrences of 13 quadrivalents during meiosis, which disjoined normally.

Mazzani and Micheletti de Zerpa (1953) observed in the subsequent generation of colchicine induced tetraploids of Sesamum, that during meiosis 5 - 10 quadrivalents

were formed and the remaining were bivalents. Irregular separation of chromosome was observed during meiosis.

Kedarnath (1954) observed in the autotetraploids of Sesamum, that during meiosis in pollen mother cell variable number of quadrivalents, trivalents, bivalents, and univalents were formed. Maximum of 4 quadrivalents were observed.

Rao et al (1944, 1945) opined that the tetraploid jute showed the typical characters and were structurally different from the diploids. The fibre structure was changed according to the chromosome number.

Nishiyama (1950) compared the growth and yield of diploids and artificially induced tetraploids of raddish. Eventhough a retardation of growth was noted in the tetraploids, the yield of the tetraploid was consistently higher than that of the diploid varieties.

Tandon and Chinoy (1950) observed that the colchicine induced tetraploids Amaranthus blitum had thicker stem, more number of branches and larger leaves and prolonged vegetative growth.

Tandon (1951) showed that in colchicine treated Brassica oleraceae var. botrytis, the branching was profuse, as compared to the complete absence of branching in the diploid.

Derman (1954) induced tetraploidy in grapes by colchicine technique and succeeded in selecting bunch grapes with better sized berries.

Srivastava (1956) induced tetraploidy in sesame (Sesamum orientale, L.) and studied its breeding behaviour. The percentage of tetraploids produced by colchicine treatment was only 4%. The tetraploids of the C_1 generation possessed simple, wavy and very dark green leaves. The number of stomata was reduced with a corresponding increase in size. Flowering date was delayed for one week and the size of flower was larger. The diameter of pollen grain was increased. There was no significant difference in fertility with the diploids. Fruits were longer, broader and hairy.

The tetraploid plant possessed triploid and diploid branches.

In the C_2 generation, out of 181 seeds raised from the tetraploid plant 176 were autotetraploids and 5 were mixoploids. Tetraploid seeds from some treatment failed to germinate. The gigas characters of tetraploids were seen in C_2 generation also. He states that the high fertility of these autotetraploids is similar to those of Buck wheat.

In the meiosis of the tetraploids, varying numbers of quadrivalents and bivalents were formed at diakinesis

and metaphase-I.

Kundu and Sharma (1956) induced tetraploidy in Corchorus olitorium, L. and found that the vigour in growth of the 4n jute is only slightly below than that of the normal plants. They have also found that in tetraploids of jute the meiotic irregularities are few and the pollen fertility is sometimes higher than that of normal plants.

Sharma and Dutta (1957) found that the seeds from autotetraploids of Coriandrum sativum failed to germinate.

Sen and Cheda (1958) induced tetraploidy in 5 varieties of Black gram by colchicine technique. They found that the induced polyploids were either complete polyploids, branch polyploids, or sectorial polyploids with many or few random polyploid sectors. The polyploids possessed bigger flowers, pollen and stomata. The pods were shorter but the seeds were bigger and heavier. Pollen fertility was 75 - 80% C_1 and C_2 plants were less vigorous and the flowering was delayed. Not much meiotic abnormalities were observed. Quadrivalents were few. Some pollen mother cells gave 3, 5, 6, 7 or 8 spores instead of tetrads.

Fraudson (1959) observed that tetraploids of Red clover surpassed diploids in persistence, yield of dry matter and crude protein, but rather low in fertility.

Kovacs - schneider (1959) studied the vegetative development of tetraploid tomatoes and found that polyploid showed early development of new shoots but flowered and set fruit at the same time as the diploids. They possessed smaller fruits with fewer seeds.

Chopra and Swaminathan (1960) observed the seed fertility in autotetraploid water melon was considerably lowered.

Aleksic (1961) found in tetraploid Capsicum annum smaller and lighter fruits with thicker and higher dry matter content.

Janaki Ammal (1962) observed that the tetraploid Rauvolfia serpentina, B. showed an increase in alkaloid content, and larger size of flower and vegetative parts.

Vakili (1962) induced tetraploidy and octoploidy in Musa balbissiana and Musa balbissiana x Musa accuminata hybrids, by colchicine treatment. The tetraploid had thicker leaves and shorter petioles than the diploids. Many of the octoploids and tetraploids roverted to tetraploids and diploids respectively.

Raman and Kesavan (1963) induced autotetraploidy in Arachis duranensis and studied the chromosome associations

at diakinesis and metaphase-I. In 38 pollen mother cells examined, the frequency of quadrivalents ranged from 0 - 8 the most frequent association being 6 IV + 8 II. In a few cells trivalents were noticed (6 IV + 2 III + 5 II and 8 IV + 2 III + 1 II), but a corresponding number of univalents were not present. A single case of 20 II was observed. At anaphase-I, 20/20 separation was most frequent.

Gopalakrishnan and Shastry (1964) obtained tetraploid Oryza australiensis by colchicine treatment. The tetraploid sector was clearly distinguishable on the basis of their dark green foliage, stouter foliar veins, larger spikelets and elongated tip of palea.

Sankaran (1964) studied the morphological and cytological behaviour of autotetraploid Sorghum vulgare (2n = 40) and its hybrids with S. halepense. The tetraploid resembled its fertile counterpart and exhibited increased size of grain. Maximum of 10 IV were formed during meiosis, despite of this the tetraploid was stable and seed fertile.

Visweshwara and Chinnappa (1965) induced autotetraploidy in Coffea canephora (2n = 22). Gigas characters were noticed. Flowering was profuse but fruit set was low. Somatic chromosome number was found to be 2n = 44. At meiosis 22/22 separation of chromosome was observed at anaphase-I.

Pollen fertility was increased in tetraploid (96%) than in diploids (79.7%). Meiotic irregularities were few. Bivalents were more common.

Mehra et al (1966) induced polyploidy in Methra (Trifolium foenum-graecum, L.) 2x = 16, 4x = 32. Chromosome doubling, large stomata, pollen and seed, increased seed sterility, larger floral parts, and smaller pods were seen in tetraploids. The flowering was delayed by 3 - 4 weeks in tetraploids. In the C_2 generation the strains showed difference in their response to polyploidy. Increase in forage yield was noticed. Seed set was low. Meiosis showed a mean frequency of 3.84 IV + 1.63 III + 5.60 II + 1.63 I maximum of 6 quadrivalents were observed. Increase in essential oil content was noticed.

Crossing autotetraploids and diploids and study of the behaviour of the autotriploids.

The problem of fertility in induced autopolyploids has been proposed as a serious objection. The problem of sterility is more in case of triploids. But in many cases triploidy has been beneficially utilised in crop plants.

Triploidy was first reported by Gates (1908) in Oenothera. Characteristically, many triploids are quite seed sterile as a result of both unbalanced gametes and un-

balanced physiological and genotypical make up. There are numerous exceptions, such as fertile unbalanced polyploids, and fertile triploid aster hybrids discussed by Avers (1954), triploid Hyacinthus orientalis (Darlington, 1939), triploid Leucopogon juriperium (Smith and White, 1948), and triploid potatoes cited by Swaminathan and Howard (1953). The triploid selection of Tea (Thea sinensis) and Mulberry tree (Morus alba) are widely grown in Japan. There are natural triploid fruit crops like Banana, where seed sterility is beneficial.

Triploidy has been most beneficially utilized in the case of sugar beet and watermelon.

Mc Clintock (1929) explained the behaviour of an autotriploid maize (Zea mays) 2n = 30 originated in a diploid culture. The triploids were more vigorous than their diploid sibs. During meiosis 10 III at diakinesis and metaphase I, were formed. There was a tendency for members of the extra set of chromosome to be disassociated from their homologues at diakinesis and metaphase I, thus forming 9 III + 1 II + 1 I or 8 III + 2 II + 2 I.

Steere (1932) reported short chains of 3 chromosomes during meiosis as most common in autotriploid Petunia.

Muntzing (1933) reports 11 bivalents at Metaphase I in triple chain type or Y shape with triple terminal chiasmata in autotriploid Solanum tuberosum.

Upcott (1935) studied chromosome association of autotriploids in Lycopersicum and has recorded 50, 39.1 and 10.7 per cent of chain, 'Y' and frying pan shaped trivalents.

Ramanujam (1937), Moringa and Fukushima (1935) have opined that the autotriploids in rice (Oryza sativa) were with broader leaves, stouter tillers, larger floral parts and more vigorous than diploids. Cells with 10 III were most frequent. Pairing between non-homologous chromosome was also noticed. The fertility of triploid ranged from 0 - 24%.

Kihara (1951) also in Japan has succeeded in producing commercial triploid varieties of water melon. The triploid fruits were bigger, seedless and resistant to wilt disease. The 4x x 2x cross resulted in 3x seeds which were slightly thinner than the 4x seeds.

Matsumura (1953) made a comparative study of diploid, triploid and tetraploid sugar beet, in Japan; and revealed that the 3x plants were superior to both 2n and 4x plants. The weight of tuber and percentage of sugar is more in triploids. The tetraploid was used as the female parent in crossing.

Srivastava (1956) made crosses between diploids and autotetraploids of sesame (S. orientale, L.) and observed that the cross 2x (♀) x 4x (♂) yielded only empty capsules or capsules with shrivelled seeds. Capsule development was only 24%. In the reciprocal cross, where the original diploid from which the autotetraploid was produced was used as male parent, capsule development was 60% and the seeds were normal looking. When the male parent was other than the original diploid parent, out of the 50 crosses made only few, small, empty seeds were obtained.

Fraudson (1959) reported that the triploids of Red Clover were superior to diploids in dry matter and yield.

Toyao (1960) reported that the tetraploids of Tea plants were self sterile but gave rise to triploids when pollinated with pollen from 2x plant. The 2x (♀) x 4x (♂) was ineffective.

Karibasappa (1961) studied an autotriploid of rice and its progeny. The mean frequency of chromosome association during meiosis was found to be 9.4 III + 2.5 II + 2.5 I.

Krishnamoorthy (1963) studied the morphology and fertility of allotriploids in cotton and their breeding behaviour in a cross between Gossypium hirsutum (2n = 52) and G. raimondi (2n = 26). The triploid resembled its tetraploid

parent. For most of the quantitative characters the triploid was intermediate in expression. During meiosis the triploids show 13 II and 13 I at diakinesis and metaphase I in 88% of the pollen mother cells. The occurrence of more than 13 II and the higher associations like trivalents and quadrivalents were not seen. The formation of normal tetrads was as low as 24.7%.

Ramanujam, Omura and Koga (1964) reported that the allotriploids in rice were obtained only with much difficulty by crossing a diploid with a tetraploid.

Shastry and Misra (1964) studied the cytology of a triploid Oryza hybrid from a cross between tetraploid Oryza sativa (4x = 48 ♀) x O. barthi (2x = 24 ♂). In the 115 pollen mother cells studied at Metaphase I, hexa, penta, quadri, tri, bi and univalents were observed, trivalents being the most predominent of the association. A maximum of 12 trivalents were observed in a single pollen mother cell. Among 115 pollen mother cells studied trivalents were seen in all, quadrivalents in 48 cells, pentavalents in 11 cells, and hexavalents in 13 cells. The shape of multivalents varied widely ie. rings, chains, Y shaped, H shaped, Dumb-bell shaped, frying pan shaped etc.

These types of configurations can be expected when 3 chromosomes are either completely or cryptic structurally homologous.

Hybridization (interspecific) in Sesamum.

Between species with n = 13 x n = 32.

Garu (1934) made crosses, but failed.

Dadlani (1958) explained the reason of failure as due to early collapse of hybrid endosperm and so shrivelled and non-viable seeds only were obtained.

Mazzani (1957) made successful crosses by embryo culture. The F_1 was weak and resembled S. radiatum with 2n = 64. Meiosis was regular. This is presumed to be developed by diploid parthenogensis in the maternal plant.

Between species with n = 32 x n = 16.

S. occidentale (n = 32) x S. laciniatum (n = 16)

Ramanathan (1950) made reciprocal crosses, but the seeds were shrivelled and non-viable. Fruit set was found to be nil in reciprocal crosses.

S. radiatum (n = 32) S. angolense (n = 16)

Nakamura and Sato (1958) made crosses and obtained 2 hybrids. They were intermediate and showed hybrid vigour. At meiosis one hybrid (2n = 48) showed 17 II and 14 I. Other hybrid (2n = 64) showed 12 III + 4 II + 20 I. This hybrid might have obtained by fertilization of normal egg by an unreduced gamete of S. angolense.

MATERIALS AND METHODS

MATERIALS AND METHODS

A. MATERIALS

Seeds:

Seeds of Sesamum indicum, L. of strain TMV-2, obtained from the suspected polyploids of colchicine treatment conducted by Nair (1965) were used for the present investigation. He had treated the seeds and seedlings with colchicine at different concentrations and at varying duration of time. From the C_1 generation he had collected suspected polyploids from 14 different treatments. They are -

No.	Original treatment No.	Colchicine treatment	
1	3	0.05% for 6 hours	Seed treatment
2	5	0.05% for 9 hours	
3	7	0.10% for 3 hours	
4	9	0.10% for 6 hours	
5	15	0.15% for 6 hours	
6	17	0.15% for 9 hours	
7	21	0.20% for 6 hours	
8	23	0.20% for 9 hours	
9	6	0.05% for 9 hours	Seedling treatment
10	16	0.15% for 6 hours	
11	18	0.15% for 9 hours	
12	22	0.20% for 6 hours	
13	24	0.20% for 9 hours	
14	20	0.20% for 3 hours	

These 14 types of seeds together with the control (diploid) formed the material for the study of the C_2 generation.

The tetraploids obtained from the C_2 generation were crossed with the diploid reciprocally. The 4x (♀) x 2x (♂) cross, only was successful and from this cross 47 seeds were obtained. The reciprocal cross of 2x (♀) x 4x (♂) produced only shrivelled and nonviable seeds.

The selfed seeds from the tetraploid plant together with the crossed seeds and the selfed seeds from its parent diploid plant formed the material for the study of C_3 generation and the crossed progeny.

B. METHODS

In order to study statistically the relative merits of the polyploids obtained from the different treatments in comparison with the diploid, a randomised block design with 15 treatments and 4 replications were laid out for the study of the C_2 generation.

For the study of the C_3 generation and the triploids, a randomised block design with 6 treatments and 8 replications were laid out. In the first 4 treatments seeds from tetraploid parent, in treatment 5 seeds from diploid parent, and in treatment 6 the crossed seeds from tetraploid x diploid were used.

The following characters were studied:

1. Germination percentage.

Germination trials were conducted separately. The different types of seeds from the suspected polyploids and also of diploids were kept in moist petridishes, 100 seeds in each from each treatment. The germination percentage was calculated after allowing the seeds to germinate for ten days. Counting of the germinated seeds was done every day.

Because of the shortage of the crossed seeds, they were sown directly in pots - 5 seeds each in eight pots - and the rate of germination and percentage of germination were recorded.

Date of commencement of germination in the case of different treatments was also recorded.

2. Height of plants.

The height of individual plants were measured from the third week of sowing to the day of harvest at weekly intervals. Thus the rate of growth and the total height of individual plants at the time of harvest were recorded.

3. Leaf characters.

(a) Mean area of leaves: Measurement of area of

leaves was made in sq. cms. using graph paper technique (Darrow's method). For this ten leaves were collected from each treatment, from the sixth node of the plants, when they were 40 days old.

(b) Mean thickness of leaves: The thickness of leaves was measured out from the sample collected from the middle of the plants, when they were 45 days old. It was recorded in micron (μ) as measured from 100 hand sections for each treatment.

4. Size and distribution of stomata.

A sample of 10 leaves was collected at random from each treatment. The tissue from the lower surface was pealed off, and stained with 0.5% safranin. The length and width of 100 randomly selected stomata were measured for each treatment, using a standarised ocular micrometer. The frequency of stomata per unit area was determined, by counting their number from 100 fields at random for each treatment. The data for size and distribution of stomata were analysed statistically.

5. Number of flowers.

Flower counts were taken daily from each plant under different treatment, and the final data were statistically analysed. Date of flowering of individual plant was

also recorded.

6. Size and sterility of pollen grains.

The size and sterility of pollen grains were studied from entirely random samples for each treatment. Ten plants were selected at random from each treatment. The pollen grains from one flower, from each plant, were stained with Glycerine-acetocarmine. Diameter of 100 pollen grains was measured in micron (μ) for each treatment, using a standarised ocular micrometer.

For the estimation of sterility, ten fields were scored for sterile and fertile grains from each slide at random.

7. Cytological observations.

Suspected polyploids were separated on the basis of their gigas character peculiar to polyploids, as well as other characters like leaf thickness, size and distribution of stomata, pollen size and sterility, late flowering etc. The flower buds of appropriate size from these plants as well as from other plants were fixed separately in a 3:4:1 mixture of abolute alcohol, chloroform, and propionic acid, which has been previously saturated with ferric chloride. The fixation was done between 11 a.m. and 12 noon for 24 hours. The maximum division was noticed between 11.15 a.m.

and 11.45 a.m. After keeping in the fixative for 24 hours the buds were stored in 70% ethyl alcohol. Anthers were squashed in a drop of 1% propiono-carmine. Gentle tapping and judicious warming favoured excellent spreading and differential staining of the chromosome and cytoplasm in the microsporocytes.

The chromosome number and their behaviour during meiosis were photographed and also camera lucida drawings were made from meiosis of pollen mother cells.

8. Crossing tetraploid with diploid.

Only one plant was found to be a tetraploid cytologically also and this was crossed with diploid reciprocally.

(a) Technique of crossing. As the flowers are bisexual and naturally self pollinated, emasculation was done so that the flower becomes functionally female. The time of anthesis is between 6 a.m. and 7 a.m. So emasculation was done in the previous evening. Emasculation in the case of sesame is easy since the stamens are epipetalous. The mature flower buds which would open on the next morning, were selected and the corolla tube was carefully pulled out without damaging the pistil, the androecium coming out along with the corolla tube. The emasculated bud was then covered with a paper cover to prevent contamination. Similarly flower buds

from the pollen parent were bagged to prevent contamination. On the next day by about 7 a.m. newly opened flower from the male parent plant was taken and the anthers were rubbed on the pistil of the emasculated flower, after removing the paper cover. Enough pollen grains were liberated so that all the ovules would get fertilized. After pollination the paper cover was replaced, and a label showing details of male and female parents and date of pollination was tied to the node of the plant. The paper cover was retained for one week. The seeds were collected from the reciprocal crosses, separately, at the time of harvest.

9. Selfing tetraploid and diploid plants.

The selfed seeds of tetraploid and diploid parents of the cross, were collected.

The technique of selfing is easy as the plant is naturally self pollinated. Mature flower buds, which would open on the next day were selected, and they were covered with a paper cover on the previous evening of anthesis. A label bearing date of selfing was tied at the node of the plant. The cover was retained for one week. The selfed seeds of tetraploid and diploid were collected separately at the time of harvest.

10. Number and size of capsule.

The total number of capsules in each plant was counted at the time of harvest and the data were statistically analysed.

For determining the size of capsules a sample of 10 capsules was selected randomly from each treatment, the length and girth were measured and the data were compared.

11. Yield of seeds.

At the time of harvest, a sample of 10 capsules was collected at random from each treatment and seeds from these capsules were counted and data compared.

12. Weight of 1,000 seeds.

Weight of 1,000 seeds from each treatment was recorded in grams for each treatment and the data were statistically analysed.

Besides these characters studied for different types, the characters of confirmed polyploid plants were studied and compared with those of diploid.

13. Study of triploids.

The C_3 generation was studied with the suspected triploid plants from the seeds of tetraploid and diploid

cross, together with the selfed seeds of the parents. Besides studying the characters mentioned previously, the individual plants of the suspected triploids and selfed progeny of the tetraploid, were studied cytologically.

Statistical procedure.

The recorded data pertaining to the different characters under investigation were subjected to statistical analysis. Analysis of variance was worked out for seven characters namely height of plants, number of branches, number of flowers, numbers of stomata, pollen size, leaf thickness and number of pods. The comparison of other characters under different treatments was made by calculating the mean and standard error.

RESULTS

EXPERIMENTAL RESULTS

C_2 GENERATION

1. Percentage of germination.

The seeds of the 14 suspected polyploid types, obtained from the C_1 generation, showed a slightly reduced percentage of germination than the control type (diploid). However, the time taken by the different types to complete the germination, did not show any significant difference. The data pertaining to the results of the germination trial are given in Table I.

The maximum number of seeds germinated was during 3rd and 4th day in the case of the suspected polyploid types, whereas in the case of diploid, the maximum germination was noticed on 2nd and 3rd day.

2. Growth of plants.

The growth of plants under different types in the C_2 generation did not show much variation from the control. In general the suspected polyploid types exhibited a vigorous growth habit and a longer growth period. The types 3, 5 and 10 were slower in early growth rate, but at the later period the types 3, and 5 have surpassed the control in plant height.

TABLE - I

C_2 - Percentage of germination

Types	Percentage of seeds germinated for every 24 hours							Total percentage of germination
	1	2	3	4	5	6	7	
Control	10	20	22	9	4	-	-	65
1	2	12	15	12	1	1	-	43
2	1	12	15	12	2	-	-	42
3	1	13	15	12	1	-	-	47
4	2	13	17	14	-	1	-	47
5	1	14	15	11	1	2	-	44
6	3	14	17	24	1	-	-	59
7	1	15	25	15	1	-	-	57
8	1	13	16	22	1	-	-	53
9	-	14	14	12	1	1	-	42
10	2	13	16	23	1	-	-	55
11	2	14	16	24	1	-	-	57
12	1	22	15	22	1	-	-	61
13	-	22	13	21	1	-	-	57
14	2	12	26	13	1	-	-	54

The graphical representation of the data pertaining to the rate of growth is shown in Figures 1(a) and 1(b).

3. Size and shape of leaves.

The suspected polyploids were similar to the diploid in the case of leaf shape. Generally, the leaves upto the 8th node of the main axis were broad having serrated margin. Leaves towards the tip were entire and narrow. Three plants of type 14 and two plants of type 5, were having only entire leaves. Two plants from types 1 and 5 produced 2 trilobed leaves (Plate IX) while others produced only simple leaves. The suspected polyploid types showed an increase in leaf size than the diploid.

4. Height of plant.

The analysis of variance for height of plants under different types (Appendix I) did not show a significant F ratio. The suspected polyploid types did not differ significantly in plant height compared to diploid. The mean height of plant among the different types varied from 78.40 cm. to 97.40 cm. Type 1, 2 and 10 showed a reduction in height than the control. The data for the mean height of plants under different types are given in Table II. Graphical representation of the data is given in Figure 2.

TABLE - II

C_2 - Height of plant

Types	Mean height (cm.)
1	78.40
2	84.25
3	97.75
4	92.25
5	93.62
6	94.95
7	91.62
8	91.00
9	89.25
10	88.17
11	95.95
12	97.40
13	94.97
14	95.55
Control	88.68

TABLE - III

C_2 - Number of branches

Types	Mean number of branches
1	6.57
2	4.87
3	4.56
4	4.96
5	6.12
6	4.30
7	4.10
8	4.30
9	4.02
10	3.67
11	5.42
12	4.55
13	3.62
14	4.47
Control	4.37

TABLE - IV

C_2 - Area of leaves

Types	Mean leaf area (sq. cm.)
1	67.5 ± 5.18
2	67.9 ± 4.16
3	67.1 ± 3.56
4	71.2 ± 3.25
5	67.8 ± 5.75
6	67.9 ± 3.87
7	65.7 ± 3.87
8	71.5 ± 5.68
9	71.0 ± 4.25
10	68.5 ± 4.71
11	66.9 ± 4.41
12	68.8 ± 2.75
13	70.9 ± 4.87
14	66.0 ± 3.09
Control	68.1 ± 5.96

TABLE - V

C_2 - Leaf thickness

Types	Mean thickness of leaves (in μ)
1	234.15 ± 3.52
2	254.55 ± 3.91
3	253.50 ± 2.70
4	263.40 ± 3.70
5	279.00 ± 4.65
6	242.25 ± 3.25
7	246.00 ± 3.24
8	241.80 ± 3.13
9	245.40 ± 3.03
10	234.30 ± 2.35
11	238.80 ± 3.28
12	244.50 ± 3.16
13	235.20 ± 2.62
14	253.35 ± 3.06
Control	246.30 ± 2.73

C.D. (5%) for comparison of means = 0.4704

5. Number of branches.

Analysis of variance for the different types is given in Appendix VIII. The F ratio was found to be significant. The types 1 and 5 showed significant increase in the number of branches. The mean number of branches among the different types ranged between 3.62 and 6.57. Table III contains the mean number of branches in the different types. Graphical representation of the data is given in Figure 3.

6. Leaf characters.

(a) Area of leaves. Among the suspected polyploid types, only the types 4, 8, 9, 10, 12 and 13 have showed an increase in leaf area compared to the diploid. The variation in mean leaf area under different types was only between 71.5 and 65.7 sq. cm. The data pertaining to the mean area of leaves under different types are given in Table IV.

(b) Thickness of leaf. The analysis of variance for the data is given in Appendix II. The F ratio was significant. Types 2, 3, 4 and 5 showed significant increase in leaf thickness compared to the control, whereas types 1, 11 and 13 showed a significant reduction. The data regarding the mean thickness of leaves under different types are given in Table V. The thickness varied from 234.15 to 279.00 microns among the different types.

TABLE - VI

C_2 - Number of stomata

Types	Mean number of stomata per unit area
1	23.49 ± 0.086
2	23.18 ± 0.099
3	24.25 ± 0.121
4	23.21 ± 0.178
5	22.19 ± 0.148
6	22.81 ± 0.117
7	23.37 ± 0.250
8	22.56 ± 0.126
9	22.70 ± 0.107
10	23.27 ± 0.117
11	23.41 ± 0.100
12	23.18 ± 0.095
13	22.90 ± 0.091
14	21.42 ± 0.127
Control	23.84 ± 0.086

C.D. (5%) for comparison of means = 0.3324

TABLE - VII

C_2 - Size of stomata

Types	Mean width of stomata (in μ)	Mean length of stomata (in μ)
1	15.04 ± 0.23	23.32 ± 0.16
2	17.52 ± 0.14	25.94 ± 0.26
3	14.49 ± 0.15	28.25 ± 0.28
4	15.21 ± 0.22	26.42 ± 0.31
5	17.83 ± 0.18	26.25 ± 0.22
6	19.32 ± 0.21	26.22 ± 0.22
7	15.97 ± 0.25	25.90 ± 0.20
8	17.56 ± 0.16	25.87 ± 0.24
9	17.18 ± 0.21	27.60 ± 0.12
10	15.93 ± 0.13	25.08 ± 0.03
11	15.28 ± 0.13	24.25 ± 0.24
12	17.31 ± 0.09	26.32 ± 0.24
13	15.69 ± 0.22	26.42 ± 0.25
14	18.21 ± 0.14	26.42 ± 0.23
Control	15.07 ± 0.28	24.21 ± 0.16

7. Size and distribution of stomata.

(a) Number of stomata per unit area. The F ratio for the data under different types was found to be significant. The analysis of variance is given in Appendix III. Among the suspected polyploids, only type 3 showed a significant increase in number of stomata, compared to the diploid. The mean number of stomata of the different types (Table VI) varied from 21.42 to 24.25 per unit area.

(b) Size of stomata.

(i) Width of stomata. The suspected polyploid types showed, in general, an increase in width of stomata compared to the diploid, except types 1 and 3 which had stomata of smaller width than control. The width of the stomata among the different types varied from 14.49 to 19.32 microns.

(ii) Length of stomata. All the suspected polyploid types except type 1 showed an increase in length of stomata. The range in length among different types was between 23.42 and 28.25 microns.

The data pertaining to the mean width and length of stomata for the different types are given in Table VII.

8. Number and size of flowers.

The analysis of variance for the data on production of flowers by different types did not show any signifi-

TABLE - VIII

C_2 - Number of flowers

Types	Mean number of flowers
1	55.92
2	58.05
3	78.57
4	69.42
5	89.37
6	58.05
7	59.55
8	65.05
9	56.37
10	58.10
11	69.75
12	69.27
13	67.85
14	65.35
Control	59.95

cant variation (Appendix IV). The flowers produced by the suspected polyploid types were slightly larger than those produced by diploid (Plates XI - XIII). There was no marked difference in blooming date among the different types. The suspected tetraploid plant of type 5 showed significant delay of 10 days for commencing flowering than the diploid. The mean number of flowers produced by different types (Table VIII) varied from 55.92 to 89.37. Graphical representation of the data is given in Figure 4.

9. Cytological observation.

The behaviour of chromosomes at meiosis was studied in all the suspected polyploid plants of the 14 types as well as the control. Except one plant of type 5, all plants were similar to the control (diploid). The formation of 13 regular bivalents at diakinesis and metaphase-I was noticed in the pollen mother cells (Plates XIV b, c). The seperation of chromosomes at anaphase-I and II was also regular ie. 13 + 13. In the exceptional plant of type 5, few pollen mother cells at anaphase-I stage showed 26 chromosomes each at two poles (Plate XIV a) instead of 13 each in diploid. This indicated the tetraploid nature of the plant. In the same plant pollen mother cells with the diploid number of 2n = 26 was also noticed, which formed 13 bivalents at metaphase-I and 13 chromosomes each at the poles during

TABLE - IX

C_2 - Pollen size

Type	Mean diameter of pollen (in μ)
1	70.72 ± 0.26
2	68.96 ± 0.36
3	68.24 ± 0.30
4	70.72 ± 0.27
5	71.07 ± 0.34
6	67.93 ± 0.28
7	70.13 ± 0.36
8	68.58 ± 0.31
9	68.51 ± 0.31
10	68.68 ± 0.23
11	68.55 ± 0.36
12	70.75 ± 0.32
13	68.41 ± 0.33
14	70.03 ± 0.36
Control	68.20 ± 0.28

C.D. (5%) for comparison of means = 0.254

anaphase-I. This has created doubt regarding the true tetraploid nature of the plant. However this plant was used as tetraploid parent in crossing with the diploid.

Chromosome association at diakinesis and metaphase-I was studied in a large number of pollen mother cells. The maximum association noticed was bivalents. No univalents or any higher associations were noticed.

Persistence of one or more secondary nucleoli in the pollen mother cells was a peculiarity during all the stages of meiotic divisions. In the microspores also one or more nucleoli were observed.

10. Size and sterility of pollen.

(a) Size of pollen.

The analysis of variance for the data under different types is given in Appendix V. The F ratio was found to be significant. Types 1, 4, 5, 7, 12 and 14 showed a significant increase in pollen size compared to the control. The mean diameter of pollen grain among different types varied from 67.93 to 71.07 microns. Table IX represents the data for mean diameter of pollen under different treatments.

(b) Extent of pollen sterility.

The X^2 analysis of extent of pollen sterility

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TABLE - X

C_2 - Pollen sterility

Types	Pollen sterility %
1	5.59
2	6.85
3	5.03
4	3.52
5	3.92
6	4.07
7	9.37
8	6.67
9	6.19
10	4.97
11	7.64
12	6.66
13	12.34
14	7.31
Control	6.08

TABLE - XI

C_2 - Number of capsules

Types	Mean number of capsule
1	41.65
2	36.47
3	55.95
4	45.45
5	54.12
6	36.22
7	35.72
8	38.00
9	32.77
10	31.30
11	36.32
12	39.52
13	40.20
14	42.67
Control	38.80

(Appendix VI) did not show any significant different for the suspected polyploid types compared to control.

The percentage of sterility among the different types varied from 3.52 to 12.34, whereas the control showed a sterility of 6.08%. Table X shows the percentage of pollen sterility among the different types.

11. Number and size of capsules.

(a) Number of capsules.

The analysis of variance for the different types is given in Appendix VII. The F ratio was not significant. The mean number of capsules varied from 31.30 to 55.95 among the different types, whereas the control had a mean of 38.80. The data relating to mean number of capsule in different types are given in Table XI. Graphical representation of the data is given Figure 4.

(b) Size of capsules.

Length of capsule. The suspected polyploid types showed a slight increase in length of capsules compared to diploid. The length among the different types ranged from 2.43 to 2.70 cm. whereas the control had a length of 2.41 cm.

Girth of capsules. The data of mean girth of capsules did not show much variation. The mean girth of

TABLE - XII

C_2 - Capsule size

Types	Length of capsule (cm.)	Girth of capsule (cm.)
1	2.61 ± 0.010	3.93 ± 0.010
2	2.70 ± 0.011	4.03 ± 0.009
3	2.62 ± 0.013	3.88 ± 0.013
4	2.50 ± 0.008	3.62 ± 0.007
5	2.43 ± 0.013	3.90 ± 0.001
6	2.54 ± 0.011	3.65 ± 0.011
7	2.46 ± 0.011	3.65 ± 0.011
8	2.46 ± 0.010	3.91 ± 0.009
9	2.51 ± 0.012	3.68 ± 0.011
10	2.46 ± 0.009	3.79 ± 0.016
11	2.52 ± 0.015	3.78 ± 0.014
12	2.50 ± 0.012	3.78 ± 0.011
13	2.58 ± 0.007	3.70 ± 0.014
14	2.57 ± 0.009	3.79 ± 0.009
Control	2.41 ± 0.007	3.71 ± 0.014

TABLE - XIII

C_2 - Number of seeds

Types	Number of seeds per 10 capsules
1	852
2	847
3	861
4	870
5	856
6	857
7	853
8	850
9	842
10	865
11	869
12	859
13	850
14	848
Control	865

TABLE - XIV

C_2 - Weight of seeds

Types	Weight of 1,000 seeds (in gram)
1	2.37
2	2.45
3	2.42
4	2.40
5	2.50
6	2.35
7	2.32
8	2.36
9	2.29
10	2.46
11	2.38
12	2.45
13	2.38
14	2.45
Control	2.25

capsules ranged from 3.62 and 4.03 cm., among the different types, the control showing a mean girth of 3.71 cm. The Table XII represents the data of mean length and girth of capsules in different types.

12. Yield of seeds.

The number of seeds counted from a sample of 10 capsules collected at random from each type revealed that there is not much variation in the yield of seed among the different types and control. The data pertaining to the number of seeds per 10 capsules, for the different types are given in Table XIII.

13. Weight of 1,000 seeds.

The seeds of the suspected polyploid types showed a slight increase in the size compared to the control. Consequently there was a slight increase in seed weight also.

The results given in Table XIV confront the mean weight of 1,000 seeds in different types. The weight of 1,000 seeds ranged from 2.25 to 2.50 grams among the different types.

14. Cross between tetraploid and diploid.

The suspected polyploid plants of the different types were marked based on the appearance and they were

crossed reciprocally with diploid. On cytological examination only one plant was found to have the tetraploid chromosome number of 2n = 52. Even this plant was not a pure tetraploid. 4x and 2x chromosome numbers were found during meiosis of the pollen mother cells. However the seeds from the reciprocal crosses with this plant were collected for further studies. Out of the 5 crosses made using this suspected tetraploid plant as female parent only one capsule was developed and set seeds. The seeds were normal in appearance (Plate XV a). In the reciprocal cross using this suspected 4x plant as male and 2x plant as female, 2 capsules were developed out of 5 crosses made. One of these capsules was malformed and did not contain seeds. The other capsule was normal in appearance, but the seeds were shrivelled and nonviable (Plate XV b).

The viable seeds from these crosses were carried forward for the C_3 generation together with the selfed seeds from the suspected tetraploid parent and diploid parent.

C_3 GENERATION

The seeds obtained from the cross using the suspected tetraploid plant as female parent only were viable. The 4x x 2x crossed seeds together with the selfed seeds of the parents were raised and studied in the C_3 generation.

1. Percentage of germination.

The percentage of germination of the selfed and crossed seeds from the suspected tetraploid and diploid parents, showed considerable variation. The total percentage of seeds germinated in the 3 types is given in Table XV. The crossed seeds showed the maximum percentage of germination, and the selfed seeds of the suspected tetraploid showed least percentage of germination.

TABLE - XV

C_3 - Percentage of germination

Types	Total % of seeds germinated
1. Suspected Tetraploid (4x)	21.3
2. Control (Diploid - 2x)	43.4
3. 4x x 2x Cross	62.5

2. Growth of plants.

The progeny of the 4x plant showed a vigorous growth than the progeny of the 2x plant and 4x x 2x Cross. The rate of growth of the 3 types were almost similar upto the 8th week after sowing. After that the plants from the

4x parent continued growth at a faster rate than the other 2 types. The progeny from the crossed seeds showed an intermediate growth rate compared to the parents. The graphical representation of the rate of growth is shown in Figure 5.

3. Size and shape of leaves.

The shape of the leaves was similar in all the 3 types. The basal leaves upto the 8th node were broader and with serrated margin, compared to the leaves towards the tip which were narrow and entire. No lobed leaves were observed.

4. Height of plants.

The analysis of variance for the different types is given in Appendix IX. The F ratio was found to be significant. The progency of the suspected tetraploid plant showed significant increase in height compared to those of the diploid. The progeny of the 4x x 2x cross did not show any significant different in height compared to the parents. It was intermediate to the parents in plant height. The data of mean heights of plants under different types are given in Table XVI. Graphical representation of the data is given in Figure 6.

TABLE - XVI

C_3 - HEIGHT OF PLANTS

Types	Mean height (in cm.)
4x	103.68
2x	94.23
4x x 2x	98.18

C.D. (5%) for comparisons of 4x mean = 5.85

C.D. (5%) for comparisons of 2x and 4x x 2x means = 7.67

5. Number of branches.

The F ratio was found to be significant in the analysis of the variance of the data for the 3 types (Appendix XVI). The 3 types showed significant difference in the mean number of branches. The maximum number of branches was noticed in the progeny of the suspected tetraploid, and the minimum in diploid. The progeny of the 4x x 2x cross was intermediate between the parents. Table XVII contains the data for the mean number of branches in the different types. The graphical representation of the data is given in Figure 7.

TABLE - XVII

C_3 - Number of Branches

Types	Mean number of branches
4x	4.52
2x	2.77
4x x 2x	4.06

C.D. (5%) for comparison of 4x mean = 0.23

C.D. (5%) for comparison of 2x and 4x x 2x means = 0.36

6. Leaf characters.

(a) Area of leaves.

The difference in mean leaf area among the 3 types was only negligible. The plants from the suspected tetraploid parent had the maximum leaf area followed by the diploid and the 4x x 2x crossed progeny. Table XVIII gives the data for mean height of plants among the 3 types.

TABLE - XVIII

C_3 - Area of leaves

Types	Mean leaf area (sq. cm.)
4x	70.46 ± 4.59
2x	69.95 ± 4.68
4x x 2x	69.82 ± 1.97

(b) Thickness of leaves.

The analysis of variance for the different types (Appendix X) showed a significant F ratio. The 3 types showed significant difference in leaf thickness. The progeny of the suspected tetraploid plant showed maximum leaf thickness and those of the diploid the minimum. The progeny of the cross was intermediate to the parents in leaf thickness. Table XIX represents the data for the mean thickness of leaf of the 3 types.

TABLE - XIX

C_3 - Leaf thickness

Types	Mean thickness (in μ)
4x	274.20 ± 3.45
2n	250.35 ± 2.73
4x x 2x	253.50 ± 3.07

C.D. (5%) for comparison of 4x mean = 0.49

C.D. (5%) for comparison of 2x and 4x x 2x means = 0.62

7. Size and distribution of stomata.

(a) Number of stomata per unit area.

The analysis of variance for the 3 types is given in Appendix XI. The F ratio was found to be significant. The 3 types showed significant difference in the mean number of stomata per unit area. The progeny of the suspected tetraploid parent showed the minimum number. The diploid progeny had the maximum number and the progeny of the cross was intermediate to the parents. Table XX gives the data for mean number of stomata.

TABLE - XX

C_3 - Number of stomata

Types	Mean number of stomata
4x	23.30 ± 0.101
2x	24.00 ± 0.126
4x x 2x	23.48 ± 0.159

C.D. (5%) for comparison of 4x mean = 0.294

C.D. (5%) for comparison of 2x and 4x x 2x mean = 0.352

(b) Size of stomata.

In general the progeny of the suspected tetraploid parent possessed larger sized stomata. The progeny of the diploid parent had stomata of smaller size and that of the 4x x 2x cross had stomata of intermediate size compared to the parents. The results given in Table XXI confronts to the mean width and length of stomata.

TABLE - XXI

C_3 - Size of stomata

Types	Mean width (in μ)	Mean length (in μ)
4x	17.59 ± 0.17	27.73 ± 0.24
2x	16.76 ± 0.26	23.14 ± 0.25
4x x 2x	16.90 ± 0.24	24.08 ± 0.21

8. Number and size of flowers.

The F ratio of the analysis of variance of the data on flower production did not show any significance (Appendix XII). The progeny of the suspected tetraploid parent showed maximum flower production while those of the diploids showed the minimum (Figure 8). Table XXII gives the data on mean number of flowers produced by the 3 types.

The flowers produced by the progeny of the suspected tetraploid parent were slightly larger in size compared to that produced by the progeny of diploid and 4x x 2x cross, which were almsot similar in size (Plate XVII). The date of blooming also did not vary markedly among the

3 types. In general, the progeny of the diploid started flowering slightly earlier than those of the suspected tetraploid and 4x x 2x cross.

TABLE - XXII

C_3 - Number of flowers

Types	Mean number of flowers produced
4x	64.70
2x	60.86
4x x 2x	60.72

9. Cytological observations.

The selfed progeny of the suspected tetraploid and diploid parents and the progeny of the 4x x 2x cross, behaved cytologically in the same manner. All the 30 plants raised from the crossed seeds, were examined cytologically. Pollen mother cells showing regular meiosis were noticed in all the plants. During meiosis, formation of 13 bivalents at diakinesis and metaphase-I with normal disjunction at anaphase-I and II were observed. The chromosome number remained consistently as 2n = 26, proving the diploid nature of the plants. Similar chromosome number and behaviour were observed in the progeny of the suspected tetraploid parent and the

diploid parent. This confirmed the diploid nature of all the plants in the C_3 generation, and also proved that the suspected tetraploid parent of C_2 generation was not a genuine tetraploid. Chromosome associations, above bivalents, were not observed in any of the pollen mother cells.

10. Size and sterility of pollen.

(a) Size of pollen.

The analysis of variance for the pollen size of the different types showed a significant F ratio (Appendix XIII) The progeny of the suspected tetraploid produced significantly larger pollen grains compared to the progeny of the diploid and 4x x 2x cross. The pollen grains of progeny from the cross showed an intermediate size between the parents. The data pertaining to the mean diameter of pollen grains are given in Table XXIII.

TABLE - XXIII

C_3 - Pollen size

Types	Mean diameter (in u)
4x	70.89 ± 0.32
2x	67.86 ± 0.25
4x x 2x	68.55 ± 0.33

C.D. (5%) for comparison of 4x mean = 0.196

C.D. (5%) for comparison of 2x and 4x x 2x mean = 0.803

(b) Extent of pollen sterility.

The x^2 analysis of the extent of pollen sterility did not show any significant difference between the 3 types (Appendix XIV). The data on the percentage of pollen sterility (Table XXIV) among the 3 types, also did not vary markedly. The progeny of the suspected tetraploid parent exhibited maximum sterility and those of the diploid the minimum.

TABLE - XXIV

C_3 - Percentage of pollen sterility

Types	Pollen sterility %
4x	8.3
2x	6.4
4x x 2x	8.1

10. Number and size of capsules.

(a) Number of capsules.

The analysis of variance for the different types is given in Appendix XV. The F ratio was found to be significant. The production of capsules in the progeny of the 4x x 2x cross showed a significant increase compared to the

parents. The selfed progenies of the suspected tetraploid and diploid parents did not show any significant variation in capsule production. The data pertaining to the mean number of capsules produced by the different types are given in Table XXV.

TABLE - XXV

C_3 - Number of capsules

Types	Mean number of capsules
4x	30.70
2x	28.48
4x x 2x	36.67

C.D. (5%) for comparison of 4x mean = 3.11

C.D. (5%) for comparison of 2 x and 4x x 2x means = 3.95

The graphical representation of the data is given in Figure 8.

(b) Size of capsule.

The size of the capsule did not show any significant difference among the 3 types. The progeny of the suspected tetraploid parent showed a slight increase in size of

the capsule when compared to the other 2 types. The progeny of the 4x x 2x cross was intermediate in capsule size compared to the parents. The data pertaining to the mean length and girth of capsules of the 3 different types are given in Table XXVI.

TABLE - XXVI

C_3 - Size of capsule

Types	Length (cm.)	Girth (cm.)
4x	2.69 ± 0.070	3.97 ± 0.030
2x	2.57 ± 0.047	3.80 ± 0.017
4x x 2x	2.67 ± 0.068	3.83 ± 0.284

12. Yield of seeds.

The number of seeds counted from 10 capsules collected at random from each type did not show any marked difference. The data for number of seeds per 10 capsules of the 3 types are given in Table XXVII.

TABLE - XXVII

C_3 - Number of seeds

Types	Number of seeds/10 capsules
4x	892
2x	905
4x x 2x	897

13. Weight of 1,000 seeds.

The 1,000 seed weight did not show much variation among the 3 types. The date are given in Table XXVIII.

TABLE - XXVIII

C_3 - Weight of seeds

Types	Thousand seed weight (in gm.)
4x	2.62
2x	2.30
4x x 2x	2.41

The progeny of the suspected tetraploid produced heavier seeds than those of the diploid and 4x x 2x cross. The seed weight of the 4x x 2x crossed progeny was found to be between than that of the parents.

In general, in the C_3 generation the selfed progeny of the suspected tetraploid showed significant increase in many characters than those of the diploid and 4x x 2x cross. Although there was no cytological variation; in the case of capsule production which is economically more important, the progeny of 4x x 2x cross showed a significant increase over both parents. The progeny of the diploid parent was below the other 2 types in almost all characters.

DISCUSSION

DISCUSSION

The object of the present investigation was to study the nature of polyploids in C_2 and C_3 generations, as well as to study the progeny of the cross between C_2 tetraploid and diploid in Sesamum indicum, L.

Polyploidy has played a major role in the evolution of many plant groups. Its induction in plants has consequently acquired an important position among other methods as a valuable tool in plant breeding. It must however be combined with other methods for achieving specific purposes.

In Sesamum, tetraploidy has been induced by Richharia and Persai (1940), Langham (1940), Kobayashi and Shimamura (1945), Srivastava (1956) and Nair (1965). But the percentage of tetraploids recovered was only small in these cases.

Nair (1965) observed gigas characters in the C_1 plants. But the fertility was low and there was no increase in the oil content. However, for the present investigation, the seeds obtained from the suspected tetraploids of C_1 generation, have been carried forward.

In the present investigation, in the C_2 generation one plant was observed to be a suspected tetraploid. But later it was found to be a sectorial polyploid. All the other plants were diploids. Further, the sectorial polyploid on selfing gave rise to only diploids in the C_3 generation. The progeny of the cross between this sectorial polyploid and diploid also turned out to be diploids.

The results of the present investigation indicate that the C_1 tetraploids obtained by Nair (1965) were not genuine tetraploids. The tetraploid chromosome number (2n = 52) shown by some of them may be due to its branch or sectorial polyploid nature, as reported by Sen and Cheda (1958) in black gram.

The gigas characters shown by some of the C_1 plants may be due to the following reasons:-

The plants may be periclinal chimeras with 2x inner core and 4x outer layer as reported by Pal, Ramanujam and Joshi (1941) in Chillies (Capsicum annum, L.), Baker (1943) in potato and Bhaduri et al in Corchorus olitorius.

Muntzing and Runquist (1939) in their studies on Festula pratensis and Lolium perenne, have showed that

colchicine has got a growth stimulating action besides inducing polyploidy. Out of 16 different plant species treated with colchicine, they have observed chromosome doubling in only 3 species. Such an action of colchicine on the growth hormones has been reported by Havas (1938).

Germination of seeds

The 14 types of seeds from the suspected C_1 tetraploids did not show any marked difference in germination compared to the diploid. The percentage of germination of the 14 seed types ranged from 42-61 while the diploid showed 65% of germination. Such a high germinability of seed from tetraploids was recorded by Kobayashi and Shimamura (1949, 1952) and Srivastava (1956) in the same crop, Kundu and Sharma (1956) in Corchorus olitorius, L. and Sen and Cheda (1958) in black gram.

The comparatively higher percentage of germination in the C_2 generation may be due to the smaller number of diploid gametes fertilized. Further, such tetraploid seeds might not have germinated. Only the seeds developed from the normal haploid gametes are seen to be germinated, as only diploids were seen in the progeny.

In the C_3 generation also only diploids were seen in the progeny. The germinability of C_2 seeds was comparable

to the diploid. The seeds from the tetraploid x diploid cross showed even a higher percentage of germination than the diploid. The progeny of the cross was found to be all diploids.

The higher percentage of germination of the crossed seeds may be due to the extra vigour obtained by cross pollination, as Sesame is a naturally self pollinated plant.

Growth of Plants

In general the growth rate of the suspected C_2 tetraploids did not vary markedly from the diploid. The types 3 and 5 of C_2 generation were more vigorous than the diploid inspite of their early retardation of growth. Similar results were observed by Nair (1965) in the C_1 generation and by Srivastava (1956) in C_2 generation of the same crop.

The progeny of the suspected C_2 tetraploid exhibited similar vigorous growth in the C_3 generation compared to the diploid. The progeny of the tetraploid x diploid cross were intermediate to the parents in growth rate; as recorded in similar cases by Mc_Clintock (1929) in Zea mays, Ramanujam (1937), in Oryza sativa and by Krishnamoorthy (1963) in cotton.

The suspected C_2 tetraploids and the progeny in C_3 generation, as well as the progeny of the tetraploid x diploid cross showed a significant increase in the production of branches compared to the diploid. In autotetraploids of Brassica oleraceae, Tandon (1961) has recorded similar profuse branching nature.

Eventhough the progeny of the suspected C_1 tetraploids in C_2 and C_3 generation as well as the progeny of tetraploid x diploid cross were all proved to be diploid, this vigorous growth and profuse branching may be due to the following reasons:-

The progeny in C_2 and C_3 generations might have inherited the vigorous growth habit of the C_1 generation plants.

Due to cross pollination the progeny of the tetraploid x diploid cross might have obtained some extra vigour. The intermediate nature of these progeny of the cross in characters like height may be due to the expression of quantitative characters by the F_1, which is usually intermediate to the parents.

Size and distribution of stomata

The number per unit area and the size of stomata of the suspected C_2 tetraploids and the progeny in C_3

generation as well as the progeny of tetraploid x diploid cross were not significantly different from those of the diploid.

Herzch (1951) has recorded similar results in tetraploids of Vicia villosa. But Graner (1941) in Manihot utilissima, Langham (1942) and Srivastava (1956) in sesame, Kumar and Abraham (1942) in Phaseolus radiatus and Knight (1957) in Theobroma cocoa have recorded results contradictory to the present observation.

The diploid nature of the progeny of C_1 and C_2 suspected tetraploids (except one sectorial polyploid in C_2 generation) and that of the tetraploid x diploid cross may be the reason for the similarity, in the size and number of stomata, with the diploid.

Area and thickness of leaves

The progeny of the suspected C_1 and C_2 tetraploids and that of the cross between C_2 tetraploid and diploid did not show significant variation in leaf area and thickness, compared to the diploid.

The results were in contradiction with those obtained by Richharia and Persai (1940), Shimamura and

Kobayashi (1945), and Nair (1965) in Sesame, Kumar and Abraham (1942) in Phaseolus radiatus, Tandon and Bali (1957) in Linaria vulgaris, Vakili (1962) in Musa, Ammal (1962) in Rawolfia serpentina, Sobti (1963) in Menta piperata, and Vig (1964) in Cyamopsis psoralioides. In these cases the tetraploids possessed larger, coarser, darker and thicker leaves than the diploids.

The contradictory results in the present investigation may be due to the diploid nature of progeny of the C_1 and C_2 suspected tetraploids as well as that of the tetraploid x diploid cross.

Blooming behaviour, number and size of flowers

On an average 7 - 10 days delay in flowering was noticed in a few suspected C_2 tetraploids compared to the diploid. The progenies of C_2 tetraploid and tetraploid x diploid cross took 3 - 5 days more for starting flowering than the diploid. Randolph (1944) observed prolonged vegetative growth and delayed flowering in autotetraploids of maize. Similar observations were made by Tandon and Chinoy (1950) in Amaranthus blitum and Srivastava (1956) in sesame. But Kovac Schneider (1956) observed that the tetraploid tomatoes flowered at the same time as the diploids.

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The number of flowers produced by the suspected C_2 tetraploids and the progeny in C_3 generation as well as the progeny of the C_2 tetraploid x diploid cross, was slightly more than that of the diploid. Flower size in these cases, also showed a slight increase than the diploid. Parthasarathy and Kedarnath (1945) observed that autotetraploids of many crops produce bigger sized flowers in large numbers. Similar observations were also made by Visweswara and Chinnappa (1965) in Coffea canephora, Amin (1940) in cotton, Kobayashi and Shimamura (1949, 1952), Srivastava (1956) and Nair (1965) in sesame, Sen and Cheda (1958) in black gram, Mehra et al (1966) in methra, and Ramanujam (1937) in autotetraploids of rice.

The slight increase in the number of flowers produced and in the size of floral parts observed in the progeny of the suspected C_1 tetraploids and the progeny in C_3 generation as well as the progeny of the C_2 tetraploid x diploid cross may be due to their vigorous growth habit, as no genuine tetraploid was observed among them.

Cytological observation

The meiotic behaviour of the suspected C_2 tetraploids and the progeny in C_3 generation as well as the progeny of the C_2 tetraploid x diploid cross, was found to

be normal resembling that of the diploid. In one plant of the C_2 generation few pollen mother cells with the tetraploid chromosome number (2n = 52) was observed. Here also the anaphasic separation was normal ie. 26/26. But later this plant was found to be a sectorial polyploid as pollen mother cells with diploid chromosome number (2n = 26) was also observed. The selfed progeny of this sectorial polyploid in C_3 generation, as well as the progeny of its cross with diploid were found to consist only of diploids.

Ramuson and Levan (1939) observed diploid and tetraploid sectors in the same branch or even in the same flower in the case of sugar beet.

After studying the colchicine technique in black gram, Sen and Cheda (1958), concluded that the induced polyploids were either complete polyploids, branch polyploids or sectorial polyploids. Nair (1965) observed chromosome doubling in a few plants of C_1 generation of sesame.

Normal anaphasic separation in autotetraploids was observed by Kundu and Sharma (1956) in Corchorus olitorius, Visweswara and Chinnappa (1965) in Coffea conephora.

The apparent reversion of the progeny of C_1 tetraploid to diploid may be due to the following reasons:-

The suspected tetraploids of C_1 generation may not be genuine tetraploids. Instead, some of them might have been periclinal-ploid chimera. Consequently the seeds collected from such plants might have consisted of only diploid seeds. Some of the C_1 tetraploids may not be complete polyploids. They might be sectorial or branch polyploids. The seeds collected from such plants could possibly be a mixture of tetraploid and diploid seeds, of which only diploid seeds might have germinated in the C_2 generation.

Size and sterility of pollen

A few of the suspected C_2 tetraploid possessed larger pollen grains compared to the diploid. The selfed progeny of the C_2 tetraploid and the progeny of its cross with diploid also had bigger pollen grains than the diploid.

Larger size of pollen in autotetraploids has been recorded by Amin (1940) in cotton, Pal, Ramanujam and Joshi (1941) in Capsicum, Parthasarathy, Kedarnath (1945), Srivastava (1956) and Nair (1965) in sesame and Mehra et al in Trifolium foenum-graecum.

Nair (1965) observed in the C_1 tetraploids, pollen sterility ranging from 31.91 to 43.91%. On the other hand in the C_2 generation pollen sterility ranged from 3.52 to 12.34% only. In C_3 generation it was only 8.3%. The progeny of the cross between C_2 tetraploid and diploid showed a sterility of 8.1%.

There exist a great deal of diversity regarding the pollen fertility in autotetraploids. Fertility comparable to that of diploids was recorded by Ramuson and Levan (1939) in sugar beet, Langham (1942), Kohayashi and Shimamura (1949, 1952) and Srivastava (1956) in sesame, and Sen and Cheda (1958) in black gram. Kundu and Sharma (1956) observed in the case of autotetraploids of Corchorus olitorius, even an increase in fertility than diploid. A similar instance was reported by Visweswara and Chinnappa (1965) in Coffea canephora.

On the contrary considerable reduction in fertility of autotetraploid was recorded by Pal, Ramanujam and Joshi (1941) in Capsicum annum, Parthasarathy and Kedarnath (1945) and Kedarnath (1954) in sesame, Chopra and Swaminathan (1960) in watermelon and Mehra at al (1966) in Trifolium foenum-graecum. Very low fertility was recorded in the case of the progeny of tetraploid x diploid crosses,

by Moringa and Fukushima (1935) and Ramanujam (1937) in Oryza sativa.

The high fertility of the progeny studied can be attributed to the orderly meiotic behaviour. The progeny consisted only of diploid except one sectorial polyploid in the C_2 generation. Even in that plant meiosis was normal. Slight increase in sterility in some of the plants may be due to the pressence of more nucleioli in the microspores; as reported by Kumar and Abraham (1940) and Nair (1965) in sesame.

The larger size of pollen grain may be due to the larger pollen mother cells as recorded by Nair (1965). The progenies of the C_1 tetraploids were possessing morphological features of tetraploids, eventhough they were having only diploid chromosome number (2n = 26).

Crossing tetraploid and diploid

Only the cross between the C_2 tetraploid as female parent and diploid as male parent yielded viable seeds. In the reciprocal cross (2x ♀ x 4x ♂) only shrivelled and non-viable seeds were obtained.

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

The failure to form viable seeds in the reciprocal cross (2x ♀ x 4x ♂) may be due to the slow growth rate of diploid pollen tube of the C_2 tetraploid.

Even the cross 4x ♀ x 2x ♂ yielded only few seeds. When these seeds were sown and raised with the C_3 generation it was observed that the progeny consisted only of diploids, instead of triploids.

The tetraploid parent in the cross was found to be a sectorial polyploid producing both diploid and haploid gametes. In the cross only the haploid gametes might have succeeded in developing into viable seeds. The 3x embryo and its endosperm produced by the fusion of diploid and haploid gametes might have collapsed in the early developmental stage. As a result only few seeds, which were diploid, were obtained in the cross.

Capsule setting and yield

In the C_2 generation the number of flowers produced and capsules developed were proportional, and this progeny did not show any significant difference in capsule setting and number of seeds per capsule, as compared to the diploid. But the capsules and seeds produced by them were slightly bigger than that of the diploid.

The progeny in C_3 generation, as well as the progeny of the cross between tetraploid and diploid did not show significant difference in flower production compared to diploid. But the capsule setting in the progeny of the cross has showed a significant increase.

Langham (1942) in sesame observed no difference in capsule setting and seeds per capsule, between tetraploid and diploid. But the capsule and seeds of tetraploids were larger than diploids. Similar results were obtained by Kobayashi and Shimamura (1949, 1952) and Srivastava (1956) in sesame.

The larger size of capsules and seeds of the suspected C_2 tetraploids and progeny may be due to the vigorous growth habit and other morphological features, since the progenies were consisted only of diploids.

The increase in capsule setting in the progeny of the cross between C_2 tetraploid and diploid may be due to its lesser susceptibility to the caterpillar Antigastra catalaunalis, which attacks flower buds also.

Nair (1965) after studying the C_1 generation has concluded that colchicine technique in improving sesame

crop is considerably limited. After studying the progeny of C_1 and C_2 generations, it is seen that, eventhough, there is no doubling of chromosome number, there are some promising types in the C_2 generation which possess good economic qualities like bigger capsules and seeds compared to normal diploid. The progeny of the cross between C_2 tetraploid and diploid, eventhough they were diploids, have shown some excess vigour than normal diploid and also lesser susceptibility to the leaf caterpillar.

SUMMARY AND CONCLUSIONS

SUMMARY

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

Selfed seeds from the suspected tetraploids of C_1 generation studied by Nair (1965) were carried forward to study the C_2 generation. Reciprocal crosses were made between C_2 tetraploid and diploid and the progeny was studied along with the selfed progeny of the parents. The morphological and cytological behaviour of the progeny of the C_1 and C_2 tetraploids and the progeny of the cross were studied and discussed.

Evidences from the present investigation revealed that the suspected C_1 tetraploids gave rise to only diploids, except for the presence of one sectorial polyploid, in the C_2 generation. This may be either due to the failure of diploid gametes to function normally,

or due to the failure of tetraploid seeds to germinate in the C_2 generation.

Eventhough the C_2 generation plants did not show tetraploid chromosome number, they possessed morphological characters of the autotetraploids. Similar case was reported in some of the C_1 generation plants also.

This may be due to the growth stimulating action of the weak concentration of colchicine solution, apart from inducing polyploidy. Consequently seeds from such suspected C_1 tetraploids gave rise to only diploids in the C_2 generation.

The diploid nature of the progeny of the suspected C_1 and C_2 tetraploids and the sectorial polyploid nature of one plant in C_2 generation was confirmed by cytological examination of pollen mother cells at meiosis. In the sectorial polyploid, pollen mother cells with both tetraploid ($2n = 52$) and diploid ($2n = 26$) chromosome numbers were observed, whereas in all the other plants only diploid chromosome number ($2n = 26$) was observed.

The C_2 sectorial tetraploid was crossed reciprocally with the diploid. Only the cross $4x \, ♀ \times 2x \, ♂$ yielded

viable seeds. The reciprocal cross (2x ♀ x 4x ♂) yielded only shrivelled and non-viable seeds. The viable seeds were raised along with the selfed progeny of the parents. The progeny of the cross was found to be diploid instead of triploids as expected, and the progeny of the selfed seeds of the C_2 sectorial tetraploid plant also was found to be diploid.

Eventhough no tetraploid chromosome number was observed in the progeny of the C_1 and C_2 tetraploids (except the single sectorial polyploid in C_2 generation), there were some promising types with good economic characters like bigger capsules and seeds compared to the normal diploid. There was no reduction in fertility and many of the C_2 plants were showing profuse grwoth habits with bigger capsules and seeds. Branching, flowering, capsule production, yield of seeds and weight of seeds were found to be superior in these plants compared to the normal diploids.

The progeny of the cross between the C_2 sectorial polyploid and diploid were found to be superior in capsule production, than both the parents, and also superior in many characters like branching, growth and flower production compared to the normal diploid. This type has shown lesser

susceptibility to the leaf caterpillar, Antigastra catalaunalis.

It was concluded that the effect of colchicine in inducing polyploidy in Sesamum indicum was not substantiated by the observations made in the C_2 and C_3 generations of the present investigation. However the progeny of the C_1 tetraploids resembled the autotetraploids morphologically. One plant in the C_2 generation was found to be a sectorial polyploid with tetraploid and diploid sectors. The progeny of the cross between C_2 tetraploid and diploid exhibited excess vigour in capsule production and also found to be less susceptible to the leaf caterpillar. The promising types of the C_2 and C_3 generation, as well as the progeny of the cross which show better economic characters than the normal diploid, are worthwhile to be tried for selecting better types of sesame.

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* Originals not seen.

APPENDIX

APPENDIX - I

C_2 GENERATION

Analysis of variance for the height of plant

Source	S.S.	D.F.	Variance	F
Total	5,396.87	59		
Block	462.14	3	154.04	1.91
Types	1,551.32	14	110.81	1.37
Error	3,383.41	42	80.55	

APPENDIX - II

C_2 GENERATION

Analysis of variance for the thickness of leaf

Source	S.S.	D.F.	Variance	F
Total	8,099.00	1499		
Types	3,815.15	14	272.31	94.6 **
Error	4,283.85	1485	2.88	

** Significant at 1% level

APPENDIX - III

C_2 GENERATION

Analysis of variance for the number of stomata

Source	S.S.	D.F.	Variance	F
Total	3,140.51	1499		
Types	241.41	14	17.22	8.8 **
Error	2,899.10	1485	1.95	

** Signification at 1% level

APPENDIX - IV

C_2 GENERATION

Analysis of variance for number of flowers

Source	S.S.	D.F.	Variance	F
Total	16,607.52	59		
Block	399.67	3	133.22	< 1
Types	4,792.92	14	342.35	1.25
Error	11,414.93	42	271.78	

APPENDIX - V

C_2 GENERATION

Analysis of variance for pollen size

Source	S.S.	D.F.	Variance	F
Total	1,520.39	1499		
Types	148.83	14	10.63	11.35 **
Error	1,371.56	1485	0.92	

** Significant at 1% level

APPENDIX - VI

C_2 GENERATION

Pollen sterility

χ^2 Table

Treatments	χ^2 value
Type 1 vs control	0.028
Type 2 vs control	0.067
Type 3 vs control	0.133
Type 4 vs control	1.253
Type 5 vs control	1.252
Type 6 vs control	0.778
Type 7 vs control	0.426
Type 8 vs control	0.025
Type 9 vs control	0.001
Type 10 vs control	0.171
Type 11 vs control	0.255
Type 12 vs control	0.031
Type 13 vs control	2.351
Type 14 vs control	0.143

Value of χ^2_1 = 3.841

None of the differences are significant

APPENDIX - VII

C_2 GENERATION

Analysis of variance for the number of capsules

Source	S.S.	D.F.	Variance	F
Total	11,340.51	59		
Block	9.71	3	3.23	< 1
Types	2,735.00	14	195.35	< 1
Error	8,595.80	42	204.66	

APPENDIX - VIII

C_2 GENERATION

Analysis of variance for the number of branches

Source	S.S.	D.F.	Variance	F
Total	110.66	59		
Block	3.65	3	1.23	1.12
Types	38.75	14	2.76	2.53 *
Error	42.40	42	1.09	

* Significant at 5% level

APPENDIX - IX

C_3 GENERATION

Analysis of variance for the height of plant

Source	S.S.	D.F.	Variance	F
Total	4,034.14	47		
Block	1,016.93	7	145.27	
Types	780.18	2	390.09	6.62 **
Error	2,237.03	38	58.86	

** Significant at 1% level

APPENDIX - X

C_3 GENERATION

Analysis of variance for the thickness of leaf

Source	S.S.	D.F.	Variance	F
Total	3,399.92	599		
Types	371.62	2	186.81	36.64 **
Error	3,028.30	597	5.07	

** Significant at 1% level

APPENDIX - XI

C_3 GENERATION

Analysis of variance for the number of stomata

Source	S.S.	D.F.	Variance	F
Total	1,350.00	599		
Types	289.04	2	144.52	81.64 **
Error	1,061.36	597	1.77	

** Significant at 1% level

APPENDIX - XII

C_3 GENERATION

Analysis of variance for the number of flowers

Source	S.S.	D.F.	Variance	F
Total	8,867.70	47		
Block	3,108.66	7	444.09	
Types	165.46	2	82.73	< 1
Error	5,593.58	38	147.20	

APPENDIX - XIII

C_3 GENERATION

Analysis of variance for the pollen size

Source	S.S.	D.F.	Varance	F
Total	597.54	599		
Types	83.12	2	41.56	48.32 **
Error	514.42	597	0.86	

** Significant at 1% level

APPENDIX - XIV

C_3 GENERATION

Pollen sterility

X^2 Table

Types	X^2 value
4x vs 2x	0.828
4x vs 4x x 2x cross	0.026
2x vs 4x x 2x cross	0.456

Value of X^2_1 = 3.841

None of the values are significant

APPENDIX - XV

C_3 GENERATION

Analysis of variance for the number of capsules

Source	S.S.	D.F.	Variance	F
Total	1,261.54	47		
Block	371.11	7	53.15	
Types	305.89	2	152.94	9.94 **
Error	584.54	38	15.38	

** Significant at 1% level

APPENDIX - XVI

C_3 GENERATION

Analysis of variance for the number of branches

Source	S.S.	D.F.	Variance	F
Total	52.84	47		
Block	4.26	7	0.6	
Types	19.83	2	9.91	13.21 **
Error	28.75	38	0.75	

** Significant at 1% level

PLATES

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LIST OF ILLUSTRATIONS

Plates

FIGURE 1 (a)

RATE OF GROWTH OF C_2 TYPES (TYPES 1 - 7)
AND CONTROL (TYPE 15)

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C_2 GENERATION.

RATE OF GROWTH.

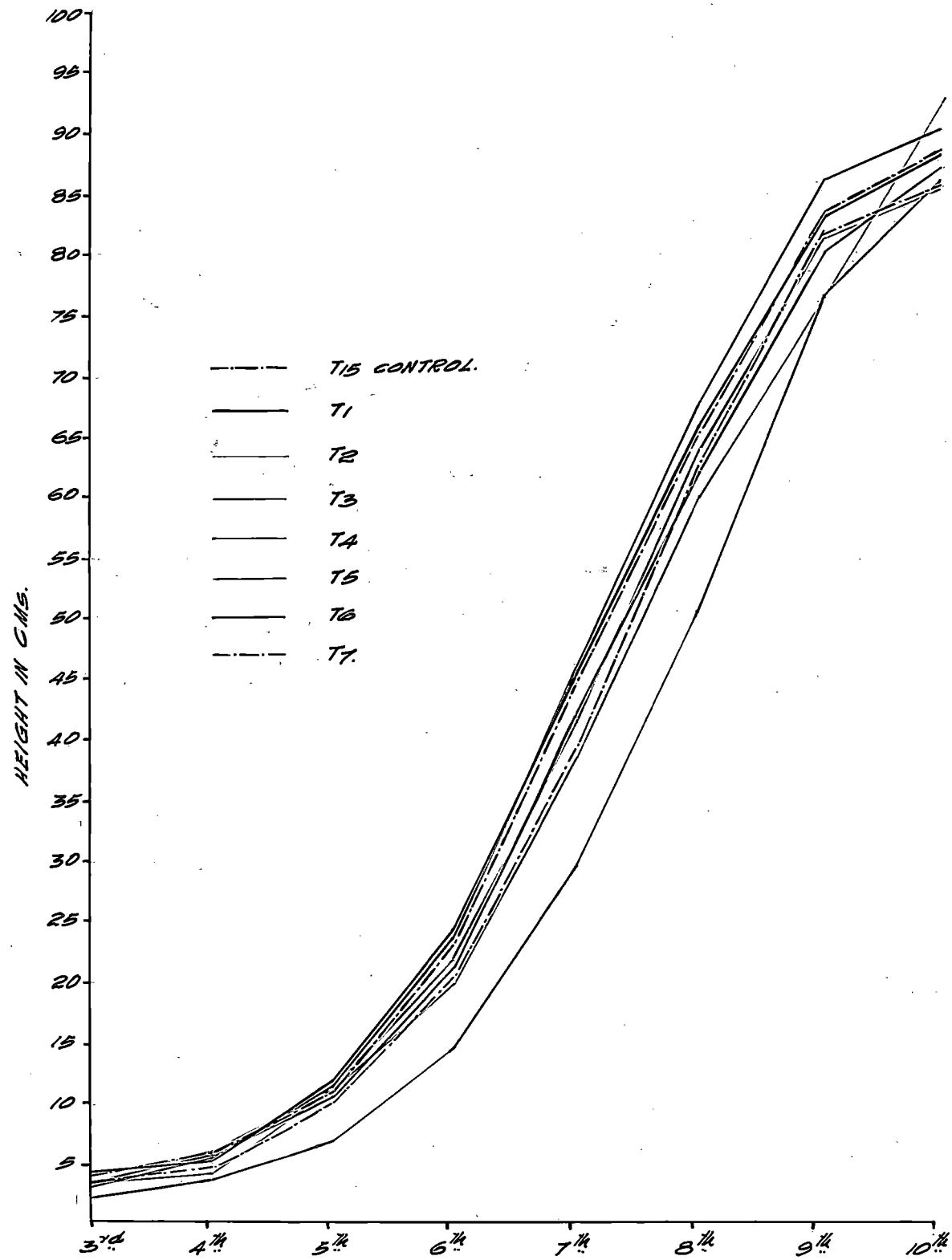

FIG: 1a.

FIGURE 1 (b)

RATE OF GROWTH OF C_2 TYPES (TYPES 8 - 14)
AND CONTROL (TYPE 15)

C_2 GENERATION.

RATE OF GROWTH.

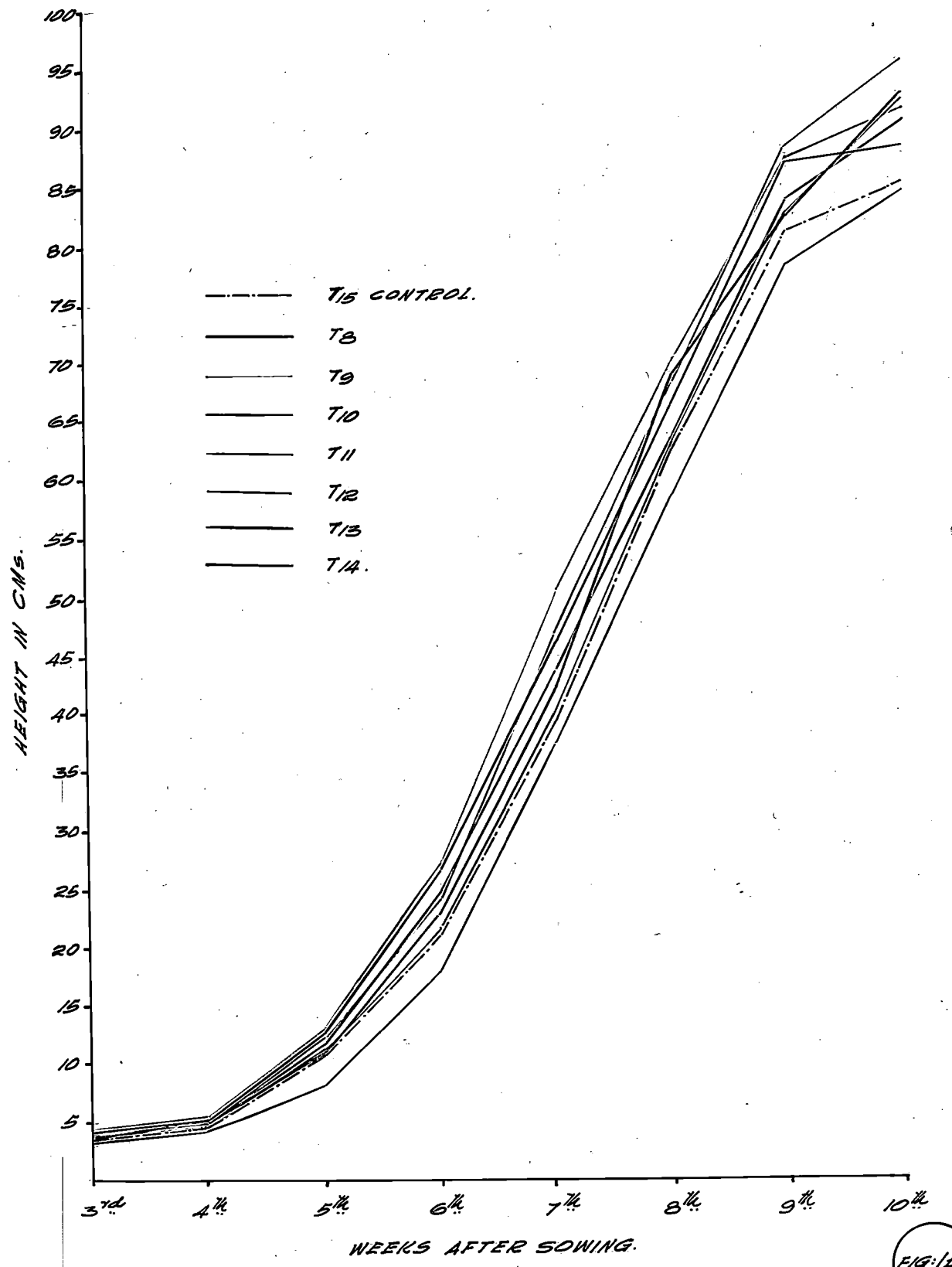

FIG: 16.

FIGURE 2

MEAN HEIGHT OF C_2 PLANTS

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LIBRARY

C_2 GENERATION.

MEAN HEIGHT OF PLANTS.

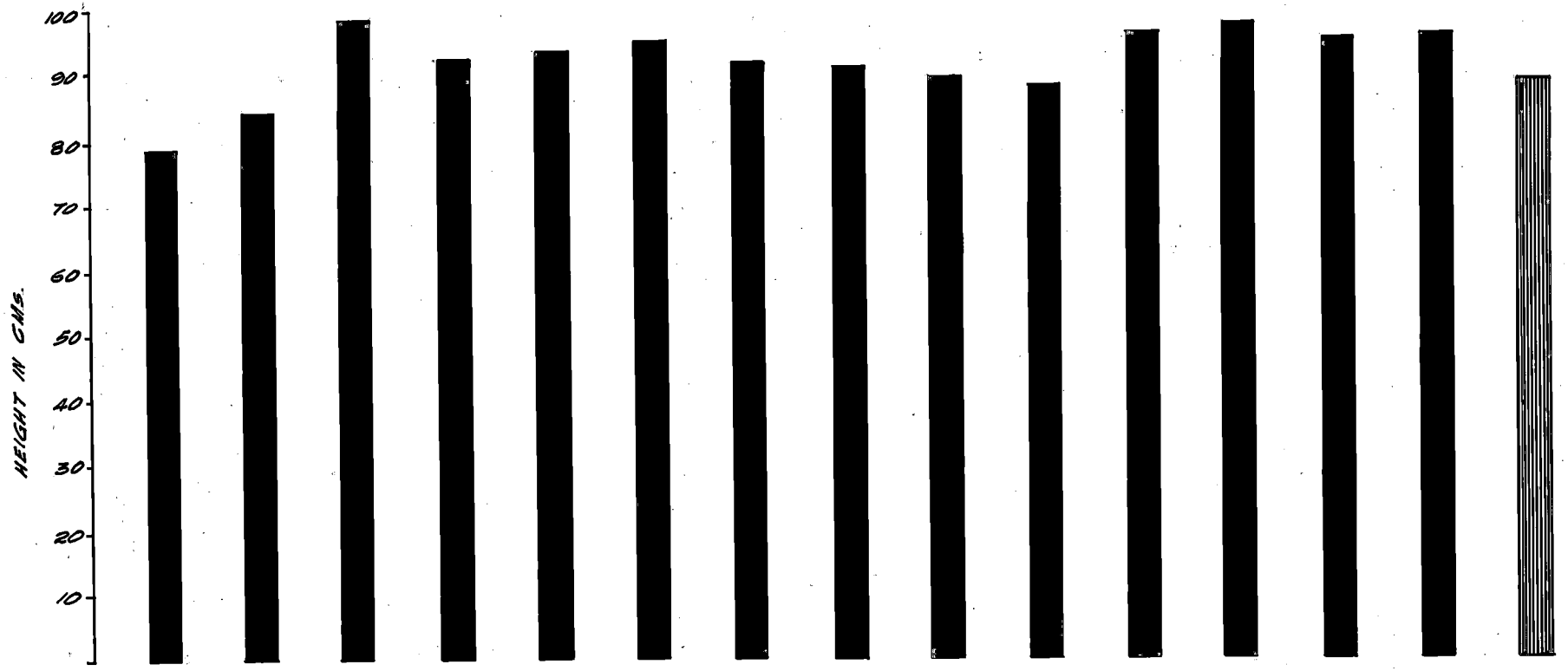

FIG: 2.

FIGURE 3

MEAN NUMBER OF BRANCHES - C_2 PLANTS

LIBRARY

C_2 GENERATION.

MEAN NUMBER OF BRANCHES.

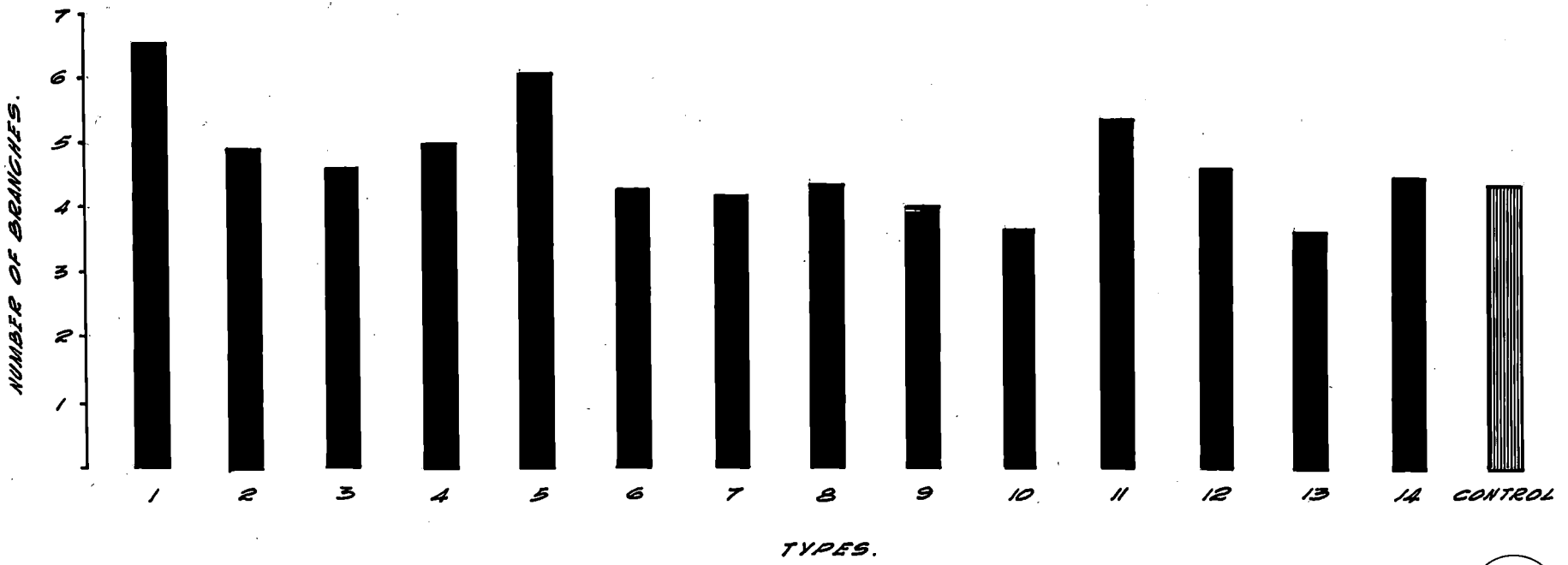

FIG: 3.

FIGURE 4

MEAN NUMBER OF FLOWERS AND CAPSULES - C_2 PLANTS

C_2 GENERATION.

NUMBER OF FLOWERS AND CAPSULES.

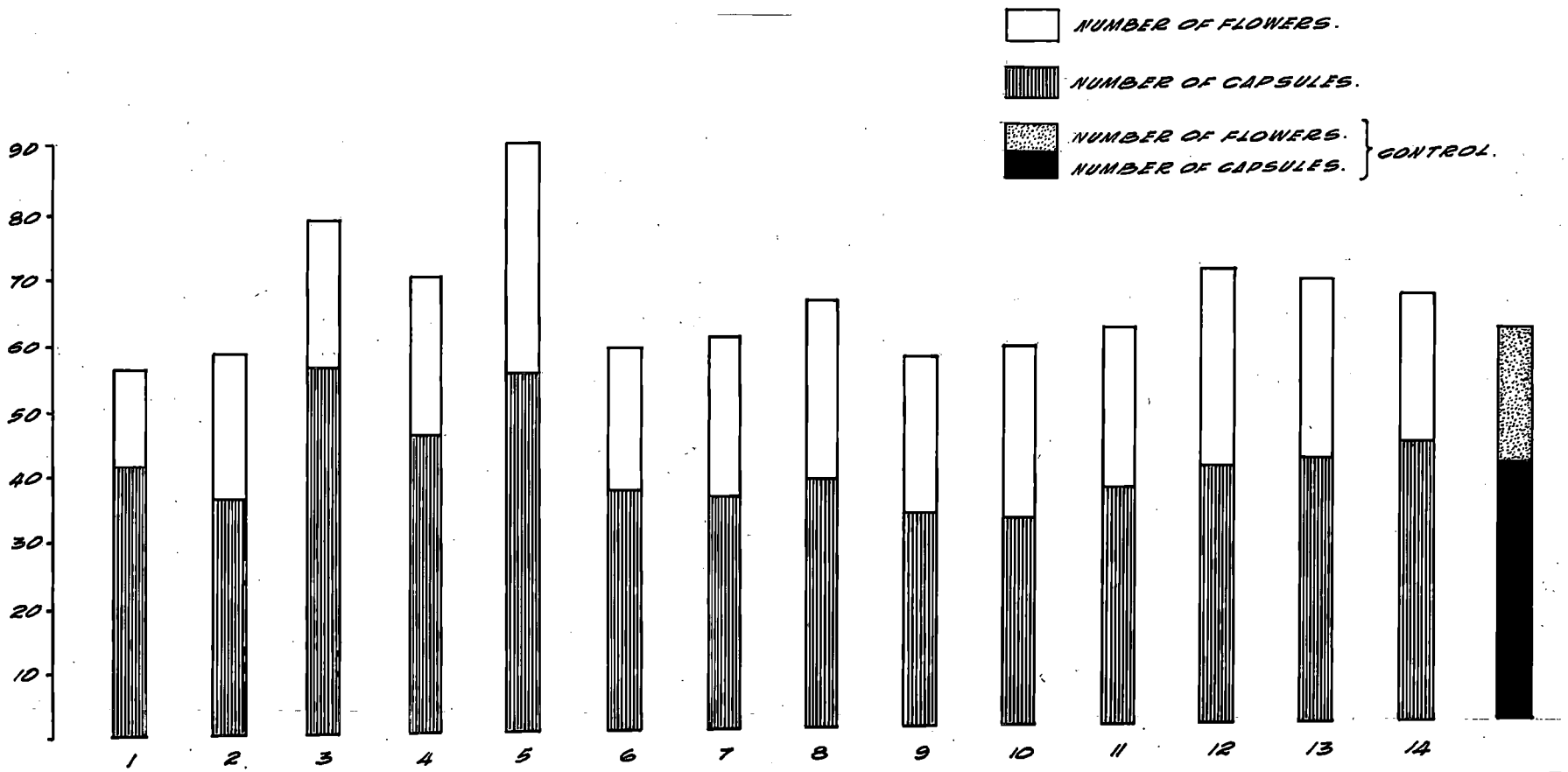

FIG: 4

FIGURE 5

RATE OF GROWTH - C_3 GENERATION

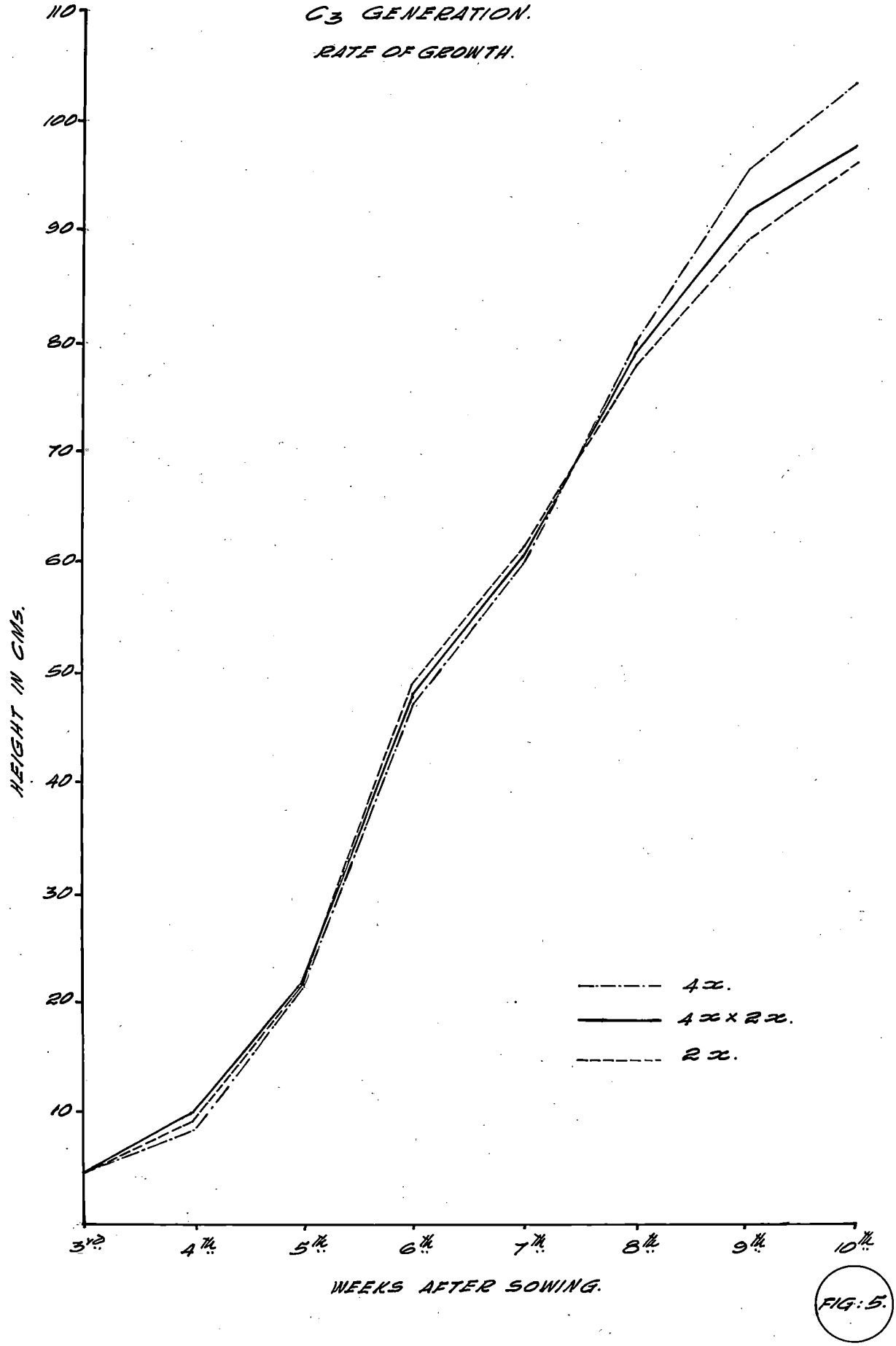
C3 GENERATION.
RATE OF GROWTH.
HEIGHT IN CMS.
110
100
90
80
70
60
50
40
30
20
10
3rd
4th
5th
6th
7th
8th
9th
10th
WEEKS AFTER SOWING.
4x.
4x x 2x.
2x.

FIG: 5.

FIGURE 6

MEAN HEIGHT OF PLANTS - C_3 GENERATION

FIGURE 7

MEAN NUMBER OF BRANCHING - C_3 GENERATION

FIGURE 8

MEAN NUMBER OF FLOWERS AND CAPSULES - C_3 GENERATION

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C_3 GENERATION.

MEAN HEIGHT OF PLANTS.

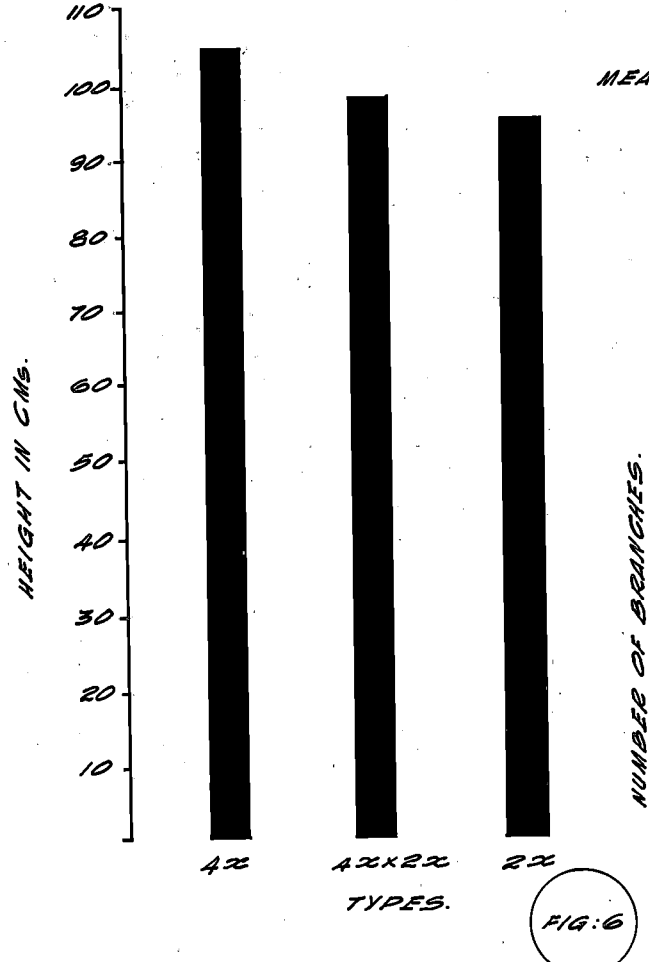

FIG:6

MEAN NUMBER OF BRANCHES.

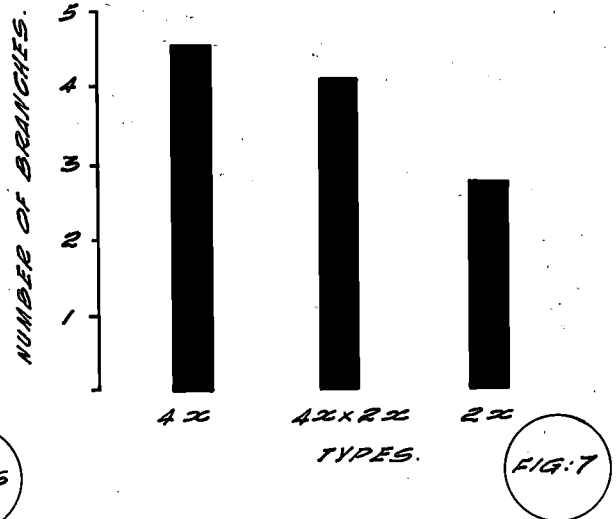

FIG:7

MEAN NUMBER OF FLOWERS AND CAPSULES.

FIG:8

PLATE I

GENERAL VIEW OF THE EXPERIMENTAL PLOT

PLATE II

COMPARISON OF C_2 TYPES (1 AND 2) WITH CONTROL (15)

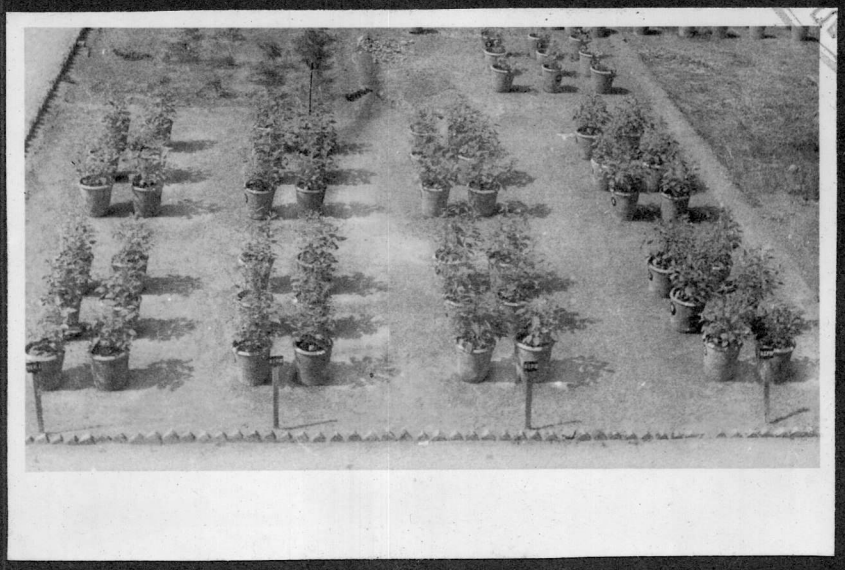

PLATE I

PLATE II

PLATE III

COMPARISON OF C_2 TYPES (3 AND 4) WITH CONTROL (15)

PLATE IV

COMPARISON OF C_2 TYPES (5 AND 6) WITH CONTROL (15)

PLATE III

PLATE IV

PLATE V

COMPARISON OF C_2 TYPES (7 AND 8) WITH CONTROL (15)

PLATE VI

COMPARISON OF C_2 TYPES (9 AND 10) WITH CONTROL (15)

PLATE V

PLATE VI

PLATE VII

COMPARISON OF C_2 TYPES (11 AND 12) WITH CONTROL (15)

PLATE VIII

COMPARISON OF C_2 TYPES (13 AND 14) WITH CONTROL (15)

PLATE VII

PLATE VIII

PLATE IX

TRILOBED LEAF IN C_2 GENERATION

PLATE X

GIGAS NATURE OF C_2 PLANT

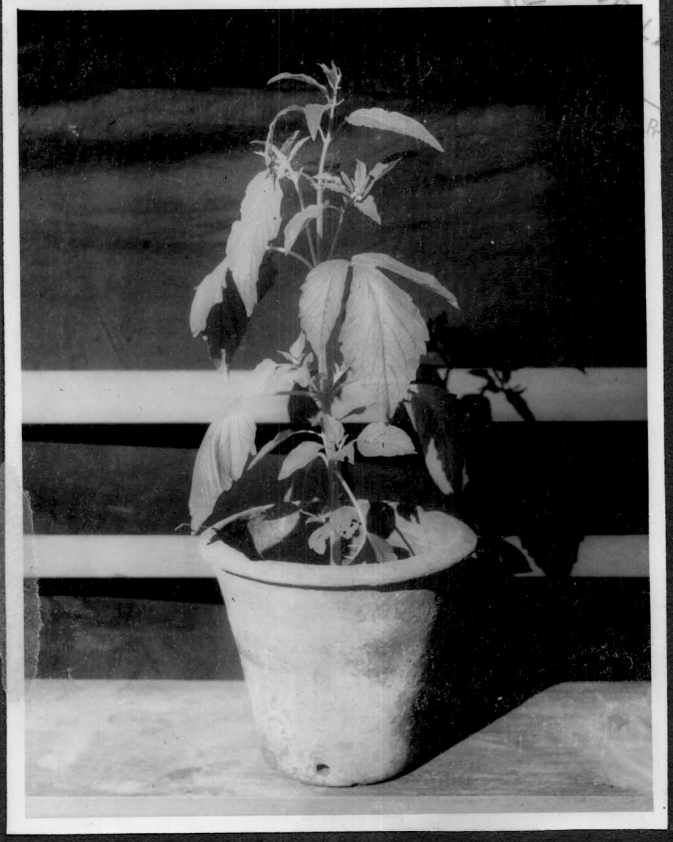

PLATE IX

PLATE X

PLATE XI

COMPARISON OF FLOWER SIZE OF C_2 TYPES (1, 2, 3 AND 4)
WITH CONTROL (15)

PLATE XII

COMPARISON OF FLOWER SIZE OF C_2 TYPES (5, 6, 7 AND 8)
WITH CONTROL (15)

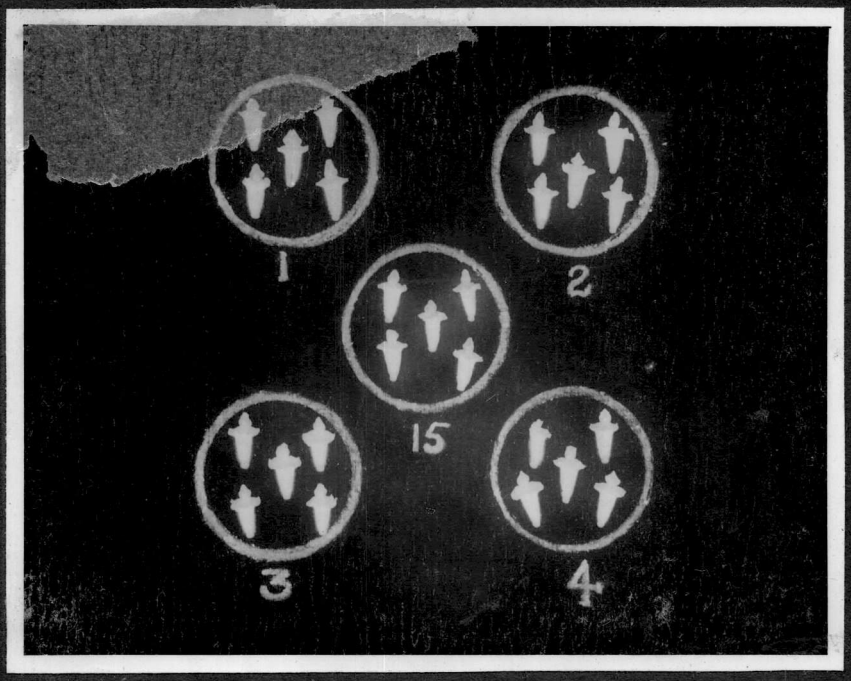

PLATE XI

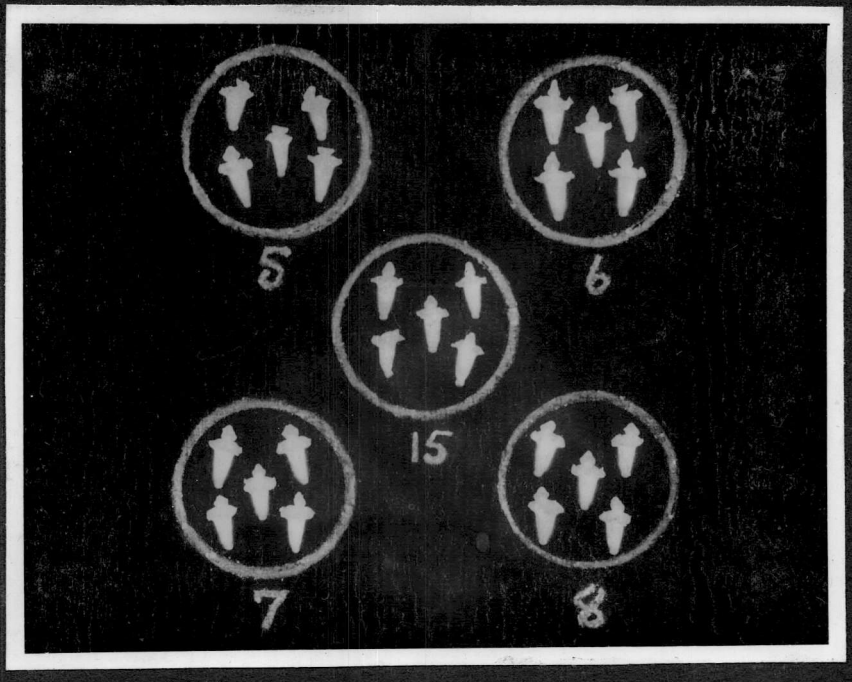

PLATE XII

PLATE XIII

COMPARISON OF FLOWER SIZE OF C_2 TYPES (9, 10, 11, 12, 13 AND 14) WITH CONTROL (15)

PLATE XIV (a)

ANAPHASE - I OF THE C_2 TETRAPLOID SHOWING 26 CHROMOSOME AT EACH POLE

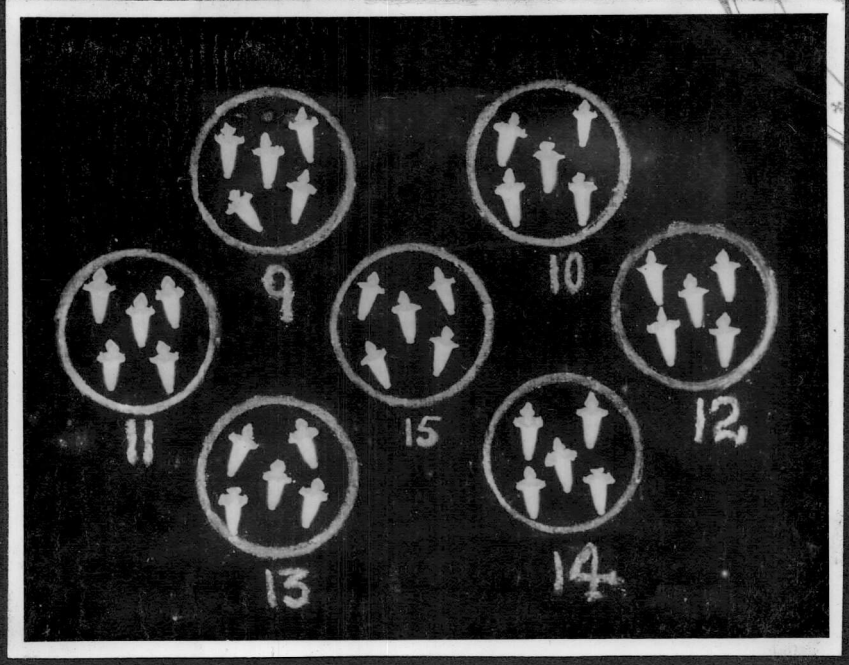

PLATE XIII

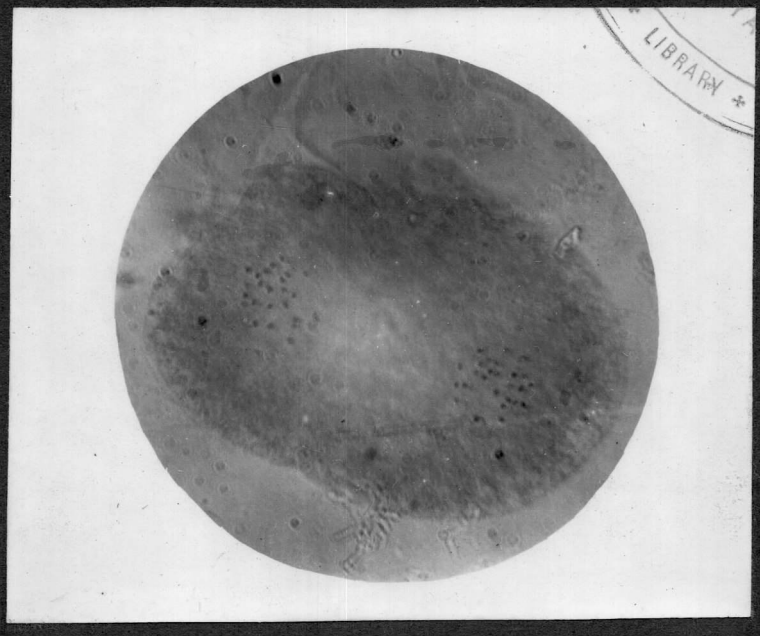

PLATE XIV (a)

PLATE XIV (b)

METAPHASE - I SHOWING 13 BIVALENTS IN DIPLOID PLANTS OF C_2 GENERATION

PLATE XIV (c)

DIPLOTENE STAGE SHOWING 13 BIVALENTS IN DIPLOID PLANTS OF C_2 GENERATION

PLATE XIV (b)

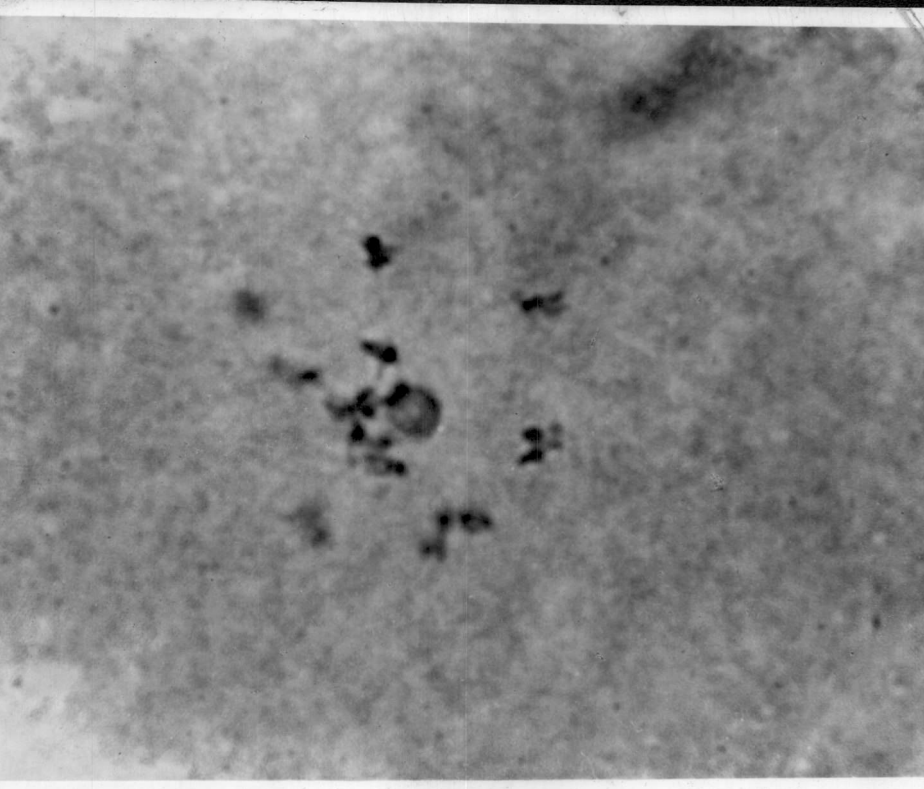

PLATE XIV (c)

PLATE XV (a)

SEEDS FROM THE 4x ♀ x 2x ♂ CROSS

In the centre (F_1) viable seeds obtained are seen

PLATE XV (b)

SEEDS FROM THE 2x ♀ x 4x ♂ CROSS

Shrivelled and nonviable seeds obtained are seen in centre (F_1)

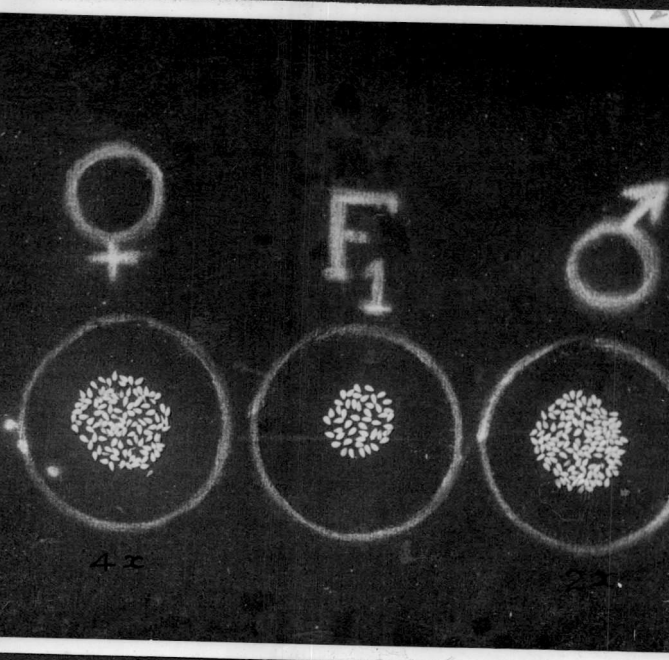

PLATE XV (a)

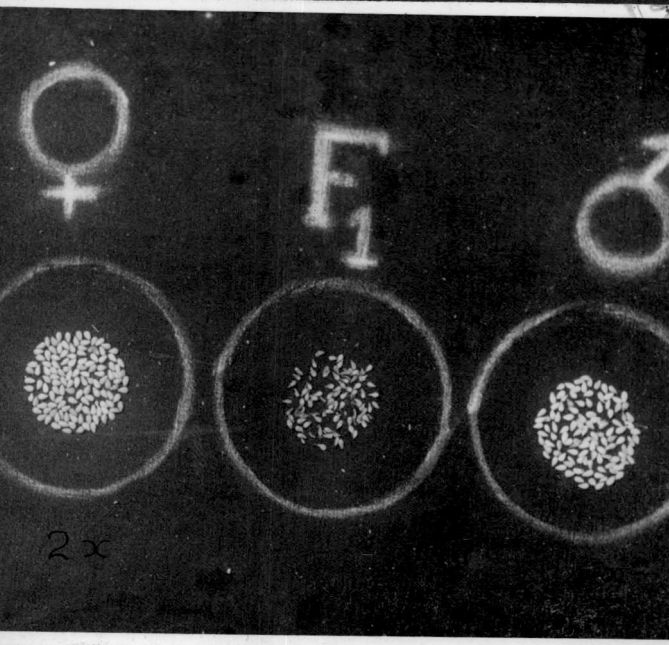

PLATE XV (b)

PLATE XVI

COMPARISON OF C_3 PLANTS (2) WITH PROGENY OF CROSS BETWEEN 4x x 2x (6) AND NORMAL DIPLOID (5)

PLATE XVII

COMPARISON OF FLOWERS

1. Flowers of the C_3 plants.
2. Flowers from the progeny of the C_2 tetraploid x diploid cross.
3. Flowers of normal diploid.

PLATE XVI

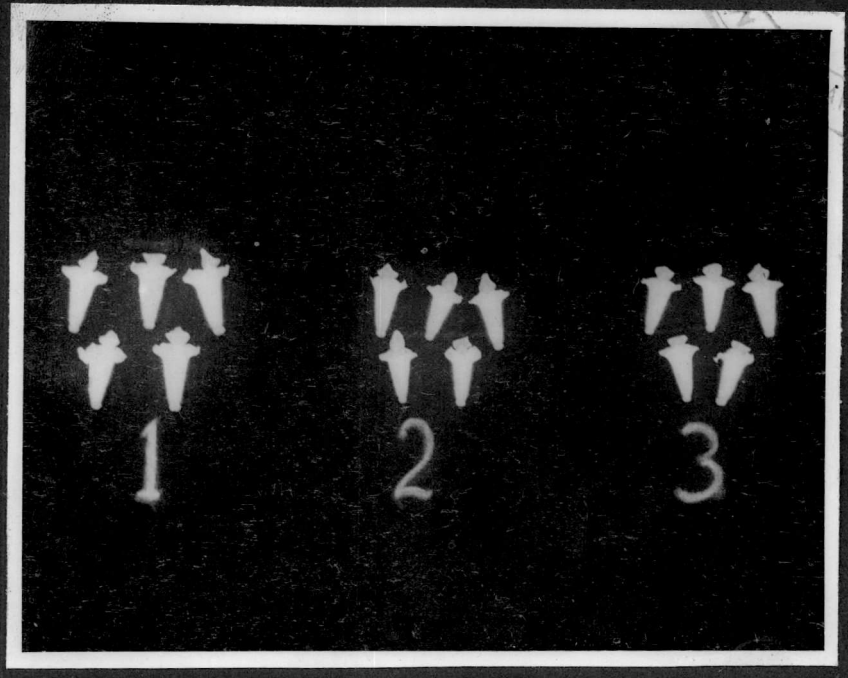

PLATE XVII