CYTO-MORPHOLOGICAL STUDIES ON COLCHICINE INDUCED POLYPLOIDS OF SESAME (*Sesamum orientale* L.)

K. MURALEEDHARAN NAYAR

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE (AGRICULTURE)

IN

AGRICULTURAL BOTANY

(CYTOGENETICS AND PLANT BREEDING)

OF

THE UNIVERSITY OF KERALA.

DIVISION OF AGRICULTURAL BOTANY

AGRICULTURAL COLLEGE & RESEARCH INSTITUTE

VELLAYANI

TRIVANDRUM.

1965

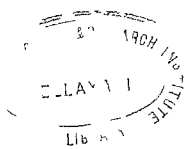
CERTIFICATE

This is to certify that the thesis submitted contains the results of bonafide research work carried out by Shri K.Muraleedharan Nayar under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

(Dr. C.K.N. NAIR)
Principal and Additional
Director of Agriculture
(Research)

(P.KUMARA PILLAI)
Vice-Principal &
Professor of Agricultural
Botany

Agricultural College and
Research Institute,
Vellayani, Trivandrum.
30--7--1965



ACKNOWLEDGEMENTS

It gives immense pleasure to the author to accord his sincere gratitude and indebtedness to Prof. P. Kumara Pillai, M.Sc., M.S. (U.S.A.), Vice Principal and Head of the Division of Agricultural Botany, and Dr.(Mrs.) Mary K. George, M.Sc., Ph.D., Junior Professor of Agricultural Botany, for their most valuable guidance and unfailing interest that they had taken throughout the progress of the present investigation.

The author is indebted to Dr.C.K.N.Nair, M.Sc., Ph.D. (Cornell), D.R.I.P. (Oak Ridge), Principal and Additional Director of Agriculture (Research), Agricultural College and Research Institute, Vellayani for the ample facilities and encouragement given for conduct of this study.

The author wishes to express his sincere thanks to Shri E.J.Thomas, M.Sc., M.S.(Iowa), Junior Professor of Agricultural Statistics, Agricultural College and Research Institute, Vellayani for his valuable advice and constant help rendered in the analysis and interpretation of the data.

The author is extremely thankful to the Oil Seeds Specialist, Agricultural College and Research Insti-

tute, Coimbatore, for supplying the seed material for the present study.

Finally, the author wishes to acknowledge with gratitude to the staff of the Botany Division, to all of his collegues and friends for the valuable help rendered by them which enabled him to complete the present endeavour successfully.

AUTHOR

C O N T E N T S

INTRODUCTION

The phenomenon of polyploidy in higher plants 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 realization that many of the most valuable crop plants, such as wheat, oats, cotton, tobacco, potato, banana, coffee and sugarcane are natural polyploids, and that many of the polyploids possessed superior economic properties over and above the diploids, led to a more exhaustive exploration into the scope of its being introduced artificially into the field of plant science, with a view to evolve promising crop varieties.

The foremost successful attempt on the artificial production of a polyploid, was made by Winkler (1916) through decapitation method. He was able to obtain a tetraploid form of Solanum nigrum from the regenerated callus tissues. Trials of many workers in order to evolve a more effective and less strenuous measure for the artificial induction of polyploids continued for a while. Cold or heat shocks, decapitation, treatments with chemicals such as acenaphthene, chloral hydrate,

ethyl mercuric chloride, benzene, veratrine sulphate etc., are some of the methods tried. However, the colchicine technique proposed by Blakeslee and Avery (1937) became universaly popular, by virtue of many of its superior qualities. Colchicine is a very effective polyploidizing agent, which can be applied to a wide range of plants with considerable ease.

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. The presence of conspicuously large and showy flowers in artificially induced tetraploids of Iberis amara (Bali and Tandon, 1957), and Cosmos bepinnatusea (Chhonkar et al, 1957), suggest the strong possibility of selection of a desirable type for introduction into the horticultural trade. Tetraploidy has been induced in fruit crops, vegetables, oil seed crops, legumes, fodder crops, tuber crops, fibre crops

spices yielding plants and in cereals. Compared with the diploid, more number of larger leaves were produced by the tetraploid Amaranthus blitum obtained by Tandon and Chinoy (1950). In addition, its vegetative cycle was observed to be prolonged.

Greater range of variability as regards size, dry matter, protein content and sugar content etc. were found in tetraploid sugarbeets and mangel (Peto and Boyes, 1940; Kloen, 1957). Glucose and corchorin content of the tetraploid seeds and leaves of Corchorus capsularis were greater (Bhaduri and Chakravorti, 1948).

Gingelly or Sesame (Sesamum orientale L.) is one of the popular oil yielding crops of India. The oil is mainly used for culinary purposes. The scope of polyploidy breeding in this crop through colchicine - technique has been studied by Richharia and Persai (1940), Kobayazhi and Shimamura (1948) and Srivastava (1956). The data obtained from these earlier works being quite inadequate, the present study has been undertaken with a view to throw more light on the following aspects.

1. To evaluate the economic aspects on the practical utilization of the colchicine - technique, in the improvement of the sesamum crop in India.

2. To make a comparison between the performance of diploids and artificially induced tetraploids with respect to the rate of growth, flower production, fruit set, fruit size, seed yield, seed weight and oil content of seeds, and

3. To study the morphological and cytological abnormalities associated with colchicine technique.

REVIEW OF LITERATURE

Colchicine was known from very early days as a specific remedy for gout. Its value as a tool in the various branches of biological research was recognised only recently.

Colchicine is a poisonous alkaloid, generally extracted from the seeds and corms of the autumn crocus, Colchicum autumnale L., belonging to the family Liliaceae. In its natural habitat, at Colchis, near the Black Sea, it grows abundantly. Various other species of Colchicum are also found to be containing colchicine, but only in limited proportions.

Colchicine in biological research:

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.

An early experimenter in plants with colchicine was Charles Darwin (1875). His studies on its effects on certain insectivorous and sensitive plants, led to no valid conclusions.

Organised botanical research with colchicine began in the United States of America, Sweden and Japan from 1937 onwards, following the reports on the unusual observations made in colchicine-treated animal cells.

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 in polyploidy 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 concentrations 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, and sugar cane are found to be natural polyploids and these polyploids possessed superior economic qualities over and above 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.

Methods of colchicine application in plants:

The principle underlying the colchicine treatment in plants is that, the alkaloid must be brought in contact with actively dividing cells. This is achieved by:-

1. Treating the seeds with colchicine solution or
2. Applying colchicine to the growing tips of the seedlings, or
3. Applying colchicine to other growing regions of the plant like roots, tubers, suckers, bulbs, flower buds, cuttings etc.

1. SEED TREATMENT

The concentration-duration effect of aqueous colchicine on seed treatment for inducing polyploidy was studied on different plant species by different authors.

Muntzing and Runquist (1939) treated seeds of Pinus ponderosa with 0.02% aqueous colchicine for 5 days and obtained polyploid varieties. Prior to treatment, the seeds were kept in moist sand at 5°C for 3

months. They were placed in the green house for germination and when the seed coats split, were treated with colchicine.

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

Ramanujam and Joshi (1941) produced tetraploid gram (Cicer arietinum) by treating soaked seeds with 0.25%, 0.5% and 1.0% aqueous colchicine for varying durations ranging from ½ to 24 hours. Treatment with 0.25% solution for ½ hour was found to be the best, from the point of view of survival of plants and induction of polyploids. They also succeeded in getting tetraploid chillies by treating seeds in 0.05%, 0.1%, 0.2% and 0.4% aqueous colchicine for 1 - 8 days.

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

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 tourne-

fortii 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 - 0.1% for 8 - 24 hours.

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

Srivastava (1955) studied the 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.

Sen and Hari (1955) showed that 0.25% colchicine for 6 hours, the best concentration - duration combination for inducing polyploidy in Vigna sinensis, by seed treatment.

Kundu and Sarma (1956, 1957) observed that effective concentration for induction of tetraploidy in Corchorus olitorius ranged from 0.0125% to 0.10% for 6 to 24 hours.

Sterbakov (1958) succeeded in getting 100%

polyploidy in many ornamental plants, the effective dose in Asparagus being a concentration of 0.1 - 0.2% treated for a period of 4 - 6 hours.

Srivastava (1956) produced fertile autotetraploids in a pure variety of Sesame (Sesamum orientale L.). 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 varying durations. After treatment seeds were washed thoroughly with water and allowed to germinate in soil in earthen pots. Only a few treated seedlings attained maturity, of which three were identified as tetraploids. 25 seeds treated with 0.04% and 0.06% for 2 and 6 hour period produced one triploid each, and 25 seeds treated with 0.10% for 4 hours produced one tetraploid.

Yokoyama and Matzui (1957) noted that in Tea, the optimum conditions for inducing polyploidy through seed treatment was with a concentration of 0.3% for a range of 180-240 hours. Better results were obtained when the temperature fluctuated over a fairly wide range, than when it remained relatively constant.

Klown (1957) induced polyploidy in 4 varieties of sugarbeet and in 3 varieties of mangel by trea-

ting germinated seeds with a 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 rasp berries by treating germinating seeds at the cracked seed-coat stage with 0.2% colchicine in 5% glucose solution at 86°F for 9 hours.

Autotetraploidy was induced in Agava cantala by Thombre and Desai (1960). Germinating seeds treated with 0.4% colchicine for 6 hours produced tetraploid plants with 2n = 90.

Saharov et al (1959) studied the concentration - duration effect for the induction of polyploidy in Sugarbeet var. Ramon.31. A higher concentration for a shorter duration of treatment enhanced the production of polyploids considerably.

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

Moffett and Nixon (1961) opined that tetraploids in Black wattle could most readily be induced by soaking the seeds in 0.02% - 0.03% colchicine for 48 hours. An alternative treatment with 0.01% - 0.02% colchicine for 48 hours also proved to be effective.

2. SEEDLING TREATMENT

Treatment of seedlings at various stages of development with colchicine has also proved to be an equally popular measure as the seed treatment for inducing polyploids in plants. In seedling treatment, colchicine is generally applied as aqueous solution, or in the form of a paste made in lanolin, agar or glycerine medium. The relative effectiveness of different combinations of concentration of colchicine, duration of treatment, and the mods of application varied from plant to plant.

Warmke and Blakeslee (1939) found that a longer period of treatment (2 days) was much more effective with 0.2% to 0.4% concentration, than a shorter treatment (1 day) with 0.8%, to induce polyploidy to tobacco seedlings.

By treating seedlings of Marigold at the 4 leaf-stage with 0.02% to 0.16% for 1 to 14 hours, Nebel and Ruttle (1939) succeeded in obtaining a number of tetraploid plants. They also obtained tetraploid petunias by painting seedlings in the rosette-stage with 1% colchicine in lanolin.

Shimamura (1939) induced tetraploidy in Lycopersicum esculentum, by subjecting growing points of seedlings to colchicine treatment. Colchicine of 0.2% in lanolin paste was applied 2 or 3 times a week.

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.

Beasley (1940) produced polyploids from 11 types of Gossypium by colchicine technique. A small slit was made about 2 cms. below the growing tip, and immersed in a vial containing o.2% aqueous solution of colchicine for 24 hours.

Emsweller et al (1940) could get tetraploid bulblets of Lilium formosanum by immersing the growing points in solutions of colchicine of 0.2% to 1.0% concentration 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.

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 shoot apex at the cotyledonary stage of growth with 0.3% to 0.5% colchicine-emulsion.

Toole and Bamford (1945) successfully doubled eight haploid peppers to their diploid forms. Strengths of 0.1% to 1.0% colchicine emulsion were smeared on the growing tips, either in one or two applications a week, or by injecting the aqueous colchicine by means of an interval injector inserted at the nodal region.

Hofmeyer (1945) could induce tetraploidy in Carica papaya by treating seedlings with 0.1% colchicine, applied in drops six times a day for 4 or 5 days.

Kobayazhi 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.

In grapes, Dermen (1954) induced tetraploidy

by applying 0.5% colchicine in 10% glycerine in water. The buds were moistened with a small drop or so of the solution, once every two days, for a total of 3 times.

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

. Zuluuga and Gargiulo (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 glycerin.

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.

Choudhury et al (1956) produced tetraploids in two varieties of Corchorus capsularis, and three varieties of Corchorus olitorius by treatment of 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.

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% concentration, applied to the growing points of seedlings for 6 hours. In Iberis amara the same concentrations for 6 to 12 hours and in Alyssum maritimum 0.20% for 6 hours, proved successful.

Knight (1957) reported the optimal concentration time combination in inducing tetraploidy in Theobroma 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 a cross between the tetraploid Arachis hypogeae (2n = 20) was found to be sterile, and with a view to overcome the sterility, a fertile allohexaploid plant (2n = 60) was produced by doubling the chromosome number by applying 0.2% aqueous colchicine to the buds of the sterile hybrid.

Sikka et al (1958) studied the effectiveness of different concentrations of colchicine and durations of 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 a time of 8 hours, and the immersion of whole seedlings of similar age in 0.05% for 4 to 8 hours respectively. The treatment was carried out at room temperature (75° to 80°F) in the case of Melilotus seedlings.

Sen and Chheda (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.

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

Kumar (1960) treated apical growing points of seedlings of Delphinum ajacus with colchicine, by dipping in different strengths of the solution for different lengths of time. 0.1% solution for 12 hours had an appreciable effect.

Sen and Bhowel (1960) induced tetraploidy in 2 varieties of Vigna sinensis. Treatment of seedlings was much more effective than that of seeds in inducing 4x sectors.

Mohanty et al (1961) produced tetraploid plants of Niger by immersing seedlings in 0.05%, 0.1%, 0.2%, 0.3% 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) in his study on the action of colchicine on the rice seedlings observed, that 20-day old plants were better than newly germinated seedlings to induce polyploidy. 0.1% colchicine solution applied for at least 2 hours was recommended for the induction of polyploids for breeding purposes.

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

Galcenko (1961) found that the optimum concentration of colchicine for polyploid induction in cucumber consisted of a 0.05% solution applied either at 18°C for 48 - 72 hours or at 33° - 35°C for 24 - 27 hours.

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

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

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.

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.

3. OTHER METHODS

Other methods include, the application of colchicine to growing regions of the plant other than stem tips, such as roots, tubers, suckers, bulbs, flower buds, cuttings etc.

Nabel and Ruttle (1938) treated cuttings of single clones of Tradescantia reflexa with 0.2% colchicine for four hours and induced totraploidy.

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

Shimamura (1939) could induce chromosome doubling by immersing the roots of Allium cepa 1.0 to 1.5 cms. in length in 0.4% aqueous colchicine for 2 hours. The same procedure was adopted by Carpentier (1954) in groundnut using 0.005% colchicine.

Sparrow (1939) obtained polyploid strains from a white flowered clone of Antirrhinum majus by treating young potted cuttings with aqueous colchicine 0.1% to 0.25% for 7 to 42 hours.

Raman and Krishnaswamy (1950) succeeded in getting an autotetraploid Pennisetum typhoides with 28 chromosomes by forcing the aqueous solution of colchicine to get into the tissues under reduced pressure.

Illies (1956) obtained giant diploid and mixoploid pollen grains by treating flower buds of birch, with a 0.2% solution of warm colchicine. The treatment was carried out in a partial vacuum, and proved effective, especially at diakinesis.

Nakasone (1961) was able to induce tetraploidy

in vanda by treating cuttings and young shoots with colchicine. The basal ends had been immersed in aqueous colchicne of 0.5% to 1.5% concentrations for 2 to 6 hours. Most of the induced tetraploids were mixoploids.

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

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

Effect of Colchicine in plants:

Colchicine, when brought in contact with actively dividing cells, interferes with the normal morphological, physiological, histological and cytological organizations of the treated materials (Blakeslee and Avery, 1937). In plants, these effects are often noticed. Plants treated with colchicine show a wide range of morphological diversities, which are associated with similar diversities at the cellular level also.

Morphological variations:

Ever since the utilization of colchicine as a polyploidizing agent in plants, morphological varia-

tions have been reported to be accompanying frequently. The first noted effect is delayed germination followed by a retardation in the growth rate. This has been pointed out by Ramanujam and Joshi (1941) in Cicer arietinum, who compared the early growths in the colchicine treated and untreated plants.

Works of many authors showed that the early retarded growth due to colchicine in many cases, is often followed by a considerably enhanced growth rate. (Pandey in Linus usitatissimum L., 1956; Srivastava in Sesamum orientale L., 1956; Saxena and Nanda in Phlox, 1960). The tetraploids are observed to take a longer time to reach maturity and flowering stage than the diploids. This was supposed to be due to their retarded growth rate (Kumar and Abraham in Phaseolus radiatus, 1942; Bali and Tandon in Alyssum maritimum, 1958).

Smith (1939), while inducing polyploidy in Nicotiana species and species hybrids by colchicine treatment 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 as compared to sterile ones.

Sando (1939) observed in colchicine treated tetraploid Fagopyron tataricum, the surface of the hull to be more rough with more prominant lateral ridges than those in the diploids.

Amin (1940) studied some of the important characteristic features of colchicine treated cotton. Swollen hypocotyledonary stem, retardation of initial growth, broader leaf lobes, prominent leaf veins, broader bracts, bigger glands, bigger flowers, bigger polpen, bigger seeds etc. were some of the characteristic features observed.

Graner (1941) found that the colchicine treated plants of Manihot utilissima possessed an average stomata length of 40 μ, had larger lobes which were suddenly constricted at the tip, in contrast to the leaves of the control, where the lobes were narrower throughout.

Ramanujam and Joshi (1941) while studying the morphology of the colchicine treated gram (Cicer

arietinum) showed that increased concentrations and longer durations caused a more pronounced swelling and a greater delay in the growth of the plumules. Many of the surviving seedlings showed characteristic abnormalities in growth in the early stages, such as curling and twisting of stem and leaves and roughening of their surface.

Shifriss (1942) noted the most distinguishing peculiarity in the tetraploid Cucumis sativus, as the accentuated serration of leaf margin. He also noted in the octoploid and other higher polyploid forms, a pronounced difference in the size of corolla, between staminate and pistillate flowers borne by the same plant.

Kobayazhi and Shimamura (1945) observed the morphological and cytological behaviour in colchicine-induced tetraploids of sesame after a period of seven years. The tetraploids were found to be extremely robust and possessed thicker stem, larger leaves and bigger stomata, flowers and seeds compared to that in diploids. Further, in the tetraploids they obtained a high fertility both in the pollen grains and the seeds.

Warmke (1945), reported that the induced tetraploid forms of Taraxacum kok-saghyz, produced more

than four times as much rubber as that produced by the normal diploids.

Rao et al (1944, 1945) opined that the tetraploid jute showed the typical gigas characters and were structurally different from the diploids. The fact that differences in the structure of the ultimate fibre are associated with changes in chromosome number, is of great economic importance as the quantity of jute depends primarily upon the nature of ultimate fibres.

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

Tandon and Chinoy (1950) observed that 30 day old colchicine treated plants of Amaranthus blitum had thicker stems, more number of leaves and branches, and larger leaves than those of the diploid plants. Larger, darker green and greater number of leaves per plant and prolonged vegetative growth in colchicine treated plants suggest a possibility of producing superior types of this, through colchicine technique.

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 control. There was a lot of variability in the size of the head, and time of its emergence in the treated plants.

Dermen (1954) induced tetraploidy in 10 varieties of grapes by colchicine technique and was successful in selecting bunch grapes with better sized berries.

Srivastava (1956) comparing diploid and tetraploid sesame observed that the tetraploids had larger leaves, larger stomatal cells, larger flowers, thicker stems, larger capsules and larger pollen grains. The main difficulty in the use of autotetraploids in a plant breeding programme was due to their sterility and poor seed set.

Spasojevic (1956) on studying colchicine induced tetraploids in bean (Phaseolus vulgaris L.) found that they were late in flowering and ripening. However, they produces larger seeds.

Sharma and Datta (1957) observed that in

Coriandrum sativum, the tetraploid plants were larger and darker in colour than the diploids. No polyploid seed had been successfully germinated.

Chopra and Swaminathan (1960) observed the seed fertility in autotetraploid watermelon was considerably lowered. By crossing 4x and 2x plants, one fruit was obtained with 67 seeds which germinated only upon removal of testa. The single triploid fruit contained many embryoless seeds.

Aleksic (1961) found in tetraploid Capsicum annum, the fruits were smaller and lighter, had thicker and higher dry matter content, than the fruits of diploid plants.

Galcenko (1961) enumerated certain outstanding characteristics observed in polyploid cucumber. They include short and thick stems, leaves which were deformed with coarse serrations, and more plumpy but short fruits. The growth habit also varied. Some were bushy, while others had a particularly short stem with determinate growth habit. A high degree of sterility was also noticed in the pollen grains.

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

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.

Cytological effects of colchicine:

Colchicine, the poisonous alkaloid is a well reputed polyploidizing agent. In spite of number of abnormalities seen in plants treated with colchicine, the technique has been found to be remarkably suitable for the artificial induction of polyploidy.

Colchicine when brought in contact with cells produces abnormalities in their morphologic and physiologic behaviour. The effects have been studied by many scientists during mitosis and meiosis.

Colchicine was first recognized as a mitotic poison. Its activity, produced cells with multiplied chromosomal complements. Similar effect was obtained when many other agencies were employed. Their effect which simulated those of colchicine was termed as C-mitotic activity.

Vaarama (1947) recorded that in the root-tips of Ribus nigrum, even a very low concentration of colchicine (0.01%) produced C-mitotic activity.

Smith and Hiner (1960) found that typical C-mitotic effect in Allium cepa could be obtained by treating the root tissues by 0.025% - 0.8% colchicine for periods varying from 3 to 12 hours.

One of the general effects of colchicine is found to be an overall decrease in the mitotic index.

Carpentier (1954) treated roots and rootlets of groundnut with 0.005% concentration of colchicine, and obtained C-mitotic cells. However, the proportion of cells in division to the total, was very much reduced.

Evans et al (1957) studied the effect of colchicine in broad beans and observed a general arrest

of the mitotic phenomenon, resulting from the accumulation of many cells at metaphase. Concentrations of 0.10%, 0.05% and 0.025% colchicine affected more or less the same extent of metaphasic accumulations. Prolonged treatments for periods over 6 hours produced considerable slow down in the rate of cells entering into mitosis, their interphases being prolonged.

Damon (1958) reported an arrest of cell division at metaphase with the production of star, exploded and ball metaphase types in the shoot cells of sorghum seedlings, which were treated with different concentrations of colchicine.

The most widely accepted effect of colchicine is the inhibition of the spindle mechanism.

Wada (1940, 49, 50) treated Tradescantia with concentrations of 0.05% - 0.10% colchicine and observed that the spindle fibres failed to develop after prophase.

Hindmarsh (1952) was of the opinion that colchicine of concentrations above 0.10% destroyed the spindle in all stages of mitosis, and thus totally inhibited their formation.

Davidson (1957) pointed out colchicine-induced chromosomal breakages in Vicia faba. This was in addition to the other effects mentioned above.

At meiosis, colchicine was found to interfere with the spindle mechanism, as in the case of mitosis. This, invariably was found to result in an unequal separation of chromosomes at anaphase, resulting in the formation of genetically unbalanced cells. In many cases the homologous chromosomes failed to synapse. Crossing over was found to be considerably suppressed. The formation of univalents and multivalents was also frequent.

Walker (1938) observed a complete as well as partial suppression of the spindle mechanism in microspore mother cells of Tradescantia paludosa, treated with colchicine. Triads, pentads, hexads, octads etc. were found to result from the partial suppression of the spindle.

Srivastava (1956) observed that in some plants of tetraploid sesame, 95% of the pollen grains were sterile. This was due to an unequal separation of the chromosomes to the opposite poles. At anaphase I, the chromosomes were observed at the poles in 26 + 26, 24 + 28, and 22 + 30 rates.

Apart from polyploids, colchicine produced occasional aneuploids after unequal separation of the chromosomes at anaphase I. Franzke and Ross (1952) reported such occurrences in sorghum.

Normal pairing of homologous chromosomes was found to be adversely affected by colchicine.

Sparrow (1942) studied that in Antirrhinum majus, colchicine delayed meiotic initiation upto eight weeks or more, after treatment. The high frequency of univalent formation (37%) was considered to be due to a failure of synapsis between homologous chromosomes. Further, crossing over was reported to be totally suppressed in the pollen mother cells after treatment.

Multivalent formation was observed by many authors as one of the universal phenomena associated with colchicine treatment in plants.

Ramanujam and Joshi (1941) studied the meiotic behaviour in the tetraploid Cicer arietinum, in comparision to the diploid. In the diploids, normal bivalents were noticed, whereas in the tetraploids, quadrivalents and trivalents were noticed in varying

frequencies. Chromosome bridges and fragments were realized in a few cells at anaphase I.

Iyengar (1944) found that in cotton, colchicine induced triploids showed marked variation in the production of multivalent configurations. However, the hexaploid showed very little deviation from the normal diploids.

Masima and Uchiyamada (1955) studying the cause of sterility in autotetraploid rice, observed that it was highly correlated to a high frequency of quadrivalent configurations, noticed at diakinesis and metaphase I.

Sen and Chheda (1958) observed a fairly low frequency of quadrivalents in tetraploid Phaseolus mungo; triads, pentads, hexads and septads were counted in larger number.

Islam (1960) found comparatively lesser number of quadrivalents in the tetraploids of Annona squamosa L. Trivalents and univalents were noticed instead. Lagging chromosomes were also seen at anaphases I and II.

Raman and Kesavan (1963) studied the chromosome associations at diakinesis and metaphase I in autotetraploid Arachis duranensis. In the 38 pollen mother cells

examined, the frequency of quadrivalents ranged from 0 to 8, the most frequent association being $6_{IV} + 8_{II}$. In a few cells with trivalents, a corresponding number of univalents was not present.

MATERIALS AND METHODS

A. MATERIALS

Seeds:

Seeds of Sesamum orientale L. of strain TMV 2 was selected for the present investigation. Of the five different strains collected for preliminary studies, TMV 2 was specially selected on the basis of its comparatively high germinability above the remaining four. This material was supplied by the Oil Seeds Specialist, Agricultural College and Research Institute, Coimbatore.

Colchicine:

Colchicine, manufactured by the British Drug House, was used for all the treatments.

B. METHODS

It is proposed to conduct treatments with four different concentrations of colchicine for three separate durations, through two modes of application.

Concentration of Colchicine

1. 0.05% - C1
2. 0.10% - C2
3. 0.15% - C3
4. 0.20% - C4

Duration of application

1. 3 Hrs. - T1
2. 6 ,, - T2
3. 9 ,, - T3

Modes of application

1. Seed Treatment - S
2. Seedling Treatment - P

TREATMENT COMBINATIONS

TABLE 1

Pot No.	Concentration.	Duration	Mode of application.	Treatment combination.
1	0.05%	3 Hrs.	Seed treatment	C_1T_1S
2	,,	3 ,,	Seedling ,,	C_1T_1P
3	,,	6 ,,	Seed ,,	C_1T_2S
4	,,	6 ,,	Seedling ,,	C_1T_2P
5	,,	9 ,,	Seed ,,	C_1T_3S
6	,,	9 ,,,	Seedling ,,	C_1T_3P
7	0.10%	3 Hrs.	Seed treatment	C_2T_1S
8	,,	3 ,,	Seedling ,,	C_2T_1P
9	,,	6 ,,	Seed ,,	C_2T_2S
10	,,	6 ,,	Seedling ,,	C_2T_2P
11	,,	9 ,,	Seed ,,	C_2T_3S
12	,,	9 ,,	Seedling ,,	C_2T_3P
13	0.15%	3 Hrs.	Seed treatment	C_3T_1S
14	,,	3 ,,	Seedling ,,	C_3T_1P
15	,,	6 ,,	Seed ,,	C_3T_2S
16	,,	6 ,,	Seedling ,,	C_3T_2P
17	,,	9 ,,	Seed ,,	C_3T_3S
18	,,	9 ,,	Seedling ,,	C_3T_3P
19	0.20%	3 Hrs.	Seed treatment	C_4T_1S
20	,,	3 ,,	Seedling ,,	C_4T_1P
21	,,	6 ,,	Seed ,,	C_4T_2S
22	,,	6 ,,	Seedling ,,	C_4T_2P
23	,,	9 ,,	Seed ,,	C_4T_3S
24	,,	9 ,,	Seedling ,,	C_4T_3P
25				Control 1
26				Control 2

Fig. 1. Plan of lay out of the Experiment.

Design: 26 x 4. Randomized Block

REP I

25 8 10 1 7 3 2 17 15 9 23 11 14

20 6 24 26 5 11 18 16 13 8 22 12 19

REP III

6 13 4 10 15 15 19 24 8 21 1 12 9

22 22 11 20 22 11 17 23 26 18 12 16 3

REP II

3 8 15 23 5 3 3 11 20 11 1 25 14

8 20 8 8 20 20 8 22 16 10 13 12 4

REP IV

1 20 21 12 26 24 18 6 4 16 11 23 9

8 25 7 10 3 2 11 19 22 12 12 11 17

4

2

2

PLAN OF LAYOUT OF THE EXPERIMENT

FIG 1

In order to study statistically, the relative merits of the different concentrations, durations, applications, and their different combinations, a Randomised Block Design with four replications was used in this experiment.

The plants were raised in pots, under identical conditions. The arrangement of pots is as shown in Fig.1. The pot number corresponding to the various treatment combinations is presented in Table I.

Seed Treatment:

Seeds soaked in distilled water for 15 hours were allowed to dry on blotting paper for 30 minutes, before they were transferred to small vials containing colchicine solutions of desired concentration. After 30 minutes they were arranged in petridishes containing filter paper moistened with the same concentration of colchicine. After the durations prescribed for the treatment, the seeds were thoroughly washed in distilled water and kept on wet blotting paper for initial germination. After twelve hours, the seeds were sown in pots. For control, seeds soaked in distilled water for the desired time duration were used.

Seedling Treatment:

Seeds were directly sown in pots without prior soaking, as in seed treatments, and the apical buds of 12-day old seedlings were subjected to treatment. The application was carried out as follows:-

The Colchicine solution of the appropriate concentration was applied drop by drop, using a glass dropper so as to wet the plugs, made up of sterilized absorbent cotton wool, kept at the region of apical buds. Considerable care was taken to see, that the plugs were kept moist throughout the time of treatment. After the treatment, the plugs were removed and the seedlings were thoroughly washed with distilled water. Plants treated identically, but with distilled water instead of the Colchicine solution, were utilized as Control.

The following characters were studied:-

1. Germination Percentage:

Germination trials were conducted separately. The different concentration-duration combination of treatments were given to the lots of 100 seeds, which were washed thoroughly after the application of colchicine. They were allowed to germinate on moist filter papers, kept

in patridishes. The germination percentage was calculated after allowing ten days for seeds to germinate. In addition, counting of germinated seeds was made every day.

2. Early deformity of seedlings:

Seedlings showed considerable range of abnormalities. The studied aspects involved the early growth of the plant, and the nature of hypocotyl, cotyledons and leaflets.

3. Height of plants:

The height was measured in the case of individual plants from the 15th day of sowing to the day of harvest. Weekly measurements were recorded, so as to study the rate of growth and the total height of individual plants at harvest.

4. Leaf characters:

a) Mean area of leaves: Measurement of area of leaves was made in sq. cms., using graph paper technique. For this, ten randomly selected leaves were collected from the middle 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 middle of the plants, when they were 45 days old. It was recorded in micra (μ) as measured from 100 hand sections for each treatment.

5. Size and distribution of stomata:

A sample of 10 leaves was collected randomly from each treatment, and 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 standardized ocular micrometer. The frequency of stomata per unit area was determined, by counting their number from 100 fields taken at random for each treatment. The data for size and distribution of stomata were analysed statistically.

6. Number of flowers:

Daily flower counts were made from each plant, under different treatments and the final data were statistically analysed.

7. Cytological observations:

By observing the gigas characters peculiar to polyploids, the suspected polyploids were labelled separately. The flower buds of appropriate size were

fixed in a 3:4:1 mixture of absolute alcochol, chloroform and propionic acid, which has been previously saturated with ferric chloride. The fixation was done between 11 and 12 A.M. for 24 hours, after which the material was stored in 70% ethyl alcochol. Anthers were squashed in a drop of 1% propionocarmine. Gentle tapping and judicious warming favoured excellent spreading and differential staining of the chromosomes and cytoplasm in the microsporocytes.

The chromosome number and their behaviour such as formation of multivalents, secondary associations etc., of the polyploids were photographed and camera lucida drawings were made from such preparations. The behaviour of chromosomes in diploids was also studied.

8. Size and sterility of pollen grains:

The size and sterility of pollen grains were studied from an entirely random sample. 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 micra (μ), for each treatment, using a standardized ocular micrometer.

For the estimation of sterility, ten fields were scored for sterile and fertile grains from each slide at random. In this way 100 microscopic fields were scored for each treatment from which the percentage of sterility was calculated.

9. Number and size of fruits:

The total number of fruits, set in each plant was counted and the data were analysed statistically.

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

10. Yield of seeds:

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

11. Weight of 1000 seeds:

Weight of 1000 seeds from each treatment was recorded in grams and the data were statistically analysed.

12. Estimation of oil content:

The oil from the polyploid seeds as well as in seeds from other treatments was extracted by percolation method, and the results were recorded in percentages.

After weighing accurately 0.3 grams of dry seeds from each treatment, they were grind with 1 gram of specially prepared glass powder and an equal quantity of anhydrous sodium sulphate, in a dry glass mortar. The mixture was transferred to a percolator which provided with a pad of cotton wool at the bottom, over which stood ¼" thickness of anhydrous - sodium sulphate. An aluminium cup with 4 pieces of 1" square filter paper was weighed, and brought under the percolator. 5-7 cc. of the solvent Carbon - Tetrachloride was poured to the percolator to wet the contents. More and more (about 20 cc.) of the solvent was added to the percolator to extract the oil fully. The material collected in the dish was allowed to evoporate in an air oven and weighed again until consecutive values agreed. From the difference in weight, the percentage of oil was calculated.

Statistical procedure:

The recorded data pertaining to the different

characters under investigation were subjected to statistical analysis. The effect of different treatments on the sterility of pollen grains was calculated by applying chi-square test. Analysis of variance was worked out for four characters namely height of plants, number of stomata, number of flowers and number of fruits. The effect of different treatments on other characters under study were determined by calculating the mean and standard error.

EXPERIMENTAL RESULTS

1. (a) Percentage of Germination:

Generally the strain TMV2 of Sesamum orientale L. possesses a remarkably high percentage of germination. Seeds treated with colchicine of varying concentration-duration combinations showed a slightly reduced percentage of germination.

(b) Time taken for germination:

Seeds treated with colchicine in most cases took a longer time for germination. The pertaining data on this aspect and the percentage of germination are appended in Table II

2. Early deformity of seedlings:

The colchicine treated plants were symptomatized by a number of morphological abnormalities. They include retarded growth, the presence of unusually thick cotyledons, development of malformed leaves which were thick, dark and crinkled and considerably stunted stem (Plate I). Of the total 480 plants treated, 85 showed such symptoms of early deformity. Cytological studies were later conducted on these isolated deformed plants,

TABLE II

Concentration-duration combination.	Percentage of seeds germinated for every 24 hours										Total percentage of germination
	1	2	3	4	5	6	7	8	9	10	
Control	13	50	85	92	95	-	-	-	-	-	95
0.05% 3 Hrs.	--	33	77	84	87	90	-	-	-	-	90
6 ,,	--	15	77	88	89	90	-	-	-	-	90
9 ,,	--	13	72	83	86	--	90	91	-	-	91
0.10% 3 Hrs.	--	24	85	89	90	91	92	--	-	-	92
6 ,,	2	25	80	86	87	89	--	--	-	-	89
9 ,,	--	10	56	83	85	--	86	--	87	-	87
0.15% 3 Hrs.	--	37	45	80	84	90	--	91	92	-	92
6 ,,	--	8	51	67	68	71	72	73	75	-	75
9 ,,											
0.20% 3 Hrs.	--	21	79	86	88	--	89	--	--	-	89
6 ,,	--	12	63	73	80	82	--	84	--	-	84
9 ,,	--	4	42	80	82	84	--	86	--	-	86

and it was found that 27 out of these 85 were polyploids.

The growth of seedlings:

The growth of seedlings was found to be practically retarded for the first five days in the case of 3 hours, treatment, and for seven days in the case of 6 and 9 hours treatments. The leaves developed immediately after the treatment were small, thick and malformed and irregularities were noted in their arrangements. In two plants raised from 0.20% for 9 hours and in one plant from 0.15% for 6 hours of seed treatments, three to five leaves seemed to emerge from each node instead of the usual two in untreated plants. Newly formed leaves in the abnormal plants were thicker, darker in colour and had coarser texture than those in the untreated.

Size and shape of early leaves:

Changes in the size and shape of leaves were also noted and recorded. Five plants raised from 0.10% colchicine for 3 hours, and two from 0.20% for 9 hours of seed treatments and 4 seedlings treated with 0.15% for 9 hours, produced only bilobed leaves for the first two weeks. First two leaves produced by a plant in

0.15% for 4 hours seed treatment were trilobed, the 3rd and 4th leaves produced by a plant in 0.10% for 3 hours seed treatment were bifoliate and 2nd, 4th and 5th leaves produced by a plant in 0.20% for 9 hours seod treatment were found to be trifoliate.

3. Height of plants:

The overall height of the treated plants at the time of harvest showed significant increase, when compared to that of the control.

Comparing the effect of different concentrations, a slight increase in height was observed in 0.20% (Plate 2B) followed by 0.15%. Between duration, there was a significant increase, in the case of 9 hours treatment. The effect of interaction between concentrations and durations was also found to be highly significant. The difference, however, in the case of seed and seedling treatments was found to show no significance.

The analysis of variance for the height of plants for different treatments is given in Table III.

Graphical representations of the height of plants under different concentration-duration combination are given in Figs.2 and 3.

TABLE III

Analysis of variance

For the height of plants at the time of harvest

Source	Sum of squares	D.F.	Variance	F.ratio
Total	16177.85	103		
Block	74.49	3	24.80	0.72
Treatment	13530.10	25	541.20	15.78**
Seed Vs. Seedling	0.66	1	0.66	0.019
Between concentrations	7231.41	3	2410.47	70.3**
Between durations	1205.02	2	602.51	17.6**
Treated Vs. control	2208.02	1	2208.02	64.3**
Interaction between concentration x duration	1495.65	6	249.27	7.27**
Error	2573.36	75	34.30	

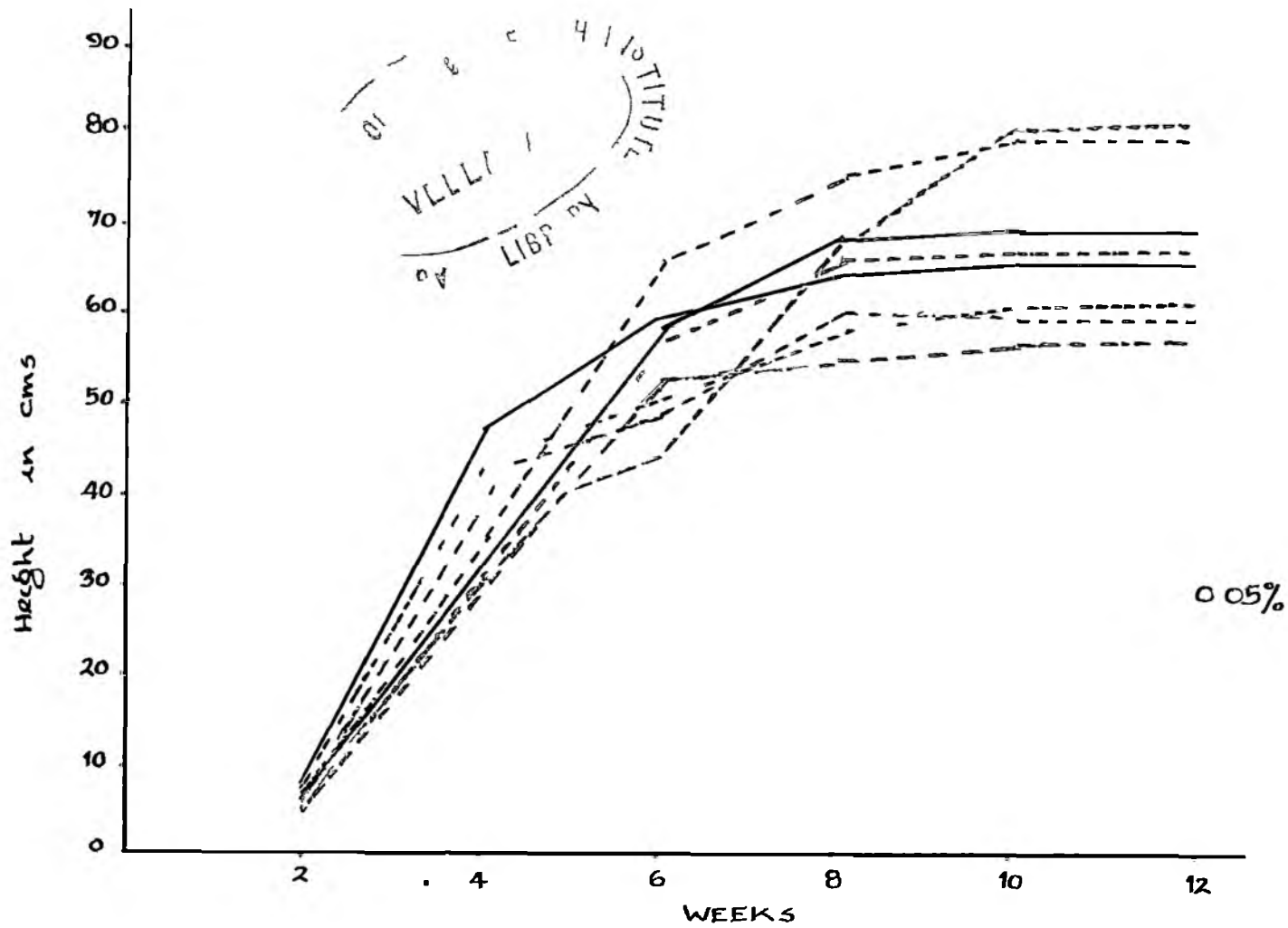
** Significant at 1% and 5% levels.

Critical difference = 5.7428

Ranking treatments

C4T2 C4T3 C3T3 C4T1 C2T2 C3T2 C1T3 C3T1 C2T1 C1T2 C1T1 C2T3 COTO

Fig. 2. Graphical representation of the total height of plants under 0.05% and 0.10% concentrations.



control 1
3 hrs
3 hrs
6 hrs
6 hrs
9 hrs
9 hrs
Control 2

Height of Plants

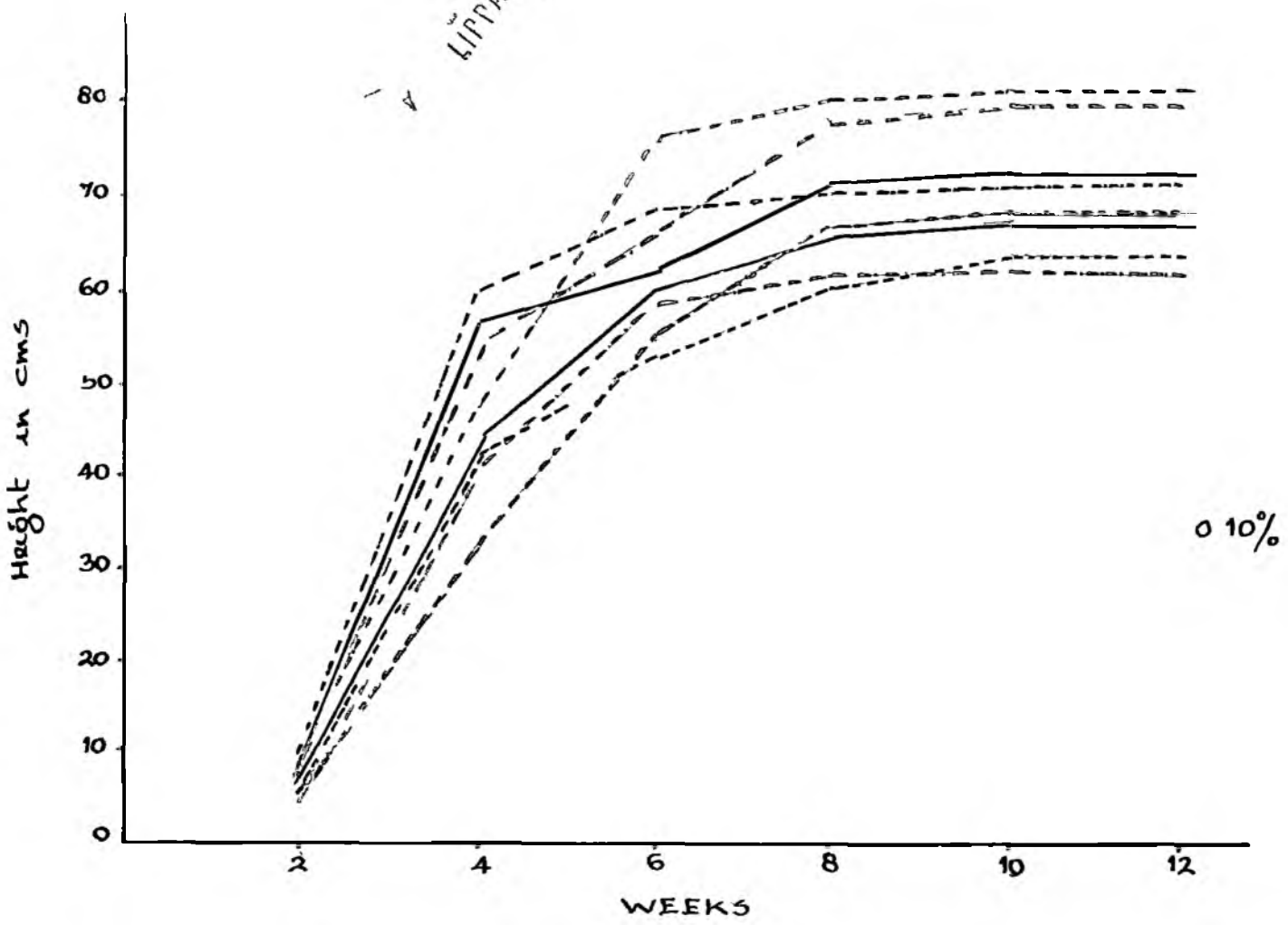
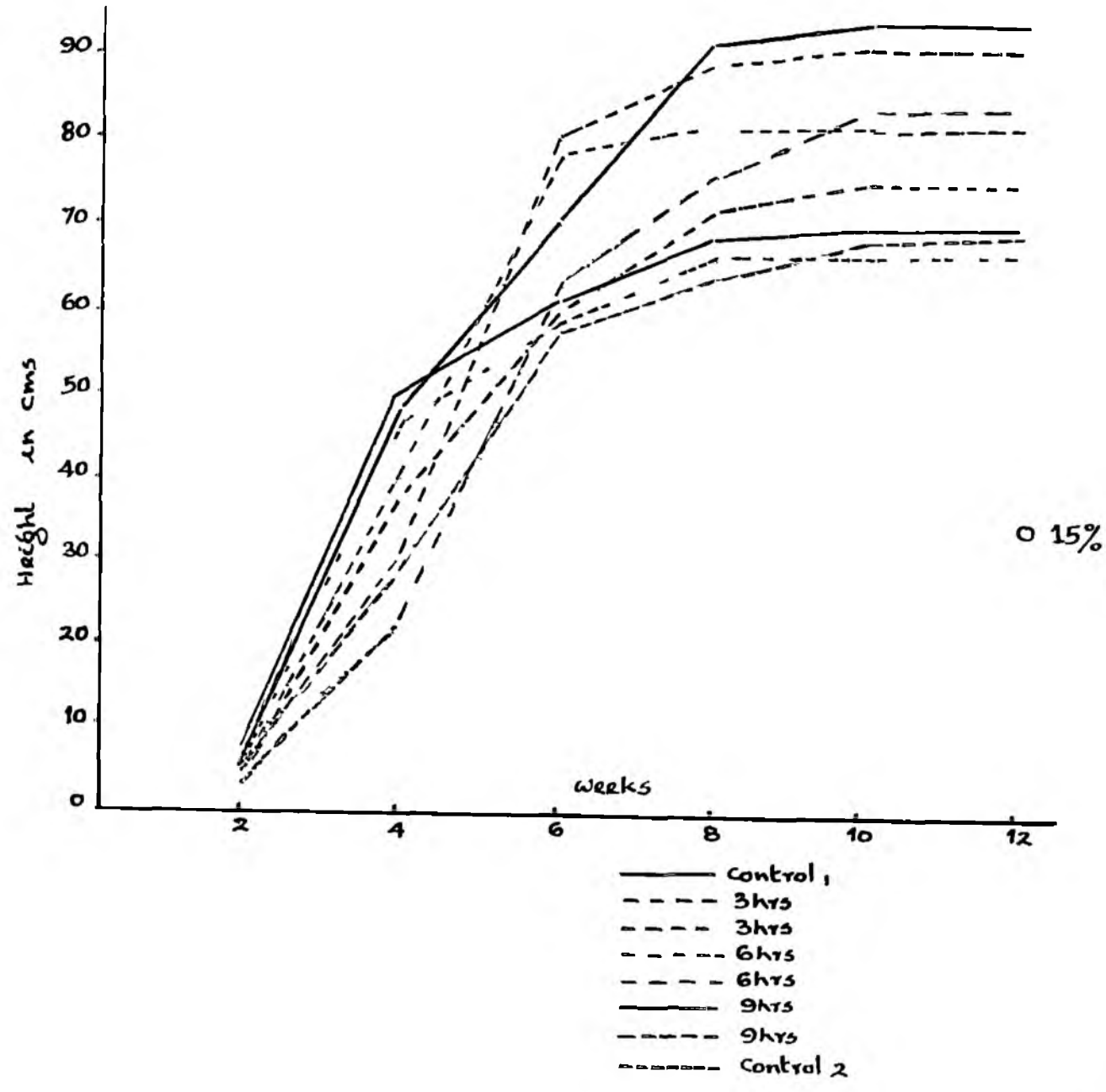


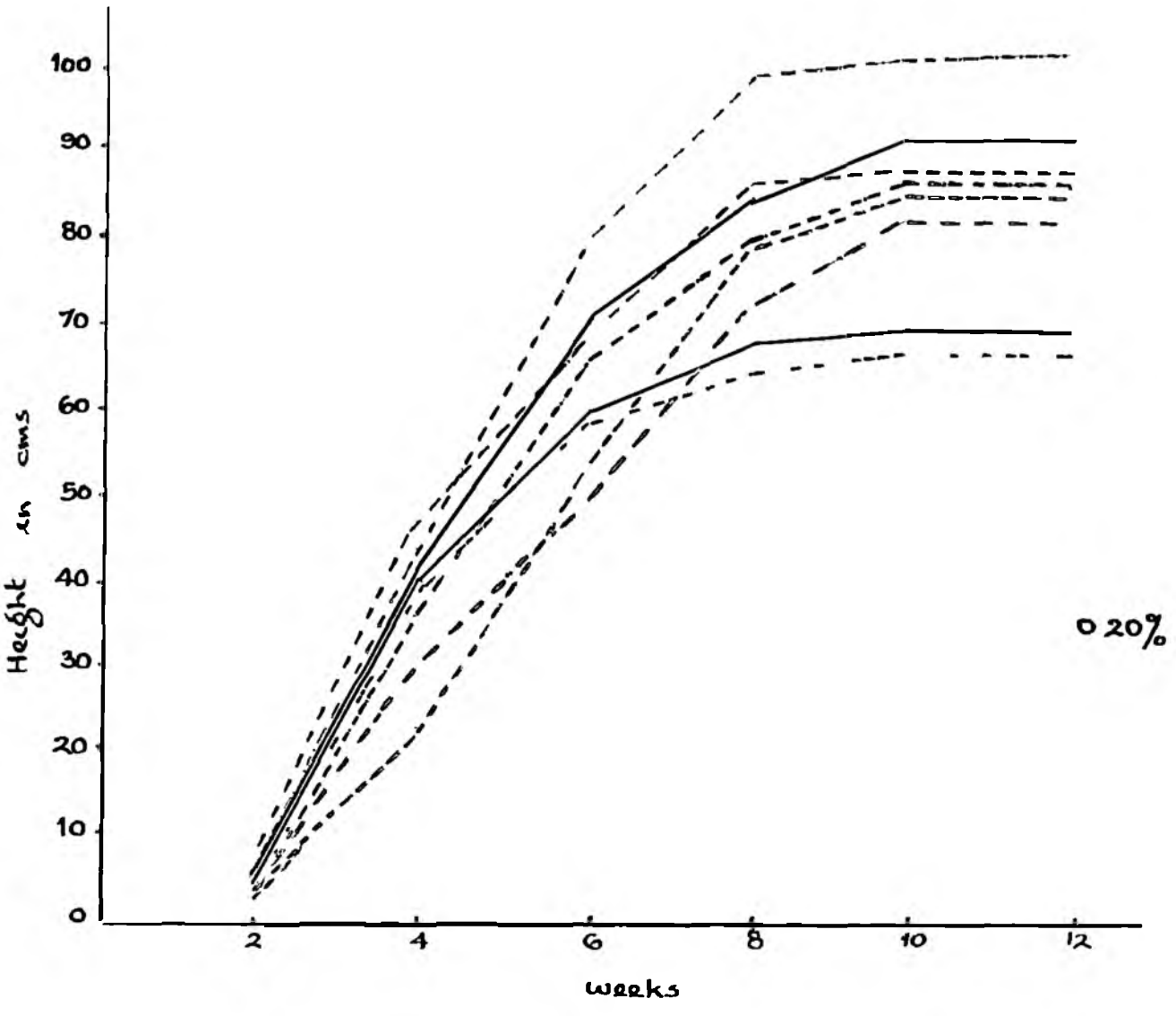
FIG 2

Fig. 3. Graphical representation of the total height of plants under 0.15% and 0.20% concentrations.



0 15%

Height of Plants



0 20%

FIG 3

4. Leaf characters:

(a) Area of leaves:

The treated plants possessed larger and broader leaves than the diploid plants under control (Plate 2A). The results given below, show the comparison of the mean area of leaves in different treatments.

Mean area of leaves in Sq. cms.

1. Between Diploid and Tetraploid.	Diploid	- 36.15 ± 1.652
	Tetraploid	- 68.76 ± 0.732
2. Treated Vs. Control	Treated	- 66.207 ± 0.923
	Control	- 36.15 ± 1.652
3. Between Concentrations	0.05%	- 64.33 ± 1.820
	0.10%	- 66.558 ± 0.410
	0.15%	- 66.542 ± 0.154
	0.20%	- 69.059 ± 0.336
4. Between Durations	3 hours	- 65.874 ± 1.960
	6 ,,	- 64.863 ± 0.390
	9 ,,	- 67.291 ± 1.106
5. Seed Vs. Seedling	Seed	- 68.00 ± 1.112
	Seedling	- 64.417 ± 0.934

The mean area of leaves in the diploid and tetraploids were found to be 36.15 sq. cms. and 68.76 cms. respectively (Table VIII). In different treatments, the

mean area varied from 54.50 to 75.61 sq. cms. Further the comparison of different concentrations showed no significant difference between 0.10% and 0.15% treatments. Of the four different concentrations 0.20% was found to be superior. Among the different durations, 6 hours treatment was found to be least effective. Seed treatment produced an increased leaf area than the seedling treatment.

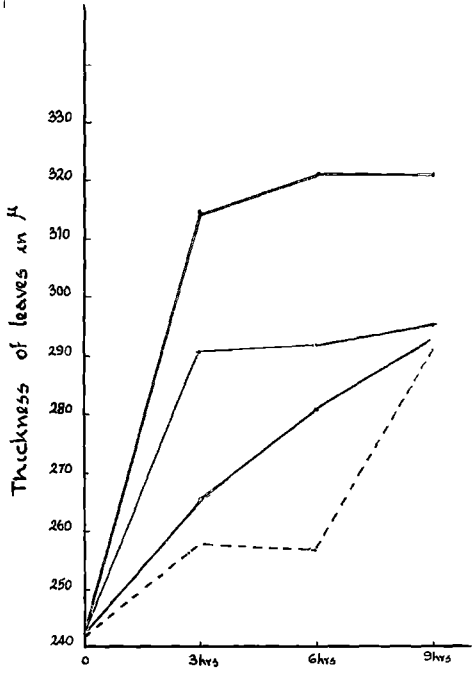
(b) Thickness of leaves:

The thickness of leaves was measured from transverse sections. It was evident that in tetraploid plants, leaves were characterised by thicker leaves than those in the diploids (Plates 3 and 4). The data for the mean thickness of leaves in different treatments were compared.

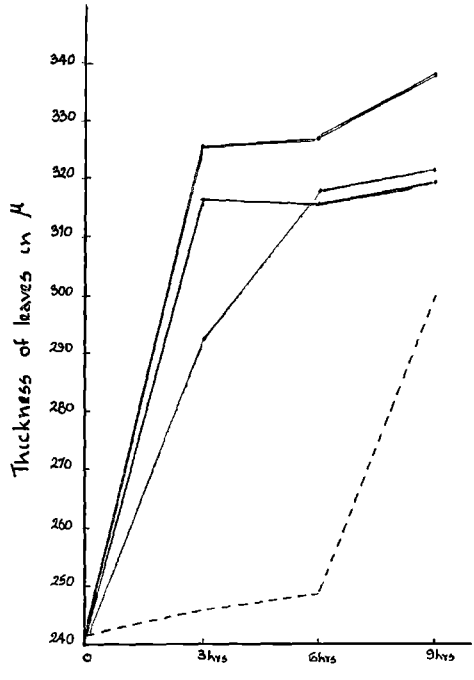
Mean thickness of leaves in μ

1.	Between diploid and tetraploid	Diploid	- 241.74	± 2.451
		Tetraploid	- 307.04	± 5.517
2.	Between treated and control	Treated	- 295.61	± 6.358
		Control	- 241.74	± 2.451
3.	Between concentrations	0.05%	- 265.44	± 15.572
		0.10%	- 297.04	± 9.530
		0.15%	- 293.88	± 12.008
		0.20%	- 323.90	± 11.127

Fig. 4. Graphical representation of the mean thickness of leaf.



SEED TREATMENT



SEEDLING TREATMENT

THICKNESS OF LEAF

FIG 4

4. Between durations	3 hours	-	287.718	± 8.051
	6 ,,	-	293.09	± 10.112
	9 ,,	-	303.36	± 6.410
5. Seed Vs. Seedling	Seed	-	287.56	± 8.697
	Seedling	-	303.94	± 4.171

The mean thickness of leaves in diploids and tetraploids were found to be 241.74 μ and 307.04 u respectively (Table VIII) and in different treatments it varied from 246.48 μ to 338.12 μ (Table XI). Maximum thickness of leaves was obtained in 0.20% for 9 hours-seedling treatment. Between durations, 9 hours, and between the stages of applications, seedling treatment was found to be the best.

Fig.4 gives the graphical representation of the mean thickness of leaves in various treatments.

5. Size and distribution of stomata:

Considerable increase was noticed in the size of stomata in treated plants, but their distribution per unit area was scanty (Plates 5 and 6).

(i) Size: In the leaves of treated plants, both the length and width of stomata showed an increase, when compared to that in diploids kept under control.

(a) Length: The tetraploids had a mean stomata length of 28.93 μ. In control it was only 22.40 μ (Table VIII). The recorded data on the mean length of stomata, in all the treatments were statistically compared.

Mean length of stomata in μ

1. Between diploid and tetraploid	Diploid	-	22.40 ± 2.685
	Tetraploid	-	28.93 ± 1.730
2. Treated Vs. Control	Treated	-	28.61 ± 2.014
	Control	-	22.40 ± 2.685
3. Between concentrations	0.05%	-	26.810 ± 4.125
	0.10%	-	28.960 ± 2.430
	0.15%	-	28.735 ± 1.970
	0.20%	-	28.845 ± 2.903
4. Between durations	3 hours	-	28.773 ± 2.613
	6 ,,	-	28.351 ± 2.400
	9 ,,	-	28.957 ± 1.774
5. Seed Vs. Seedling	Seed	-	28.420 ± 0.931
	Seedling	-	29.005 ± 1.110

From the result it is evident, that the colchicine treatment could increase the length of stomata. Maximum length of stomata was noted in 0.15% for 6 hours and 0.20% for 9 hours seedling treatment, closely followed

by 0.15% for 6 hours seed treatment. Comparing the stages of application, seedling treatment resulted in a slightly higher stomatal length than in seed treatment.

(b) Width: The width of the stomata in the tetraploids was also found to be increased. The mean width in the diploids was found to be 17.25 μ and in tetraploids, 19.62 μ (Table VIII).

Mean width of stomata in μ

1. Between Diploid and tetraploid	Diploid	- 17.25	± 2.271
	Tetraploid	- 19.62	± 1.540
2. Treated Vs. Control	Treated	- 20.10	± 4.46
	Control	- 17.25	± 2.271
3. Between concentrations	0.05%	- 20.05	± 5.30
	0.10%	- 19.39	± 2.795
	0.15%	- 20.51	± 2.30
	0.20%	- 20.10	± 1.730
4. Between durations	3 hours	- 20.09	± 3.316
	6 ,,	- 19.88	± 1.010
	9 ,,	- 20.19	± 2.274
5. Seed Vs. Seedling	Seed	- 19.842	± 0.784
	Seedling	- 19.953	± 1.253

In general, in colchicine treated plants, an

increase in the width of stomata was noticed.

The maximum width of stomata was obtained in 0.20% for 6 hours seedling treatment. 0.15% was found to be superior to 0.05%, 0.10% and 0.20% in increasing the width of stomata in combination with 9 hours duration. But no significant difference was noted between the seed and seedling treatments.

The data of the mean length and width of stomata in different treatments are given in Tables IX, X, XI and XII.

(ii) Number of Stomata:

The tetraploid showed larger stomata which were fewer in number per unit area (Plate 5 and 6). The data on stomata counts were statistically analysed and the results are given in Table IV. The analysis showed that there was significant difference in the number of stomata between treatment and the control. The effect of different concentrations, durations, interaction between concentrations and duration and the effect of treatment of different stages were found to be highly significant.

The minimum number of stomata was found in

TABLE IV

Analysis of variance

For the number of stomata per unit area

Source	S.S.	d.f.	Variance	F.ratio
Total	57069.19	1299		
Treatments	39392.31	25	1575.7	114.2**
Treated Vs. Control	19882.58	1	19822.58	1524.07**
Seed Vs. Seedling	75.85	1	75.85	5.4**
Between concentrations	14038.91	3	4679.64	338.00**
Between durations	1163.65	2	581.82	42.00*
Interaction between concentration x durations	2943.33	6	490.55	35.00**
Error	17676.88	1274	13.87	

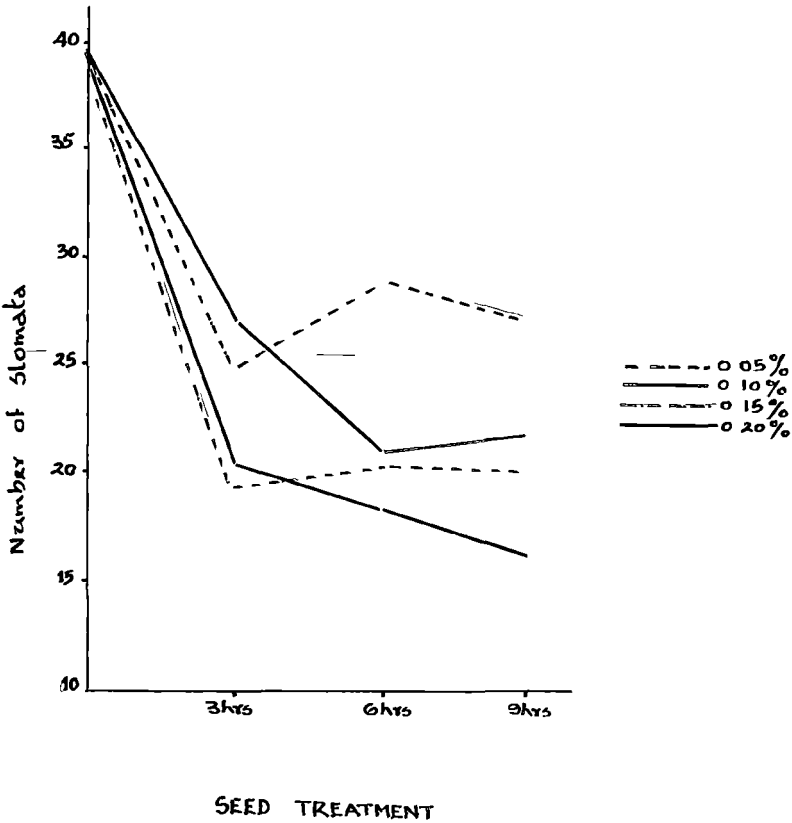
** Significant at 1% and 5% levels

Critical difference - 1.0192

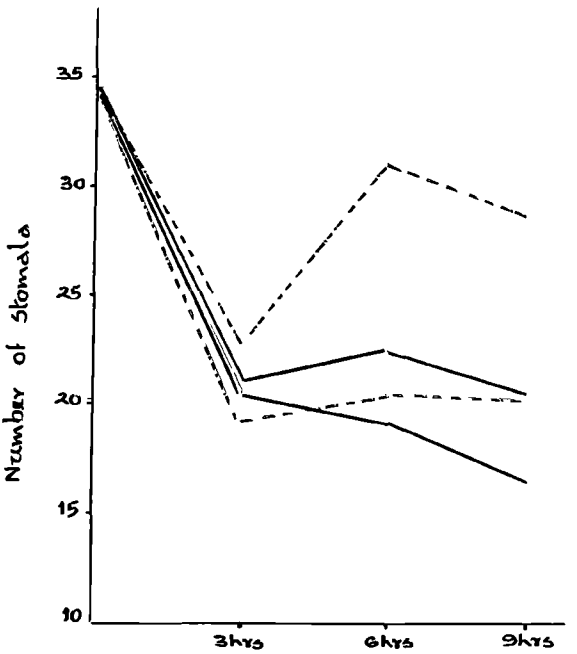
Conclusion:

C0T0, C1T2, C1T3, C1T1, C2T1, C2T3, C2T2, C4T1, C3T3, C3T2, C3T1, C4T2, C4T3.

Fig. 5. Graphical representation of the distribution of stomata.



DISTRIBUTION OF



STOMATA

FIG 5

0.20% for 9 hours seedling treatment while the maximum number was obtained in the untreated plants followed by 0.05% for 6 hours seedling treatment.

The distribution of stomata is graphically represented in Figure 5.

6. Number of flowers:

The flowers borne by tetraploid plants were conspicuously larger than those in the diploids (Plate 7), but the blooming date in the tetraploids was very much delayed. The data pertaining to flower counts in various treatments were subjected to statistical analysis, and the analysis of variance is given in Table V.

The data showed that there was significant increase in the production of flowers in the colchicine treated plants. Maximum number of flowers was produced in 0.20% for 3 hours seedling treatment. The effects of treatments at different stages of application, different concentrations and different durations and interaction between concentration and duration were not found to be statistically significant.

Total number of flowers produced in each treatment is graphically represented in Fig.6 and the average

TABLE V

Analysis of variance

For the number of flowers

Source	S.S.	d.f.	Variance	F.ratio
Total	119141.76	103		
Block	983.41	3	327.80	
Treatment	41995.01	25	1679.80	1.65*
Seed Vs. Seedling	3162.51	1	3162.51	3.11
Between concentrations	4796.28	3	1598.76	1.57
Between durations	2410.02	2	1205.01	1.18
Treated Vs. Control	16742.02	1	16742.02	16.48**
Interaction between concentrations and durations	2511.57	6	418.59	0.411
Error	76163.34	75	1015.51	

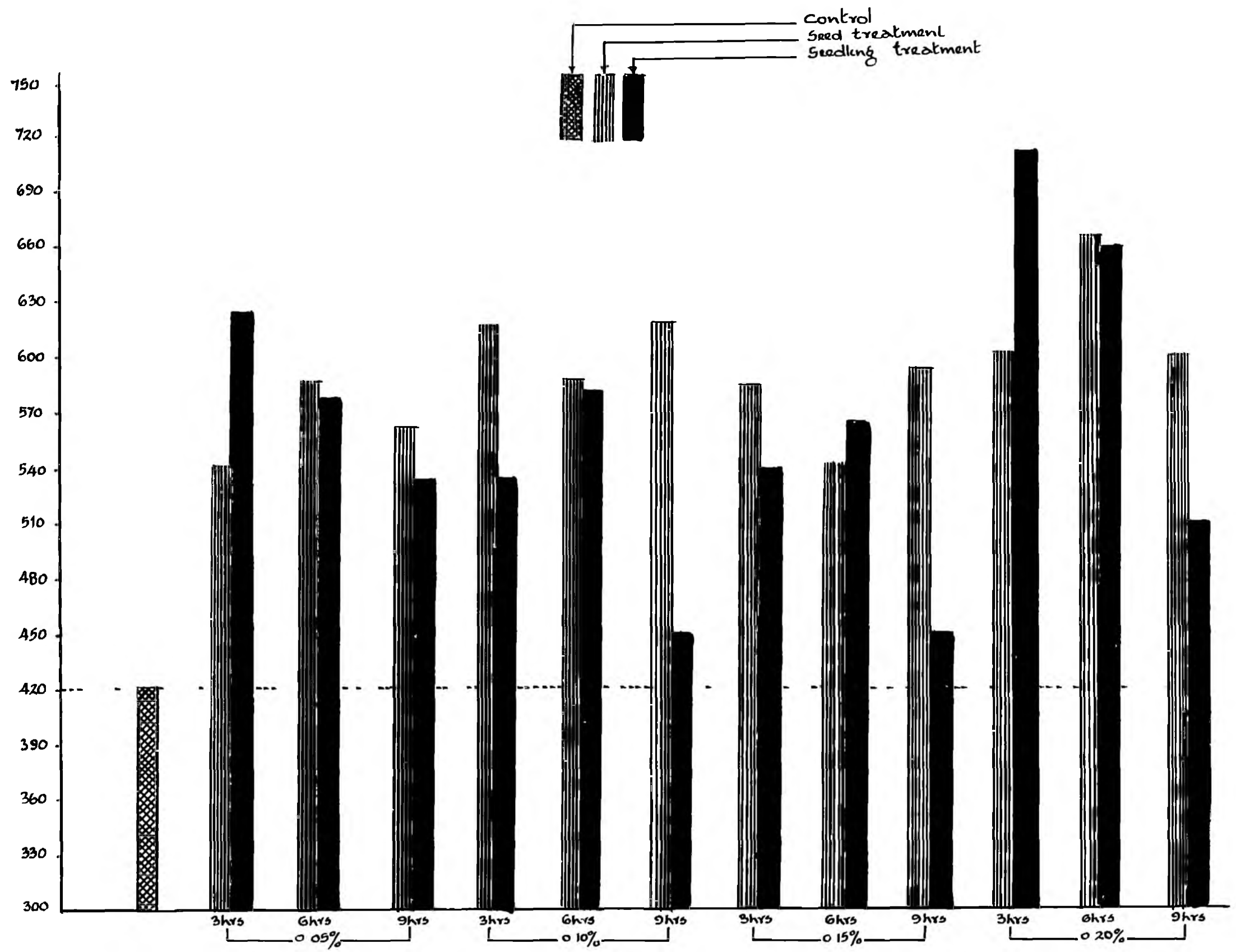
* Significant at 5% level

** Significant at 1% and 5% levels.

Conclusion:

For F ratio for treated Vs. control alone is highly significant. That is, the treatment has a significant effect on the production of flowers.

Fig. 6. Graphical representation of the total flower production under various treatments.



TOTAL NUMBER OF FLOWERS

FIG 6

number of flowers produced by plants in different treatments is included in Tables IX, X, XI and XII.

7. Cytological observations:

The behaviour of chromosomes at meiosis was studied in the treated plants as well as in the untreated plants. In general, in the diploids, the meiosis was found to be normal. The formation of 13 regular bivalents at diakinesis and metaphase I was noticed (Plate 8(a)-9a). The separation of chromosomes at anaphase I and II was also regular - 13 + 13 (Plate 8(b)-9b). In tetraploids, 26 bivalents could be counted at metaphase I which lacked the characteristic orientation at the equator of the spindle (Plate 9c). First anaphasic separation resulted in two groups of 26 chromosomes in each (Plate 10).

Chromosome association at diakinesis and metaphase I was studied in a large number of pollen mother cells. The frequencies of univalents and trivalents were more than those for higher associations (Plate 11).

Persistence of one or more secondary nucleoli was a peculiarity, throughout the meiotic stages in the

pollen mother cells of diploids as well as polyploid plants. In the plants where the pollen sterility was higher, the microspores in the tetrads varied in their relative size, and in some of the microspores, more than one nucleolus was observed (Plate 8S). Any other type of meiotic irregularity could not be observed.

8. Size and sterility of pollen:

(a) Size of pollen: The tetraploids had larger pollen grains than the diploids. From the results it was observed that the pollen grains in the tetraploids possessed a mean diameter of 63.301 μ, while it was only 53.25 μ in the diploids.

Diameter of pollen grains in μ

1. Diploid Vs. Tetraploid	Diploid	-	53.25 ± 0.630
	Tetraploid	-	63.301 ± 4.90
2. Treated Vs. Control	Treated	-	65.00 ± 2.791
	Control	-	53.25 ± 0.630
3. Between concentration	0.05%	-	58.975 ± 1.955
	0.10%	-	64.670 ± 0.790
	0.15%	-	63.505 ± 2.2925
	0.20%	-	68.703 ± 0.5320

4. Between duration	3 hours	- 61.425	± 2.471
	6 ,,	- 64.30	± 5.312
	9 ,,	- 63.910	± 4.1280
5. Seed Vs. Seedling	Seed	- 63.84	± 0.984
	Seedling	- 63.12	± 0.771

The maximum mean diameter of pollen grains was noted in 0.20% for 9 hours seed treatment, and the minimum in the untreated ones, followed by 0.05% for 3 hours seed treatment and 0.05% for 6 hours seedling treatment. Comparing the effect of concentrations, 0.20% and 0.10% were found superior to the rest of the concentrations, and between different durations, 9 hours was found to be the best. Of the two stages of applications seed treatment resulted in increased pollen diameter than the seedling treatment.

(b) Extent of pollen sterility: The analysis of the mean percentage of sterility is given in Table VI. In the tetraploids the sterility varied from 31.91 to 43.90% (Table X and XII). In the diploids, 16.00% of the pollen was found to be sterile. 0.15% for 9 hours seed treatment resulted in the highest percentage of pollen sterility. The effect of treatments in the two stages of application was not statistically significant.

TABLE VI

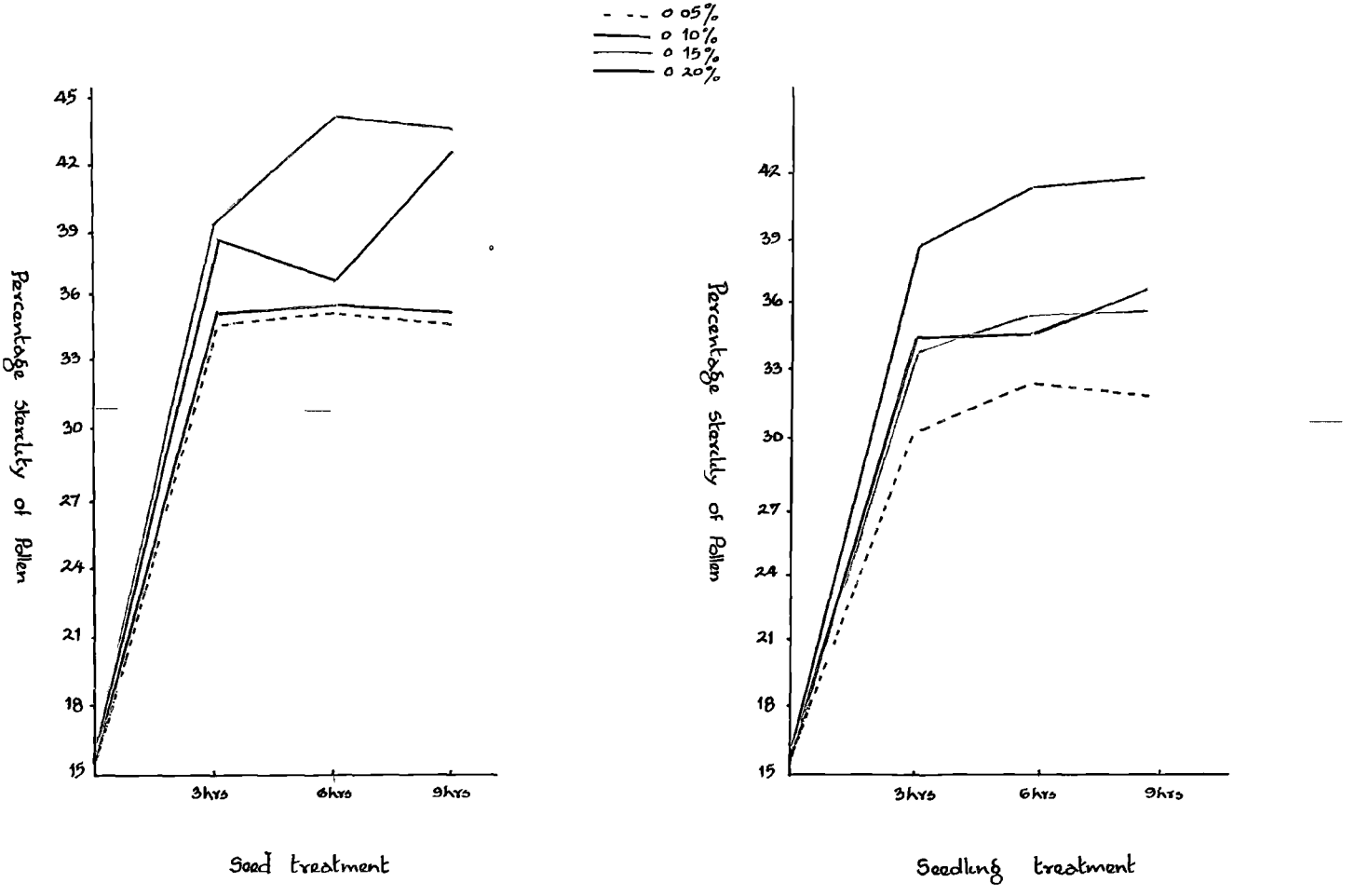
Analysis of variance

For the Sterility of pollen

Treatment	Value of X^2
Treated Vs. Control	46.35**
Seed Vs. Seedling	3.28
Between durations	77.32**
Between concentrations	80.80**

** Significant at 5% and 1% levels.

Fig. 7. Graphical representation of the percentage of sterility of the pollen grains.



STERILITY OF POLLEN

FIG 7

But the effects of concentrations and durations were found to be highly significant. Graphical representation of the percentage of sterility in different treatments is given in Fig.7.

9. Number and size of fruits:

(i) Number of fruits: The percentage of fruit set in the treated plants was found to be considerably decreased. The F-ratios for different stages of application, duration of treatment, concentrations and interaction between concentration and duration were not found to be significant. Graphical representations of the number of fruits is given in Fig.8). The analysis of variance of the data on the total number of fruits in different treatments is shown in Table VII.

(ii) Size of fruits:

(a) Length: The length of fruits was recorded in cms. The mean length of fruits in diploids and tetraploids were found to be 2.10 and 2.95 cms. respectively (Table VII).

Length of fruits in cms.

1. Between diploids and tetraploids	Diploids	-	2.10 ± 0.461
	Tetraploid	-	2.95 ± 0.317

TABLE VII

Analysis of variance

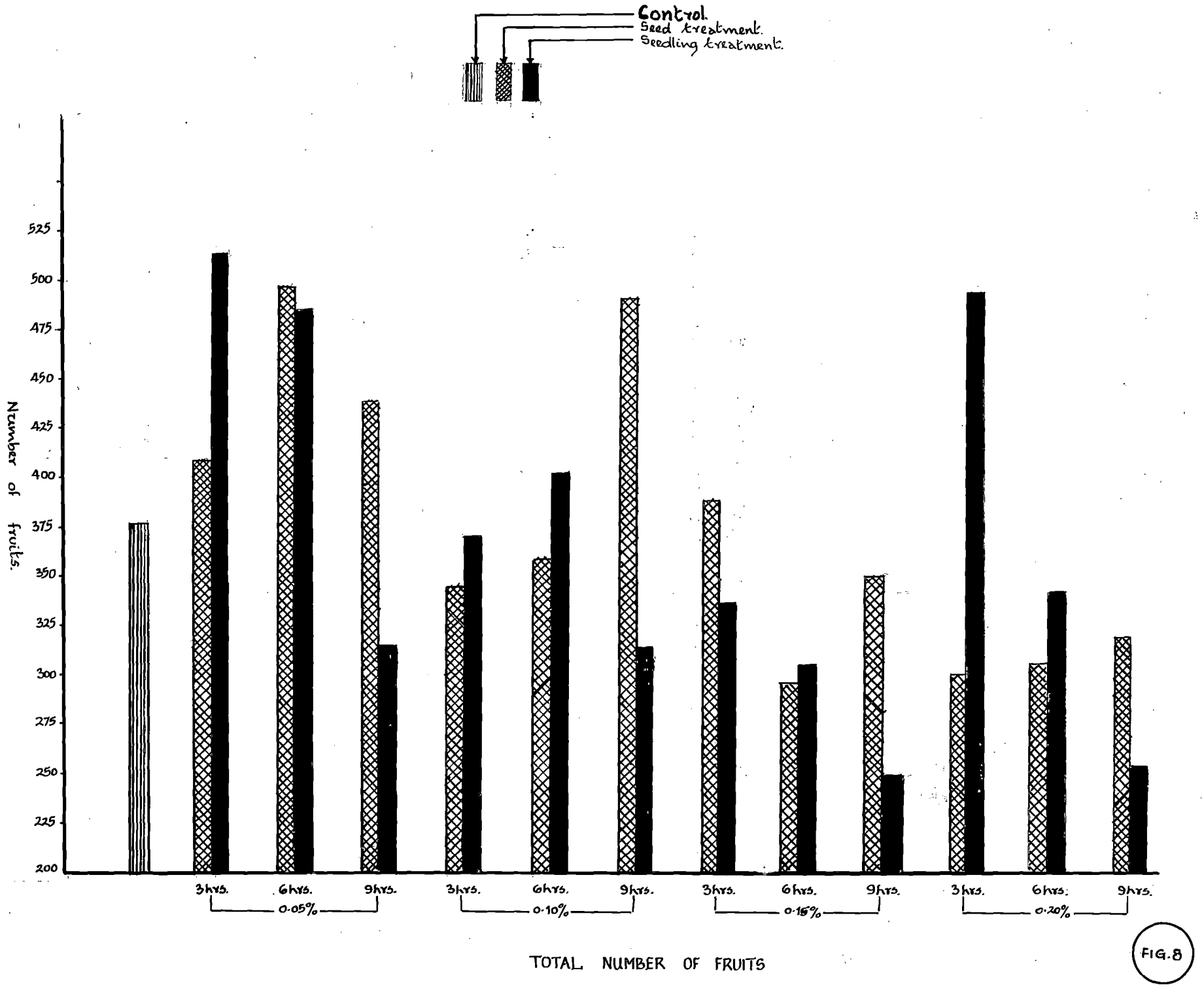
For the number of fruits

Source	S.S.	d.f.	Variance	F.ratio
Total	78584.62	100		
Block	755.77	3	251.92	
Treatment	27547.12	25	1101.88	1.64
Between seed Vs.Seedling	356.51	1	356.51	0.531
Between concentrations	3727.86	3	1242.62	1.85
Between durations	1229.69	2	614.84	0.915
Treated Vs. Control	1302.59	1	1302.59	1.94
Error	50281.73	75	670.42	

Conclusion:

The F.ratios are not significant

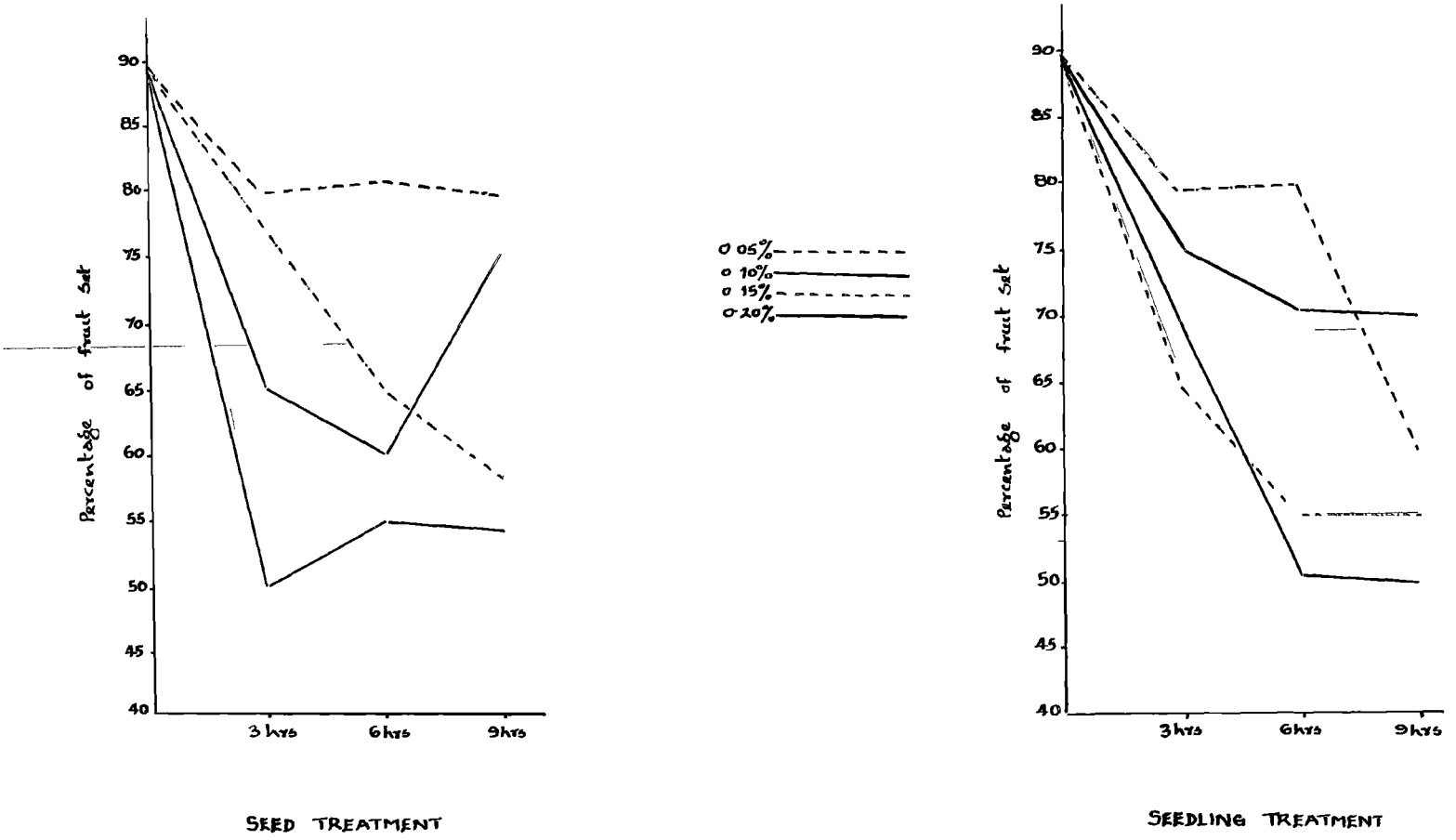
Fig. 8. Graphical representation of the total number of fruits under various treatments



TOTAL NUMBER OF FRUITS

FIG. 8

Fig. 9. Graphical representation of the percentage of fruit set.



SEED TREATMENT

SEEDLING TREATMENT

PERCENTAGE OF FRUIT SET

FIG 9

2. Between Treated and control	Treated	- 2.71	± 0.910
	Control	- 2.10	± 0.461
3. Between concentrations	0.05%	- 2.66	± 0.774
	0.10%	- 2.90	± 0.503
	0.15%	- 2.69	± 0.916
	0.20%	- 2.81	± 0.831
4. Between duration	3 hours	- 2.64	± 0.33
	6 ,,	- 2.43	± 0.135
	9 ,,	- 2.76	± 0.514
5. Seed Vs. Seedling	Seed	- 2.84	± 0.370
	Seedling	- 2.56	± 0.102

The comparative study of mean length of fruits showed that the treatment had resulted in a slight increase in the length of fruits, over the control. No appreciable difference in length was observed in different concentrations, durations and stages of applications, However, a slight increase in length was noted in 0.10% for 9 hours, seed treatment.

(b) Girth of fruits:

The data of mean girth of fruits in diploids, tetraploids and in other treatments were compared.

1. Between diploids and tetraploids	Diploids	- 3.53	± 0.263
	Tetraploid	- 4.61	± 0.195

2. Between treated and control	Treated	- 4.397	± 0.310
	Control	- 3.53	± 0.206
3. Between concentrations	0.05%	- 4.315	± 0.610
	0.10%	- 4.286	± 0.315
	0.15%	- 4.495	± 0.221
	0.20%	- 4.462	± 0.430
4. Between durations	3 hours	- 4.503	± 0.217
	6 ,,	- 4.414	± 0.253
	9 ,,	- 4.453	± 0.130
5. Seed Vs. Seedling	Seed	- 4.207	± 0.911
	Seedling	- 4.587	± 0.360

The results of comparison revealed that the fruits in diploids possessed a mean girth of 3.53 cms. while in tetraploids the girth varied from 4.13 to 4.86 cms. (Plate 12), comparison of different concentrations showed that treatment with 0.15% resulted in higher fruit girth in combination with 3 and 9 hours durations of treatments. No significant difference in girth was noted between the stages of application.

The data on the mean girth of fruits are given in Tables IX, X, XI and XII.

10. Yield of seeds:

Seed counts from a sample of ten fruits col-

lected at random from the tetraploid and diploid plants showed that there was a considerable decrease in the number of seeds in the tetraploids. The results of comparison are given below:

1. Diploid and tetraploid	Diploid	=	925.00
	Polyploid	=	863.35
2. Treated Vs. Control	Treated	=	831.12
	Control	=	925.00
3. Between concentrations	0.05%	=	782.67
	0.10%	=	897.70
	0.15%	=	826.34
	0.20%	=	817.83
4. Between durations	3 hours	=	807.63
	6 ,,	=	836.87
	9 ,,	=	830.85
5. Seed Vs. Seedling	Seed	=	869.00
	Seedling	=	800.00

Minimum number of seeds was counted in treatment with 0.05% followed by 0.20%, and maximum number in 0.10%. Treatment for 3 hour and 9 hour durations produced lesser number of seeds than treatment for 6 hours. Comparatively, the seedling treatment yielded lesser number of seeds than seed treatment.

The data on seed counts in different treatments are given in Tables IX, X, XI and XII.

11. Weight of 1000 seeds:

The seeds from the fruits in tetraploids were bigger in size than from that of the diploids (Plate 13). An increase in weight was noted as well, of such seeds. Tho following results confront the mean weight of 1000 seeds in different treatments.

Weight of 1000 seeds in grams

Between diploid and Tetraploid	Diploid	=	2.06 grams.
	Tetraploid	=	2.87 ,,
Between Treated and Control	Treated	=	2.64 ,,
	Control	=	2.06 ,,
Between concentrations	0.05%	=	2.595 "
	0.10%	=	2.491 "
	0.15%	=	2.438 "
	0.20%	=	2.975 "
Between durations	3 hours	=	2.657 "
	6 ,,	=	2.816 "
	9 ,,	=	2.731 "
Seed Vs. Seedling	Seed	=	2.540 "
	Seedling	=	2.736 "

The mean weight of 1000 seeds in tetraploids was found to be 2.817 grams, against 2.06 grams in diploids. A maximum 1000 seed weight was observed in 0.20% for 6 hours seed as well as seedling treatments. Comparatively, the seed treatment resulted in lesser 1000 seed weight than in seedling treatment.

12. Cytological identification of Tetraploids:

Based on chromosome number in the meiotic metaphasic and anaphasic preparations, twenty seven treated plants were confirmed to be tetraploids in the present study.

The following tables give the details regarding the total number of seeds or plants treated, number of suspected polyploids isolated in each group by morphological observations, and the actual number of plants those were confirmed as tetraploids by cytological analysis.

Seed Treatment				
Concentration.	Duration.	No. of seeds treated.	No. of suspected polyploids by morphological observation.	Tetraploids confirmed.
0.05%	3 Hrs.	20	3	--

0.05%	6 Hrs.	20	4	--
,,	9 ,,	20	6	2
0.10%	3 ,,	20	5	1
,,	6 ,,	20	3	2
,,	9 ,,	20	3	--
0.15%	3 ,,	20	5	--
,,	6 ,,	20	6	4
,,	9 ,,	20	3	1
0.20%	3 ,,	20	5	2
,,	6 ,,	20	8	4
,,	9 ,,	20	5	3
Total		240	56	19

Percentage of polyploid obtained = 7.91

Concentration	Duration	No. of plants treated	No. of suspected polyploids by morphological observations.	Number confirmed.
0.05%	3 Hrs.	20	1	--
,,	6 ,,	20	1	--
,,	9 ,,	20	3	1
0.10%	3 ,,	20	-	--
,,	6 ,,	20	2	--
,,	9 ,,	20	4	--

0.15%	3 Hrs.	20	3	-
,,	6 ,,	20	3	1
,,	9 ,,	20	2	1
0.20%	3 ,,	20	3	2
,,	6 ,,	20	5	2
,,	9 ,,	20	2	1
Total		240	29	8

Percentage of polyploid obtained = 3.33

13. Oil content:

The oil from a known quantity of seeds, from tetraploids as well as diploids was extracted, by the percolation method. It was inferred that the percentage of oil in the tetraploid seeds was slightly lesser than in the diploids (Tables X and XII).

TABLE VIII

Comparison between diploids and tetraploids

No.	Characters	Diploid	Tetra-ploid
1.	Height of plants at the time of harvest	67 cms.	82.51 cms.
2.	Mean area of leaves	36.15 sq.cms.	68.76 sq.cms.
3.	Mean thickness of leaves	241.74 μ	307.04 μ
4.	Average number of stomata per unit area	37.13	21.44
5.	Mean length of stomata	22.40 μ	28.93 μ
6.	Mean width of stomata	17.25 μ	19.62 μ
7.	Average number of flowers per plant	20.25	29.75
8.	Mean sterility of pollen	16.00%	38.36%
9.	Mean diameter of pollen	53.25 μ	63.30 μ
10.	Mean length of fruits	2.10 cms.	2.95 cms.
11.	Mean girth of fruits	3.53 cms.	4.61 cms.
12.	Average number of fruits per plant	17.90	17.41
13.	Average percentage of fruit set	89.50	60.33
14.	Number of seeds from 10 fruits	925	863
15.	Mean weight of 1000 seeds	2.06 gm.	2.82 gm.
16.	Mean percentage of oil content in seeds	51.337%	50.048%
17.	Chromosome number (2n)	26	52

TABLE IX

Seed Treatment

Treatments.	Mean height of plants in cms.	Mean area of leaves in cms.	Average thickness of leaves in μ.	Mean length of stomata in μ.	Mean width of stomata in μ.	Average No. of stomata	Average No. of flowers	Mean diameter of pollen in μ
Control	68.00	45.92	241.74	22.40	17.00	39.48	21.00	53.50
0.05% 3 Hrs.	80.50	66.51	259.12	26.95	19.25	24.72	26.15	58.10
6 ,,	65.51	67.62	258.80	27.65	19.14	29.20	29.45	59.59
9 ,,	74.82	71.30	290.72	27.00	19.60	28.86	28.05	59.45
0.10% 3 Hrs.	70.00	65.60	265.98	29.35	18.64	25.14	31.30	62.97
6 ,,	81.55	70.87	282.80	28.00	18.60	21.14	29.40	62.49
9 ,,	73.48	71.15	292.30	29.35	19.64	22.20	30.05	69.30
0.15% 3 Hrs.	90.25	71.68	290.34	28.00	19.95	19.16	29.40	62.37
6 ,,	83.74	61.84	290.60	29.65	20.65	20.92	26.40	63.30
9 ,,	95.00	64.40	296.50	28.00	19.75	20.76	29.45	59.65
0.20% 3 Hrs.	88.70	61.43	316.00	27.94	18.77	21.10	29.90	69.16
6 ,,	103.00	68.69	322.16	28.35	20.44	18.60	33.10	68.95
9 ,,	94.60	75.61	321.66	28.76	20.61	17.40	30.45	71.12

TABLE X

Seed Treatment

Treatments	Percentage of pollen sterility	Mean length of fruits in cms.	Mean girth of fruits in cms.	Average No. of fruits.	No. of seeds from 10 fruits.	Weight of 1000 seeds in grams.	Oil content in percentage.
Control	16.00	2.26	3.53	18.90	935	2.105	51.67
0.05% 3 Hrs.	34.71	2.62	3.76	20.80	860	2.172	48.30
6 ,,	35.23	2.80	4.16	24.43	880	2.153	51.00
9 ,,	35.05	2.71	4.23	21.84	864	3.000	50.67
0.10% 3 Hrs.	35.90	2.33	4.19	17.19	945	3.000	49.67
6 ,,	36.00	2.67	4.28	17.70	928	2.155	51.70
9 ,,	35.74	2.91	4.07	24.80	847	2.153	49.30
0.15% 3 Hrs.	39.07	2.63	4.23	19.62	750	2.000	47.30
6 ,,	43.82	2.64	4.25	17.99	720	2.340	51.00
9 ,,	43.90	2.86	4.13	14.04	982	2.740	49.33
0.20% 3 Hrs.	37.34	2.75	4.29	14.70	803	2.530	49.00
6 ,,	37.16	2.84	4.42	16.83	856	3.100	51.65
9 ,,	43.87	2.95	4.48	18.31	864	2.930	49.96

TABLE XI

Seedling Treatment

Treatment		Mean height of plants in cms.	Mean area of leaves in cms.	Average thickness of leaves in μ	Mean length of stomata in μ	Mean width of stomata in μ	Average No. of stomata	Average No. of flowers	Mean diameter of pollen in μ
Control		66.00	26.40	241.74	22.40	17.50	34.78	19.25	53.00
0.05%	3 Hrs.	72.00	73.20	246.48	26.95	18.90	22.84	31.55	58.80
	6 ,,	63.72	53.40	249.64	26.60	18.55	32.40	24.25	58.45
	9 ,,	81.40	54.50	300.20	28.35	19.60	28.64	26.45	61.60
0.10%	3 Hrs.	64.50	55.30	316.00	29.40	19.00	21.62	24.50	63.00
	6 ,,	80.68	66.10	315.58	29.05	19.25	23.81	29.00	60.20
	9 ,,	69.25	67.00	317.53	29.38	19.67	21.62	22.50	66.50
0.15%	3 Hrs.	74.33	66.00	293.88	28.70	20.44	18.74	26.00	59.20
	6 ,,	68.00	60.30	319.16	29.75	20.34	20.71	28.00	60.25
	9 ,,	84.15	64.80	319.68	29.05	20.65	21.40	22.50	60.20
0.20%	3 Hrs.	88.20	64.00	325.48	28.88	20.30	21.40	35.85	65.10
	6 ,,	80.63	68.50	325.80	29.05	20.69	18.62	33.00	68.95
	9 ,,	78.28	73.50	338.12	29.75	20.00	16.10	25.50	68.60

TABLE XII

Seedling Treatment.

Treatment	Percentage of pollen sterility	Mean length of fruits in cms.	Mean girth of fruits in cms.	Average No. of fruits.	No. of seeds from 10 fruits.	Weight of plant in gms.	Oil content in percentage.
Control	16.00	2.20	3.45	18.90	915	2.02	51.00
0.05% 3 Hrs.	31.91	2.50	4.87	24.50	660	2.20	49.00
6 ,,	32.00	2.64	4.54	23.98	630	2.68	47.33
9 ,,	32.00	2.61	4.33	15.90	800	2.55	49.00
0.10% 3 Hrs.	34.86	2.77	4.25	18.36	900	2.95	51.00
6 ,,	34.10	2.65	4.54	20.30	888	2.70	47.76
9 ,,	37.10	2.68	4.51	15.90	830	2.50	49.00
0.15% 3 Hrs.	32.54	2.55	5.79	14.90	686	2.25	51.30
6 ,,	34.00	2.66	4.18	15.10	970	2.80	51.33
9 ,,	35.65	2.80	4.33	12.30	850	2.74	49.67
0.20% 3 Hrs.	38.18	2.70	4.67	24.60	826	2.90	48.00
6 ,,	42.19	2.62	4.86	16.80	760	3.20	51.00
9 ,,	42.55	2.66	4.18	13.00	658	3.00	48.67

D I S C U S S I O N

The object of the present investigation was to conduct an evaluation of the scope for applying the colchicine technique in improving one of the major oil yielding plants - *Sesamum orientale* L., variety TMV_2.

Sesame, like many other crops plants, responded well to colchicine. This was evident from the fact that, almost all plants which were subjected to colchicine treatment, exhibited considerable extent of morphological deformities. However, as a polyploidizing agent, colchicine was less effective in sesame. Out of a total of 85 plants which showed morphological abnormalities, only 27 were found to be possessing multiplied chromosomal complements.

Polyploidy had played a major role in the natural evolution of many plant groups, and artificial induction of polyploidy in plants had consequently acquired a top position among many other methods as a valuable tool in plant breeding. It must, however, be combined with other methods for obtaining a specific purpose. (Stebbins, 1956).

In sesame, the colchicine technique had been

applied by Richharia and Persai (1940), Langham (1940), Kobayazhi and Shimamura (1945) and Srivastava (1956), with a view to evolving tetraploid strains, and obtained tetraploids. However, no further research was continued so as to evaluate the scope of utilizing this technique in the improvement of sesame. In this crop, any method of improvement could be considered desirable, only if, it enabled an enhanced production of oil.

In the present investigation, it was proved that the tetraploids could be produced in sesame through colchicine technique, but their proportion was found to be very low.

In general, almost all the treated plants were found to be affected by the drug colchicine. This was evident from their germination, early growth habit, nature of leaves and many other characters.

Germination in colchicine-treated seeds:

The strain TMV_2 possessed 95% of germination, which started from the first 24 hours after sowing and completed germination within five days. However, the seeds treated with colchicine germinated only after a period of 48 hours. The initial growth was also slow, as was evident from a

longer duration taken for full establishment of seedlings. Similar results were obtained by Smith (1939) in Nicotiana langsdorfii and N.tabaccum, Ramanujam and Joshi (1941) in Cicer arietinum, Sarma and Datta (1951) in Coriandrum sativum, Saxena and Nanda (1951) in phlox.

Colchicine is a poisonous drug, whose effect interferes adversely with the normal behaviour of the living cells. Its effect as an inhibitor of mitotic divisions (Carpentier, 1954), as an arrestor of already initiated mitotic division at the stage of metaphase (Evans et al 1957) and as a general toxic substance on the living tissues probably were the reasons for the delayed germination of seeds.

In the colchicine treated seeds, the emergence of the shoots and roots was delayed. Such observation was made earlier by Amin (1940) in cotton, Kumar and Abraham (1942) in Phaseolus radiatus, Saito (1957) in American water melons. This was again considered to be due to the effect of colchicine as a mitotic inhibitor.

Seed Vs. Seedling treatments:

From the present observation, it was evident that the seed treatment was more successful in inducing

polyploidy in sesame, than seedling treatment. The superiority of the seed treatment had been reported by Smith (1939) in Nicotiana glauca, Pal et al (1941) in Capsicum annum, Ramadujam and Joshi (1941) in Cicer arietinum L., Bragdo (1955) in Trifolium pratense, and Kundu and Sarma (1956) in Corchorus olitorius. However, a contradictory view had been proposed by Kumar and Abraham (1942) in Phaseolus radiatus, Evans (1955) in Trifolium pratense and Sen and Bhowel (1959) in Vigna sinensis, who noted, that on comparison, seedling treatment was more succesful in the induction of polyploidy. Amin (1940) reported that both seed and seedling treatments were equally effective in inducing polyploidy in cotton.

The soaked seeds normally contained more number of cells in active division. On the other hand, in seedlings, dividing cells are fewer in number and confined in specific regions. These could very well be the reasons why the emergence of polyploids from seedlings, was relatively low. For successful induction of polyploidy in any material, chances should be provided for more number of actively dividing cells, to come in contact with the drug (Stebbins, 1950).

Early deformity of seedlings:

Like many other plants, colchicine-treated sesame also showed a number of morphological abnormalities in their early growth. Of the total 480 plants treated, 85 showed symptoms of abnormalities, such as general stunting, crinkling and malformation of cotyledons and leaves. On cytological tests, only 27 out of these 85 were confirmed as true tetraploids with 2n = 52. From this, it was found that colchicine, eventhough was capable of producing morphological deformities in sesame, was a comparatively poor polyploidizing agent. This too, probably, was because of the interference of the drug with the normal biochemical path ways associated with the early growth and development of the organism.

Height of plants:

Confirmed tetraploids in the present study showed an increased height at the time of harvest especially with 0.20% for 6 hours treatment. Towards the first two weeks, the seedlings of tetraploids grew at a slower rate when compared with the normal diploids. Thereafter, they were found to surpass the diploids, and ultimately gained an increased height. This was in agreement with the results of Srivastava (1956) in the

same crop. An increased elongation of the shoots as a result of colchicine treatment, had been reported by Tandon and Chinoy (1950) in Amaranthus blitum, and Sharma and Datta (1951) in Coriandrum sativum. Contradictory results with respect to the height of plants treated with Colchicine, had been arrived at by Smith (1939) in Nicotiana rustica and N.glauca, Kundu and Sarma (1957) in Corchorus olitorius and Sen and Chheda (1958) in Phaseolus mungo.

The increased stature shown by the colchicine treated plants was probably due to the stimulation produced by the drug on the growing parts. Towards the time of application, the tissues might have been more sensitive to the toxicity, which took time to regain many of their normal features and consequently the stimulative effect might have become pronounced.

Leaf characters

Area and thickness of leaves:

It was observed in the present study, that the tetraploids possessed larger, coarser, darker and thicker leaves than those in the diploids. This was in agreement with the findings of Richharia and Persai (1940) and

Shimamura and Kobayazhi (1945) in the same crop, Kumar and Abraham (1942) in Phaseolus radiatus, Tandon and Bali (1957) in Linaria vulgaris, Vakili (1962) in Musa, Ammal (1962) in Rauvolfia serpentina, Sobti (1963) in Mentha piperata and Vig (1964) in Cyamopsis - psoralioides D.C.

In the present study it was observed that treatment with 0.20% for 9 hours resulted in the maximum area and thickness of the leaves. It was seen due to the general enlargement of cells in the epidermal, and palisade tissues of the leaves. The dark green colour of the leaves was associated with the increased proportion of chlorophyll in the palisade tissues of the leaves.

Size and distribution of stomata:

Larger but lesser number of stomata per unit area were noted in tetraploid sesame in the present investigation. This was in agreement with the findings of Graner (1941) in Manihot utilissima, Kumar and Abraham (1942) in Phaseolus radiatus, Srivastava (1956) in Sesame, and Knight (1957) in Theobroma cocoa. But such difference in the size and number of stomata could not be noticed in the tetraploids of Vicia villosa by Hertzch (1951).

It was seen that the length and width of stomata were considerably increased in the tetraploids obtained as a result of 0.20% treated for 6 to 9 hours. Their number per unit area was also found to be reduced with the same concentration-duration combination.

It was therefore assumed that colchicine had induced the enlargement of the guard cells of the stomata as in the case of other cells in the plant tissue.

Blooming behaviour, number and size of flowers:

Delayed flowering was observed in the tetraploid sesame. On an average they took a fortnight more for flowering than the corresponding diploids. The floral parts were bigger in size, with an increased number of stamens. The number of stamens varied from 4 to 7 in tetraploids whereas it was invariably 4 in the diploids. But a reduction in the number of stamens produced by the tetraploids of Corchorus capsularis was reported by Chandhuri (1956); and by Kluge (1960) in Fragaria vesca

It was observed in the present investigation that the maximum number of flowers was produced by treatment with 0.20% colchicine for a period of 3 hours. The increased production of flowers could be explained, as a

natural outcome, from the vigorous growth and branching habit due to the gigas nature of the tetraploids.

Cytological observations:

The meiotic behaviour of the tetraploids was found to be seriously affected, with the formation of varying number of multivalents in the pollen mother cells. Such observations were previously reported by Ramanujam and Joshi (1941) in Cicer arietinum, Nasima (1942) in flax, Mehta et al (1948) in berseem, Bhaduri and Chakravorti (1948) in Corchorus capsularis, Das (1953) in Melilotus alba, Srivastava (1956) in Sesamum orientale L. and Islam (1960) in Annona squamosa.

The higher percentage of pollen sterility was noted in the plants treated with 0.15% colchicine for 9 hours. The reasons for sterility might be attributed to the formation of genetically unbalanced gametes, due to the unequal separation of chromosomes during anaphase in the pollen mother cells of the tetraploids. Wherever the normal distribution of 26 + 26 chromosomes occured, fertility was resulted.

Size and sterility of Pollen:

Another reason attributed to such a higher

percentage of pollen sterility in the tetraploids was that the microspores formed in the tetrads varied in their relative size; with a larger number of nucleoli. The results fully agreed with the findings of Kumar and Abraham (1940) from their cytological analysis of sterile diploids of sesame.

Srivastava (1956) reported that the pollen sterility in the tetraploid sesame varied from 20 to 25% but in the present experiment, it was found to vary from 31.91 to 43.90%. An increase in pollen sterility in the polyploids was previously reported by Kuzdowicz (1960), in tomatoes, Saxena and Nanda (1960) in phlox, Galcenko (1961) in Cucumbers and Mehta et al (1963) in berseem.

The pollen grains produced by the tetraploid sesame were found to possess a larger diameter than in the normal diploids. Maximum diameter was noticed in 0.20%, treated for 9 hours. An increase in the size of pollen grains consequent to colchicine treatment was noted by Illies (1956) in birch, Srivastava (1956) in sesame, Armstrong and Robertson (1960) in alsike clover and Mehta et al (1963) in berseem. Stout and Chandler (1941) observed that the pollen grains in the tetraploid Petunia axillaris were larger in size with four germinal

pores, instead of three. This was supported by the findings of Bhaduri and Chakravorti (1948) in tetraploid Corchorus capsularis.

It was assumed in the present investigation, that the larger pollen grains in the polyploids of sesame might be due to the larger size of the pollen mother cells.

Fruit setting and yield:

Eventhough, the number of flowers produced by the tetraploids was higher, a considerable reduction was noticed in the percentage of fruit set because of higher percentage of sterility. The total yield was thus drastically reduced. Such reduction in yield in the colchicine-induced polyploids was previously noted by Kumar and Abraham (1942) in Phaseolus radiatus, Singh (1955) in Carica papaya, Sen and Bhowel (1959) in Vigna sinensis, Kuzdowicz (1960) in tomatoes, Sen and Vaidyabhooshan (1960) in horse gram and Fraszewska (1962) in Camelina sativa.

The fruits borne by the tetraploids of sesame were having a larger size, with larger and heavier seeds. Similar observations were made earlier by Sando (1939) in Fagopyron tataricum, Amin (1940) in cotton, Kumar and

Abraham (1942) in Phaseolus radiatus, Dermen (1943) in grapes, Kundu and Sarma (1956) in Corchorus olitorius, Srivastava (1956) in sesame, Sen and Chheda (1958) in Phaseolus mungo, Chladek and Rod (1958) in Matricaria chamomilli L. and Srivastava and Bajpai (1964) in Loquat.

The reduction in yield in the tetraploids could be offset by larger and heavier seeds, eventhough the number of seeds per fruit was a little less than in the diploids.

Oil content of seeds:

In spite of their possessing a larger size and enhanced weight, the seeds from the tetraploid plants yielded a lower percentage of oil (50.048%), as against 51.337% in the diploids.

In the light of the results obtained in the present experiment, it was concluded that the scope of colchicine-technique as one of the methods in the improvement of Sesamum orientale L. is considerably limited. Very little is known on the performance of the tetraploid as an improved variety in comparison to the normal diploid, especially with respect to the yield of oil, and more extensive experiments at the field level had to be carried out

before drawing conclusions.

Colchicine produced morphological deformities in the treated plants. But its value as a polyploidizing agent in sesame was found to be apparently low, as was evident from the extremely low proportion of polyploids identified and confirmed cytologically. Polyploids due to their abnormal behaviour at meiosis during microsporogenesis, and to the consequent production of sterile pollen, produced very few seeds which, whether breed true or not, had to be verified through other experiments, which fall beyond the scope of the present investigation. As sesame could not be propagated vegetatively and as the number of seeds produced by tetraploids was found to be low, extremely serious problems would be met with in raising and rearing polyploid populations. In addition, work had to be carried out, so as to explore more into the possibility of utilizing this technique, for the improvement of sesame, in combination with other methods, involving hybridization, selection etc.

S U M M A R Y

The present investigation was undertaken in the Agricultural Botany Division of the Agricultural College and Research Institute, Vellayani, with a view to evaluating the scope of colchicine technique in the improvement of one of the major oil yielding crop plants of India - Sesamum orientale L. (2n = 26).

In this study, colchicine in aqueous solutions of four concentrations - 0.05%, 0.10%, 0.15% and 0.20% was applied to seeds as well as seedlings in combination with three different durations viz., 3, 6 and 9 hours. Its effect as seen from the performance of plants was closely followed and discussed.

There was good indication, that like many other crop plants, sesame responded to the various treatment combinations of colchicine. It was found that the drug induced considerable amount of morphological variation in almost all treated plants. However, as a polyploidizing agent, colchicine was observed to be of relatively less active in sesame. This was confirmed on the basis of cytological observations conducted at microspo-

rogenesis. Of the total 85 plants which showed morphological deformities, only 27 were found to be polyploids - tetraploids with 2n = 52.

Treatments on seeds were found to produce more number of plants with morphological deformities and multiplied chromosome complements than the seedling treatment. Further, it was also recorded that in sesame the effect of colchicine was more effective especially at higher concentrations in prolonged treatments.

The morphological deformities included, a slightly reduced and delayed germination of seeds and general stunting and production of deformed leaves in young seedlings. This observation was proved to be in conformity with similar observations made by different authors in many other crop plants.

In the treated plants, for the first two weeks the growth was considerably inhibited. However, a marked progress was noticed from the third and fourth week onwards. The overall life, blooming, fruiting and maturity were prolonged in the case of treated plants for another two more weeks.

Blooming, though profuse, due to a high propor-

tion of pollen sterility resulted in shy bearing. Fruits were larger but contained fewer number of larger and heavier seeds. The oil content in the tetraploids was found to be slightly reduced.

In the light of the above observations, it was concluded that the scope of applying colchicine for the improvement of sesame crop is to a great extent, subject to many limitations. The proportion of colchicine induced tetraploids was found to be very low. Further, the tetraploids on comparison with the normal untreated diploids contributed poorly to the yield of oil. The difficulty with which the induced tetraploids breed true, together with the total absence of an alternative method of propagation through vegetative means, suggest that colchicine breeding directly can have little value in the improvement in this crop. However, it is also indicated that other techniques such as hybridization and selection are to be combined with colchicine technique, if any directive is to be proposed on further improvement in the crop.

REFERENCES

*Aleksic, D.	1960	The induction of tetraploidy in Red pepper. Hort. Abs. 31: 3, 638-39.
Amin, K.C.	1940	A preliminary note on Interspecific hybridization and use of colchicine in cotton. Curr. Sci. 9: 74-75
*Armstrong, J.N. and Robertson, R.W.	1956	Studies of Colchicine induced tetraploids of trifolium hybridum L. I, Cross and self fertility and Cytological observations. Canad. Jour. Agr. Sci. 26: 255-266.
Bali, P.N. and Tandon, S.L.	1957	Colchicine-induced tetraploidy in Linaria vulgaris. Curr. Sci. 8: 256-58.
----------	1957	Colchicine induced tetraploidy in Iberis amara L. Curr. Sci. 11: 867-68.
----------	1958	A preliminary study of Colchicine induced polyploids of Alyssum maritimum L. Curr. Sci. 10: 407-408.
Beasley, J.O.	1940	The production of polyploids in Gossypium. Jour. Hered. 31: 39-48.
Bhattacharjee, S.K.	1956	Study of autotetraploid - Cajanus cajan. Caryologia 9: 149-59.
Blakeslee, A.F. and Avery, A.G.	1937	Methods of inducing chromosome doubling in plant with Colchicine. Sci. 86: 408.
Bouharmont, J.	1961	Action of colchicine on the rice seedling. Cytological observations. Cellule 61: 313-15.
Bargdo, M.	1955	Production of polyploids by colchicine. Euphytica. 4: 76-82.

Carpentiers, S.	1954	Action of colchicine on the roots and rootlets of the groundnut. Rev. Cytol. Paris. 15: 153-78.
*Chandra Das, B.	1953	Cytology of megasporogenesis as a basis for sterility in autotetraploid sweet clover - Melitotus alba. Iowa St. Coll. Jour. Sci. 27: 153-54.
*Charles Darwin	1875	Cited by O.J. Eigsti and Pierre Dustin Jr. Colchicine in agriculture, medicine, biology and Chemistry. The Iowa State College Press, Ames, Iowa, U.S.A.
Chaudhuri, S.D., Eunus, A.M. and Jabbar, M.A.	1956	Studies on Colchicine induced tetraploids of jute. Euphytica 5: 339-46.
*Chladek and Rod, J.	1958	The importance of polyploidy in the breeding of Matricaria chamomilla Hort. Abst. 29 (1): 1544.
*Chopra, V.L. and Swaminathan, M.S.	1960	Induction of Polyploidy in watermelon. Proc. Ind. Acad. Sci. Sect. B. 51: No.2. 57-65.
*Damon, E.G.	1958	Investigation into the cytogenetic effects of colchicine on varieties of Sorghum vulgare. Proc. Okla. Acad. Sci. 38: 9-13.
Davidson, D.	1957	Relation of colchicine to chromosome breakage. Nature. Lond. 180: 1059.
*Dereskevicus, K	1956	Use of Colchicine in Tomato breeding. Bot. Zurnal. 41: 1482.
Dermen, H.	1954	Colchiploidy in grapes. Jour. Hered. XLV. 159-72.
Eigsti, O.J. and Dustin, P.	1934	Colchicine - in Agriculture, Medicine, Biology and Chemistry. The Iowa State College Press, Ames, Iowa, U.S.A.

Emsweller, S.L. and Philip Brierley	1940	Colchicine-induced tetraploidy in Lilium. Jour. Hered. 31: 223-30.
Evans, H.J., Neary, G.J. and Tonkinson, S.M.	1957	The use of colchicine as an indicator of mitotic rate in broad-bean root meristems. Jour. Genet. 55: 487-502.
Galcenko, N.B.	1961	Production of Polyploids in green hous cucumber varieties by Colchicine treatment. Cytologia. 3: 108-112.
Gopalakrishnan, R.& Shastry, S.V.S.	1964	Induced polyploidy in Oryza offinalis and Oryza australiensis. Curr. Sci. 33: 504.
Graner, E.A.	1941	Polyploid Cassava (Manihot utilissima) induced by Colchicine treatment. Jour. Hered. 32: 281-88.
*Hertzsch, W.	1951	Observation on polyploid Vicia villosa. Pflamzenz. 30: 210-217.
*----------	1954	Observations on artificially produced polyploid forage plants - Alsike clover (Trifolium hybridum) Canad. Jour. Agr. Sci. 34: 201.
*Hindmarsh, M.M.	1952	The effect of colchicine on the spindle of root tip cells. Proc. Linn. Soc. N.S.W. 77: 300-306.
*Hofmeyer	1945	Further studies of tetraploidy in Carica papaya L. South Afr. Jour. Sci. XLI. 225-230.
*Hull, J.W. and Britton, D.M.	1958	Development of colchicine induced and natural polyploid breeding lines in the genus Rubus (Tourn).L. Bull. Md. Agric. Exp. Sta. A.91: 63.
*Illies, Z.M.	1956	Changes in the size of birch pollen after treatment of the flowers with colchicine. Forstgen. Forstp. 5: 112-15.

Islam, A.S.	1960	Unproductivity in the Colchicine-induced Tetraploids of Annona squamosa L. Nature. 187: 343-44.
Iyengar, N.K.	1944	Cytological investigations on hexaploid cottons. Ind. Jour. Agric. Sci. 14: 142-51.
Janaki Ammal, E.K.	1962	Tetraploidy in Rauvolfia Serpentina Benth. Curr. Sci. 12: 520-21.
*Kloen, D.	1957	Polyploid sugarbeets and Mangels. Land. Tijd. Wage. 69: 44-52.
*Kluge, K.	1960	The behaviour of the diploid form of Fragaria vesca; var. Semperflorens as compared with the tetraploid form in respect of flowers and fruits. Arch. Gart. 7: 589-606.
Knight, R.	1957	Induced polyploidy in Theobroma Cocoa L. and related species. J. Hort. Sci. 32: 1-8.
*Kobayazhi, T. and Shimamura, T.	1947	Artificial polyploids of Sesame. Jap.Jour. Genet. 22: 29.
Kumar, L.S.S. and Abraham, A.	1941	A cytological study of sterility in Sesamum orientale L. The Ind. Jour. Genet. Pl. breed. I. 41-59.
----------	1942	A study of colchicine-induced polyploidy in Phaseolus radiatus L. Jour. Uni. Bombay. 11: Part III.
Kumar, S.	1960	Preliminary studies on Colchicine induced polyploids in larkspur (Delphinium ajacus). Sci. and Cult. 25: 334.
Kumar, L.S.S., D'Cruz, R. and Oke, I.G.	1957	A synthetic allohexaploid in Arachis. Curr. Sci. 26: 121-22.
Kundu, B.C. and Sarma, M.S.	1956	Studies on Colchicine-induced tetraploids of Corchorus olitorius L. The Jour. of Ind. Bot. Soc. XXXV.1. 11-25.

*Kuzdowicz, A.	1960	The problem of fertility and yield in autotetraploids of the genus Lycopersicon. Zuchter 30: 6-11.
Langham, D.G.	1940	Fertile Tetraploids of Sesame, Sesamum indicum L. induced by Colchicine. Science. 95: 204.
*Lelakis, P.	1958	Induction of Polyploidy in Vitis Vinifera L. by application of Colchicine. Bl. breed. Abst. 28: 3404.
*Lutz, A.M.	1907	A preliminary note on the chromosomes of Oenothera lamarckiana and one of its mutants, Oenothera gigas. Science, n.s.26: 151-52.
*Masima, I and Uchiyamado, H.	1955	Studies on the breeding of highly fertile 4-ploid rices. Georgia 2: 49.
Mirov, N.T. and Stockwell, P.	1939	Colchicine treatment of Pine Seeds. Jour. Hered. 30: 389-90.
Mohanty, R.N., Satpathy, D. and Misra, B.	1961	Preliminary observations on induction of polyploidy in Niger. Ind. Oil seeds Jour. V: 87-90.
*Moffett, A.A. and Nixon, K.M.	1960	Induced tetraploidy in black wattle. Hort. Abstr. 31: No.2. 361.
Muntzing, A. and Runquist, E.	1939	Note on some colchicine-induced polyploids. Hereditas. 25: 491-95.
*Nakasone, H.Y	1961	Artificial induction of polyploidy in orchids by the use of colchicine. Hort. Abst. 31:(3) 640.
Nebel, B.R. and Ruttle, M.L.	1939	The cytological and genetical significance of colchicine. Jour. Hered. 28: 3-9.
Nirad Kumar Sen and Chheda, H.R.	1958	Colchicine induced tetraploids of five varieties of Black gram. Ind. Jour. Genet. 8: Pl. br. 18: 238-48.
*Nishiyama, I. and Takasugi, K.	1960	Studies on artificial polyploid plant. XII. Investigations on the growth and yield of an autotetraploid radish. Heredity, 4: 399.

Pal, B.P., Ramanujam, S. and Joshi, A.B.	1941	Colchicine induced polyploidy in crop plants. II - Chilli (Capsicum annum L.) Ind. Jour. of Genet. and Pl. breed. 1: 28-39.
*Pandey, K.K.	1956	Studies in autotetraploids of linseed (Linum usitatissimum L.) I. Growth rate. Lloydia. 19: 120-28.
Rao, K.R., Sanyal, A.T. and Datta, J.	1944	Colchicine treatment of Jute. Sci. and Cult. X: 86-89.
Ramanujam, S. and Deshmukh, M.J.	1945	Colchicine induced polyploidy in crop plants - III - oleiferous brassicae. The Ind. Jour. of Genet. and Pl. breed. 5: 63-81.
Ramanujam, S. and Joshi, A.B.	1941	Colchicine induced polyploidy in Crop plants. I. Gram. (Cicer arietinum L). Ind. Agri. Sci. 11: 835-49.
Raman, V.S. and Kesavan, P.C.	1963	Induced autotetraploidy in Arachis duranensis. The Ind. Jour. Genet. and Pl. breed. 23: 320-24.
Richharia, R.H and Persai, D.P.	1940	Tetraploid Til (Sesamum orientale L). from colchicine treatment. Current Sci. 9: 542.
*Saharov, V.V. and Mansurova, V.V.	1959	Obtaining Polyploid sugarbeet. Proc. Acad. Sci. U.S.S.R., 128: 1283-85.
*Saito, K.	1957	Studies on the induction and utilization of polyploidy in some cucurbits. Jap. Jour. Breed. 6: 217-20.
Sando, W.J.	1939	A colchicine induced tetraploidy in Buck wheat (Fagopyron tataricum) Jour. Hered. 30: 271-72.
Saxena, S.S. and Nanda, J.S.	1960	A note on colchicine treated phlox. Curr. Scie. 29: 281.
*Schank, S.C. and Knowles, P.F.	1961	Colchicine induced polyploids of Carthamus tinctorius L. (Safflower) Crop. Sci. 1: 242-45.

Sen, N.K. and Bhowal, J.G.	1960	Colchicine induced tetraploids of six varieties of Vigna sinensis. Ind. Jour. Agric. Sci. 30: 194-61.
*Sen, N.K. and Ghose, A.K.	1960	Studies on the tetraploids of six varieties of Green gram. Proc. Nat. Inst. Sci. India. 26: Part.B. 291-99.
*Sen, N.K. and Hari	1956	Colchicine induced tetraploids of Vigna sinensis. Ind. Jour. Agri. 25: 101-104.
Sen, N.K. and Vaidyabooshan.	1960	Colchicine induced tetraploids of horse gram - Dolichos biflorus Nature. Lond. 187: 310-12.
*Sharma, A.K. and Datta, P.L.	1957	Artificial polyploidy in coriander. (Coriandrum sativum L.). Cervologia 10: 152-58
Shifriss, O.	1942	Polyploids in the genus Cucumis - Preliminary account. Jour. Hered. 33: 144-52
*Shimamura, T.	1939	Experiments of inducing tetraploid in tomatoes by means of colchicine. Jap. Jour. Genet. 14: 304-308.
Singh, R.N.	1953	Colchicine induced Polyploidy in Papaya (Carica papaya). Sci. & Cult. 19: No.1. 44.
----------	1955	Further studies in colchicine induced polyploidy in Papaya (Carica papaya). Ind. Jour. Hort. 12: No.2. 63-71.
Sikka, S.M., Swaminathan, M.S. & Mehta, R.K.	1958	Induced polyploidy in Egyptian and Indian clovers. Nature. 181: 32-33.
Smith, H.H.	1939	The induction of polyploidy in Nicotiana species and species hybrids by treatment with colchicine. Jour. Hered. 30: 290-306.
*Smith, D.L. and Hiner, L.D.	1960	A comparison of the effect of colchicine and some purified veratrum alkaloids on nuclear division in roots of Allium cepa. Jour. Amer. Pharm. Ass. Sci. Ed. 49: 538-42.

Sobti, S.N.	1964	Induced polyploidy in English Black mint. Curr. Sci. 5: 149-50.
Sparrow, A.	1942	Colchicine induced univalents in Diploid Antirrhinum majus L. Science. 26: 363-64.
*Spasojevic	1956	Induction of autotetraploidy in the bean (Phaseolus vulgaris). Red. Tran. Inst. Physiol. Dev. Genet. Selet. Beo. 4: 117-34.
*Srivastava, R.N.	1955	Varietal differences among polyploids obtained from different strains of gram (Cicer arietinum L) Proc. Bihar. Acad. Agric. Sci. 4: 59-66.
----------	1956	Production of fertile autotetraploids in Sesame and their breeding behaviour. Jour. Hered. 47: 241-44.
*Srivastava, V.K. and Bajpai, P.N.	1964	The effect of colchicine on fruit set and pulp/seed ratio of Loquat (Eriobotrya japonica L.). Med. Agric. Jour. 51: 394-97.
Stebbins, G.L.(Jr.)	1950	Variation and Evolution in plants. Columbia Univ-rsity Press., New York. 16: 298-379.
----------	1956	Artificial polyploidy as a tool in plant breeding. Brookhaven Symposia in Biol gy. Genetics in Plant Breeding. # 9: 37-52.
*Sterbakov, V.K.	1956	Inducing polyploidy in ornamental plants. Hort. Abstr. 30 (3): 540.
Stout and Chandler	1941	Change from self incompatability to self-compatability accompanying chan e from diploidy to tetraploidy. Science. 94: 118.
Straub	1940	Production of colchicine induced tetraploids - Pisum sativum Pl. breed. Abst. 12: 98.

Tandon, S.L. and Chinoy, J.J.	1950	Colchicine induced polyploidy in Amaranthus blitum. Sci. & Cult. 15: 398.
Tandon, S.L.	1951	Colchicine induced polyploidy in Brassica oleraceae. var. Botrytiss L Sci. & Cult. 16: 483-84.
Thombre, M.V. and Desai, M.C.	1960	A preliminary note on autotetraploidy in Agave contala L. Sci. & Cult. 26: 276-77.
Toole, M.G. and Bamford, R.	1945	The formation of diploid plants from haploid peppers. Jour. Hered. 36: 67-70.
*Vârama, A.	1947	Morphological and cytological studies on colchicine-induced tetraploids in Ribus nigrum. Suom. Maa. Scur. Tulk. 67: 55-92.
Vakili, N.G.	1962	Colchicine-induced polyploidy in Musa. Nat. Lond. 194: 453-54.
Vig, B.K.	1962	A note on a mixoploid plant obtained from colchicine treated Guar (Cyamopsis psoralioides D.C.) Sci. and Cult. 28: 434-35.
*Wada, B.	1940, 1949 & 1950	Cited by O.J.Eigsti and P.Dustin Jr. Colchicine in agriculture, medicine biology and chemistry. The Iowa State Col. Press. Ames. Iowa, U.S.A.
*Walker, G.W.R.	1938	The effect of colchicine on microsporogenesis in cultured anthers of Tradescantia paludosa. Amer. Jour. Bota. 44: 690-96.
Warmke, H.E. and Blakeslee, A.F.	1939	Induction of simple and multiple polyploidy in Nicotiana by Colchicine treatment. Jour. Hered. 30: 419-32.
Warmke, H.E.	1945	Experimental polyploidy and rubber content in Taraxacum Kok-saghyz. Bot. Gaz. 106(3): 316-324.

*Yokoyama, S. and Matsui, H.	1957	On the induction of polyploidy in the Tea plant. The influence of Secondary condition during colchicine treatment on the occurrence of Tetraploidy. Tea Res. Jour. 9: 8-11.
*Zuluuga, P.A. and Gargiulo, A.	1954	Polyploidy in Vitis vinifera. Rev. Fac. Cien. Agarar. 4(2): 1-13. bibl. 15, illus.

---

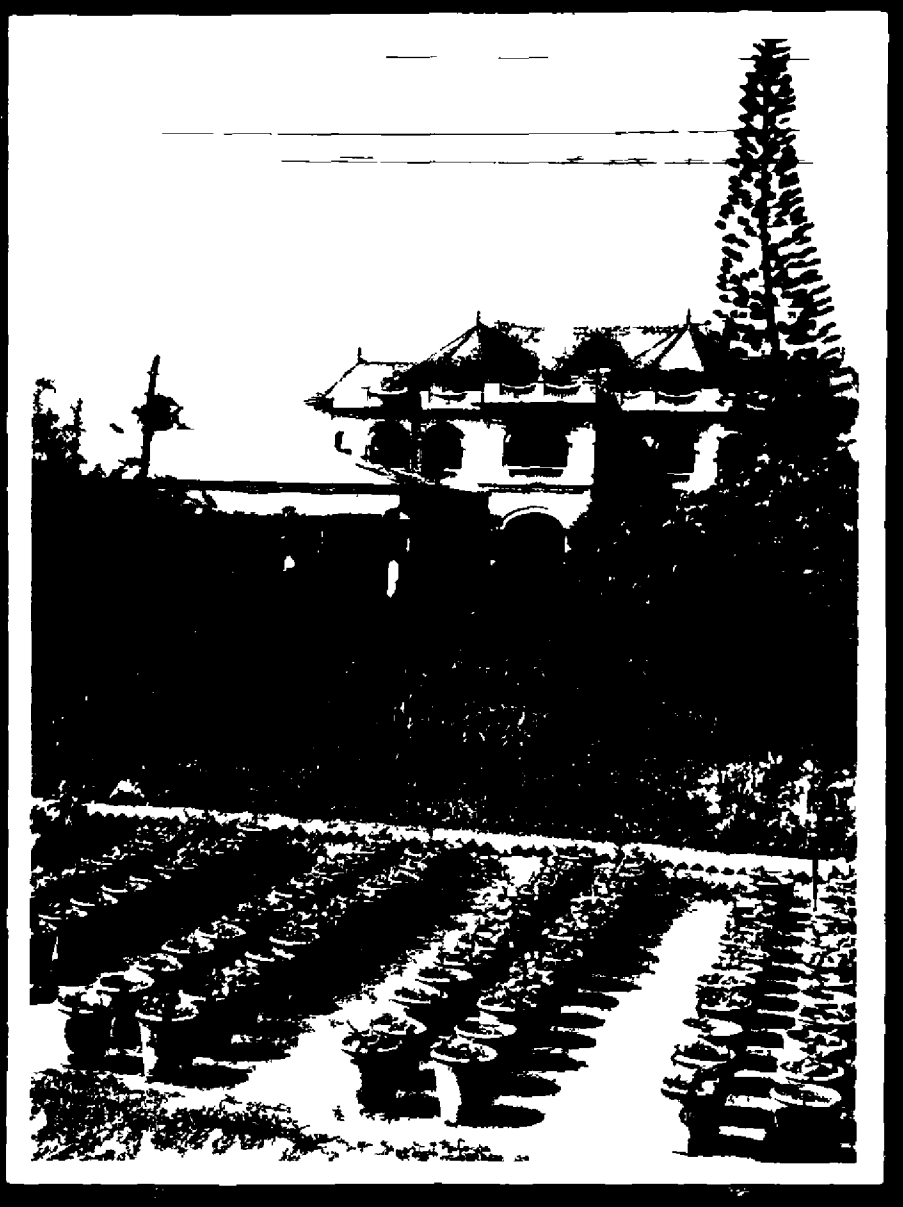
*Originals not seen

A GENERAL VIEW OF THE EXPERIMENTAL PLOT

Plate. 1. Early deformities shown by plants treated with 0.20% colchicine for 9 hours

23 = 0.20% - 9 Hrs. - Seed treatment

24 = 0.20% - 9 ,, - Seedling treatment



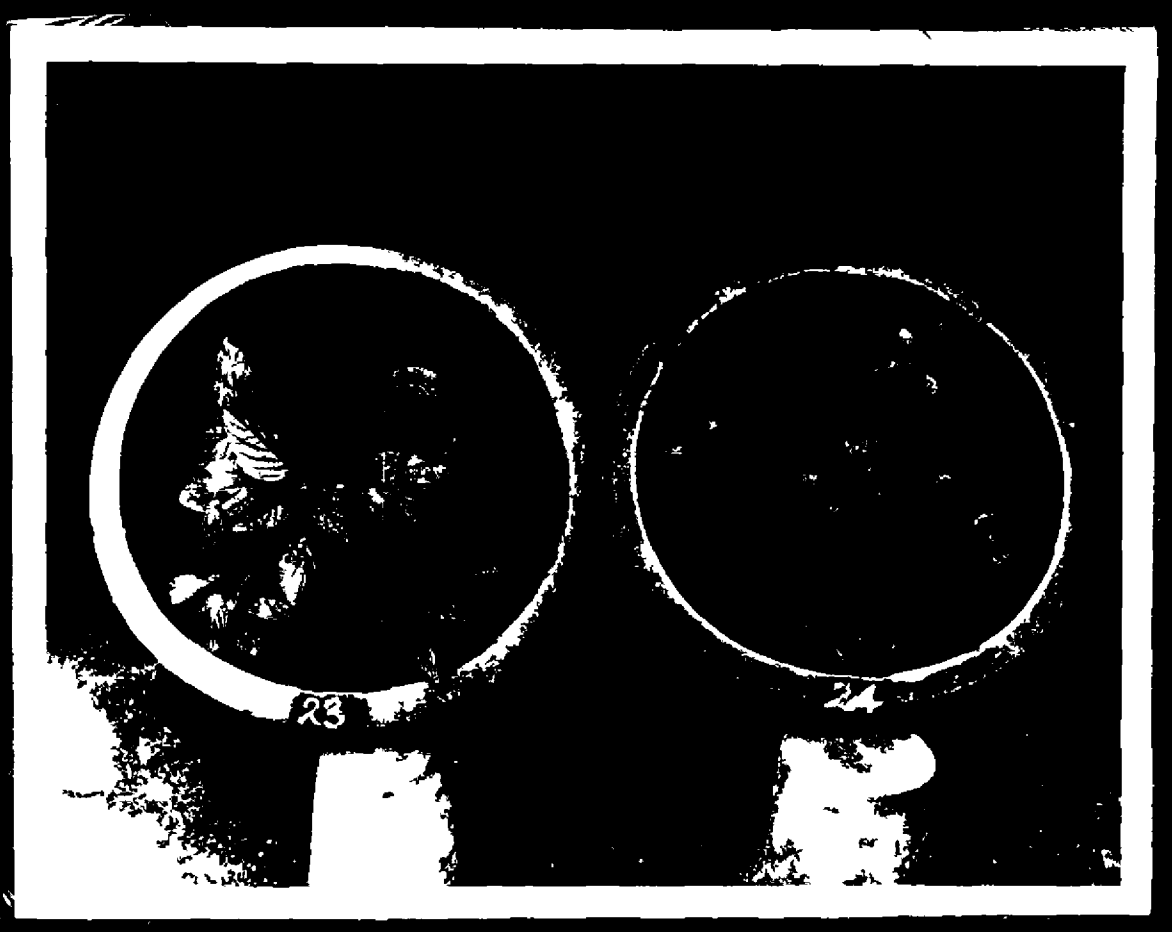


PLATE 1

Plate 2A. A tetraploid plant obtained from seeds treated with 0.20% for 9 hours, showing gigas characters. 25 = Control

2B. Tetraploid and diploid plants showing differences in the external characters. viz. nature of branching and height.

PLATE 2 A

PLATE 2 B

Plate 3. Leaf thickness of the diploid plant

4. Leaf thickness of the tetraploid plant

PLATE 3

PLATE 4

Plate 5. Size and distribution of stomata in diploids.

6. Size and distribution of stomata in tetraploids.

PLATE 5

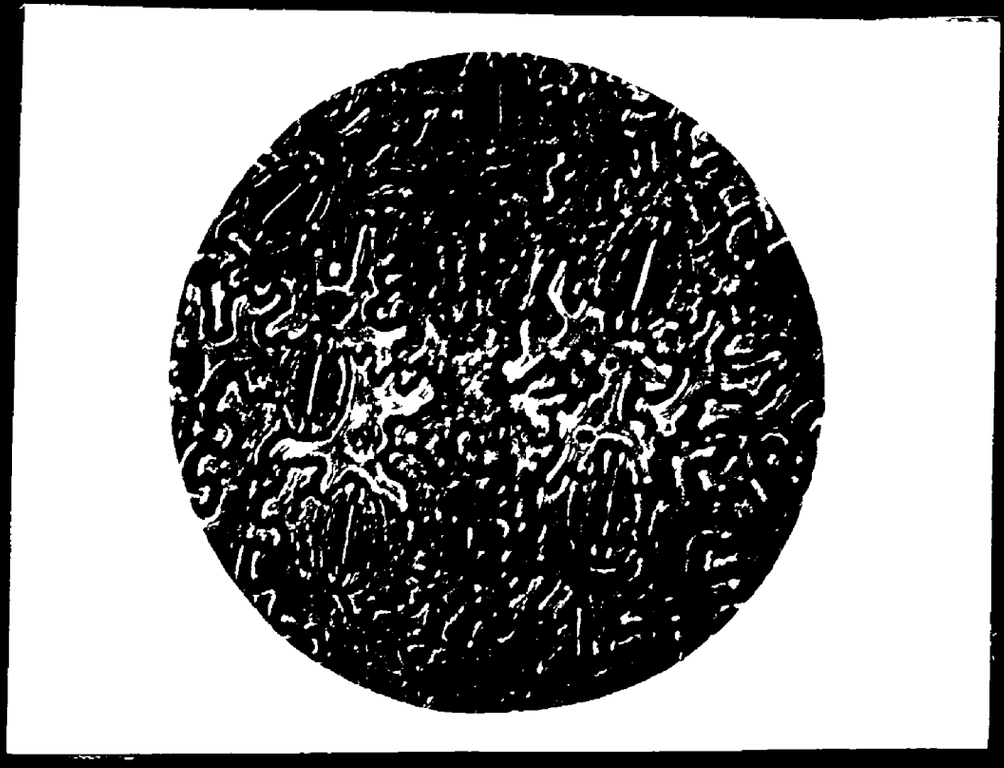


PLATE 6

Plate 7. Size of corolla in the tetraploid and diploid (C) plants.

8. (1 & 2) First meiotic metaphase of the Pollen Cells of the diploid plant, showing 13 bivalents properly oriented (Camera lucida drawing 10 x 63).

(3) First anaphasic separation in the diploid plant, showing 13 chromosomes in each pole. Two small round dots represent the secondary nucleoli (Camera lucida drawing 10 x 63).

(4) Second metaphase of the diploid plants with 26 bivalents (13 + 13). A small dot in the centre is the secondary nucleolus (Camera lucida drawing 10 x 63).

(5) Pollen grain smear from a tetraploid plant with 43.90% pollen sterility, showing variation in their size and an increased number of nucleoli (Camera lucida drawing 10 x 63).

(F) Pollen grain smear from normal diploids showing single nucleolus in the nucleus (Camera lucida drawing 10 x 63).

9A. Smear preparation from the normal diploid plants to show the characteristic orientation of 13 bivalents at the equator during first metaphase.

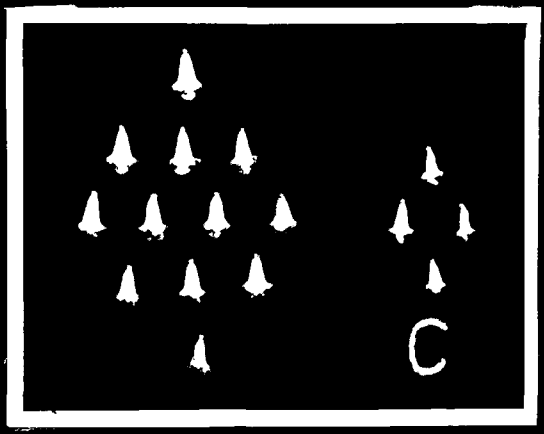


PLATE 7

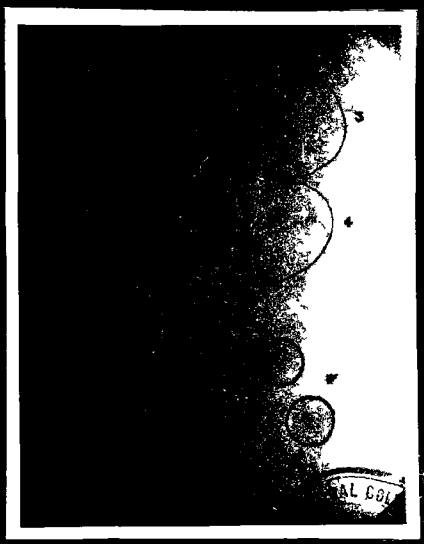


PLATE 8

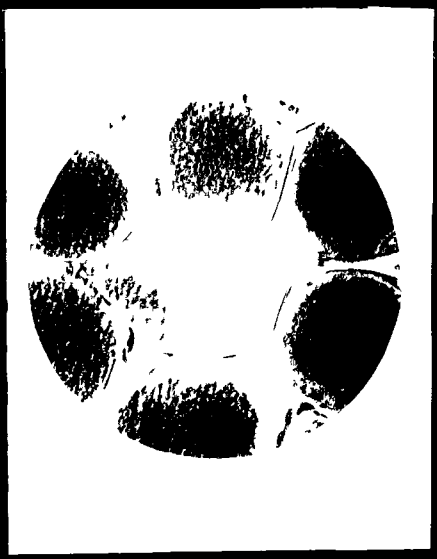


PLATE 9 A

Plate 9B. Early anaphase I in diploids to show normal separation of 13 bivalents

C. First meiotic metaphase of the tetraploid plant showing 26 bivalents (3 out of focus).

10. First meiotic anaphase of the tetraploid plant showing 26 chromosomes in each pole.

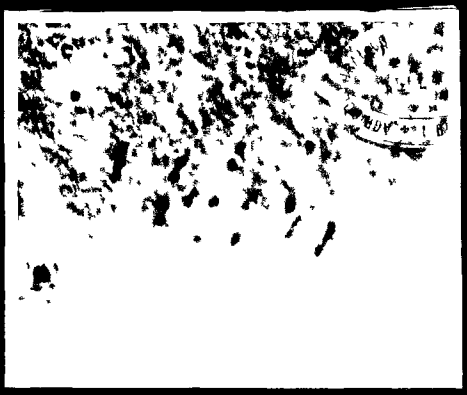


PLATE 9 B

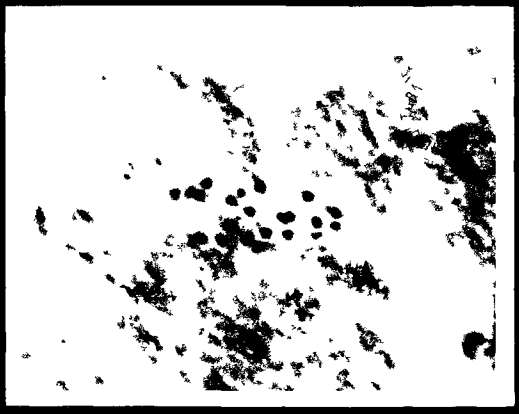


PLATE 9 C

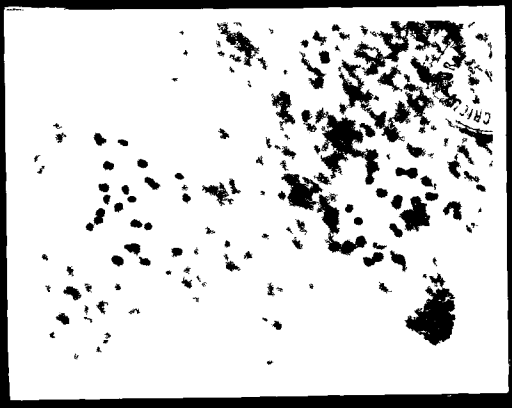


PLATE 10

CAMERA LUCIDA DRAWINGS FROM SMEAR PREPARATIONS IN TETRAPLOIDS (10 x 63)

Plate 11 A. First meiotic metaphase, with 26 bivalents, scattered in the cytoplasm.

B & C. First anaphase showing 26 chromosomes in each pole. Small round dots represent the secondary nucleoli.

D. Diakinesis - $2_{IV} + 4_{III} + 13_{II} + 6_{I}$

E. do $1_{IV} + 5_{III} + 12_{II} + 9_{I}$

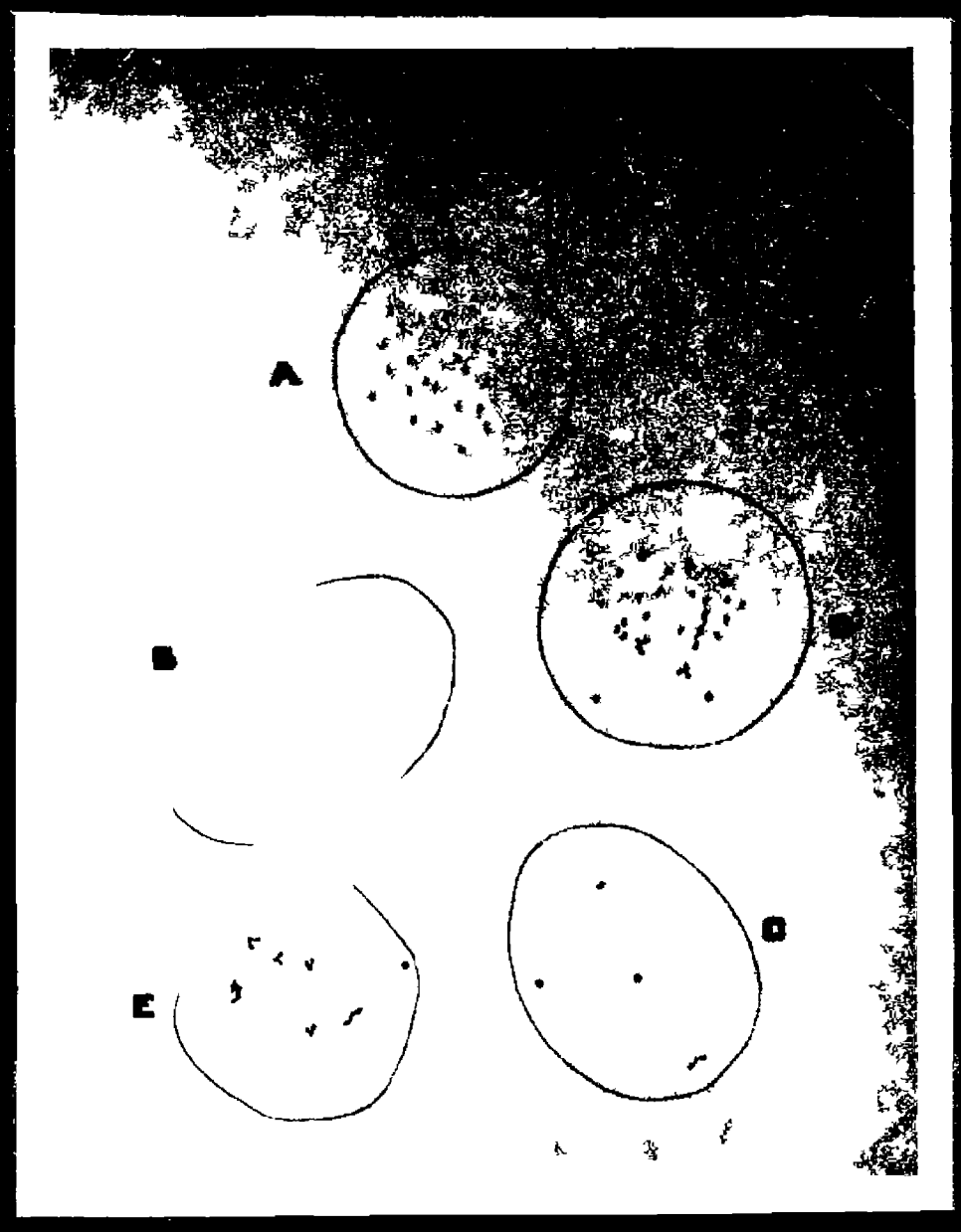


PLATE 11

Plate 12. Difference in the size of fruits in diploids (C) and tetraploids.

13. Difference in the size of seeds in diploids and tetraploids.

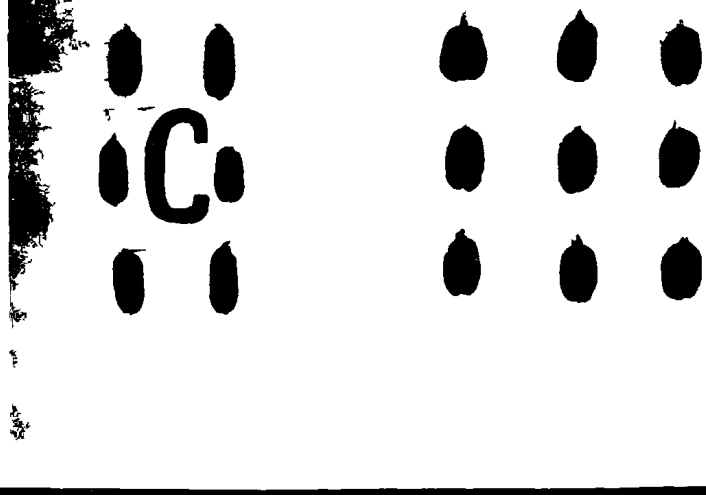


PLATE 12

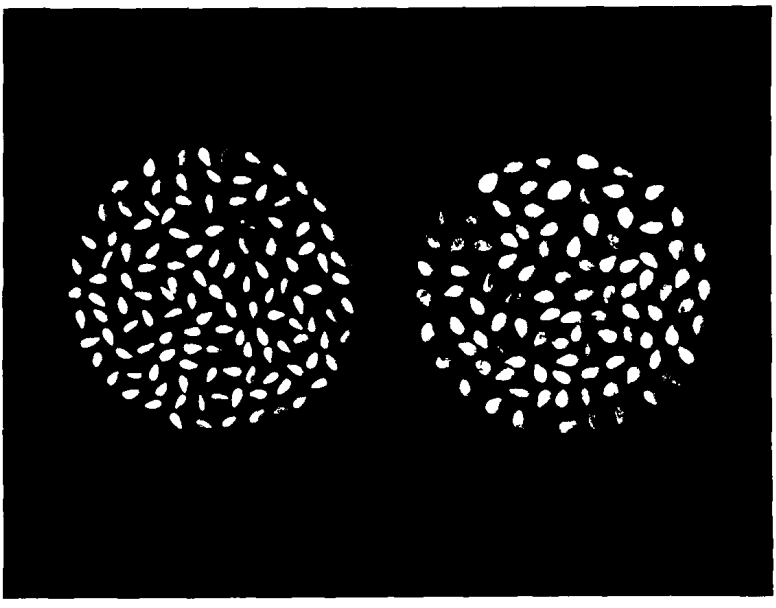


PLATE 13