CYTO-MORPHOLOGICAL STUDIES ON COLCHICINE INDUCED POLYPLOIDS OF CHILLIES (*Capsicum frutescens L.*)

BY

S INDIRAMMA

THESIS

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C E R T I F I C A T E

This is to certify that the thesis submitted contains the results of bonafide research work carried out by Kumari S. Indiramma 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 &
Research Institute,
Vellayani, Trivandrum,
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C O N T E N T S

			Page
I.	INTRODUCTION	...	1-3
II.	REVIEW OF LITERATURE	...	4-30
III.	MATERIALS AND METHODS	...	31-39
IV.	EXPERIMENTAL RESULTS	...	40-53
V.	DISCUSSION	...	54-62
VI.	SUMMARY	...	63-65
	REFERENCE		
	PLATES		

INTRODUCTION

INTRODUCTION

Following the discovery of polyploidy in Oenothera by Lutz (1907) it was found to be one of the most widespread and distinctive features of large number of plant species. The fact that many crop plants like wheat, oats, cotton, tobacco, potato, banana, coffee and sugarcane are high polyploids led to the induction of polyploidy in plants with a view to producing more promising varieties.

The first successful attempt was made by Winkler (1916) in through decapitation method and he obtained a tetraploid form of Solanum nigrum from the regenerated callus tissue. Cold or heat shock, decapitation treatments with chemicals such as acenaphthene, chloral hydrate, ethyl mercuric chloride, benzene, veratrive sulphate etc., are some of the methods tried. However Blakeslee and Avery (1937) are credited for the first discovery of colchicine technique which became universely popular, because colchicine can be applied to a wide range of plants with considerable ease.

The alkaloid colchicine is extracted from the seeds and corms of the autumn crochus, Colchicum autumnale belonging to the family Liliaceae.

Induced polyploidy has immense use in plant breeding. The presence of larger and showy flowers in polyploids of Iberis amara (Bali and Tandon, 1957) suggests the strong possibility of selection of a desirable type for the introduction into horticultural trade. Tetraploidy has been introduced in fruit crops, vegetable crops, legumes, fodder crops, fibre crops, spices yielding plants and in cereals. Tandon and Chinoy (1950) obtained in Amaranthus blitum larger and more number of leaves in the tetraploid and vegetative cycle was found to be prolonged.

Greater range of variability with regard to size, dry matter, protein content and sugar content etc. were found in tetraploid sugar beets and mangel (Peto and Boye, 1940, Kloon, 1957).

Chilli (Capsicum fruitiscense) is widely used in India for culinary purposes. The scope of polyploidy breeding by colchicine treatment in this crop has been indicated by Pal et al (1939) and (1941) and Aleksic (1960).

The present study was undertaken with a view to throw more light into the following aspects:-

1. To evaluate the efficiency of colchicine as a polyploidising agent in chillies.

2. To make comparison between the performance of diploids and artificially induced tetraploids with respect to the rate of growth, flower production, fruit set and yield.

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

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Early history

The history of colchicine, the drug of ancient and modern materia medica is rooted in the myths and in the written records of ancient, Egypt, India and Greece and runs its course through the ages into the world of today. It is known from very early days as a specific remedy for gout.

Colchicine is a poisonous alkaloid extracted from the seeds and corms of the autumn crocus Colchicum autamnale L. belonging to lily family. It grows abundantly in its natural habitat near the black sea ie., at Cochis. More than 50 species are known which contain colchicine.

Colchicine in biological research

Dustin (1934) in Belgium introduced colchicine in biological research as a valuable tool. He recognised it as a mediotic poison. Charles Darwin (1875) was an early experimenter on the effect of colchicine on plants. He applied the drug on insectivorous and sensitive plants. But he reported no valid conclusions. Modern period of

research with colchicine began in 1889 when Pernice showed a disproportion of metaphase, and arrested anaphases in colchicine treated mitotic cells of gastric gland of dog.

Dustin (1934) suggested the possibility of colchicine as a tool for cancer chemotherapy. Dustin et al (1937) very closely established that colchicine acted upon mitosis both in animal and plant tissues.

Nebel and Ruttle (1939) demonstrated that colchicine acted upon mitosis and that it was an important tool in inducing polyploidy in plants.

Blakslee and Avory (1937) through their work on Datura and other plant species established the fact that colchicine was a new and effective tool for making experimental polyploids. Its high solubility in water and non-lethality to plant at concentrations which can induce polyploidy are the major advantages of colchicine as a tool in polyploidisation in plants.

Method of application

The principle underlying the colchicine treatment is that the alkaloid must be brought into contact with actively dividing cells. This is effected by treating presoaked seeds with colchicine solution to the

meristematic region of the plant or young seedling. The method consists of treating the seeds with colchicine solution, applying colchicine to the growing tips of young seedlings and applying colchicine to the other growing regions of the plant like roots, tubers, suckers, bulbs, flower buds, cuttings etc.

Seed treatment

Various workers studied the effect of aqueous colchicine solution on different plants species in inducing polyploidy.

Muntzing and Runquist (1939) treated seeds of Pinus ponderosa by keeping them in moist sand at 5°C for three months in green house. The seeds were treated with 0.25 percent aqueous solution of colchicine for five days when seed coat split, resulting in polyploid varieties.

Ramanujan and Joshi (1941) produced tetraploid gram (Cicer arietinum) by treating soaked seeds with 1, 0.25 and 0.5 percent aqueous colchicine for varying duration ranging from half to twenty four hours. Treatment with 0.25 percent solution for half an hour was found to be the best. They also got tetraploid chillies by colchicine treatment of seeds. The effective

treatment was treating seeds with 0.05, 0.1, 0.2 and 0.4 percent aqueous colchicine for one to eight days.

Rao et al (1944) produced tetraploid strains of Corchorus olitorius by treating dry seeds with colchicine solutions of 0.05 percent to 1 percent concentration for 12 to 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 compestris Var. Sarson, Brassica tournefortii and Brassica juncea by treating dry seeds in aqueous colchicine of 0.1 to 0.4 percent strength for 24-48 hours and germinated seeds in 0.025 to 0.1 percent for 8-24 hours.

In Taraxacum kok-saghyz, Warmke (1945) could induce polyploidy by immersing seeds in 0.05-0.8 percent colchicine for one, two and four days in covered petri dishes at room temperature.

Hertzsch (1954) obtained tetraploid forms of Oryza sativa by immersing germinated seeds in 0.05 percent colchicine.

Kluge and Kramer (1955) on treating seeds of Vaccinium myrtillum, Var. corymbosum and diploid and

tetraploid varieties of Fragaria vesca with 0.1 to 0.5 percent solutions for period ranging from 27-72 hours noted an accelerated germination and increased germinability in the case of seeds that were stored for some time. In the case of diploid F. vesca the seeds gave best response to 0.3 percent colchicine solution for 72 hours.

Eifler (1955) reported that tetraploids were obtained by treating seeds of Picea abies and Betula verucosa with 0.25 and 0.2 percent colchicine solution respectively.

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

Johnson (1956) reported that treatment of seed of Betula verucosa and an F1 hybrid between Betula japonica and B. verucosa with 0.2 percent colchicine solution for 3 weeks gave tetraploid individuals.

Srivastava (1956) produced fertile autotetraploids in 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 0.1 percent aqueous solution of

colchicine for varying durations. After treatment the seeds were washed thoroughly. 25 seeds treated with 0.04 and 0.06 percent for 2 and 6 hours period produced one tetraploid each, and 25 seeds treated with 0.1 percent for 4 hours produced one tetraploid.

Yokoyama and Matzui (1957) observed that in tea the optimum conditions for inducing polyploidy through seed treatment was with concentration of 0.3 percent 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.

Knight (1957) treated Theobroma cocao with 0.6 percent colchicine in agar for 24 hours soon after germination.

Kundu and Sarma (1956-57) observed that effective concentration for induction of tetraploidy in Corchorus olitorius ranged from 0.0125 percent to 0.10 percent for six to twentyfour hours.

Kloen (1957) induced polyploidy in 4 varieties of sugarbeet and 3 varieties of mangel by treating germinated seeds with 0.1 to 0.2 percent colchicine solution at 28°C for different durations.

Smith (1958) induced tetraploidy in Barley varieties by soaking germinated seeds in 0.1 percent colchicine solution.

Hull and Britton (1958) obtained a number of polyploid blackberries and raspberries by treating germinating seeds at the cracked seed coat stage with 0.2 percent colchicine in 5 percent glucose solution at 86°F for 9 hours. Franzke et al (1959) obtained many diploid mutants induced by treating germinating seeds of sorghum with 0.5 percent colchicine.

Saharov et al (1959) treated seeds of sugarbeet with 0.05 percent aqueous solution of colchicine for 48 hours. Cytological analysis showed that 2 out of 150 plants were tetraploids. They suggested that a higher colchicine concentration 0.1 to 0.2 percent and shorter period of treatment, 2-6 hours might increase the number of polyploids.

Thombre and Desai (1960) treated seeds of Agave cantala with 0.4 percent colchicine for 6 hours and obtained tetraploids.

Cicin et al (1960) obtained polyploids by treating seeds of winter rye with 0.5 percent colchicine solution for 24 hours.

Aleksic (1960) reported that tetraploid chillies were obtained by treating seeds with 0.8 percent colchicine solution.

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

Stulzynski et al (1963) reported that seed treatment was effective in obtaining polyploids in rye grass.

Thankamma (1964) treated soaked seeds of cluster beans with aqueous solution of colchicine. The concentrations used were 0.1, 0.25 and 0.5 percent for 3, 6 and 9 hours. 0.5 percent colchicine solution for 9 hours treatment was more effective in producing 6 polyploids. 0.1 percent solution of colchicine for 3 hour treatment was unsuccessful.

Nair (1965) obtained polyploid sesamum by treating the soaked seeds with 0.05, 0.1, 0.15, 0.2 percent colchicine solutions for 3, 6 and 9 hours. The effective treatment was 0.2 percent and 0.15 percent colchicine solution for 6 hours each. These concentrations gave 8 polyploids out of the total 19 polyploids obtained.

Seedling treatment

Seedling treatment with colchicine at various stages of development has also been proved to be as effective as the seed treatment and in some crops more effective than the seed treatment. 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 effect of different concentrations of colchicine, duration of treatment and mode of application may vary depending upon the plant on which it is treated.

Warmke and Blakeslee (1939) found that a longer period of treatment (2 days) with 0.2 to 0.4 per cent colchicine solution was much more efficient than a shorter treatment (1 day) with 0.8 percent colchicine to induce polyploidy in tobacco seedlings.

Noble and Rattle (1939) obtained tetraploid marigold plants by treating seedlings with 0.02 to 0.16 percent colchicine solution for 1-14 hours at the 4 leaf stage. They also obtained tetraploid petunias by painting in the rosette stage with 1 percent colchicine in lanolin.

Shimamura (1939) induced tetraploidy in Lycopersicum esculentum by subjecting the growing points of seedlings

to colchicine treatment. Colchicine of 0.2 percent in lanolin paste was applied 2 or 3 times a week.

Straub (1940) observed that the application of 0.025 percent colchicine in cotton wads for two successive mornings to the growing tips of shoots in young seedlings was an effective method to induce tetraploidy in Pisum sativum.

Beasley (1940) produced polyploids from eleven types of Gossypium by colchicine technique. A small slit was made about 2 cm below the growing tips and immersed in a vial containing 0.2 percent aqueous solution of colchicine for 24 hours.

Emsweller et al (1940) get tetraploid bulblets of Lilium formosanum by immersing the growing points in solutions of colchicine of 0.2 percent to 1 percent concentration for 2 hours.

Langham (1940) treated the axillary buds of sesame with 0.5 percent colchicine and with 0.4 percent colchicine emulsion. In both the treatments severe burning and drying back of 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) reported that the more effective method of inducing polyploidy in Phaseolus radiatus was by treating the apical buds of seedlings with 0.4 percent colchicine in agar.

Shifriss (1942) recommended a 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 percent colchicine emulsion.

Tool and Bamford (1945) successfully doubled eight haploid forms of pepper to their diploid forms. Strengths of 0.1 to 1 percent colchicine emulsion were smeared on the growing tips, either in one or two applications in a week, or by injecting the aqueous colchicine by means of an interval injector inserted at the nodal region.

Hofmeyer (1945) treated the seedlings of Carica papaya with 0.1 percent colchicine applied in drops 6 times a day for five days.

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

Dermen (1954) obtained tetraploidy in grapes by applying 0.5 percent colchicine in 10 percent glycerine

in water. The buds were moistened with a small drop or so of the solution once for every two days for a total of three times.

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

Zulueta and Gargiulo (1954) obtained a tetraploid variety of Vitis vinifera by injecting newly formed buds with 0.1 to 0.3 percent colchicine in 5 percent pure glycerine. Carletto (1954) obtained diploid cells with 2n=40 in Cocao by treating the seedling with 0.375 percent to 0.6 percent colchicine solution.

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

Sedimayr (1955) treated young seedlings of sugar-beet with 0.2 percent colchicine solution and obtained tetraploids.

Choudhury et al (1956) produced tetraploids in two varieties of Corchorus capsularis, and three varieties of Corchorus olitorius by treating seedlings with 0.1 to 0.2 percent colchicine for 24 hours.

Verga (1956) obtained 50-70 percent tetraploidy in beet by treating it with 0.05 to 0.1 percent aqueous solution of colchicine at the apex of the seedling at the cotyledonary stage.

Dereskevicus (1956) induced polyploidy in tomatoes by applying 0.01 to 1.0 percent colchicine solution at the growing point of seedlings.

Bali and Tandon (1957) reported that the most effective method of treatment in obtaining polyploidy in Linarius vulgaris was by treating the growing point of seedlings with 0.1 to 0.2 percent colchicine solution for six hours. In Iberis amara the same concentrations for 6-12 hours and in Alyssum maritinum 0.2 percent for 6 hours proved successful.

Knight (1957) reported the optimum concentration time combination in inducing polyploidy in Theobroma cocoa to be 0.6 percent colchicine in agar, applied to apical buds for 24 hours.

Kumar et al (1957) obtained a fertile allohexaploid Arachis by treating the sterile triploid hybrid, obtained in a cross between 2 tetraploid Arachis hypogaea with 0.2 percent aqueous solution of colchicine to the buds of the sterile hybrid.

Sikka et al (1959) obtained tetraploid Trifolium by immersing 4 day old seedlings in 0.1 percent colchicine. Treatment with 0.05 percent colchicine for eight hours at room temperature gave a high percentage of mixaploids. In Melilotus indica the best treatment was 0.05 percent colchicine for 4 hours at room temperature.

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

Kumar (1960) treated apical growing points of seedlings of Delphinum ajacus with colchicine, by dipping in different strengths of solution for different lengths of time. 0.1 percent solution for 12 hours was very effective.

Mohanty et al (1961) produced tetraploid plants of Nigor by immersing seedlings in 0.05, 0.1, 0.2 and 0.3 percent colchicine solutions for 4, 8 and 12 hours. Treatment of 0.1 percent for 4 hours and 0.05 percent for 8 hours gave best results.

Bouharmont (1961) in his study on the action of colchicine on rice seedlings observed, that 20 day old plants were better than newly germinated seedlings to

induce polyploidy 0.1 percent colchicine solution applied for at least 2 hours was recommended for the induction of polyploidy in rice.

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

Vig (1963) obtained autotetraploids in Pb IV type of Cyamopsis psoralioides D.C. by treating young seedlings with 0.2 percent colchicine in lanonine paste.

Srivastava and Bajpai (1964) treated 25 healthy branches of loquet (Eriobotrya japonica L) with 0.12 per cent to 1 percent colchicine for 6 to 24 hours, and induced polyploidy.

Thankamma Pillai (1964) treated seedlings of cluster beans with colchicine. Concentrations and durations were 0.1, 0.25 and 0.5 percent and 3, 6 and 9 hours respectively. The seedling treatment was found to be effective. Maximum number of polyploids was obtained in the seedling treatment with 0.5 percent colchicine for 9 hours.

Nair (1965) treated sesame seedlings with colchicine solution. The concentrations were 0.05, 0.1,

0.15 and 0.2 percent colchicine solution in varying durations of 3, 6 and 9 hours. The effective concentration-duration combinations were found to be 0.2 percent at 3 hours and 0.2 percent at 6 hours.

Other methods

Other method of application includes the application of colchicine to growing regions of plants other than stem tip such as root tip, tubers, tillers, flower buds, cuttings etc.

Nebel and Ruttle (1939) treated cuttings of single clone of Tradescantia reflexa with 0.2 percent colchicine for four hours and induced tetraploidy.

Pal et al (1939) obtained polyploid chillies by treating the roots of seedling with 0.05 - 0.4 percent colchicine for 4-6 hours at the time of transplanting.

Shimamura (1939) induced polyploidy by immersing the roots of Allium cepa (1.0 to 1.5 cm in length) in 0.4 percent colchicine for 2 hours. Sparrow (1939) obtained polyploid strains of white flowered clone of Antirrhinum majus by treating cuttings with aqueous colchicine of 0.1 to 0.25 percent for 7-42 hours.

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

Tozyo (1954) obtained tetraploid mulberry plants by treating the buds with 0.4 percent colchicine.

Watanalu et al (1955) treated wheat with 0.05 percent colchicine and obtained amphidiploids.

Carnahan and Hill (1955) made a cross between an induced tetraploid Festuca elator and Lolium perinne. A hybrid with 21 chromosome was obtained. Treatment of F1 tillers with aqueous solution of colchicine resulted in the production of autoallohexaploids.

Illis (1956) reported that giant diploid and mixaploid pollen grains were obtained by treating flower buds of birch with 0.2 percent warm colchicine solution. The treatment was carried out in a partial vacuum and was effective especially at diakinesis.

Kothecker and Fielder (1957) reported that by grafting method and keeping the air humidity at 20 percent and temperature 20°C with colchicine solutions of 0.5 and 0.1 percent for 72 to 96 hours and using tomato as root stalk successful polyploids of Solanum paradoxa were produced.

Osmone (1958) treated flower buds of tea plant with colchicine. Of the 53 buds thus treated, 7 developed fruits with one seed. The resultant seedlings were triploids. The most effective treatment was 0.05 percent colchicine solution for three days.

Palmarcuk (1958) obtained a fertile amphidiploid derived from the cross Secale cereale x Agropyron glaucum by treating the clones of hybrid with colchicine solution, the effective concentration being 0.05 percent.

Papaioannou (1960) obtained polyploids of tobacco by treating 6 lines of Nicotiana with colchicine solution. The most effective concentration was 0.5 percent solution applied for 24 hours.

Nakasone (1961) induced tetraploidy in Vanda by treating cuttings and young shoots with colchicine. The basal end was immersed in aqueous colchicine of 0.5 - 1.5 percent concentrations for 2-6 hours. Most of the induced polyploids were mixaploids.

Ammal (1962) got tetraploid Rauvolfia serpentina L. by subjecting cuttings of the plant to colchicine treatment. Sobti (1963) obtained tetraploid strains of Mentha piperata by treating the suckers with 0.1 percent colchicine for 24 hours.

Effect of colchicine on plants

Colchicine interferes with the morphological, physiological, histological and cytological organisation of treated materials when it is brought in contact with actively dividing cells (Blakeslee and Avery, 1937).

Morphological variation

Whenever colchicine is used as a polyploidising agent, morphological variations have been reported to be accompanying frequently.

Works of many authors showed that the early retarded growth due to colchicine in many cases, was often followed by a considerably enhanced growth rate. (Pandey, 1956 in Linum usitatissimum L., Srivastava, 1956 in Sesame orientale L. and Saxena and Nanda, 1960 in Phlox). The tetraploids were 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, 1942 in Phaseolus radiatus, Bali and Tandon, 1958 in Alyssum maritimum).

Smith (1939) induced polyploidy in Nicotiana species and found that the autotetraploids of Nicotiana rustica, N. tabacum and N. glaucua were characterised by

smaller plant habit, and the presence of smaller and thicker leaves.

Warmke and Blakeslee (1939) found 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 Fagopyron tataricum the surface of the hull to be more rough with more prominent lateral ridges than those in diploids.

Amin (1940) found in colchicine treated cotton, abnormalities such as swollen hypocotyledonary stem, prominant leaf veins, broader bracts, bigger glands, bigger flowers, pollens and seeds.

Graner (1941) found that colchicine treated plants of Manihot utilissima possessed larger lobes which were suddenly constricted at the tip where as in the normal plants leaves were narrower.

Ramanujam and Joshi (1941) who compared the early growths in the colchicine treated and untreated Cicer arietinum reported the delayed germination and retardation of growth rate in treated plants. They 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 abnormalities 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 on the accentuated serration of leaf margin. He also noted in the octoploid and other higher polyploid forms, a pronounced difference in the size of the corolla, between staminate and pistillate flowers borne by the same plant.

Kobayashi and Shimamura (1945) observed the morphological and cytological behaviours in colchicine induced polyploids of sesame after a period of 7 years. The tetraploids were found to be robust and possessed thicker stem, larger leaves and bigger stomata, flowers and seeds compared to those in diploids.

Rao et al (1944, 45) reported that the tetraploid jute showed the typical gigas characters and structurally different from the diploids.

Nishiyama (1950) compared the growth and yield of diploids and colchicine induced polyploid strains of raddish. Though the growth rate was retarded, the yields of tetraploids were higher than those of the diploids.

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

Tandon (1951) reported that colchicine treated plants of Brassica oleraceae Var. Botrytis, showed profuse branching as compared with complete absence of branching in the diploid plants.

Derman (1954) obtained tetraploid grapes with better sized berries by colchicine treatment.

Pisarev (1955) reported that the hybrids derived from wheat x rye, when treated with colchicine gave high yield in grain and straw, higher protein content and resistance to diseases and pests.

Srivastava (1956) comparing diploid and tetraploid sesamum found that the tetraploids had larger leaves, larger stomatal cells, larger flowers, thicker stems, larger capsules and larger pollen grains, but poor in fertility and seed set.

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

Johnsson (1956) treated seeds of Betula verucosa and an F1 hybrid between B. japonica and B. verucosa with 0.2 percent colchicine solution. The individuals obtained were having an abnormal growth due to excessive branching.

Sharma and Dutta (1957) observed that in Coriandrum sativum the tetraploid plants were larger and darker in colour than the diploids. But no polyploid seed germinated.

Libedeva (1959) found that colchicine induced polyploid potatoes were less vigorous. The colchicine treated seedlings did not differ from the diploids. They were self sterile. There was an increased yield linked with decreased starch content.

Cytological effect of colchicine

Colchicine acts upon mitosis with great efficiency, high specificity and total selectivity. The reaction of the drug depends upon (1) specific concentration (2) given exposure period (3) particular mitotic stage where the

chemical contacts the nucleus (4) cellular type and (5) environment favourable for mitosis. The alkaloid mostly arrests the metaphase. As a result chromosomes accumulate in pairs ("Colchicine pairs"). Mitosis with an arrested metaphase due to the action of colchicine is termed as colchicine mitosis (abbreviated C. mitosis).

An arrest at metaphase reduces the number of anaphase or telophase, ultimately the restitution nucleus is formed when the chromosomes enter the interphase without forming daughter nucleus.

Vasrama (1947) reported that in root tips of Ficus nigrum even a very low concentration of colchicine i.e., 0.01 percent produced C. mitotic activity.

Smith et al (1960) found that typical C. mitotic activity in Allium cepa could be obtained by treating the root tissue by 0.025 to 0.8 percent colchicine for a period varying from 3-12 hours.

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

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

Dustin (1934) reported that 0.005 percent of colchicine when applied to Allium root tip for 46 hours increased the percentage of tropokinesis.

Harpsteed et al (1954) reported that arrangement of chromatin was irregular which was detected in the analysis of pairing relationship at pachytene in colchicine induced varieties in sorghum.

Muntzing (1955) obtained a colchicine induced Triticale group which showed cytological abnormalities like restitution nucleus, prophase crumpling, arrested metaphase, bridge formation in anaphase etc.

Evan et al (1957) reported that in broad bean root meristem 0.1, 0.05 and 0.025 percent colchicine solution induced metaphase accumulation. Prolonged treatments for periods of over 6 hours produced considerable slow down in the rate of cells entering in to C. mitosis, their interphase being prolonged.

Son and Chedda (1958) observed a low frequency of quadrivalents in tetraploid Phaseolus mungo. Triads,

Pentads, hexads and septads were counted in larger numbers.

Islam (1960) found comparatively lesser number of quadrivalents in the tetraploids of Anona squamosa, trivalents and univalents were noticed. Laggards were also noticed at anaphase I and II.

Clement et al (1961) found in colchicine treated alfalfa, that sporogenesis was normal in dihaploids which were colchicine treated to double the chromosome number.

Raman and Kesavan (1963) studied the chromosome association at diakinesis and metaphase I. In autotetraploid of Arachis duranensis, in 38 pollen mother cells examined, the frequency of quadrivalents ranged from 0 to 8, the most frequent association being 6 IVs and 8 IIs. In a few cells with trivalents a corresponding number of univalents was not present.

Thankamma (1964) reported that colchicine induced tetraploids of cluster beans showed 14 bivalents at metaphase. Some cells showed 13 bivalents. She also found that octoploid plants showed 28 bivalents during diakinesis.

Nair (1965) observed that colchicine induced tetraploids of sesame showed multivalent association during meiosis, which seriously affected the meiotic behaviour of the tetraploids.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation was conducted in the Division of Agricultural Botany, Agricultural College and Research Institute, Vellayani during the year 1966-67.

A. Materials

Seeds of chillies, *Capsicum frutiscence*, Datta series No. 2, were taken from the collection maintained in the Agricultural College, Vellayani. Seeds were tested for viability as well as for germinability before being used for the investigation.

Chemical

Colchicine (U.S.P.) batch No. 2578 made in Switzerland was used.

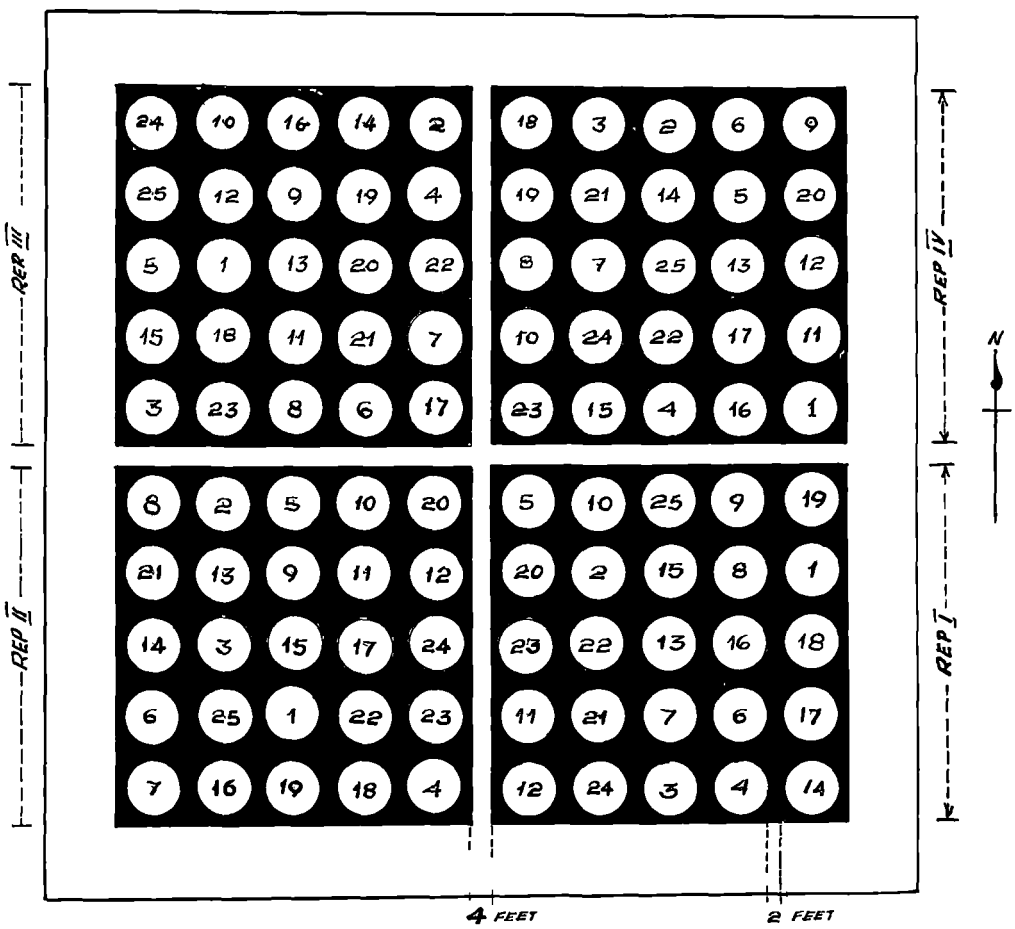
B. Method

Two types of treatments ie., seed treatment and seedling treatment were tried. Aqueous solution of colchicine at four concentrations ie., 0.15, 0.25, 0.35 and 0.45 percent and three durations ie., 3, 6 and 9 hours were employed for the studies. A pot culture experiment using a randomised block design, with four

replications and 25 treatments was conducted. Three replications were used for statistical analysis and the 4th one was kept for cytological investigations.

Treatment details

Treatment No.	Concentraction	Duration	Mode of application
T_1 (Control)	Distilled water application		
T_2	.15%	3 Hrs.	Seed treatment
T_3	.15%	6 ,,	,,
T_4	.15%	9 ,,	,,
T_5	.25%	3 ,,	,,
T_6	.25%	6 ,,	,,
T_7	.25%	9 ,,	,,
T_8	.35%	3 ,,	,,
T_9	.35%	6 ,,	,,
T_{10}	.35%	9 ,,	,,
T_{11}	.45%	3 ,,	,,
T_{12}	.45%	6 ,,	,,
T_{13}	.45%	9 ,,	,,



DESIGN OF EXPERIMENT

Treatment No.	Concentration	Duration	Mode of application
T_{14}	.15%	3 Hrs.	Seedling treatment
T_{15}	.15%	6 ,,	,,
T_{16}	.15%	9 ,,	,,
T_{17}	.25%	3 ,,	,,
T_{18}	.25%	6 ,,	,,
T_{19}	.25%	9 ,,	,,
T_{20}	.35%	3 ,,	,,
T_{21}	.35%	6 ,,	,,
T_{22}	.35%	9 ,,	,,
T_{23}	.45%	3 ,,	,,
T_{24}	.45%	6 ,,	,,
T_{25}	.45%	9 ,,	,,

Sowing

Pots of uniform size were filled with standard potting mixture. They were labelled according to treatments and arranged as per the statistical design. 20 seeds were dibbled in each pot at uniform depth. After one week's growth 5 uniform and healthy seedling were retained in each pot. The removed seedlings under seed

treatment were planted separately for cytological examination. One month after seedling treatment, the plants were again thinned to two in 3 replications and the seedlings were kept without further thinning on replication 4. The plants thus removed from the pots were planted in the field and were also cytologically examined.

Seed treatment

Seeds were directly immersed in the respective solutions in petri dishes for the scheduled periods of time, washed with distilled water and sown in respective pots.

Seedling treatment

Seeds were sown in pots on the same day as in the case of the seed treatment. The apical buds of 15 day old seedlings under each treatment were treated with colchicine at the scheduled concentrations and for scheduled periods of duration.

The solution was dropped using a dropper on the apical bud of the seedlings plugged with cotton. More solution was added to keep the cotton plug moist. After the scheduled periods of treatment the cotton plugs were

removed and the buds were thoroughly washed with distilled water.

Characters studied

The following characters were studied.

Germination percentage

Germination percentage was estimated separately. 50 seeds from each treatment were taken, washed well with distilled water and were allowed to germinate on moist filter paper kept in petri dishes. The germination counts were taken every day for 6 days and germination percentage was worked out from the data.

Early deformity

Abnormalities shown by the plants were observed and recorded.

Height of the plants

Plant height was recorded in cm measuring from soil level to the tip of the apical bud. This was done from the 10th day of sowing till the day of harvest. Data on height of plants recorded before harvest were statistically analysed.

Number of stomata

A random sample of 10 leaves were taken from each treatment for this study. The tissue was peeled off from the lower surface of the leaf and stained with 0.5 percent safranin. The slides were prepared and examined under the microscope. The length and width of 100 randomly selected stomata were measured for each treatment. Number of stomata per microscopic field was also recorded and in each treatment 100 such observations were made. The data for the distribution of stomata were analysed statistically.

Area and thickness of leaves

Measurement of mean area of leaves was made in sq.mm. using graph paper technique. For this, randomly selected leaves were used from the middle of the plants when they were 50 days old.

The thickness of leaves was measured out from the sample collected. It was recorded in micron (μ) as measured from 100 hand sections for each treatment. Measurements were confirmed to the central portion of the leaves in all cases.

Number of flowers

Flower counts were made daily from each plant under different treatments from all replications and the total number of flowers under each treatment was compared by statistical analysis.

Cytological observations

By observing the peculiar characteristics of polyploids the suspected polyploids were labelled. The flower buds of all the treated plants were fixed in 1:3 acetic alcohol. The fixation was done at 9 a.m. for 6 hours. After fixation the material was stored in 70 per cent alcohol. Anthers were squashed in a drop of 2 percent aceto carmine. Gentle tapping and judicious warming resulted in the uniform spreading of chromosomes. The chromosome number and their behaviour such as abnormalities or regularities during meiotic behaviour were studied and the stages were photographed. The behaviour of chromosomes of tetraploid and diploid were compared.

Pollen size and sterility

The size and sterility of pollen grains were studied from an entirely random sample. The pollen grains from the flower of each plant were stained with glycerine aceto

carmine. Diameter of 100 pollen grains from each treatment was measured in micron (u), using a standard ocular micrometer. For estimation of sterility 30 microscopic fields were taken. Well filled and darkly stained pollen grains were classified as fertile and shrunken and unstained ones as sterile.

Number of fruits

The total number of fruits set in each plant was counted and the data were subjected to statistical analysis. The percentage of fruit set was also worked out.

Yield

Yield of fruits, fresh weight as well as dry weight of all the treatments were recorded and the data were analysed. Weight of 1000 seeds from each treatment was recorded in grams.

Statistical procedure

The recorded data, for different characters under study were subjected to statistical analysis. The effect of different treatments on sterility of pollen was calculated by applying the X^2 test. Analysis of variance

was worked out for height of plants, number of stomata, number of flowers, number of fruits and dry weight of fruits. The effect of other treatments on other characters under study was determined by calculating the mean and standard error.

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

Percentage of germination

The seeds of Capsicum frutiscence variety Dutta series No. 2 possess a high percentage of germination. Seeds treated with colchicine showed no reduction in germination (Table I).

Time taken for germination

The seeds treated with colchicine in general showed no delay in germination. The data on germination percentages at 24 hours intervals are presented in Table I.

Early deformity of seedling

No plants under seed treatment showed any deformity of the seedlings. The colchicine treated seedlings were characterised by a number of morphological abnormalities such as retarded growth at early stage, crinkling and lobing of leaves, thicker and dark purple coloured leaves etc. (Table II, Plate I). Out of the 480 treated plants 142 showed such abnormalities. All the treatments except T_{14} ie., treatment with lowest concentration for the shortest duration showed crinkling

o

Table I

Germination of colchicine treated seeds

Treatments	Germination percentage after						
	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	116 Hrs.	140 Hrs.	Total
T_1		8	4	22	44	8	88
T_2		10	8	28	6	34	86
T_3		18	14	12	10	4	94
T_4		28	10	40	18	-	96
T_5		18	24	24	6	22	94
T_6		32	16	10	4	30	92
T_7		28	8	20	20	20	88
T_8		-	12	34	8	26	80
T_9		20	8	44	14	4	90
T_{10}		-	12	46	-	30	88
T_{11}		8	6	68	6	4	88
T_{12}		8	12	42	18	14	94
T_{13}		6	12	36	12	24	90

of leaves for one week after treatment. The growth of seedlings was found to be retarded for the first 4 days in the case of 3 hours' treatments and one week for 6 hours' and 9 hours treatments in all the concentrations. The leaves developed immediately after the treatment were small, thick and malformed. With regard to lobing of leaves the different treatments produced different results. T_{14} ie., the lowest concentration for the shortest duration produced leaves with no lobing. T_{25} ie., the highest concentration for the maximum duration produced leaves with a maximum of eight lobes. T_{18}, T_{19}, T_{20} and T_{24} produced leaves with varying lobes of 3-5. The other treatments produced leaves with the maximum of 2 lobes.

Height of plants

The final plant height at the time of harvest showed no significant difference when compared with the control. The analysis of the data is given in Table III.

Leaf characters

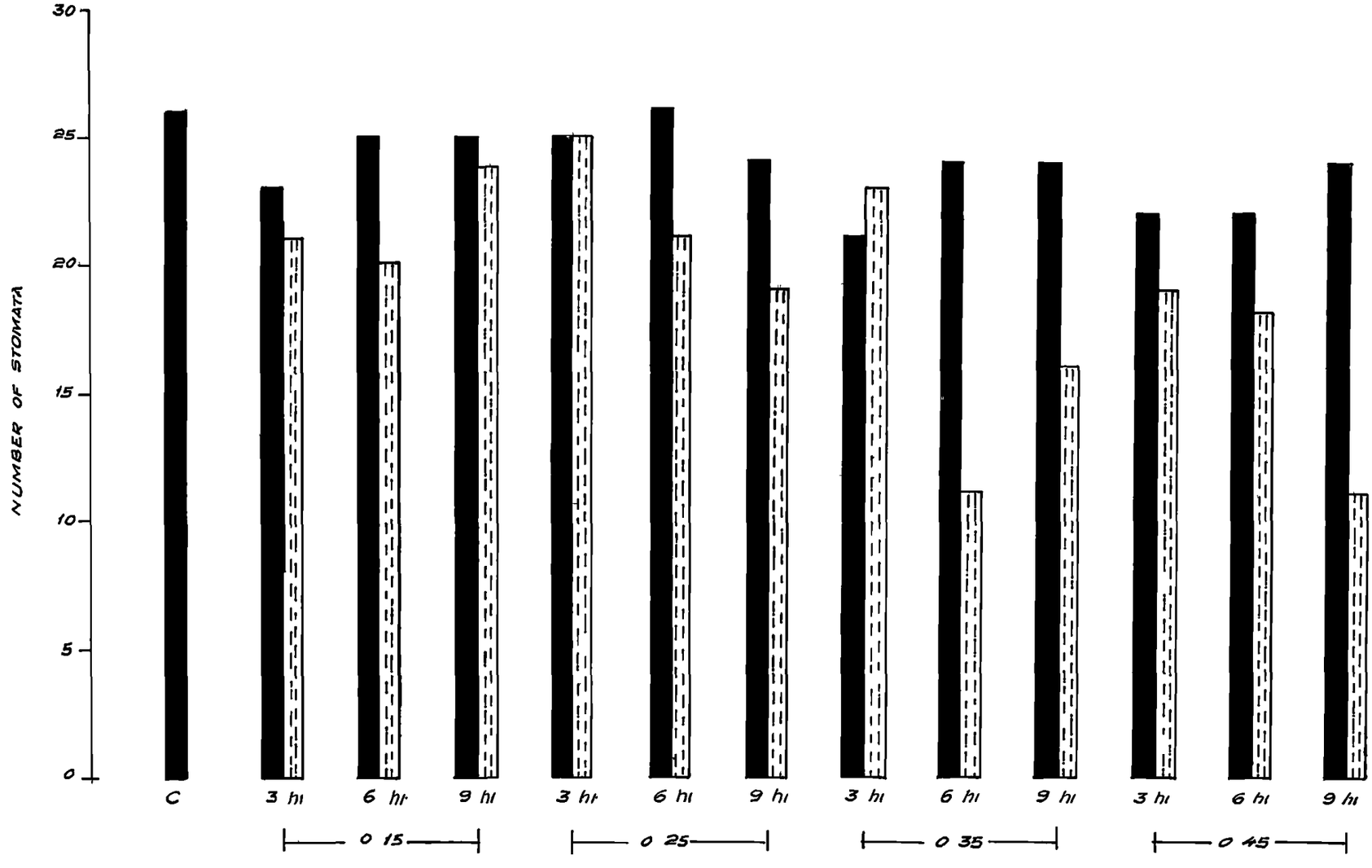
Size and distribution of stomata

Considerable increase was noticed in the size of the stomata in the treated plants. With regard to the

Table II

Early Deformity

Treatment	Total No. of plants	Dobbing	Crinkling	Purple dots	Smooth	Rough
T_{15}	20	12	16	-	14	6
T_{16}	20	16	7	-	12	8
T_{17}	20	12	5	-	15	5
T_{18}	20	12	5	-	15	5
T_{19}	20	16	6	1	16	4
T_{20}	20	9	2	-	20	0
T_{21}	20	33	7	-	9	11
T_{22}	20	17	10	-	9	11
T_{23}	20	8	10	-	10	10
T_{24}	20	5	8	-	14	6
T_{25}	20	19	10	-	9	11



FREQUENCY OF STOMATA

SEED TREATMENT —

SEEDLING TREATMENT —

FIG 2

Table III

Height of plant at the time of harvest

Analysis of variance

Source	S.S.	Df	V	F	F
Total	1010303.16	74			
Block	996579.4	2	498289.7	223.98	
Treatment	3045.16	24	126.88	1	
Error	10678.6	48	222.47		

F. ratioes are not significant

number of stomata per unit area the treatment mean was not statistically different from the control. But the tetraploid plant showed significant reduction in the number of stomata per unit area.

Size

Both the length and width of stomata were found to be increased in the leaves of the treated plants when compared to those of the control.

Length

The tetraploid had a mean stomata length of 69.430 ± 0.398 μ while in the control it was only 21.81 ± 0.01617 μ. The data in respect of the length of stomata are given in Table X. The maximum mean length of stomata ie., 67.430 ± 0.298 μ was obtained in T_{25} followed by T_{19} having 45.74 ± 0.418 μ.

Mean length of stomata in μ

Between deploid and tetraploid	Diploid	22.110 ± 0.0167
	Tetraploid	69.430 ± 0.398
Between concentration	0.15%	25.69 ± 0.1804
	0.25%	27.94 ± 0.2332

	0.35%	24.88 ± 0.1452
	0.45%	31.25 ± 0.2882
Between duration	3 hours	27.852 ± 0.1496
	6 hours	28.028 ± 0.1716
	9 hours	28.078 ± 0.0616
Seed Vs. Seedling	Seed	26.07 ± 0.1166
	Seedling	32.428 ± 0.2882

Width

The width of stomata in the polyploid was also found to be increased. The mean width of stomata in the tetraploid was 47.078 ± 0.812 µ while in diploid it was 20.350 ± 0.11518 µ. Maximum mean width of stomata was obtained in T which was 45.078 ± 0.719 µ followed by T_{16} having a mean width of 31.450 ± 0.275 µ. The details of width of stomata in the various treatments are given in Table X. Plate II shows the size of stomata in the control as well as in the polyploid plant.

Mean width of stomata in µ

Between diploid and tetraploid	Diploid	19.250 ± 0.1518
	Tetraploid	47.078 ± 0.812

Between concentrations	0.15%	17.67 ± 0.01
	0.25%	20.83 ± 0.1364
	0.35%	21.054 ± 0.011
	0.45%	27.126 ± 0.3168
Between durations	3 hours	20.372 ± 0.099
	6 hours	20.526 ± 0.0946
	9 hours	32.032 ± 0.2266
Seed Vs. Seedling	Seed	19.734 ± 0.770
	Seedling	23.10 ± 0.209

Number of stomata per unit area

The data on stomatal counts were statistically analysed. Eventhough the treatment differences were statistically not significant the higher treatments showed fewer stomata per unit area. The polyploid had only 11 stomata per unit area while it was 26 in the control. (Table IV, Plate III, Fig. 2).

Mean area of leaves

The treated plants possessed larger leaves than the control. The mean area of leaves ranged from 181 mm in treatment 4 to 281 mm in treatment 22 whereas it was 219 mm in the control. Difference in the mean area of

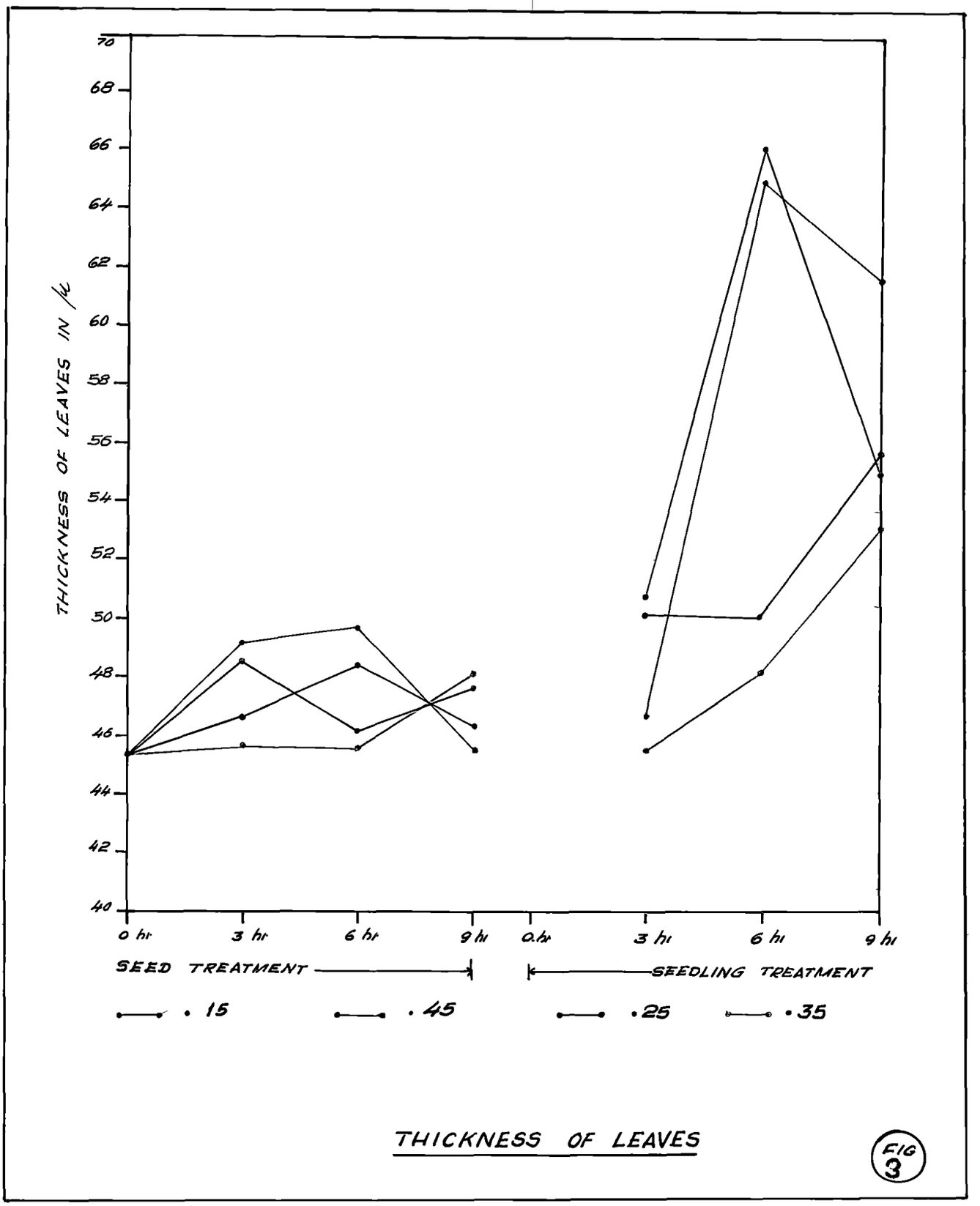
Table IV

Number of stomata per unit area

Analysis of variance

Source	S.S.	Df	V	F
Total	228844933.0	2459		
Treatment	48606.5	24	2025.27	0.213
Error	2283586.8	2475	9226.62	

F values are not significant



THICKNESS OF LEAVES

FIG 3

Table V

Analysis of variance for mean area of leaves

Source	S S	Df	V	F
Total	589069.39	74		
Replication	98658.35	2	49329.17	9.58**
Treatment	243368.06	24	10140.34	1.97*
Seed Vs Seedling	36405.01	1	36405.01	7.07**
Seed Vs Control	404.32	1	404.32	0.08
Seedling Vs Control	9014.78	1	9014.78	1.75*
Between duration	4982.53	2	2491.26	0.48
Between concentration	72753.86	3	35173.31	6.83**
Between duration for seed treatment	2184.00	2	1092.00	0.21
Between concentration for seed treatment	72753.86	3	24251.29	4.71**
Between duration for seedling treatment	20039.05	2	10019.52	1.95*
Between concentration for seedling treatment	58494.55	3	19498.18	3.79*
Error	247042.98	48	5146.73	

** Significant at 1% and 5% level

Between concentration C.D. 63.35

T_3 T_5 T_2 T_4 T_6 T_7 T_8 T_9 T_{10} T_{11} T_{12} T_{13} T_{14}

T_{15} T_{16} T_{17} T_{18} T_{19} T_{20} T_{21} T_{22} T_{23} T_{24} T_{25}

Between concentration for seed treatment C.D. 68.07

T_1 T_2 T_3 T_4 T_5 T_6 T_7 T_8 T_9 T_{10} T_{11} T_{12} T_{13} T_{14} T_{15}

T_{16} T_{17} T_{18} T_{19} T_{20} T_{21} T_{22} T_{23} T_{24} T_{25}

Between duration for seedling treatment C.D. 41.66

T_{15} T_1 T_2 T_3 T_4 T_5 T_6 T_7 T_8 T_9 T_{10}

T_{11} T_{12} T_{13} T_{14} T_{16} T_{17} T_{18} T_{19} T_{20} T_{21} T_{22}

T_{23} T_{24} T_{25}

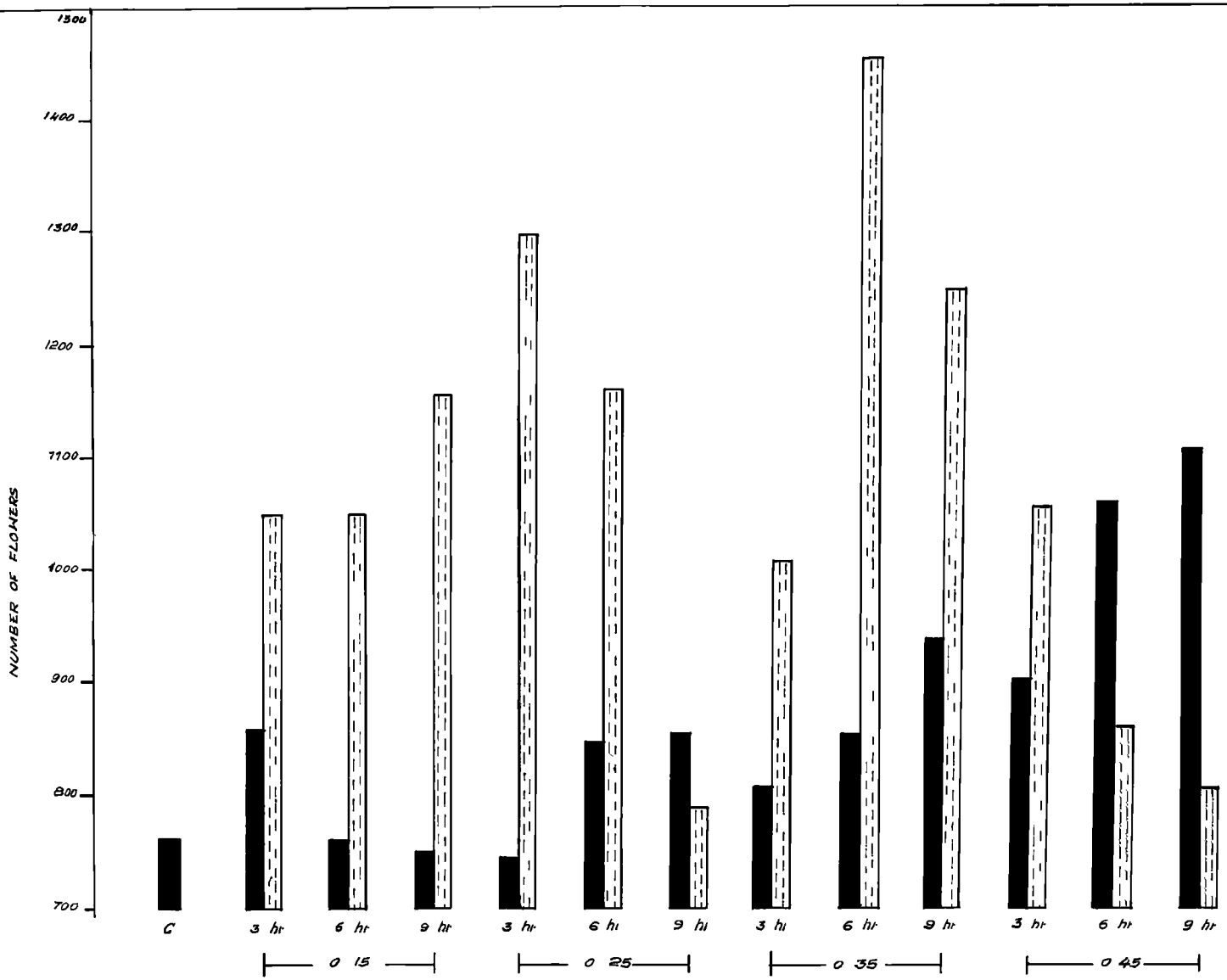
leaves between the different treatments was significant. The data are presented in Table V. The maximum mean area of 281 mm was obtained under treatment 22 (0.35 per cent solution for 9 hours in seedling treatment). The plant which was later confirmed to be a tetraploid possessed a mean leaf area of 198 mm as compared with the mean leaf area of 219 mm of the control. (Table IX).

Thickness of leaves

The data for mean thickness of leaves in different treatments are given in Table X. In the tetraploid the mean leaf thickness was found to be 64.30 ± 0.26624 while in diploid it was only 45.46 ± 0.1716 μ. (Table IX, Plate III, Fig. 3). Between seedling treatments it was found to be significantly superior. Between concentrations the concentration 0.35 percent and between durations, the duration of 9 hours were found significantly superior. The maximum mean thickness of leaves was obtained in T_{25} (0.45 percent for 9 hours seedling treatment).

Mean thickness of leaves in μ

Between diploid and tetraploid	Diploid	42.46 ± 0.1716
	Tetraploid	64.30 ± 0.26624



TOTAL NUMBER OF FLOWERS

SEED TREATMENT

SEEDLING TREATMENT

FIG 4

Between concentrations	0.15%	50.49 ± 0.2552
	0.25%	49.126 ± 0.3686
	0.35%	53.90 ± 0.3696
	0.45%	50.116 ± 0.2562
Between durations	3 hours	51.788 ± 0.2662
	6 hours	50.402 ± 0.2024
	9 hours	59.51 ± 0.3564
Seed Vs Seedling	Seed	48.18 ± 0.0182
	Seedling	52.14 ± 0.2486

Number of flowers

The data for total number of flowers produced are presented in Table VI and Fig. 4. The mean total number of flowers produced ranged from 112 in T_4 to 193 in T_{22} while it was 128 in the control. The treatment differences were not statistically significant. The tetraploid plant produced 200 flowers while in control it was 128. (Table X and IX).

Cytological observations

Meiosis was studied in the treated plants as well as in the untreated plants. Only one plant under treat-

Table VI

Number of flowers

Analysis of variance

Source	S.S.	Df	V	F
Total	1440184.35	74		
Replication	597625.79	2	298812.89	28.36
Treatment	336754.35	24	14031.43	1.33
Error	505804.21	48	10537.59	

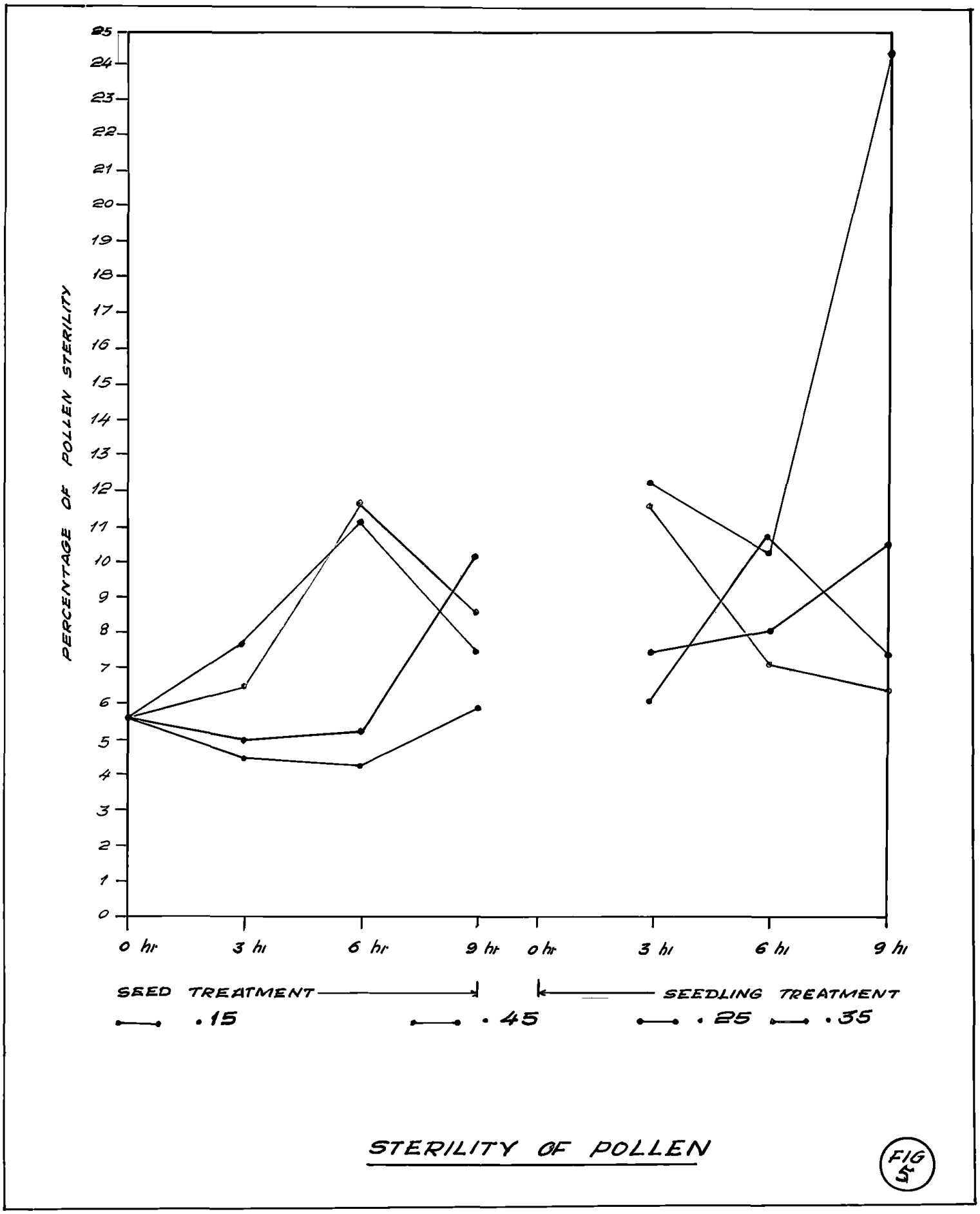
F r tios are not significant

ment 25 was found to be tetraploid with 2n = 48. In diploids the meiotic division was normal with the formation of 12 IIs at diakinises and metaphase I and regular seperation at anaphase I and II (Plate IV). The early stages of diakinises and metaphase I in the pollen mother cells of the tetraploid were normal with the formation of 24 IIs, but abnormalities like laggards and bridges were seen at anaphase I and II (Plate V). During anaphase I, 3 laggards were seen in one cell and in another cell 2 laggards and 3 bridges were seen. During telophase in some cells only 3 groups of chromosomes were noticed. In some others 5 groups were noticed. In some cases besides these 5 groups, 2 laggards were seen located at the centre of the cell.

Size and sterility of pollen

Size of pollen

The tetraploid had larger pollen grains than the diploids. The pollen grains in tetraploid possessed a mean diameter of 46.970 ± 0.0187 μ while it was 22.836 ± 0.3718 μ in diploids. (Fig. 5). The mean diameter of pollen grains under the various treatments is given in Table X.



STERILITY OF POLLEN

FIG 5

Table VII

Analysis of variance for the sterility of pollen

Treatment	Value of X^2
Treated Vs control	59.2**
Seed Vs Seedling	4041.56**
Between durations	121.36**
Between concentrations	236.64**

Significant at 1% and 5% level

Diameter of pollen grains in μ

Between diploid and tetraploid	Diploid	22.836 ± 0.3718
	Tetraploid	46.970 ± 0.0187
Between concentrations	0.15%	24.002 ± 0.0374
	0.25%	24.41 ± 0.0352
	0.35%	23.958 ± 0.0352
	0.45%	25.52 ± 0.0066
Between durations	3 hours	23.958 ± 0.033
	6 hours	24.332 ± 0.0374
	9 hours	25.058 ± 0.0187
Seed Vs Seedling	Seed	24.64 ± 0.029
	Seedling	25.21 ± 0.0418

The maximum mean diameter of pollen grains was noted in 0.25 percent concentration followed by 0.35, 0.45 and 0.15 percent respectively. Between duration maximum size was noticed in 9 hours treatment followed by 6 hours and 3 hours respectively of the 2 stages of treatment. Mean diameter of pollen was greater in seedling treatme t than in seed treatment.

Extend of pollen sterility

The mean percentages of sterility and its analysis

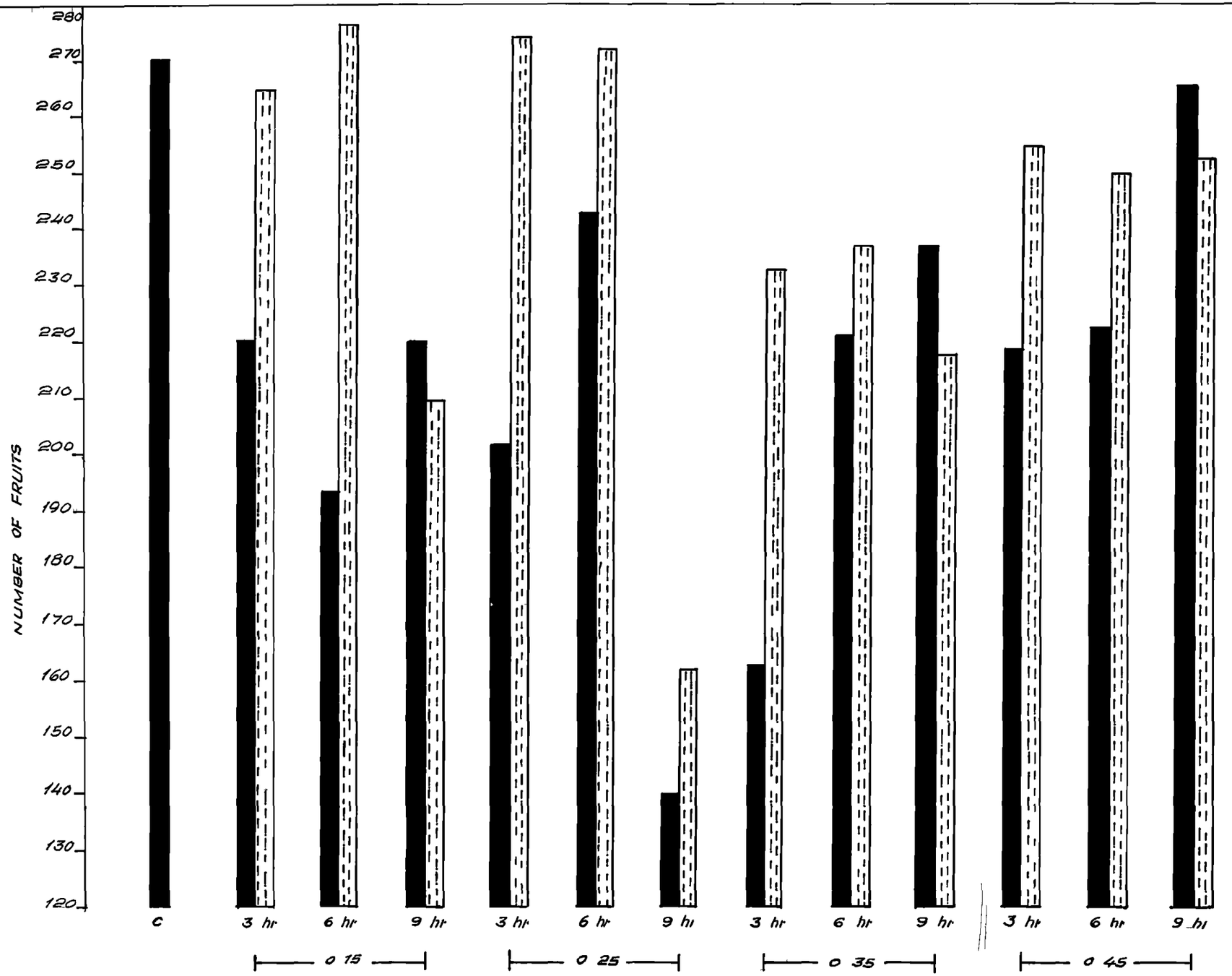
Table VIII

Number of fruits

Analysis of avariance

Source	S.S.	Df	V	F
Total	34928.00	74		
Treatments	9405.65	24	391.90	1.24
Replications	10346.64	2	5173.32	16.36
Error	15176.63	48	316.17	

F ratioes are not significant

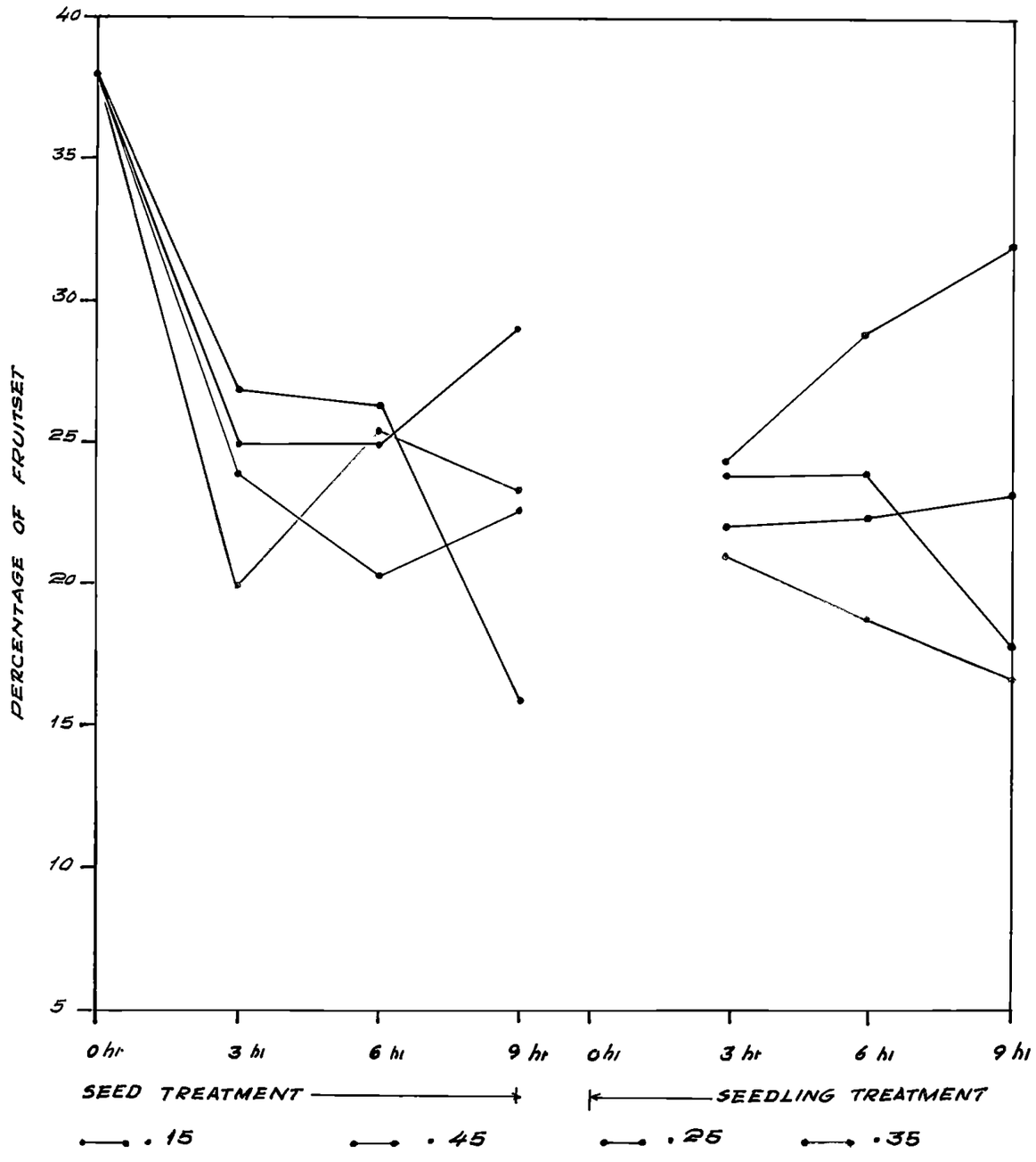


TOTAL NUMBER OF FRUITS

SEED TREATMENT

SEEDLING TREATMENT -

FIG 6



PERCENTAGE OF FRUITSET

FIG 7

are given in Table VII. (Fig. 5). In the tetraploid, the percentage of sterility was 44.2 whereas in diploid the sterility of pollen grain was only 5.1 percent. 0.5 0.45 percent for 9 hours treatment at seedling stage resulted in the highest percentage of sterility of pollen grains. The seedling treatment was remarkably superior to seed treatment in producing pollen sterility.

Number of fruits

Mean number of fruits produced and the percentage of fruit set under the different treatments are given in Table X. (Fig. 6). The mean number of fruits varied from 32 in treatment 3 to 46 in treatment 15 as compared to 45 in the control. The treatment difference was not found to be statistically significant. (Table VIII). The percentage of fruit set ranged from 16.3 in treatment 7, to 31.3 in treatment 25, as compared to 37.7 in the control. (Fig. 7). The tetraploid plant produced 120 fruits with a fruit set of 60 percent compared to the control producing 45 fruits and fruit set of 37.7 percent.

Yield of fruits

The yield data of ripe fruits pertreatment before and after drying in the various treatments are given in Table XI.

Table XI

Yield of fruits in grams

Treat-ments	Fresh weight	Dry weight	Treat-ments	Fresh weight	Dry weight
T_1	242	169.5	T_{14}	307	265.0
T_2	300	220.0	T_{15}	318	276.0
T_3	272	196.5	T_{16}	252	210.4
T_4	300	219.2	T_{17}	316	273.0
T_5	244	191.6	T_{18}	314	271.2
T_6	285	241.8	T_{19}	214	261.0
T_7	182	140.1	T_{20}	275	232.7
T_8	233	162.1	T_{21}	299	246.5
T_9	263	220.0	T_{22}	280	219.2
T_{10}	269	246.8	T_{23}	297	254.7
T_{11}	292	218.8	T_{24}	292	243.1
T_{12}	265	222.8	T_{25}	390	258.0
T_{13}	308	266.0			

The yield of ripe fruits before drying ranged from 182 g in treatment 7 and 300 g in treatment 25 while it was 242 g in the control.

The yield of ripe fruits after drying ranged from 140 g in treatment 7 to 258 g in treatment 25 while it was 169.5 g in the control.

Polyploid plant gave a yield of 125 g of ripe fruits before drying and 99.5 g after drying. There was significant difference between the fresh and dry weight of fruits between polyploid and control.

Weight of 1000 seeds

The following results give the weight of 1000 seeds in different treatments.

	1000 seed weight
T_1	2.98 g
T_2	2.90 g
T_3	2.80 g
T_4	3.00 g
T_5	3.00 g
T_6	2.90 g

T_7	2.80 g
T_8	2.90 g
T_9	3.20 g
T_{10}	3.10 g
T_{11}	3.10 g
T_{12}	3.20 g
T_{13}	2.90 g
T_{14}	3.20 g
T_{15}	3.20 g
T_{16}	2.95 g
T_{17}	3.00 g
T_{18}	3.10 g
T_{19}	3.10 g
T_{20}	3.00 g
T_{21}	3.10 g
T_{22}	3.00 g
T_{23}	3.10 g
T_{24}	3.10 g
T_{25}	3.50 g
Tetraploid	3.90 g

Cytological identification of tetraploid

Based on meiotic chromosome number in the meiotic metaphasic and anaphasic preparations, only one plant was confirmed to be tetraploid in the present study. The following data 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 confirmed as polyploid by cytological analysis.

Seed treatment

Concentration	Duration	No. of seeds	No. of suspected polyploids	Tetraploid confirmed
0.15%	3 hours	20	-	-
0.15%	6 ,,	20	-	-
0.15%	9 ,,	20	-	-
0.25%	3 ,,	20	-	-
0.25%	6 ,,	20	-	-
0.25%	9 ,,	20	-	-
0.35%	3 ,,	20	-	-
0.35%	6 ,,	20	-	-
0.35%	9 ,,	20	-	-

Concentration	Duration	No. of seeds	No. of suspected polyploids	Tetraploid confirmed
0.45%	3 hours	20	-	-
0.45%	6 ,,	20	-	-
0.45%	9 ,,	20	-	-
	Seedling treatment			
0.15%	3 ,,	20	-	-
0.15%	6 ,,	20	16	-
0.15%	9 ,,	20	16	-
0.25%	3 ,,	20	12	-
0.25%	6 ,,	20	12	-
0.25%	9 ,,	20	10	-
0.35%	3 ,,	20	9	-
0.35%	6 ,,	20	13	-
0.35%	9 ,,	20	17	-
0.45%	3 ,,	20	8	-
0.45%	6 ,,	20	10	-
0.45%	9 ,,	20	19	1-

Table IX

Comparison between Diploid and Tetraploid

No. of characters	Diploid	Tetraploid
Height of plant at the time of harvest	50.00 cm	64.50 cm
Mean area of leaves	219.00 mm	198.00 mm
Mean thickness of leaves	45.54 μ	64.30 μ
Average number of stomata per unit area	26	11
Mean length of stomata	21.89 μ	69.43 μ
Mean width of stomata	20.35 μ	47.078 μ
Mean diameter of pollen	22.86	46.97
Average number of flowers per plant	128	200
Mean sterility of pollen	5.7 %	44.4 %
Average number of fruits per plant	45	120
Mean weight of 1000 seeds	2.98 g	3.9 g
Chromosome number	2n = 24	2n = 48

Table X

Seed Treatment

Treatments		Percentage of germination	Mean height of plant cm	Average number of stomata	Mean length of stomata in μ	Mean width of stomata in μ	Average area of leaf in sq. mm.
Control		88	55	26	25.30	17.82	219
0.15%	3 Hrs.	86	54.8	23	21.89	20.35	206
	6 ,,	94	55.0	25	24.64	18.37	225
	9 ,,	96	56.6	25	22.55	28.04	181
0.25%	3 ,,	94	46.8	25	24.64	18.59	213
	6 ,,	92	60.5	26	21.34	17.93	224
	9 ,,	88	56.8	24	22.60	22.22	228
0.35%	3 ,,	80	60.6	21	30.58	22.22	255
	6 ,,	90	57.3	24	28.20	17.93	265
	9 ,,	88	60.0	24	24.75	17.71	268
0.45%	3 ,,	88	63.3	22	28.60	20.35	240
	6 ,,	94	64.0	22	27.83	13.15	197
	9 ,,	90	53.0	24	31.90	21.82	203

Table X (Contd..)

Treatments		Average thickness of leaf in μ	Average No. of flowers	Percentage of pollen sterility	Mean diameter of pollen in μ	Average No. of fruits	Percentage of fruit set
Control		45.54	128	5.7	22.836	45	37.7
0.15%	3 Hrs.	45.54	129	4.4	23.47	36	25.4
	6 ,,	46.86	129	4.3	23.10	32	24.9
	9 ,,	48.42	123	6.2	23.95	36	29.3
0.25%	3 ,,	46.42	150	4.9	23.60	33	27.2
	6 ,,	48.62	142	5.1	23.36	40	26.9
	9 ,,	46.31	135	10.1	21.60	23	16.3
0.35%	3 ,,	47.96	144	6.6	24.90	27	20.02
	6 ,,	45.98	157	11.7	23.29	36	25.5
	9 ,,	48.29	151	8.8	23.27	37	23.9
0.45%	3 ,,	49.30	178	7.8	25.65	36	24.1
	6 ,,	49.85	175	11.7	23.10	37	20.8
	9 ,,	45.67	175	7.1	23.65	44	23.7

Table X (Contd..)

Seedling treatment

Treatments		Mean height of plant cm	Average No. of stomata	Mean length of stomata in μ	Mean width of stomata in μ	Average area of leaf in sq. mm.	Average thickness of leaves in μ
0.15%	3 Hrs.	65.83	21	26.29	17.82	190	59.4
	6 ,,	66.50	20	32.58	20.72	206	66.2
	9 ,,	61.66	21	29.35	31.45	245	55.5
0.25%	3 ,,	62.5	25	26.24	22.85	259	51.04
	6 ,,	66.5	21	29.86	20.63	245	51.34
	9 ,,	69.0	19	45.74	23.1	253	55.94
0.35%	3 ,,	61.5	23	31.45	22.9	249	45.71
	6 ,,	61.8	11	27.83	21.34	259	48.33
	9 ,,	69.3	16	26.74	20.13	281	53.21
0.45%	3 ,,	61.83	19	27.74	21.67	231	46.75
	6 ,,	58.0	18	28.82	21.78	276	65.50
	9 ,,	63.17	11	67.43	45.078	276	61.84

Table X (Contd..)

Treatments		Average No. of flowers	Percentage of pollen sterility	Mean diameter of pollen in μ	Average No. of fruits	Percentage of fruit set
0.15%	3 Hrs.	175	5.9	24.22	44	24.0
	6 ,,	175	10.8	23.65	46	25.1
	9 ,,	193	7.4	25.54	35	18.1
0.25%	3 ,,	215	7.7	23.27	45	22.1
	6 ,,	196	8.1	24.97	45	23.9
	9 ,,	131	10.7	24.95	28	10.8
0.35%	3 ,,	178	11.7	22.22	38	21.8
	6 ,,	214	7.1	25.01	41	19.2
	9 ,,	242	6.6	24.402	36	17.3
0.45%	3 ,,	193	11.8	25.21	42	24.1
	6 ,,	143	10.2	25.89	41	29.1
	9 ,,	134	24.2	31.22	42	31.3

DISCUSSION

DISCUSSION

The object of the trial was to evaluate the scope for applying the colchicine technique in improving chillies.

Chillies responded well to colchicine, which was evident from the fact that most of the treated plants showed various types of morphological abnormalities. But it was less effective in inducing polyploidy. Out of the 480 plants treated, 142 showed such abnormalities but out of this only 1 plant showed doubling of chromosome number. Pal et al (1938) and (1941), Ramanujam and Joshi (1941) and Aleksio (1960) induced polyploidy in chillies by applying colchicine technique.

In the present investigation it was proved that the tetraploids could be produced in chillies by colchicine application. But their proportion of production was too low with the concentration employed in this study.

In general, many of the treated plants showed that they were affected by the alkaloid colchicine. This was evident from their morphological abnormalities.

Germination in colchicine treated seeds

The seeds treated with colchicine started germination after 24 hours as in the control and the initial growth rate was also normal. In certain cases the treated seeds showed greater germination percentage. Kluge and Kramer (1955) obtained accelerated germination and increased germinability in colchicine treated Vaccinium myrtellium and V. corymbosum, diploids and tetraploids varieties of Fragaria vesca, while Pal et al (1941) recorded reduced germination percentage in seed treatment of chillies.

Seed Vs Seedling treatment

From the present study it was clear that the seedling treatment was more successful in inducing polyploidy in chillies, than seed treatment. The success of seed treatment in inducing polyploidy was reported by various workers like Muntzing (1939) in Pinus pondorosa, Pal et al (1941) in chillies, Ramanujam and Joshi (1941) in Cicer arietinum, Srivastava (1956) in Sesame and Nair (1965) in Sesame.

But according to Warmke (1939) in Tobacco, Shimamura (1939) in Lycopercicum esculantum, Beasley

(1940) in Gossypium, Langhas (1940) in Sesame, Kumar and Abraham (1942) and Kumar (1945) in Phaseolus, Tool and Bamford (1945) in pepper and Thankamma (1964) in cluster beans seedling treatment was found to be superior to seed treatment.

Early deformity of seedlings

The data presented in Table II show that in general all the concentrations of colchicine employed in the present study for the seedling treatment resulted in some early deformities of the treated seedlings. Except with the lowest concentration of 0.15 percent for 3 hours durations the treatments were effective. On a comparison of the different durations it was seen that the initial retardation of growth was found to persist for longer periods with the longer durations of treatment. It was also observed that though the increase in leaf lobing was not proportionate to the increase in concentration or duration, the abnormal leaves with the maximum lobings were obtained under the treatment receiving the highest concentration for the longest duration.

Early deformities in colchicine treated plants including retardation of growth were recorded by Pandey (1956) in Linum usitatissimum, Srivatsava (1956) in

Sesamum orientale. Ramanujam and Joshi (1941) found that increased concentrations and longer durations of treatment caused more pronounced retardation of growth rate in colchicine treated Cicer arietinum. Similar results with chillies were reported by Pal et al (1941).

In the present study only 142 out of 480 treated seedlings produced morphological abnormalities. Even among these 142 seedlings only one plant under T_{25} was confirmed as a polyploid and hence it could be observed that all the lower concentrations and shorter duration could produce only some physiological disturbances in the treated plants.

There was no early deformity in plants from seeds treated with colchicine. Pal et al (1941) recorded slower growth, leaf curling and other abnormalities in seedlings of chillies from colchicine treated seeds. The results in this study indicated that colchicine treatment of chilli seeds should be done for more than 9 hours in any case if some effects of this chemical are to be obtained even with a concentration of 0.45 percent.

Height of the plants

The final plant height was not found to be much affected by any of the treatments. In the tetraploid which was confirmed after cytological observations it was found that there was considerable increase in the plant height compared to the diploid. Towards the first week there was a slow growth rate in the tetraploid than the diploid, but later there was an increase in height. This is in agreement with the result of Srivastava (1956) in sesame and Tandon and Chinoy (1950) in Amaranthus blitum.

The fact that the tetraploid obtained showed increased stature than any other plant treated with the same concentration and duration of the chemical suggests that the mechanism involved is not a general stimulus but an increase in cell size brought about by chromosome doubling.

Size and distribution of stomata

On a comparison of the different treatments it was found that there was a general increase in stomatal size both in length and width in the plants under the seedling treatments. With regard to the distribution

of stomata, though the differences between treatments were not significant, there was a general reduction in the number of stomata per unit area in the plants treated with higher concentration.

The polyploid plant obtained showed significant increase in stomatal size both in length and breadth and a significant reduction in the number of stomata per unit area compared to the control. Gramer (1941) in Manihot utilissima and Kobayashi (1945) and Srivastava (1955) in sesame also obtained similar results.

Area and thickness of leaves

It was observed from the present investigation that the tetraploid possessed thicker and darker leaves with rough surface than the diploids. The lower surface of leaves was hairy. But the leaf area was comparatively smaller than the corresponding diploids. This is in agreement with the record of Smith (1939) in Nicotiana. Tandon and Chinoy (1950) observed thicker and darker leaves with increased leaf area in polyploids of Amaranthus blitum. The rough leaf surface in polyploids was recorded by Sando (1939).

Blooming behaviour and number and size of flowers

Delayed flowering by 27 days was observed in tetraploid chilli. The floral parts were in general bigger in size compared to the control. Delayed flowering of polyploid was reported by Kumar and Abraham (1942) in phaseolus, Srivastava (1955) in sesame, Spasojevic (1956) in beans and Bali and Tandon (1958) in Alyssum maritinum.

Cytological observations

The meiotic behaviour of the tetraploid was found to be normal during diakinesis and metaphase I with 24 bivalents. Pal et al (1941) in chillies, Sen and Ghedda (1958) in Phaseolus mungo, Islam (1960) in Anona squamosa, Raman and Kesavan (1963) in Arachis duranensis, Thankamma (1964) in cluster beans and Nair (1965) in sesame recorded higher associations at diakinesis and metaphase I.

Meiotic abnormalities like laggards and bridges during anaphase, and triads, pentads and excluded chromosomes during telophase were observed in the tetraploid obtained in the present study. Such abnormalities were recorded by Muntzing (1954) in Triticales, Sen and

Chedda (1958) in Phaseolus mungo and Islam (1960) in sesame.

Size and sterility of pollen

The tetraploid obtained in the present study was characterised by a high percentage (44.2 percent) of pollen sterility. Pal et al (1941) recorded 30-90 per cent pollen sterility in tetraploid chillies.

Srivastava (1956) in sesame and Saxena and Nanda (1960) in phlox also recorded increased pollen sterility in polyploids.

The pollen grains produced by the tetraploid chillies were larger in size than the corresponding diploids. Increase in size of pollen grains of tetraploids was recorded by Pal et al (1941) in chillies, Illis (1956) in birch, Srivastava (1956) in sesame, Armstrong and Robertson (1960) in Alsike clover.

Fruit setting and yield

There was an increase in the percentage of fruit set in the tetraploid even though there was greater pollen sterility. Total fruit yield was thus increased.

Other workers like Kumar and Abraham (1942) in *Phaseolous radiatus*, Singh (1955) in *Carica papaya*, Sen and Bhowal (1959) in *Vigna sinensis* and Nair (1965) in sesame however, recorded reduction in fruit set and yield. The fruits of tetraploid were smaller than the corresponding diploids. Production of smaller fruits in tetraploid chillies was recorded by Aleksic (1961).

SUMMARY

S U M M A R Y

The present study was undertaken in the Division of Agricultural Botany at the Agricultural College and Research Institute, Vellayani with a view to evaluating the scope of colchicine technique in the improvement of chillies (*Capsicum frutescens*, 2n = 24).

Four concentrations - 0.15, 0.25, 0.35 and 0.45 percent - of aqueous solutions of colchicine in combination with three different durations ie., 3, 6 and 9 hours and two modes of application ie., seed and seedling treatments were tried and the effect of the drug as seen from the growth and performance of the plants was discussed.

The observations of the present study can be summarised as follows:-

1. At the concentrations and durations of treatment employed in this study germination of colchicine treated chilli seeds was not affected.

2. Seedling treatment of colchicine was more effective for induction of polyploidy in *C. frutescens*, than seed treatment.

3. Seedling treatment with colchicine resulted in early deformities like retarded growth, leaf lobing and crinkled appearance of leaves, whereas plants from treated seeds were normal.

4. It was found that early deformities immediately following the application of colchicine cannot be taken as an indication of induction of polyploidy as only one out of 142 plants with early deformities was confirmed as a polyploid in this study.

5. The early retardation in plant height due to colchicine application was found to be made up during the later stages of plant growth.

6. The tetraploid C. frutescens showed taller plant habit, thicker leaves, larger stomata in lesser number, delayed blooming, larger flowers in larger number, high pollen sterility and increased yield of fruits compared to the diploids.

7. The meiotic abnormalities observed in the pollen mother cells of tetraploid C. frutescens were laggards and bridges at anaphases I and II and occurrence of triads, pentads and excluded chromosomes at telophase.

8. The fact that only one polyploid plant could be obtained in the present study indicates that colchicine at concentrations tried is not much effective in Capsicum frutescens as a polyploidising agent.

9. The fact that previous workers have obtained polyploids in Capsicum annum with concentrations of upto 0.4 percent and that in the present study with Capsicum frutescens even a concentration of 0.45 percent was not much effective suggests the possibility that different species of Capsicum may respond differently to colchicine treatment.

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*Originals not seen.

Plate I (A) Early deformities shown by plants treated with 0.4% colchicine for 9 hours. ie., T_{25}

(B) Control plants

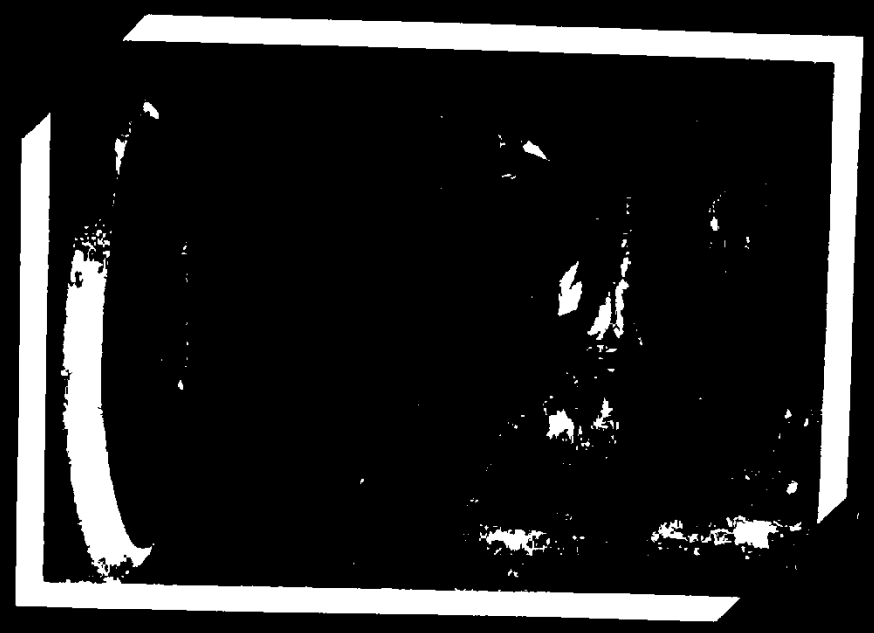
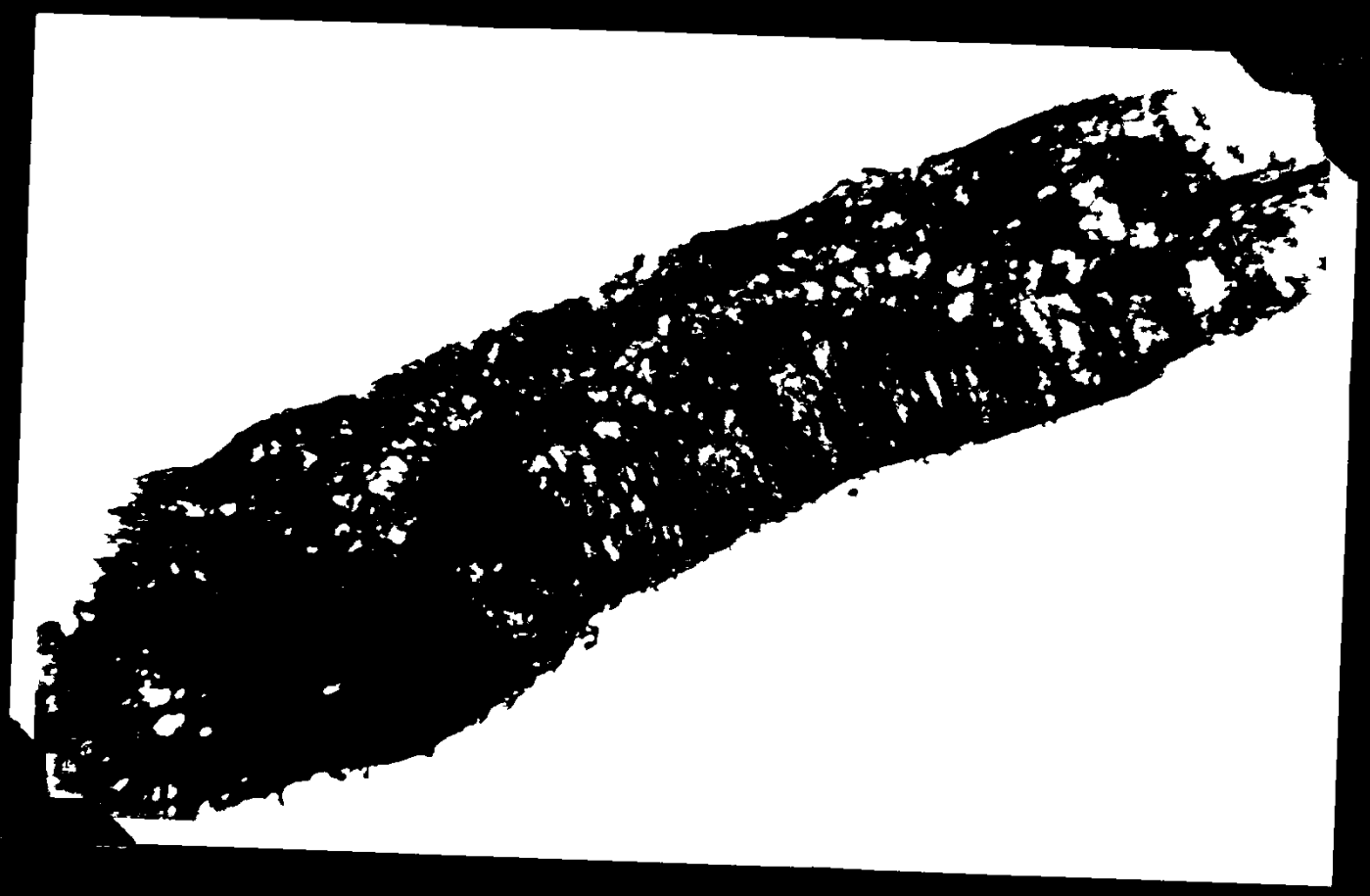


Plate II (A) Size and distribution of stomata in diploids. (Magnification : 150).

(B) Size and distribution of stomata in tetraploid

Plate III (A) Leaf thickness of the diploid plant (Mag. 100)

(B) Leaf thickness of the tetraploid plant



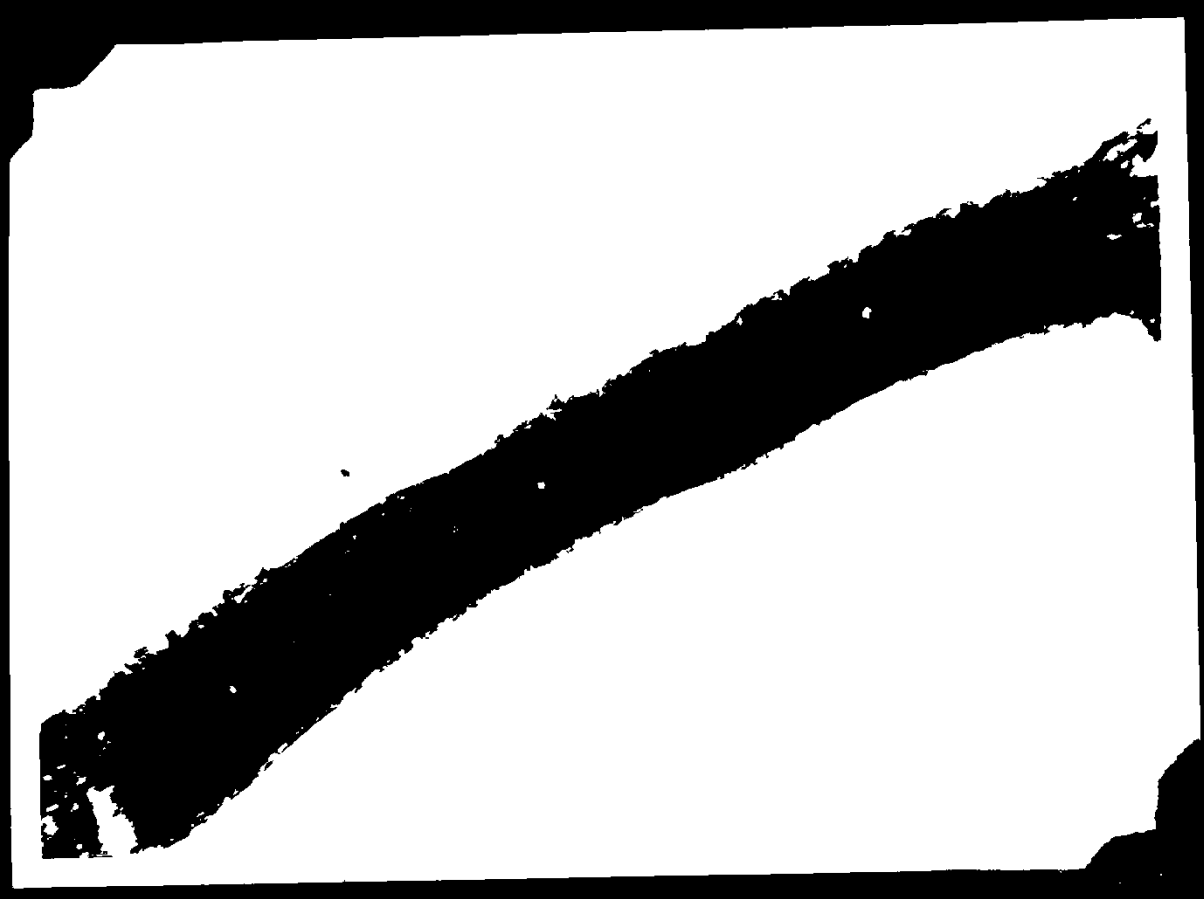


Plate IV (A) Meiotic division showing 12 bivalents in control (Magnification 1000 μ).

Plate V (A) Early meiotic anaphase in the polyploid

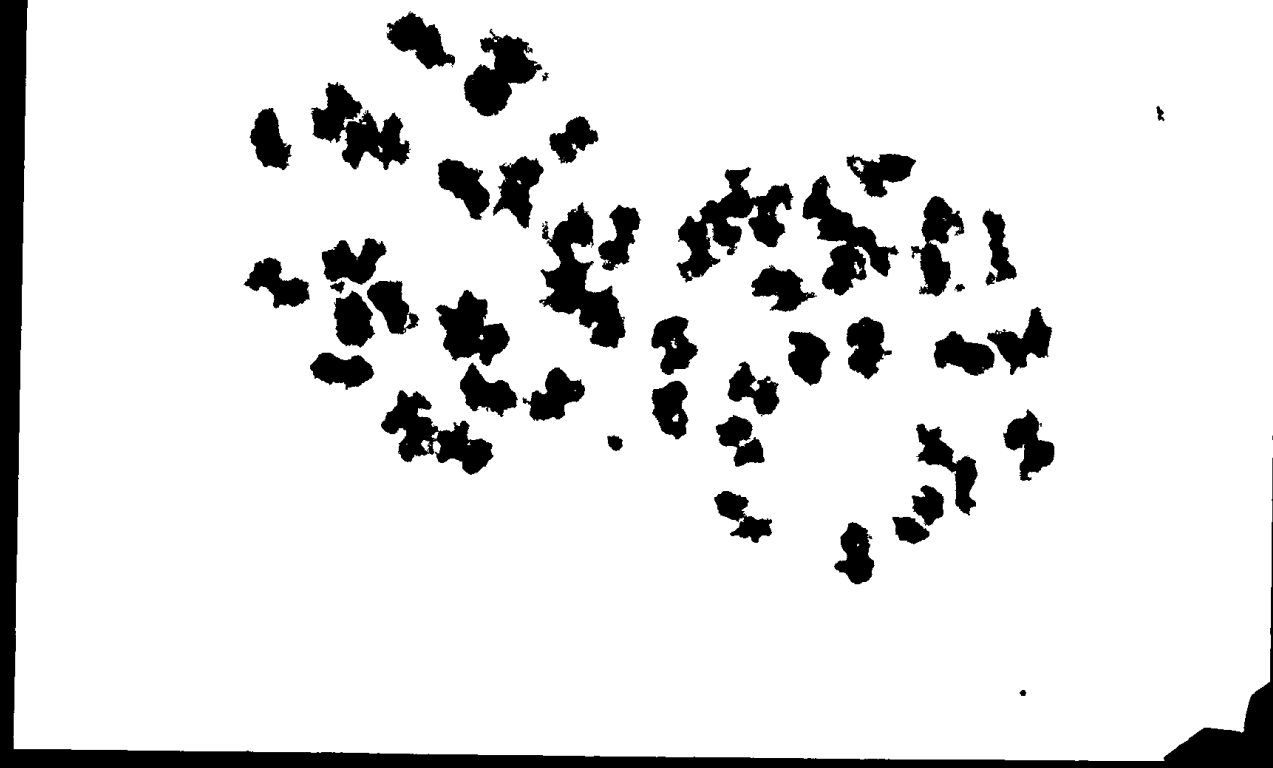


Plate V (B) Late anaphase 1st in polyploid

Plate V (C) Meiotic anaphase 1st in the polyploid showing 2 laggards.

Plate V (D) Meiotic anaphase 1st in polyploid showing 1 laggard

(E) Meiotic telophase II in polyploid showing 5 groups of chromosomes.