STUDIES ON THE INDUCED POLYPLOIDS OF CLUSTER BEANS (*Cyamopsis Psoralioides* D. C.)

P. K. THANKAMMA PILLAI

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
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C E R T I F I C A T E

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

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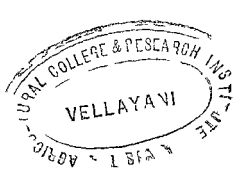

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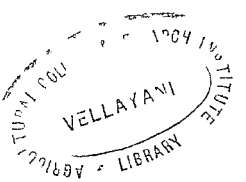

C O N T E N T S

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INTRODUCTION

Polyploidy is now widely recognised as one of the principal methods for the formation of new species among the higher plants. It is not only one of the best known evolutionary processes, but also the most rapid method known for producing radically different genotypes. Blakeslee and Avery (1937) by their classical work on Datura and other plant species discovered that polyploidy could be induced in plants through the action of the drug colchicine. The discovery of this wonder drug paved the way for the rapid development in the formation of novel and superior plant types that could completely revolutionize the field of agriculture.

Colchicine though commonly extracted from the seeds and corms of the autumn crocus (Colchicum autumnale. L) is also present in many species of Colchicum and in certain other liliaceous plants. Increased and universal interest in colchicine developed among the botanists following the announcement that this chemical could induce chromosomal doubling.

Intensive work in this new field has been in progress and a number of artificial polyploids

have been produced in a variety of garden plants as well in crop plants. Tetraploids of several legumes have been obtained by different workers, although economic utility have been achieved only in some fodder legumes. Among seed legumes tetraploidy has been induced in gram (Ramanujam and Joshi,1941), green gram (Kumar and Abraham, 1942), black gram (Sen and Chedda,1958), horse gram (Sen and Bhowel, 1959) Soybean (Tang and Loo, 1940), and beans (Spasojevic, 1956), but reduced fertility proved to be the main handicap for their economic cultivation. Several fodder legumes have surpassed their diploids in yield. Tetraploid strains of red clover are now being grown by farmers for its high forage value as well as higher yield. (Levan 1942). Encouraging results have been obtained with such crops as subterranean clover (Hutton and Peak, 1954), Egyptian clover (Sikka et al, 1958), alsike clover (Bragdo, 1955) and Indian clover (Sikka et al, 1958).

Clusterbeans or guar (Cyamopsis psoralioides (L) D.C. Syn: C. tetragonaloba Taub) is one of the popular vegetable and fodder crops in India. Researches

for the improvement of the crop have so far been directed mainly towards its agronomical and pathological aspects. Plant breeding methods are comparatively recent introductions in this field. Vig (1962,'63) tried to induce polyploidy in guar, but could succeed only in getting a mixoploid and a tetraploid each time.

Since knowledge on the methods of induction as well as on the cytomorphological characters of the colchiploids in guar (Cyamopsis psoralioides) has been limited, an attempt has been made in the present investigation to study these aspects in a detailed and exhaustive manner.

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REVIEW OF LITERATURE.

1. EARLY HISTORY.

The history of colchicum, the drug of ancient and modern materia medica, is rooted in the myths and the written records of ancient civilizations, and runs its course through ages into the world of today. More than 50 species containing colchicine are known, of which the most notable are Colchicum autumnale and C. luteum.

Colchicine as a mitotic poison and a tool for biological research was discovered in 1934 at Brussels in Belgium in the laboratory of Prof.A.P. Dustin Sr, who for a long time had been investigating means of altering mitosis. The horizons of Colchicine research widened quickly when botanists learned how effectively the drug could be used in their work as well. To the layman the interest in this drug arose when references to cancer entered in the discussion and when the evolution of new varieties of plants stimulated new programmes in the field of agriculture.

Charles Darwin (1875), an early experimenter on the effect of colchicine on plants, applied the drug to

"insectivorous" and sensitive plants. The reaction in leaf movements were tested but no conclusive results were obtained for colchicine.

Dixon and Malden (1908), who prepared an excellent report on the effect of colchicine on the blood picture, were cited as the pioneers in the field of colchicine research, until Pernice's report was discovered. The modern period of research with colchicine began in 1889, when Pernice described metaphasic arrest produced by this drug, even though the significance of this phenomenon was not then fully realised.

Dustin (1934) called attention to the possibilities of colchicine as a tool for cancer chemotherapy. Dustin et al (1937) very closely established that colchicine act upon mitosis, both in animal and plant tissues. Nebel and Ruttle (1937) clearly demonstrated that colchicine acted upon mitosis and showed that this drug was an important tool for inducing polyploidy in plants.

Of the many methods tried to double the chromosome number uniformly and thus obtain seeds with the doubled number, the application of the alkaloid colchicine is proved to be the most efficient and the one so far found to be of the greatest general application. Gavaudan and associates (1937) published the first account on colchicine-

induced polyploidy. Blakeslee and Avery (1937) through their work on Datura and other plant species clearly established the fact that colchicine was a new and effective agent for making polyploids experimentally.

The principle underlying the colchicine treatment is that the alkaloid must be brought in contact with the actively dividing cells. This is effected either by treating pre-soaked seeds with colchicine solution or by applying colchicine to the growing tips of the seedlings.

II. SEED TREATMENT.

Numerous reports have been published concerning the treatment of seeds of many plants with colchicine. These treatments either produced no effects or resulted in harmful or beneficial effects. Tanaka (1950) obtained tetraploid strains of 9 varieties of egg plants by colchicine treatment. Armstrong (1951) produced tetraploid strains of annual rape by subjecting germinating seeds to colchicine treatment. Schrock (1951) reported the stimulating action of colchicine on germination and growth of birch seeds soaked in 0.2% solution for 24 hours.

Kluge and Kramer (1955) on treating seeds of Vaccinium myrtillum (2n = 24) and V. corymbosum (2n = 48) and diploid and tetraploid varieties of Fragaria vesca with 0.1% to 0.5% colchicine solution for periods ranging

from 27 to 72 hours, noted an acceleration in germination, and found increased germinability in the case of seeds that had been stored for some time. In the case of diploid F.vesca the seeds gave best response to 0.3% colchicine solution treated for 48 hours. But for tetraploid F.vesca a 0.3% or 0.5% solution treatment lasting for 72 hours was found to be most effective. The germinability of seeds of Vaccinium myrtillum that had been stored for six months at room temperature was considerably increased by treatment with colchicine - a concentration of 0.5% giving the best results.

Tomar and Khanna (1955) produced polyploids in radish by treating pre-soaked seeds with an aqueous solution of colchicine of different concentrations for varying periods.

Srivastava (1955) studied the varietal differences among polyploids obtained from different strains of gram and found that germinating seeds of black-seeded type, simple-leaved mutant, "tiny leaf" mutant, NP1, NP4, NP13 and NP 26 treated with 0.35% of colchicine gave tetraploids from all the strains except NP13.

Chakravarty (1956) produced artificial polyploids of New Zealand certified mother strains of white clover by treating seeds with colchicine solution.

Clydechander (1956) induced tetraploidy in the horticultural variety of Verbena hybrids by soaking seeds in aqueous solution of colchicine. Kawatani et al (1956) working on Chrysanthemum cinerariaefolium produced colchicine - induced tetraploids through seed treatment.

Giles et al (1956) obtained colchicine-treated tetraploids in dry and pre-germinated seeds of Solanum antipoviczii, S. verrucosum and S. longipedicelatum and further found that treatment with colchicine solution of 0.2% for 6 hours in the case of dry seeds, proved most effective and concentrations above 0.6% proved to be lethal.

Srivastava (1956) produced fertile autotetraploids in sesame and found that the most effective treatment for the induction of tetraploids in pre-soaked seeds was that with 0.06% solution for 6 hrs or with 0.1% solution for 4 hrs.

Harada et al (1957) obtained tetraploid strains of tea plant by colchicine treatment of three diploid varieties and from the progeny of 3n strains of the variety U.24.Kloen (1957) reported polyploid sugar beets and mangels that resulted by treating germinating seeds of 4 varieties of sugar beet and 3 varieties of mangel

with 0.1% to 0.2% colchicine solution at a temperature of 28° C.

Rajhathy (1957) obtained 14 tetraploid plants together with chimeras in Triticum monococcum var: flavescens by germinating seeds in a 0.02% colchicine solution and later by injecting the seedlings at the growing point with 0.2% solution. Bose et al (1958) observed a quantitative increase of the enzyme during the process of polyploidization in Cicer arietinum Linn. Smith (1958) found that in barley most of the autotetraploids were induced by soaking germinated seeds in 0.1% colchicine. Sen and Bhowel (1959) reported colchicine-induced tetraploids in six varieties of Vigna sinensis which included seed treatment.

Yokoyama and Matsui (1957) noted in tea plant that the optimum conditions for inducing polyploidy was the treatment of seeds with 0.3% colchicine solution for 180-204 hrs.

Aleksic (1960) obtained tetraploid red pepper by treating seeds with 0.8% colchicine solution. Cicin and Mahalin (1960) produced polyploid winter rye (2n = 28) by colchicine treatment.

Gargiulo (1960) studied several methods of colchicine application to induce polyploidy in commercial varieties

of hybrid lines of Vitis vinifera. The most effective concentration consisted of a 0.5% solution mixed with 10% glycerine. Saxena and Nanda (1960) found that phlox seed when treated with different concentrations of colchicine show reduction in the germination percentage.

Ammal and Bezbaruah (1962) obtained tetraploid plants of Cathranthus roseus by treating pre-soaked seeds and apical buds of about 10 day old seedlings with aqueous solution of colchicine ranging from 0.05 to 0.5% concentrations. In seed treatment the maximum number of tetraploid plants was obtained from the seeds treated with 0.2% colchicine solution for 24 hrs. Bezbaruah (1963) could get tetraploid Asclepias curassavica L: (2n = 44)by treating pre-soaked seeds with a 0.1% aqueous solution of colchicine for 24 hrs.

III. SEEDLING TREATMENT.

Treatment of the apical buds of young seedlings with colchicine solution is yet another method for the induction of polyploidy. Evans (1955) working on Trifolium pratense compared the effectiveness of different techniques commonly used and found that seedling treatment gave the best results. Armstrong and Robertson (1956) reported that tetraploids of Trifolium hybridum L. were easily produced by immersing the top of the seedling in 0.2% aqueous solution of colchicine for 12 hrs. Kumar and Abraham (1942)

obtained tetraploids in Phaseolus radiatus following seedling treatment.

Gentcheff (1947) produced polyploids in rye and barley by dipping young plants of 4-6 cms length upside down in a glass containing 0.125% aqueous solution of colchicine in such a way that the coleoptiles were immersed in the solution. Emsweller (1949) by immersing detached bulb scales in 0.2% solution of colchicine obtained polyploids in 12 spp. and 2 sp. hybrids of Lilium.

Sonnenschein (1949) induced polyploids in cucumber varieties by applying 0.1, 0.2, 0.3 and 0.4% aqueous colchicine solution to the terminal buds. Solution of 0.2 and 0.3% gave best results in producing polyploids. Batra (1952) obtained tetraploid muskmelon by treating the plants with 0.4% colchicine solution at the cotyledon stage.

Choudhury (1955) has recorded in Lycopersicum esculentum the comparative efficacy of treating the seeds, shoots or the seedling root tips with 0.2% and 0.4% colchicine solution for 48, 96, 144 and 192 hrs, in the induction of polyploids.

Choudhury et al (1956) produced tetraploid plants of two varieties of Corchorus capsularis and three of C. olitorius by colchicine treatment of seedlings.

Dereskevicus (1956) obtained polyploid tomato by applying 0.01 to 0.1% colchicine at the growing points of the seedlings. Varga (1956) recorded 50-70% tetraploidy in beet by the application of 0.05 to 0.1% aqueous solution of colchicine to the apex of seedlings in the cotyledon phase.

Braak and Zeilinga (1957) found that in Asparagum the sensitivity of the seedlings to treatment increased with age. 8 days old seedlings nearly died after the application of very weak solutions, but seedlings aged 5 days withstood a concentration of 1.6% and tetraploidy being induced in about 60% of the seedlings.

Knight (1957) reported that treatment of the apical bud with 0.6% colchicine in agar for 24 hours soon-after germination was found to be a satisfactory method for inducing tetraploidy in Theobroma cacao.

Rosenthal (1958) found in mangel plants, that treatment with colchicine to very young growing points gave the highest percentage of tetraploid C_1 plants.

Sikka et al (1959) studied the effectiveness of several methods of colchicine treatment for inducing polyploidy in Trifolium alexandrinum and Melilotus indica. In the former immersion of shoots of 4-day old seedlings

in 0.1% colchicine gave the highest percentage of pure tetraploids (30%.).Treatment of whole seedlings with 0.05% colchicine for 8 hrs at room temperature gave highest percentage of mixoploids (60.5%). Melilotus indica responded best to whole seedlings treatment with 0.05% colchicine for 4 hrs at room temperature.

Nyst (1959) studied a comparkson of the principal methods of induction of polyploidy in Oryza sativa. Two methods involving colchicine treatments of roots and two of stem were compared. One of the root treatment methods gave most rapid results, but it was thought that stem treatment method would be valuable if the techniques were improved.

Sugiyama (1959) observed that buds of potted cuttings of mulberry were treated with colchicine and subsequently the flowers were pollinated by diploid mulberry. One of the 190 seedlings from the treatment with 0.2% colchicine was triploid (2n = 42).

Chopra and Swaminathan (1960) induced polyploidy in watermelon var. asahiyamato and farrukhabadi by treating the growing points in the cotyledonary stage with colchicine.

Franzke et al (1960) obtained some diploid mutants in Sorghum which have arisen after application

of 0.5% colchicine in lanolin to coleoptiles of seedlings. Segregation in the second generation of such mutants was also investigated. Lelakis (1960) produced polyploid plants of Cannabis sativa by immersion of the tips of one week old seedlings for 3 hrs in 0.5% colchicine solution.

Nakasone (1960) tried various kinds of colchicine treatment to seeds and vegetative organs of Vanda and Dendrobium species, but tetraploidy was induced only in cuttings and young shoots of vanda.

Sen and Vidyabhushan (1960) obtained tetraploid horsegram by treating the seeds with 0.01 to 0.25% for 1/2 to 24 hrs and by apical bud treatment of seedlings with 0.1, 0.25 and 0.5% colchicine solution for 3 and 9 hrs. Apical bud treatments produced more polyploids. Jadhav (1961) induced polyploidy in Crotalaria juncea by treating the tip of the primary root with 0.025 and 0.05% colchicine.

Jain et al (1962) observed that in Antirrhinum majus var. Katrain, treatment of young seedlings at the cotyledonary stage or a little later with 0.4% solution by cotton wool plug method for 2 hrs each on two consecutive days produced nearly 20% polyploidy.

Vig (1962) obtained a mixoploid guar (Cyamopsis psoralioides) from the applicatinn of 0.1% aqueous colchicine for 12 hrs by placing a piece of wet cotton on the growing plumule at the cotyledonary stage. Vig (1963) produced autotetraploids in Pb IV type of guar (Cyamopsis psoralioides D.C) by treating young seedlings with 0.2% colchicine in lanolin paste.

IV. MORPHOLOGICAL VARIATIONS.

Morphological variations in plants, subjected to treatment with colchicine, have been reported by various authors. In most of the tetraploid plants the growth is slow at first, but later on it grows more rapidly than the diploids (Janakiammal and Bezbaruah, 1962). According to Kumar and Abraham (1942) tetraploids show a decreased growth rate and consequently take longer time to reach maturity and flowering stage than the diploids.

Warmke (1945) obtained tetraploid races of Taraxacum kok-saghyz by colchicine treatment. Nineteen tetraploids grown in green-house showed significantly larger roots but a lower rubber percentage than a similar number of green-house grown diploids. 43 field-grown tetraploid plants had both significantly larger roots and higher rubber percentage than a group of 36 comparable diploids.

Lesik (1948) in a study of the colchicine-induced amphidiploids of Avena sativa x A. abyssinica noticed the high fertility of the amphidiploids. The high degree of fertility is attributed to normal meiosis.

Shimamura and Kobayashi (1948) on comparing diploid and colchicine-induced tetraploid strains of sesame found that the tetraploids bore relatively shorter capsules. The tetraploid seeds were larger and fewer per capsule and fertility was lower. Under suitable conditions of cultivation, however the fertility of the tetraploids improved, the number of capsules borne exceeded that of the diploids, and the total yield of the seed per plant suffered no reduction.

Luongdinhcua (1950) produced artificial polyploids in rice by colchicine treatment. He found that awn development, increase in length of the ligule of the flag leaf, size of spikelets and pollen grains were found to be especially useful in distinguishing colchicine-induced tetraploids of four varieties from the original diploids. Many pollen grains of tetraploids had two or three germinal pores whereas diploid grains generally possessed only one. Varietal difference in frequency of grains with more than one germinal pore was noted among the tetraploids. Pollen and seed fertility were reduced in the tetraploids; seed set did not correspond with the

percentage of normal grains. Number of spikelets per panicle was reduced to about 40% in the tetraploids.

Singh (1955) while studying colchicine-induced tetraploids in papaya (Carica papaya) found the tetraploids to be of no economic importance since they were highly sterile and produced round fruits which were much reduced in size.

Bhattacharjee (1956) found that tetraploids of Cajanus cajan obtained from colchicine treatment had significantly lower number of branches and nodes than the diploids. The 4n and 2n plants showed only slight differences in leaf and leaflet measurements and in the total number of leaves.

Patel et al (1956) reported that colchicine treatment of F_1 bybrid seelings of Vitis vinifera X V. rotundifolia had given a number of polyploid plants which could be recognized by their increased stomatal size and vigour.

Spasojevic (1956) on studying colchicine induced-tetraploids in bean (Phaseolus vulgaris. L) found that they were late in flowering and ripening and had larger seeds. Saito (1957) found that colchicine induced tetraploid strains of American watermelon were very late maturing.

Sharma and Datta (1957) observed that in coriander (Coriandrum sativum. L.) the tetraploid plants were larger and darker in colour than diploids. Meiotic irregularities were observed, seed setting was found to be poor and no polyploid seed could be successfully germinated.

Sen and Chedda (1958) reported a retardation of growth of seedlings of blackgram (Phaseolus mungo) when the seeds were treated with colchicine solution. The tetraploid plants were smaller and more bushy than the diploids and the leaves were smaller, thicker and darker.

Zimmermann (1958) found that in forage grasses, colchicine-induced tetraploids were less vigorous and winter hardy than diploids.

Kluge (1959) recorded the following differences in a comparison of the diploid and colchicine-induced tetraploid types of Fragaria vesca var. Semperflorens.(1) Tetraploids formed fewer inflorescences per plant and accordingly had fewer flowers (2) Tetraploids had bigger flowers with the same number of petals but fewer anthers. (3) Tetraploids produced fewer pollen grains and viability of those pollen was too low to facilitate uniform and adequate pollination. (4) Seed weight in tetraploids was

twice as high as in diploids, but the viability of the former was low (20-30%). The number of seeds per fruit amounted to about 100 in diploids against 30-40 in tetraploids (5) The fruits of tetraploids were often deformed and were smaller than those of the diploids.

Mackevic (1959) observed a retardation of growth in the colchicine-induced tetraploids of Populus tremula L. and P. balsamifera . The leaves of tetraploid P.tremula were larger than those of the diploids. The growth rate was as high as in the diploid.

Armstrong and Robertson (1960) on studying the comparison between tetraploid and diploid alsike clovers with respect to pollen diameter, stoma size, number of plastids in guard cells, height, tiller number, stem thickness, leaf area, seed size and florest numbers per head, found that the tetraploid surpassed the diploid in all such characters with the exception of the tiller number. The tetraploid was significantly superior to the diploid in hay yield.

Moffett and Nixon (1960) observed in black wattle (Acacia)that tetraploids were induced readily by soaking seeds in colchicine solution and that they were less vigorous than the diploids. Although the bark thickness and tanin content were greater, yield per acre was likely to be less owing to less vigorous growth.

Thombre and Desai (1960) found that treatment of seeds of Agave cantala with colchicine, induced polyploids having thicker and broader leaves than the diploids.

Mehta et al (1963) studying the effect of 22 different colchicine treatments on C10 (early) C9 and C_{11} (medium and early) and C_2 (late) strains of berseem found that the tetraploids differ from the diploids in inflorescence size, seed setting and seed size.

Hertzsch (1951) observed that the plants obtained on treating Vicia villosa with colchicine showed various stages of polyploidy from 4n-10n. However he found, no difference in the measurements of stomata and pollen grains in the polyploid and the normal plants.

V. CYTOLOGICAL OBSERVATIONS.

The observations by Nebel (1937), Nebel and Ruttle (1938), Levan (1938) and many others showed that colchicine inhibited the formation of the spindle in a dividing nucleus without affecting the chromosome division. Due to this failure of spindle formation the daughter chromosomes do not move to opposite poles and no cytokinesis takes place. The original cell has a reconstituted nucleus with double the chromosome number.

Wada (1940, 49, 50) proved that in Tradescantia spindle fibres did not develop at prophase with concentrations of 0.05% or 0.1% colchicine. Sparrow (1942) reported

an increase in univalents about 37% among the treated plants of Antirrhinum.

Vaarma (1947) has recorded the threshold value for C-mitosis in the root tips of Ribus nigrum to be the 0.01% concentration. Hindmarsh (1952) found that colchicine above a concentration of 0.1% destroyed the spindles in all stages of mitosis and prevented spindle formation.

Carpentier (1954) by treating roots and rootlets of groundnut with 0.005% colchicine solution produced C-mitotic cells, which showed a decrease in the frequency of total number of dividing cells.

Uchikuwa (1956) reported that in colchicine-induced tetraploid strains of two Japanese varieties of Cucumis sativus meiosis and irregular and fertility low. Douglas Davidson (1957) noted the ability of colchicine to induce chromosome breakage in Vicia faba.

Evans et al.(1957) used colchicine as an indicator of mitotic rate in broad bean root meristems and found that three concentrations, 0.1, 0.05 and 0.025% were similar in capacity for inducing metaphasic accumulations. The treatments for more than 6 hrs showed an inhibitory effect which slowed down the rate of entry of cells into mitosis, the time spent in interphase being increased.

Damon (1958) observed that colchicine treatment of Sorghum seedlings caused arrest of cell division at metaphase with production of star, exploded and ball metaphase types, in the shoot apices.

Islam (1960) reported that in colchicine-induced tetraploids of Anona squamosa, microsprogenesis was highly irregular, 80% of the pollen being unstainable. Only trivalents, bivalents, and univalents, were observed. Failure in fruit formation may thus be primarily due to irregular meiosis, presumably in megasporogenesis as well as microsporogenesis.

Smith and Hiner (1960) observed typical colchicine mitotic effect in Allium cepa by treating the root tissue with concentrations of 0.025 - 0.8% colchicine for different periods varying from 3-12 hrs.

VI. EFFECT ON POLLEN GRAINS.

The response of the changes in size of pollen and the percentage of pollen sterility by colchicine treatment has received attention of many investigators. Illies (1956) reported the changes in size of birch pollen after treatment of the flowers with colchicine. Giant diploid and mixoploid pollen grains were obtained by treating flower bud with 0.2% warm solution of colchicine.

Osone (1958) treated the buds of tea plants with colchicine in which the anthers were at meiotic stage. Giant pollen grains constituted about 10.5% of the pollen produced per flower.

Walker (1957) cultured excised anthers in Taylor's medium containing 0.01 and 0.1% colchicine for 24-48 hrs. Treatment with 0.1% colchicine initiated at stages from zygotene to diplotene and treatment at a concentration of 0.01% at zygotene only, prevented pollen mother cell nuclei from progressing further than prophase.

Sen and Chedda (1958) reported that in Phaseolus mungo, the pollen fertility of the polyploids was 75-80% while that of the diploids being 95%. Vig (1962) noted that the pollen grains,obtained from a mixoploid guar plant by colchicine treatment, were highly sterile.

Inspite of so much volume of informations published, there still remains unexplored problems which appear to have promise for more discoveries.

The present investigation deals with techniques adopted in inducing polyploidy in Cyamopsis psoralioides(L) D.C. var. C.P. 78 (cluster beans) and a comparison of the diploids and tetraploids with regard to certain morphological and cytological aspects.

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MATERIALS AND METHODS

The present investigation was carried out in the Division of Agricultural Botany, Agricultural College and Research Institute, Vellayani in the year 1963-'64.

A. MATERIALS.

(1) Seed Material.

Quality seeds of cluster beans (Cyamopsis psoralioides D.C.) were taken from the collection maintained in the Division of Agricultural Botany, Agricultural College and Research Institute, Vellayani. Seeds were tested for viability as well as for germination percentage before being used for the investigation.

(2) Chemical.

Colchicine (B.D.H) was used.

Aqueous solutions at three concentrations namely 0.1% , 0.25% and 0.5%, and in three durations as 3 hrs, 6 hrs and 9 hrs. were tried.

(3) Treatments.

The different concentrations of the chemical and the durations, and stages of its application are denoted by the following symbols.

(1) Concentrations

0.1% - C_1

0.25% - C_2

0.5% - C_3

(2) Durations

3 hrs - D_1

6 hrs - D_2

9 hrs. - D_3

(3) Stages of application

Seed treatment - S_1

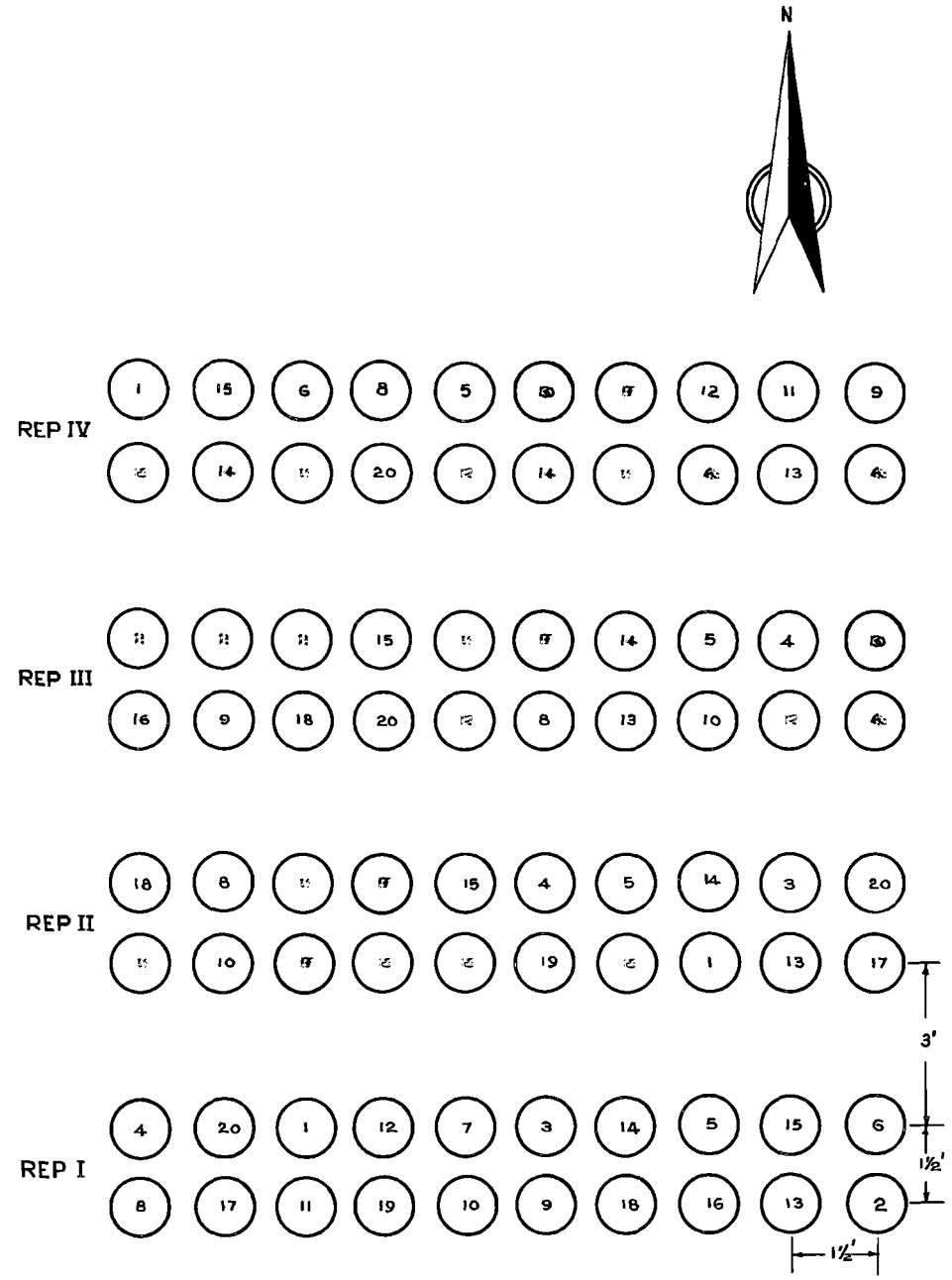
Seedling treatment- S_2

The following were the treatment combinations.

C_1 D_1 S_1	0.1%	3 hrs.	Seed treatment
C_1 D_1 S_2	0.1%	3 hrs.	Seedling treatment
C_1 D_2 S_1	0.1%	6 hrs.	Seed treatment
C_1 D_2 S_2	0.1%	6 hrs.	Seedling treatment
C_1 D_3 S_1	0.1%	9 hrs.	Seed treatment
C_1 D_3 S_2	0.1%	9 hrs.	Seedling treatment
C_2 D_1 S_1	0.25%	3 hrs.	Seed treatment
C_2 D_1 S_2	0.25%	3 hrs.	Seedling treatment
C_2 D_2 S_1	0.25%	6 hrs.	Seed treatment
C_2 D_2 S_2	0.25%	6 hrs.	Seedling treatment
C_2 D_3 S_1	0.25%	9 hrs.	Seed treatment
C_2 D_3 S_2	0.25%	9 hrs.	Seedling treatment
C_3 D_1 S_1	0.5%	3 hrs.	Seed treatment
C_3 D_1 S_2	0.5%	3 hrs.	Seedling treatment
C_3 D_2 S_1	0.5%	6 hrs.	Seed treatment
C_3 D_2 S_2	0.5%	6 hrs.	Seedling treatment
C_3 D_3 S_1	0.5%	9 hrs.	Seed treatment
C_3 D_3S_2	0.5%	9 hrs.	Seedling treatment

FIG. 1 Layout plan of the experiment

Design -20 x 4 Randomised Block



PLAN OF LAYOUT OF THE EXPERIMENT

FIG 1

B. METHODS.

(1) Lay out Randomised Block Design.

Replications - Four.

There were altogether 80 pots which were arranged in four blocks of 20 pots each.

(2) Sowing.

Identical pots filled with equal quantity of potting mixture were employed. Ten seeds were dibbled in each pot at uniform depth. On attaining one week's growth, four uniform plants were retained in each pot by removing the rest of the seedlings. The plants were irrigated both in the morning and evening on all days.

(3) Seed treatment.

The treatment consisted of:

(1) 0.1% solution for 3 hrs., 6 hrs., and 9 hrs., durations.

(2) 0.25% solution for 3 hrs., 6 hrs., and 9 hrs., durations.

(3) 0.5% solution for 3 hrs., 6 hrs., and 9 hrs., durations.

Prior to treatment, the seeds were soaked in distilled water for 12 hours. The soaked seeds were dried by keeping them spread over a bloating paper for about two hours under room temperature and were afterwards kept in the respective solutions for half an hour. These

seeds were then transferred into petridishes with filter paper and drenched with the respective colchicine solution to a level sufficient to cover half the depth of the seeds. They were kept covered for the rest of the treatment period. Treated seeds were washed in distilled water and allowed to sprout on wet filter paper and were then sown in prepared pots. Seeds soaked in distilled water and allowed to sprout on moist filter paper were used as controls and for raising plants for seedling treatment.

(4) Seedling treatment.

The apical buds of one week old seedlings grown in the experimental pots were treated with colchicine solution. The concentrations and durations employed were:
(1) 0.1% solution for 3 hrs, 6 hrs, and 9 hrs. durations.
(2) 0.25% solution for 3 hrs,6 hrs, and 9 hrs. durations.
(3) 0.5% solution for 3 hrs, 6 hrs, and 9 hrs. durations.

The solution was dropped to the apical buds of the seedlings plugged with cotton, from a glass dropper. More solution was added from time to time to keep the cotton plug moist. After the scheduled period of treatment the cotton plugs were removed and the apical buds washed with distilled water.

(5) Characters studied.

Morphological observations were recorded from the date of germination. The following characters were recorded from individual plant.

(1) Germination percentage

(2) Early deformity

(3) Height of plants.

(4) Number and size of stomata

(5) Area and thickness of leaf

(6) Number of flowers

(7) Sterility and size of pollen

(8) Meiotic behaviour

(9) Number of fruits

(10) Length of fruits

(11) Seeds from 100 fruits.

(12) Dry weight of the plant.

(1) Germination percentage.

The germination trial was conducted in the laboratory by treating the seeds withaqueous solution of colchicine, and allowing to germinate on moist filter paper placed in petridishes. 100 seeds were arranged in each petridish. The germinated seeds were removed and counted at 12 hour intervals for 15 days. Beyond this period, seeds were found not to germinate. The data were analysed statistically.

(2) Early deformity.

Abnormalities of the treated plants by way of size and thickness of cotyledons, lethality etc. were observed and recorded.

(3) Height of plants.

Elongation of the shoots was recorded in Cms. measuring from soil level to the base of the terminal bud. This was done from the 15th day of sowing till the day of the last harvest. Data on the last measurement for height were alone statistically analysed, the rest of the data being utilized in graphically representing the rate of growth of plants under each treatment.

(4) Number and size of stomata:

A sample of ten leaves was selected from each treatment for this study. The tissue was pealed off from the lower surface of the leaf and stained with safranin. Ten slides were prepared and ten fields were counted at random from each of the slide. For estimating the size, the length and width of ten stomata from each slide was measured randomly by using a standardised ocular micrometer. The data were tabulated and analysed statistically.

(5) Area and thickness of leaf.

Leaf area from a sample of ten leaves taken at random from each treatment was measured and recorded in sq. inches by using a planometer.

Thickness of the leaves was recorded by measuring ten hand sections - one section being taken from each of the ten leaves in the sample - by using a standardised ocular micrometer.

(6) Number of flowers.

The total number of flowers produced in each plant was recorded and the data statistically analysed.

(7) Meiotic studies.

One inflorescence from each of the plant was taken and fixed in a fresh mixture of acetic-alcohol (1:3) Meiotic behaviour was studied by smearing the anthers of these fixed buds in iron-acetocarmine.

(8) Sterility and size of pollen.

A sample of ten flowers was selected from each treatment and pollen from each of them was stained with acetocarmine - glycerine mixture. Fertile and sterile pollen grains were counted from 100 fields taken at random. Diameter measurements from 100 pollen grains were taken randomly by using a standardised ocular micrometer. The data were tabulated and analysed statistically.

(9) Number of fruits.

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

(10) Length of fruits.

A sample of ten fruits from each treatment was selected and the length was measured in Cms. The data were analysed statistically.

(11) Seeds from 100 fruits.

Number of seeds from a sample of 100 fruits from each treatment was recorded and statistically analysed.

(12) Dry weight of the plant.

The plants were harvested and dried to constant weight in sun. The dry weight was recorded and data analysed statistically.

6. Statistical procedure.

The recorded data pertaining to the different characters under study were subjected to statistical analysis. Analysis of variance was worked out for four characters to find out whether there was significant difference between the treatment and control.

The effect of different treatments on the sterility of pollen grains was calculated by applying large sample tests.

The effect of different treatments on the distribution and size of stotama and size of pollen grains, were calculated by using the chi-square tests.

---ooOoo---

EXPERIMENTAL RESULTS.

Data on the effect of colchicine treatments on seeds as well as seedlings, were recorded and analysed statistically for the characters, viz germination percentage, height of plants, number and size of stomata, area and thickness of leaf, number of flowers, sterility and size of pollen, number of fruits per plant, length of fruits, number of seeds per fruit, and dry weight of the plant.

1. PERCENTAGE OF GERMINATION.

About half of the treated seeds when germinated, showed an arrest of root growth by the presence of swollen tip of the roots except in treatments 0.1% - 3 hrs and 0.1% - 6 hrs. Germination started by the second day and completed within five days in the case of untreated seeds . On the other hand treated seeds took a fortnight for completion of germination.

The maximum percentage of germination was observed in untreated seeds closely followed by those with 0.1% - 3 hrs treatment. The minimum germination percentage was observed in 0.5% - 9 hrs treatment.

Percentage of germination.

(1) Between treated and control

Treated = 61.88 ± 0.485

Control = 71.00 ± 0.453

(2) Between concentrations.

0.1% = 67.33 ± 0.470

0.25% = 61.00 ± 0.448

0.5% = 57.33 ± 0.495

(3) Between durations.

3 hrs = 66.33 ± 0.473

6 hrs = 61.33 ± 0.487

9 hrs = 57.66 ± 0.495

The mean percentage of germination showed that there was a decrease in germination percentage in the treated seeds compared with the untreated ones. Comparing the three different concentrations, it is found that there was a decreasing tendency in germination percentage when the concentrations were increased. Compairing the different durations, treatment with 9 hrs showed a decrease in germination percentage. 3 hrs treatment was found superior to 6 hrs treatment.

The data of the percentage of germination are given in table 13.

2. EARLY DEFORMITY.

In some of the seedlings, vegetative parts appeared thicker and smaller. They took a much longer time to use up the stored food materials in the cotyledons as indicated by the presence of unshrivelled cotyledons

on the seedlings for many days after germination. In the case of 0.5% - 9 hrs, 5 seedlings did not survive.

Such features as retardation of growth, very small and thick leaves and shorter and thicker cotyledons were shown by 31 plants out of the 144 plants observed. These plants grew slowly and had a stunted appearance. The leaves in all the abnormal seedlings were darker and thicker. (Plates 1,2,3 and 4).

In the case of seedlings, the growth of the seedlings after treatment was practically arrested for about a week. A few leaves appeared very small and malformed. These abnormalities were noted in 44 plants out of the 144 treated. In four of these plants subjected to 0.5% - 6 hrs and 9 hrs treatment, 3-6 equally growing branches were produced simultaneously from the growing tip. In all the abnormal plants, new leaves and stems were thicker, darker green and had coarser texture as compared with the untreated ones.

3.HEIGHT OF PLANTS.

The analysis of variance shows that colchicine could induce sufficient increase in height of the plant. The mean height of plants in different treatments varied from 76.97 cms. to 104.15 cms. The analysis of variance for height of plants at the time of harvest is given in in Tables 1 and 2.

T A B L E No. I

ANALYSIS OF VARIANCE FOR THE HEIGHT OF PLANTS AT THE TIME OF HARVEST.

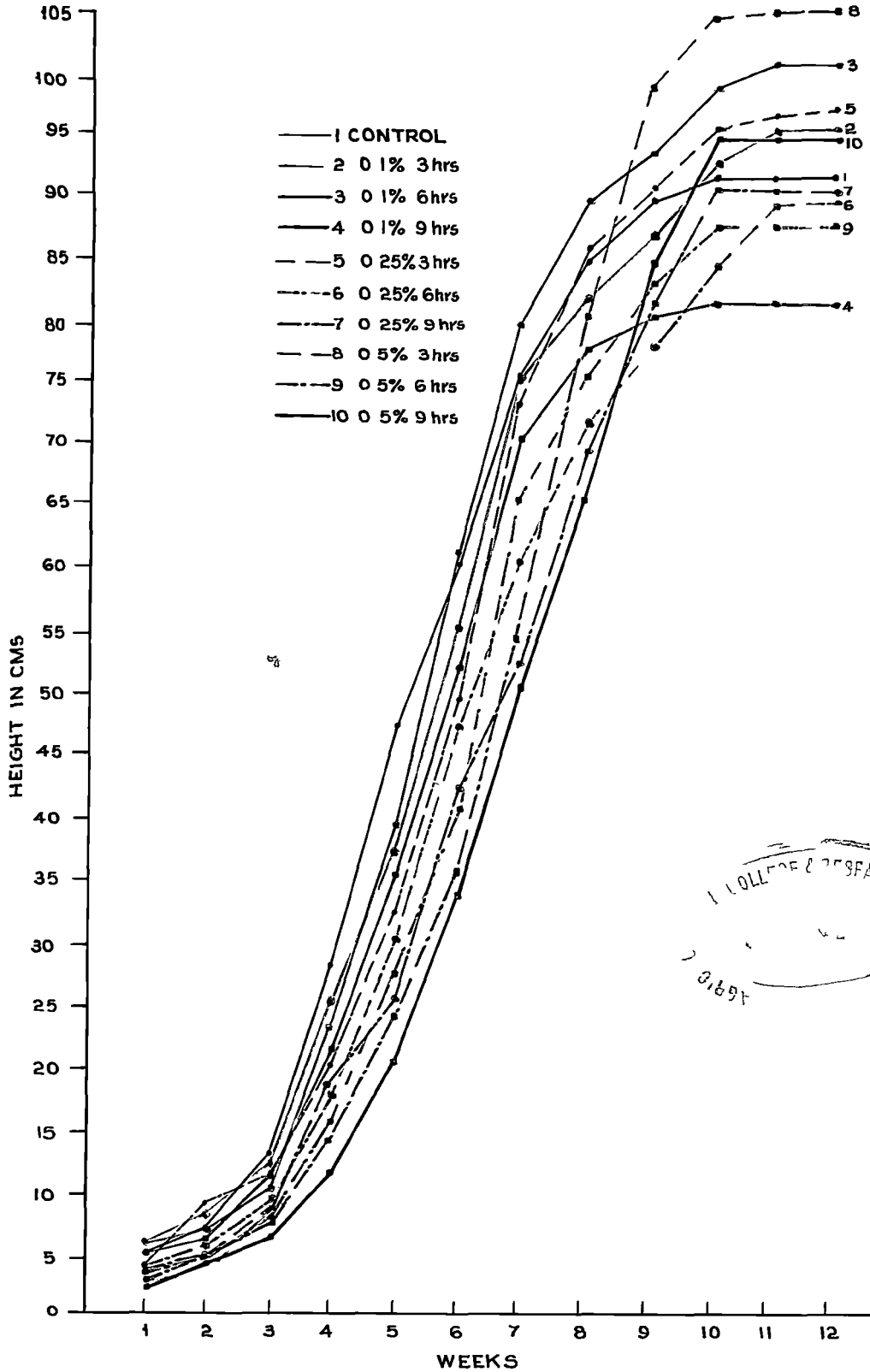
Source	Sum of squares	D.f	Variance	F-ratio
Total	11017.68	79		
Block	1535.06	3	511.68	6.26**
Treatment	4734.38	19	249.17	2.96*
Error	4748.24	57	83.30	

** Significant at 1% level

* Significant at 5% level

FIG. 2 - Graphical representation of the total height of plants under seed treatment.

FIG.3 - Graphical representation of the total height of plants under seedling treatment.



HEIGHT OF PLANTS (SEEDLING TREATMENT)

FIG·3

The analysis of the data shows that the treatment has got marked influence in increasing the height of plants. It is found from further comparison that the height of plants under various treatments as recorded at the time of harvest, showed significant increase in height compared to the control. Comparing the different stages of application, seedling treatment is found to be superior to seed treatment. The maximum height was produced by seedlings treated with 0.5% - 3 hrs closely followed by 0.1% - 3 hrs. The minimum height was found in 0.25% - 9 hrs seed treatment and 0.5% - 9 hrs seedling treatment.

Comparing the different concentrations, it is found that there was no significant difference between the various concentrations. Between duration there was a significant increase of height in 3 hrs treatment followed by 6 hrs treatment. In the ranking of various treatments it is found that there was no significant difference among them.

But there was significant difference between the treatments that gave the minimum heights.

Graphical representations of the height of plants are given in Fig. 2 and 3.

4. NUMBER OF STOMATA.

The suspected polyploids showed larger stomata but fewer in number per unit area. The cell size was also larger. Statistical analysis showed that there was significant difference in the number of stomata between the treatmen

T A B L E No. III

ANALYSIS FOR THE NUMBER OF STOMATA

Treatments	value of the test criterion
Seed Vs. seedling	6.56**
Between concentrations	24.47**
Between durations	7.09*
Treated Vs.control	26.11*

** - Significant at 1% level

* - Significant at 5% level

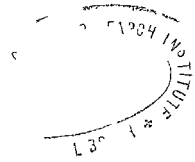
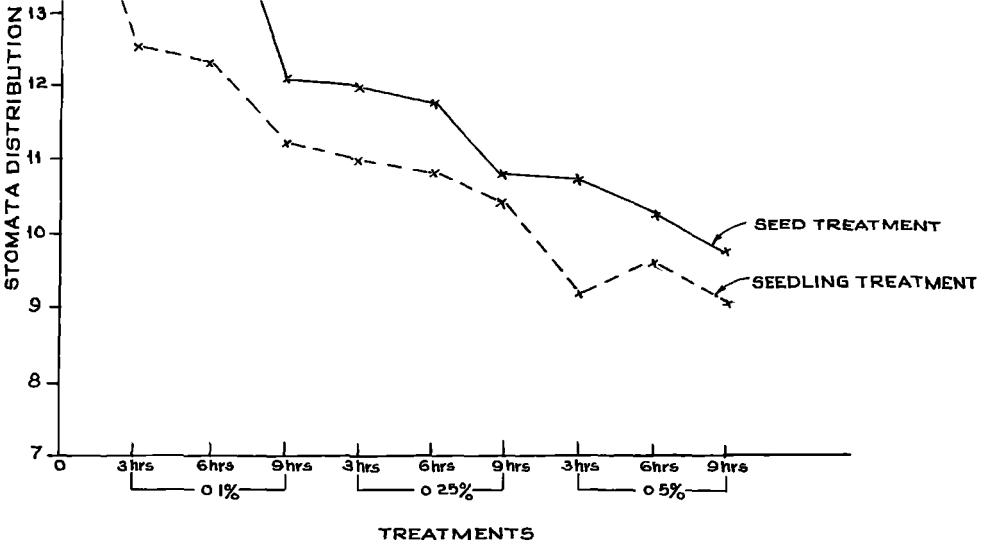
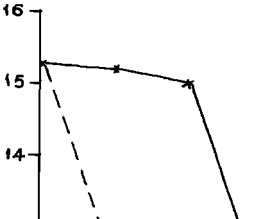


FIG.4. Graphical representation of the distribution of stomata.



DISTRIBUTION OF STOMATA

FIG 4



and the control. (Table No.3). Further comparison showed that the maximum number of stomata was found in the untreated plants. As a whole plants treated in the seedling stage showed lesser number of stomata than the seed treated ones.

Comparing the different concentrations, 0.5% solution showed the minimum number of stomata followed by 0.25%. There was not much difference between 0.1% - 3 hrs and 6 hrs treatment and control. Comparing the durations 9 hrs treatment showed lesser number of stomata followed by 6 hrs treatment.

Graphical representation of the distribution of stomata is given in Fig. 4.

5. SIZE OF STOMATA.

The colchiploids obtained showed larger stomata, but fewer per unit area. The analysis of the data showed that the treatment resulted in significant increase in the size of the stomata over the control. (Plates 5 and 6).

5(a) LENGTH OF STOMATA.

The mean length of stomata in different treatments varied from 21.84 to 29.70 μ. Comparing the different stages of application, seedling treatment was found to be superior to seed treatment. Comparing the different concentrations 0.5% was found to be superior to 0.25% and 0.1%. The effect between the concentrations was highly significant. The

T A B L E No. IV

ANALYSIS FOR THE LENGTH OF STOMATA

Treatments	value of the test criterion
Seed Vs. seedling	17.78**
Between concentrations	97.86**
Between durations	15.87**
Treated Vs. control	49.93**

** Significant at 1% level.

T A B L E No. IV

ANALYSIS FOR THE LENGTH OF STOMATA

Treatments	value of the test criterion
Seed Vs. seedling	17.78**
Between concentrations	97.86**
Between durations	15.87**
Treated Vs. control	49.93**

** Significant at 1% level.

T A B L E No. V

ANALYSIS FOR THE WIDTH OF STOMATA

Treatments	Value of the test criterion
Seed Vs. seedling	6.95**
Between concentrations	112.04**
Between durations	4.62
Treated Vs. control	32.98**

** Significant at 1% level

duration of time also showed significant increase in length of the stomata. Treatment for 9 hrs was found to be superior to 6 hrs and 3 hrs treatment. (Table 4).

5(b) WIDTH OF STOMATA.

The mean width of stomata in the different treatments varied from 16.10 to 19.54 μ . The analysis of the data given in table 5, showed that there was significant increase in width of stomata in the treated plants. In the case of stages of application, seedling treatment was found to be superior to seed treatment. Comparing the different concentrations 0.5% was seen superior to 0.25% and 0.1%. However the effect of different durations of time was found to be not significant.

6. AREA OF LEAF.

The leaves of the tetraploids were larger and broader in size than the diploids. The most conspicuous features of the leaves were the dark green colour and thickness. The leaves of the diploids invariably showed serrate margin, but tetraploids showed both serrate and entire margins.

Area of leaf in sq. inches.

(1) Seed Vs. seedling.

Seed = 5.8076 ± 0.28

Seedling = 6.3121 ± 0.266

(2) Treated Vs.control.

Treated = 6.0598 ± 0.207

Control = 4.4754 ± 0.71

(3) Between concentrations.

0.1%	=	5.1432	± 0.16
0.25%	=	5.9763	± 0.40
0.5%	=	7.0600	± 0.183

(4) Between durations

3 hrs.	=	5.8798	± 0.441
6 hrs.	=	6.0025	± 0.362
9 hrs.	=	6.2972	± 0.270

The area of leaves in different treatments varied from 4.4754 to 7.7869 sq.inches. The maximum leaf size was obtained in 0.5% - 3 hrs - seedling treatment. The average leaf area of untreated plants was found to be 4.4574 sq inches closely followed by those in the case of seed treatment with 0.1% - 3 hrs and 6 hrs, which were 4.500 and 4.6760 sq. inches respectively. Comparing the different stages of application seedling treatment was found to be superior to seed treatment. Comparing the different concentrations, 0.5% was found superior to 0.25% and 0.1% but between 0.25% and 0.1% there was not much difference. Comparing the durations,9 hrs and 6 hrs treatments were found superior to 3 hrs treatment.

The data of the mean area of leaf are given in tables 13 and 15.

7. THICKNESS OF LEAF.

Transverse section of leaflets of comparable age and stage of development showed that tetraploids have

thicker leaves, larger cells and plastids than the diploids (Plates 7 and 8). The cells of palisade and spongy parenchyma were bigger. The mean thickness of leaves in different treatments was compared and is given below.

Thickness of leaf in μ

(1) Seed Vs.seedling

Seed	=	269.7992	± 8.53
Seedling	=	288.59	± 7.69

(2) Treated Vs. control.

Treated	=	275.5311	± 12.9
Control	=	228.60	± 2.93

(3) Between concentrations.

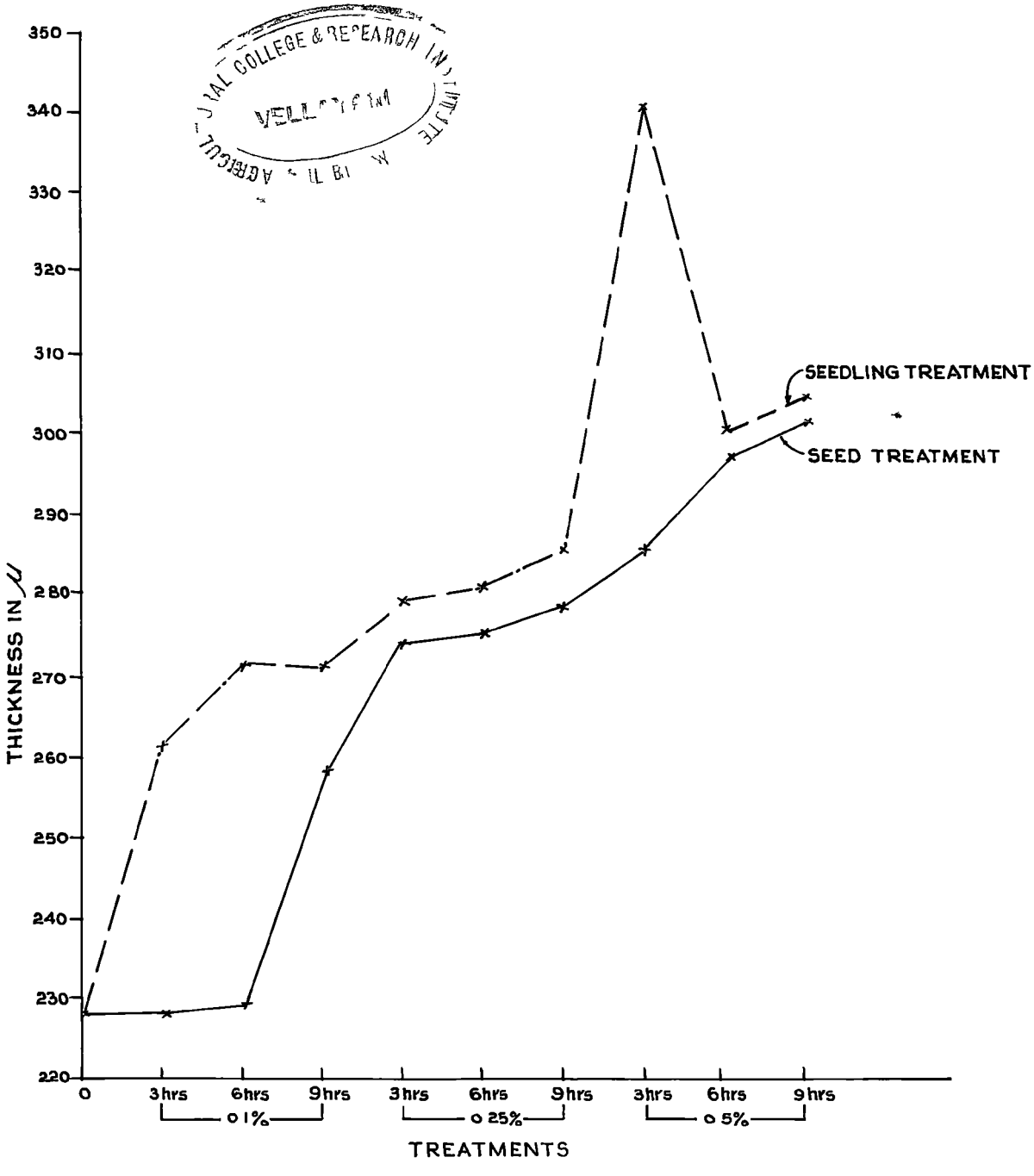
0.1%	=	253.27	± 7.2
0.25%	=	279.2635	± 2.29
0.5%	=	305.257	± 7.2

(4) Between durations.

3 hrs.	=	278.7303	± 14.75
6 hrs.	=	275.931	± 9.2
9 hrs.	=	282.9959	± 8.16

The thickness of leaf in different treatments varied from 226.10 μ to 343.9 μ . The maximum mean thickness was found in 0.5% - 3 hrs - seedling treatment. Between different stages of application, seedling treatment was found superior to seed treatment. Further comparison

FIG.5 GRAPHICAL REPRESENTATION OF THE MEAN THICKNESS OF LEAF.



THICKNESS OF LEAF

FIG 5

showed that treatment with 0.5% solution showed an increase in thickness followed by 0.25%. Comparing the durations 9 hrs treatment was found superior to 6 hrs. and 6 hrs treatment was found superior to 3 hrs. (Tables 13 and 15).

Graphical representation of the thickness of leaf is given in figure 5.

8. NUMBER OF FLOWERS.

Delayed flowering was noticed in tetraploids. On an average they took 10-15 days more for blooming than the diploids. The flower and all the floral parts were bigger in size. The analysis of variance for the number of flowers is given in tables 6 and 7.

The maximum number of flowers was produced in the case of 0.5% - 9 hrs - seedling treatment closely followed by 0.5% - 3 hrs - seedling. The minimum number of flowers was found in 0.1% - 6 hrs - seed treatment.

The analysis of the data showed that there was significant increase in the number of flowers in the treated plants. Comparing the different stages of application seedling treatment was found superior to seed treatment. The effect of duration of time in the production of flowers was not significant. Comparing the different concentrations, 0.5% was found superior to 0.25% and

T A B L E No. VI

ANALYSIS OF VARIANCE FOR THE NUMBER OF FLOWERS

Source	Sum of squares	D.f	Variance	F-ratio
Total	10632.80	79		
Block	79.30	3	26.25	0.325
Treatment	5892.30	19	245.32	3.00**
Error	4661.20	57	81.77	

** Significant at 1% level.

TABLE NO. VII

ANALYSIS OF VARIANCE FOR THE NUMBER OF FLOWERS

Source	Sum of squares	D.f.	Variance	F-ratio
Total	10632.80	79		
Block	79.30	3	26.65	0.325
Treatment	5892.30	19	245.32	3.00**
Seed Vs Seedling	533.55	1	533.55	6.49*
Between concentrations	3069.08	2	1534.54	18.75**
Between durations	362.33	2	181.16	2.21
Treated Vs control	584.00	1	584.00	7.13*
Interaction (Between concentration and durations.)	948.79	4	237.19	2.89*
Error	4661.20	57	81.77	

** Significant at 1% level

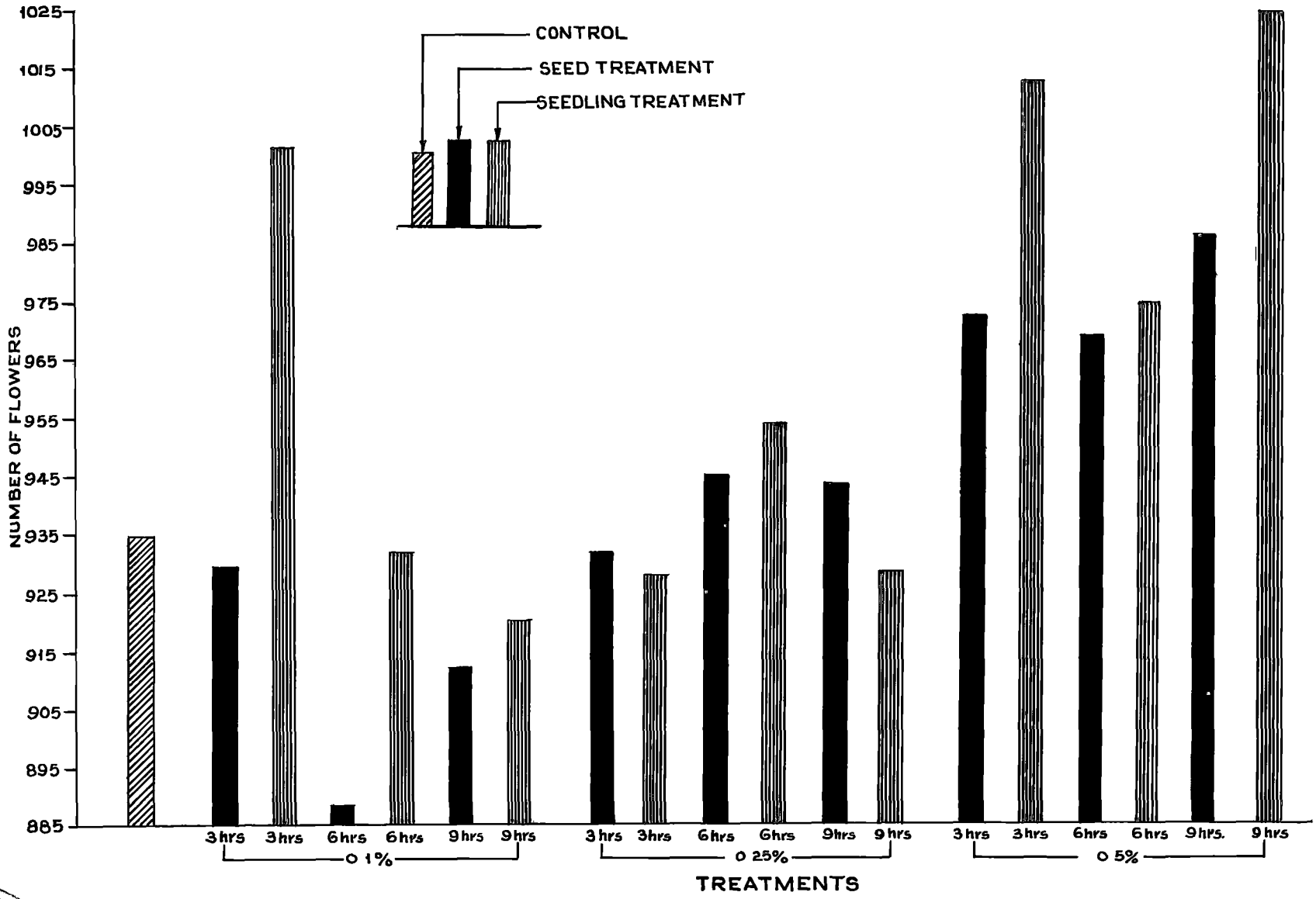
* Significant at 5% level

Critical difference = 12.793161

Conclusion:

18 14 2 17 16 13 15 10 (19 20) 11(4 7)1(8 12)1,9 6 5 3

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



TOTAL NUMBER OF FLOWERS

FIG·6

0.1%. It was found from further comparison that the effect of the interaction between concentration and duration of time was also significant.

Graphical representation of the number of flowers is given in Fig.6.

9. STERILITY OF POLLEN.

The percentage of sterility of pollen grains was more in tetraploid plants than in the diploids.(Tables 14 and 16).

In the normal diploid plants pollen sterility seldom exceeded 7.82%. In the case of treatments the pollen sterility varied from 8.31% to 52.04%. The highest percentage of sterility was shown by 0.5% - 9 hrs - seedling treatment. The analysis of the percentage of sterility in different treatments are given in Table 8.

Comparing the different stages of application seedling treatment was found superior to seed treatment. 0.5% - 9 hrs treatment showed the highest percentage of sterility. The effect of concentrations and durations were found highly significant. Treatment for 9 hrs was found superior to 6 hrs and 3 hrs treatment and 0.5% solution was found superior to 0.25% and 0.1%.

Graphical representation of the percentage of sterility is given in Fig.7.

T A B L E No. VIII

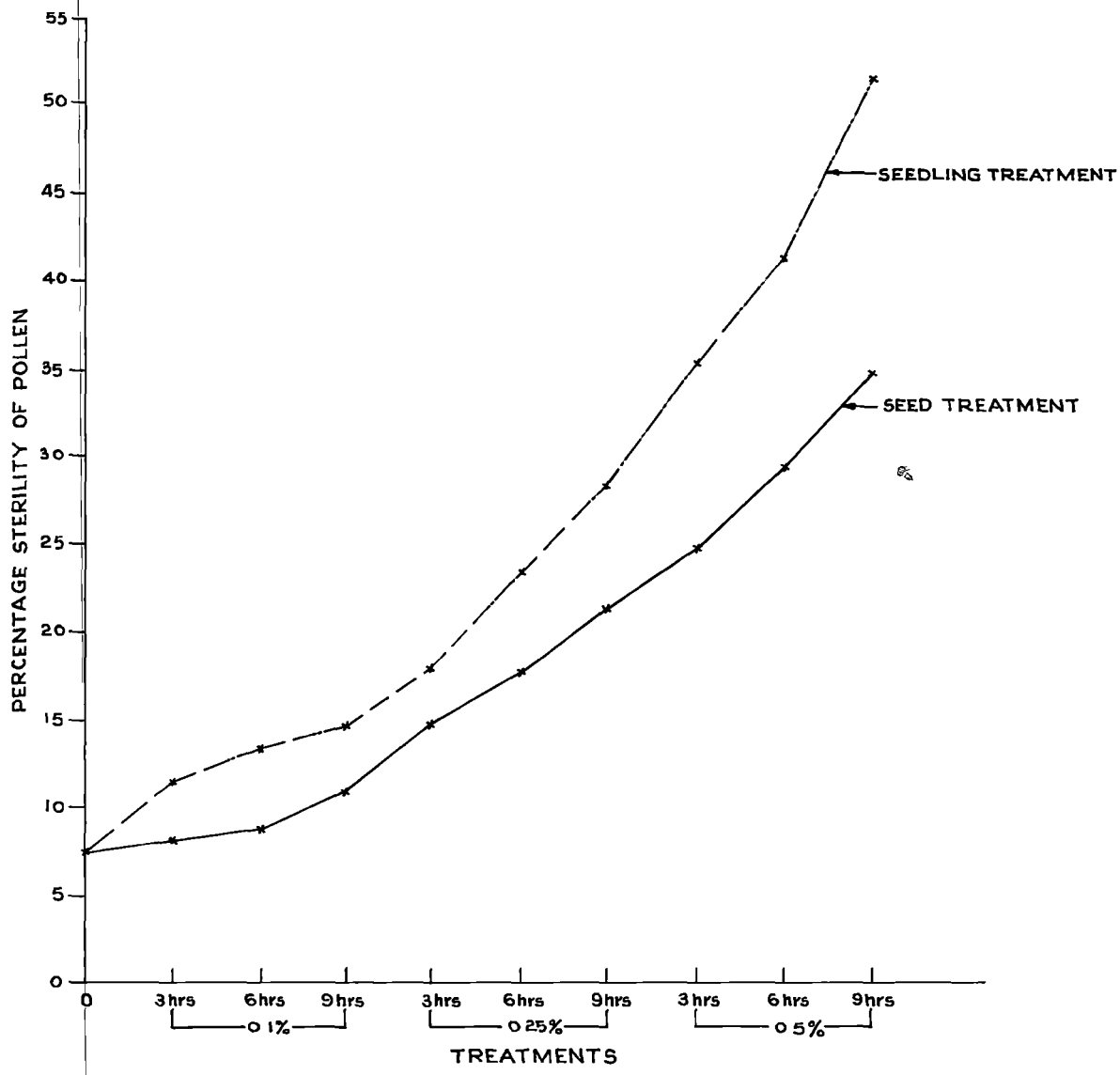
ANALYSIS FOR THE PERCENTAGE OF STERILITY OF POLLEN

Treatments	Value of the test criterian
Seed Vs. seedling	2.225*
Between concentrations	28.54**
Between durations	24.504**
Treated Vs.control	5.11**

** Significant at 1% level

* Significant at 5% level.

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



STERILITY OF POLLEN

FIG 7

10. SIZE OF POLLEN.

The tetraploids showed larger pollen grains than the diploids. The analysis of the diameter of pollen grains in different treatments are given in Table 9.

The mean diameter of pollen in the untreated plants was found to be 32.12 μ which was closely followed by seed treatment with 0.1% - 3 hrs and 6 hrs which showed 32.50 μ and 32.36 μ respectively. The diameter of pollen in different treatments varied from 32.12 μ to 36.94 μ. The maximum mean diameter was found in 0.5% treatment especially in seedling application for periods of 3 hrs and 9 hrs. Comparing the different stages of application, the seedling treatment was found superior to seed treatment. Comparing the effect of duration of time, there was no significant difference between the various durations. Comparing the different concentrations 0.5% was found superior to 0.25% and 0.1%.

11. MEIOSIS.

In all the diploid plants meiosis was normal which showed 7 bivalents at diakinensis and in the first and second anaphase seven chromosomes per pole (Plate 9). Lagging chromosomes, unequal separation or other meiotic irregularities were not observed. In tetraploids 14 bivalents were observed, even though some of the cells showed only 13 bivalents. (Plate 10). Octoploid plants with 28 bivalents at diakinensis were also observed in

T A B L E No. IX

ANALYSIS FOR THE DIAMETER OF POLLEN GRAINS

Treatments	Value of the test criterion
Seed Vs seedling	14.38**
Between concentrations	28.58**
Between durations	5.94
Treated Vs. control	72.64**

** Significant at 1% level.

4 plants. The number of flowers and fruits borne by the octoploid plants were fewer than that of the diploids and in the tetraploids it was greater (Plate 11 and 12). The growth of the terminal bud was inhibited at a certain stage of the life of the octoploids.

Tripolar spindle in the second anaphase was observed in a few cells of the tetraploids. However any other type of meiotic irregularities could not be observed.

12. NUMBER OF FRUITS.

Most of the colchiploids produced greater number of fruits even though a few of them were shy bearers. The data on the number of fruits corresponding to different treatments were analysed statistically and the analysis of variance is given in tables 10 and 11. The treatment was found to be significant.

Comparing the effect of seed and seedling treatments, it is found that seedling treatment was found superior to seed treatment in increasing the number of fruits, but the difference in effect between different concentrations was not significant.

Comparing the different durations, it is found that 3 hrs treatment has produced greater number of fruits than 6 hrs and 9 hrs treatments whereas 6 hr is better than 9 hrs treatment. The interaction between

T A B L E No. X

ANALYSIS OF VARIANCE FOR THE NUMBER OF FRUITS

Source	Sum of squares	D.f.	Variance	F-ratio
Total	11692.80	79		
Block	36.50	3	12.16	0.001
Treatment	5368.81	19	282.57	2.54*
Error	6287.50	57	110.30	

* Significant at 6% level

T A B L E No. XI

ANALYSIS OF VARIANCE FOR THE NUMBER OF FRUITS

Source	Sum of squares	D.f.	Variance	F-ratio
Total	11692.80	79		
Block	36.50	3	12.16	0.001
Treatment	5368.81	19	282.57	2.54*
Seed Vs Seedling	813.39	1	813.39	7.32*
Between concentrations	618.85	2	309.34	2.78
Between durations	2045.85	2	1022.92	9.21**
Treated Vs control	2275.58	1	2275.58	20.50**
Interaction (Between concentrations and durations)	618.69	4	154.47	1.21
Error	6287.50	57	110.30	

** Significant at 1% level

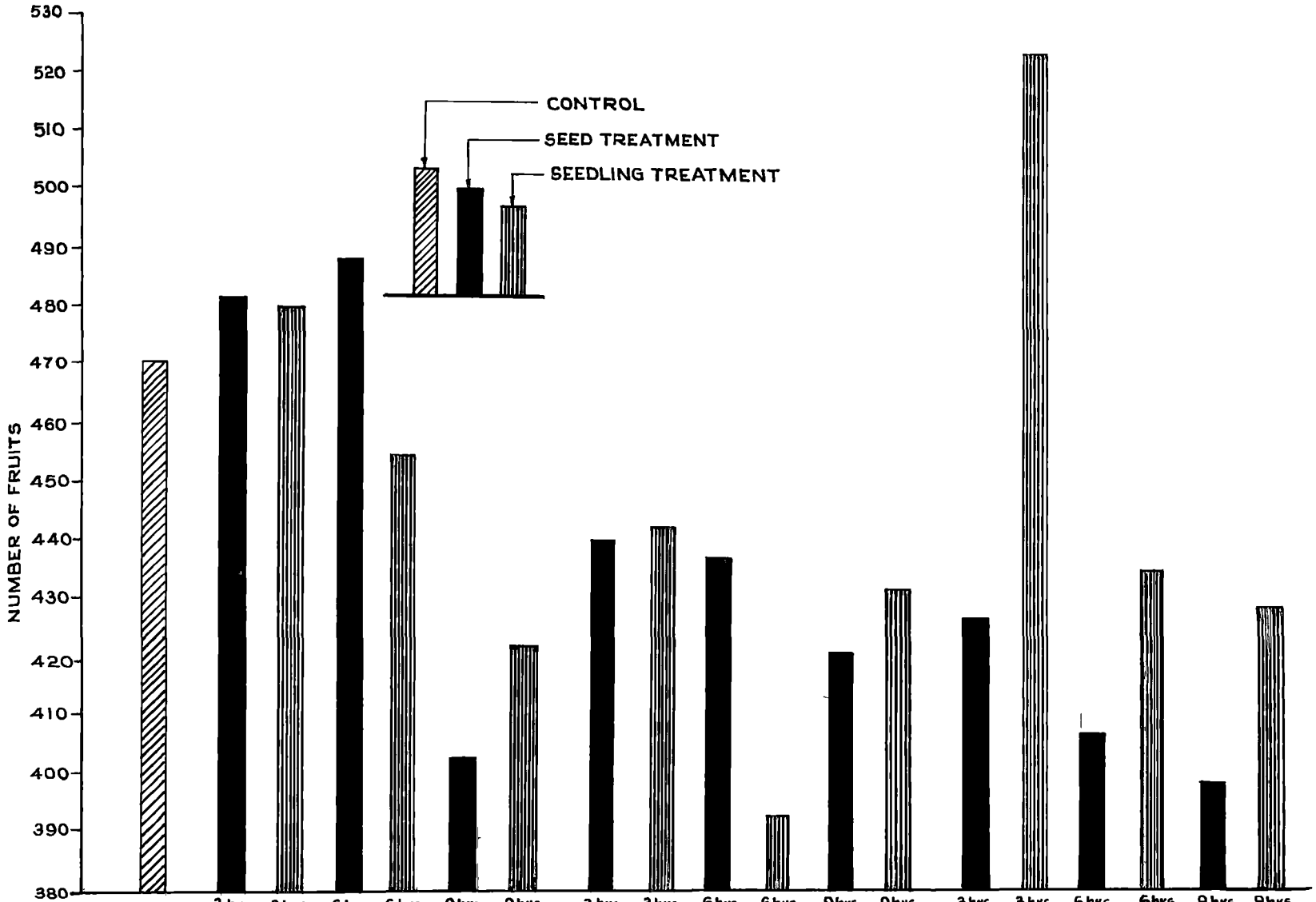
* Significant at 5% level

Critical difference = 14.9083.

Conclusion:

14 3 19 1 2 20 4 16 8 7 9 12 18 13 6 11 15 5 17 10

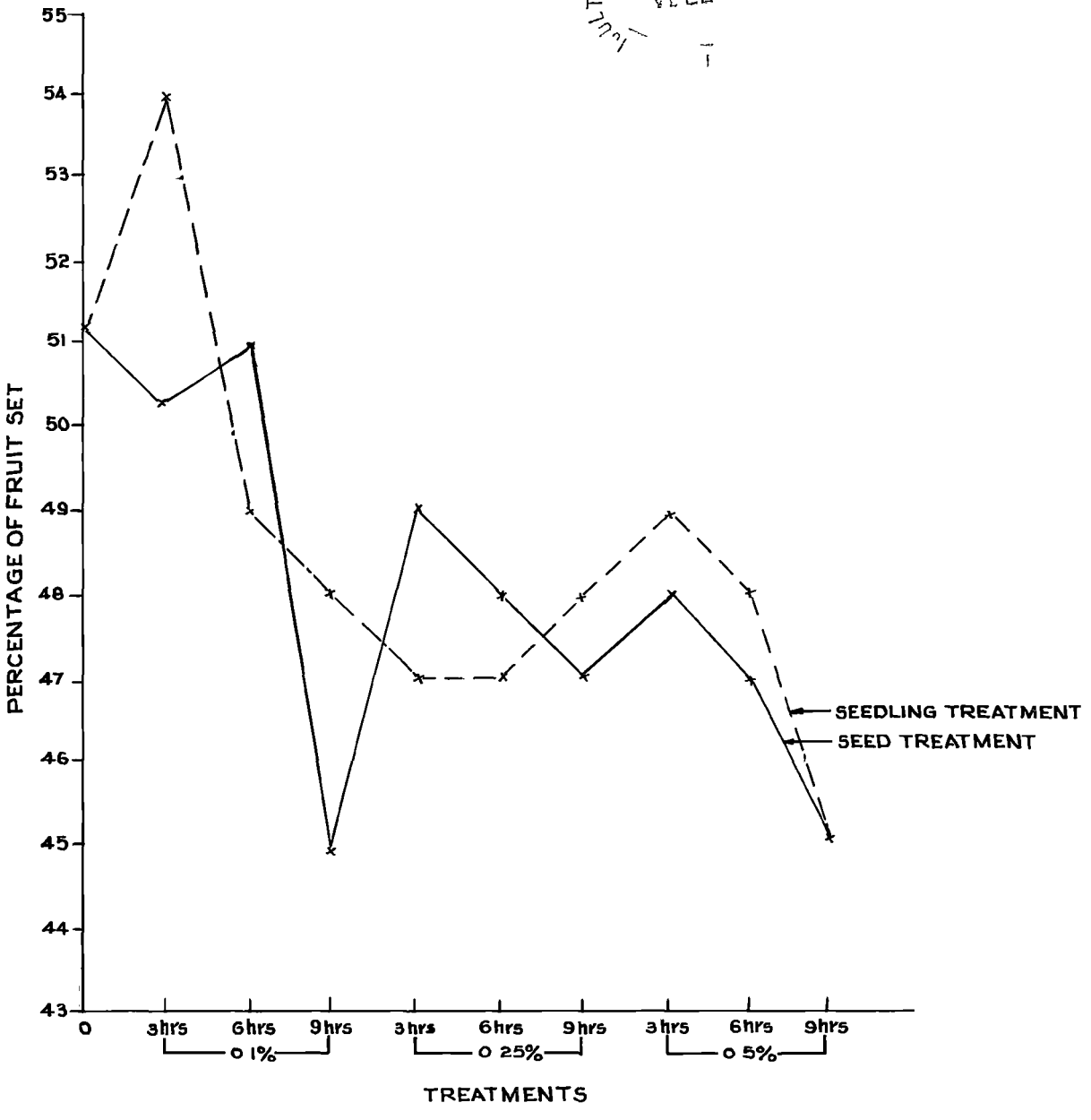
FIG.8. Graphical representation of the total fruits under various treatments



TOTAL NUMBER OF FRUITS

FIG 8

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



PERCENTAGE OF FRUIT SET

FIG 9

concentration and duration of time was not significant. The differences in effect among treatments were not significant.

Graphical representation of the number of fruits is given in Fig. 8.

13. LENGTH OF FRUITS.

A sample of 10 fruits from each treatment was measured and found that there was not much difference in length of fruits between the various treatments. The mean length of fruits in different treatments was compared and are given below.

Length of fruits in Cms.

(1) Seed Vs. seedling.

Seed = 5.24 ± 0.0046

Seedling = 5.37 ± 0.01

(2) Treated Vs.control.

Treated = 5.23 ± 0.223

Control = 4.84 ± 0.015

(3) Between concentrations.

0.1% = 5.02 ± 0.133

0.25% = 5.36 ± 0.01

0.5% = 5.53 ± 0.008

(4) Between durations.

3 hrs. = 5.26 ± 0.01

6 hrs. = 5.27 ± 0.13

9 hrs. = 5.39 ± 0.009

The mean length of fruits in the treated plants was found to be 5.23 cms and that of the untreated was 4.84 cms. Further comparison showed that there was not much difference between seed and seedling treatments. Comparing the different concentrations and also durations significant difference could not be observed.(Tables 14 and 16).

14.YIELD OF SEEDS.

Seeds from a sample of 100 fruits from each treatment were counted and the comparison between the various treatments are given below.

Yield of seeds.

(1) Seed Vs. seedling.

Seed = 695.22 ± 6.74

Seedling = 692.00 ± 3.09

(2) Treated Vs. control.

Treated = 693.61 ± 25.07

Control = 705.00 ± 10.71

(3) Between concentrations.

0.1%	=	699.16	±	6.41
0.25%	=	693.00	±	3.21
0.5%	=	688.66	±	4.19

(4) Between durations.

3 hrs.	=	696.33	±	4.34
6 hrs.	=	694.16	±	3.31
9 hrs.	=	694.33	±	3.42

A study of the data reveals that the colchicine treatment showed a decrease in the yield of seeds. Comparing the effect of seed and seedling treatments there was not much difference. Between different concentrations, 0.1% produced the maximum number of seeds followed by 0.25% and the minimum number of seeds having produced by 0.5%. Comparing the effect of durations of time, 3 hrs treatment produced the maximum number of seeds followed by 6 hrs, while 9 hrs treatment produced the minimum number of seeds. (Tables 14 and 16).

15. DRY WEIGHT OF THE PLANT.

Analysis of the data on the dry weight of the plants in different treatments revealed that the results did not show any increase in weight of plants over the control. The analysis of variance is given in Table 12.

On comparing the different treatments, it is found that the maximum dry weight was produced by 0.5% -

T A B L E No. XII

ANALYSIS OF VARIANCE FOR THE DRY WEIGHT OF THE PLANT.

Source	sum of squares	D.f.	Variance	F-ratio
Total	4560.975	79		
Block	372.20	3	124.06	2.42*
Treatment	1567.34	19	82.49	1.69
Error	2921.935	57	51.25	

* Significant at 5% level

Critical difference = 10.13518

TABLE No. XIII

SEED TREATMENT.

Treatments	% of Germination	Mean height of plants in cms.	Average Number of stomata	Mean length of stomata in μ	Mean width of stomata in μ	Average area of leaf in sq. inches	Average thickness of leaf in μ
0.1% -- 3 hrs	70	91.82	15.10	21.84	17.30	4.500	227.94
0.1% -- 6 hrs	68	86.70	15.02	21.48	16.94	4.6760	229.27
0.1% -- 9 hrs	64	89.74	12.14	25.10	17.30	5.3905	258.60
0.25% --3 hrs	64	96.82	12.07	25.28	17.50	5.6110	274.59
0.25%-- 6 hrs	60	87.20	11.85	25.56	17.30	5.6885	275.93
0.25%-- 9 hrs	59	76.97	10.89	26.40	17.60	6.0945	278.59
0.5% -- 3 hrs	65	90.67	10.75	27.04	17.70	6.1280	285.26
0.5% -- 6 hrs	57	95.72	10.32	28.22	18.60	7.0690	297.25
0.5% -- 9 hrs	50	86.42	9.96	28.60	18.70	7.1110	301.25
Control	71	91.45	15.29	21.84	17.38	4.4754	228.60

T A B L E No. XIV

S E E D T R E A T M E N T

Treatments	Average number of flowers	Percentage of sterility of pollen	Mean diameter of pollen in μ	Average number of fruits	Mean length of fruits in ems.	Seeds from 100 fruits	Dry weight of plants in gms.
0.1% -- 3 hrs	58.10	8.31	32.50	30.13	4.94	695	129.50
0.1% -- 6 hrs	58.60	8.83	32.32	30.50	4.83	720	129.55
0.1% -- 9 hrs	57.20	11.16	35.20	27.50	5.04	695	150.30
0.25%-- 3 hrs	58.25	15.53	35.40	28.60	5.22	698	146.70
0.25%-- 6 hrs	58.50	18.18	35.50	29.00	5.28	700	141.10
0.25%-- 9 hrs	59.00	21.38	35.72	29.92	5.40	688	129.10
0.5% -- 3 hrs	60.80	25.15	35.82	32.60	5.46	685	107.10
0.5% -- 6 hrs	60.60	29.23	36.40	29.70	5.48	690	131.70
0.5% -- 9 hrs	62.50	35.38	36.56	30.17	5.54	682	118.90
Control	58.00	7.82	32.12	29.70	4.84	705	149.07

TABLE NO. XV

SEEDLING TREATMENT

Treatments	Mean height of plants in cms.	Average number of stomata	Mean length of stomata in μ	Mean width of stomata in μ	Average area of leaf in sq.in.	Average thickness of leaf in μ	Average number of flowers.
0.1% -- 3 hrs	99.82	12.52	25.18	16.10	5.2310	261.26	62.51
0.1% -- 6 hrs	91.32	12.33	25.78	17.40	5.4652	271.93	58.00
0.1% -- 9 hrs	80.75	11.10	26.34	17.50	5.5965	270.59	57.5
0.25%-- 3 hrs	90.22	11.00	27.62	17.60	6.0220	279.93	58.6
0.25% --6 hrs	78.65	10.98	28.00	17.22	6.0450	281.26	59.2
0.25% --9 hrs	86.86	10.55	28.30	17.98	6.3970	285.26	58.5
0.5% -- 3 hrs	104.15	9.29	29.70	19.54	7.7869	343.91	67.13
0.5% -- 6 hrs	88.72	9.82	29.56	19.22	7.0715	299.92	61.06
0.5% -- 9 hrs	83.00	9.32	29.56	19.40	7.1940	303.92	67.30
Control	91.45	15.29	21.84	17.38	4.4754	228 .60	58.00

TABLE No. XVI

SEEDLING TREATMENT

Treatments	Percentage of sterility of pollen	Mean diameter of pollen in μ	Average number of fruits.	Mean length of fruits in cms.	Seeds from 100 fruits	Dry weight of plants in gms.
0.1% -- 3 hrs	11.52	35.30	34.40	5.04	710	121.90
0.1% -- 6 hrs	13.35	35.40	28.30	5.05	690	140.70
0.1% -- 9 hrs	14.28	35.68	28.25	5.27	685	125.60
0.25%-- 3 hrs	18.96	35.72	28.30	5.30	690	144.60
0.25% --6 hrs	23.34	35.82	30.07	5.45	680	126.90
0.25%-- 9 hrs	28.83	36.04	30.13	5.54	702	124.00
0.5% -- 3 hrs	35.96	36.94	37.30	5.61	700	177.90
0.5% -- 6 hrs	41.10	36.56	30.13	5.55	685	144.20
0.5% -- 9 hrs	52.04	36.58	30.50	5.56	686	132.20
Control	7.82	32.12	29.70	4.84	705	149.07

3 hrs - seedling treatment followed by the control. The minimum dry weight was found in 0.5% - 3 hrs - seed treatment closely followed by 0.5% - 9 hrs - seed treatment.

Comparative effects of the different treatments on the production of colchiploid plants.

Treatments.	No. of plants treated	No. of colchiploids produced.
0.1% - 3 hrs - seed treatment	16	0
0.1% - 3 hrs - seedling treatment	16	4
0.1% - 6 hrs - seed treatment	16	0
0.1% - 6 Hrs - seedling treatment	16	2
0.1% - 9 hrs - seed treatment	16	3
0.1% - 9 hrs - seedling treatment	16	4
0.25%- 3 hrs - seed treatment	16	4
0.25%- 3 hrs - seedling treatment	16	5
0.25%- 6 hrs - seed treatment	16	4
0.25%- 6 hrs - seedling treatment	16	5
0.25%- 9 hrs - seed treatment	16	4
0.25%- 9 hrs - seedling treatment	16	4
0.5% - 3 hrs - seed treatment	16	5
0.5% - 3 hrs - seedling treatment	16	8
0.5% - 6 hrs - seed treatment	16	5
0.5% - 6 hrs - seedling treatment	16	6
0.5% - 9 hrs - seed treatment	16	6
0.5% - 9 hrs - seedling treatment	16	6
Total plants treated	288	75

Out of the 288 plants treated 75 were colchiploids . The maximum number of colchiploids obtained in the seedling treatment with 0.5% solution for a period of 3 hrs. As a whole seedling treatment gave more colchiploids than seed treatment.

---ooOoo---

DISCUSSION

Investigations carried out by different workers on the effect of colchicine on germination, growth, flowering, fruiting and final yield in various plants have given varying results. The results of a similar study taken up on cluster beans and which are presented in the preceding chapter are discussed below.

(1) Percentage of germination.

The first observation made during this investigation was the lower percentage of germination produced by colchicine treatment, especially with 0.5% solution for a period of 9 hrs.

The results of the present study showed that treatment 0.1% for periods of 3 hrs and 6 hrs showed no reduction in germination percentage, but treatment with 0.25% for 9 hrs, and 0.5% for 6 and 9 hrs showed slight reduction in the percentage of germination. A reduction in germination percentage in colchicine treated seeds was previously reported by Sharma and Datta (1957) in Coriandrum sativum, Saxena and Nanda (1960) in phlox, and Sen and Vidyabhushan (1960) in horse gram. However, Kluge and Kramer (1955) in Vaccinium myrtillum and V.corymbosum and Schrock (1951) in birch had noticed an increase in germination percentage by colchicine treatment.

Hyun (1956) was of opinion that the inhibition of germination was probably due to deleterious effect of colchicine on the enzyme system of seed. The effect was likely to be more deleterious in large seeds which would imbibe more colchicine solution.

It is possible that the slight reduction in germination percentage noticed at higher concentrations for longer durations in the present study may be due to enzymatic disturbance caused by colchicine.

(2) Seedling treatment.

The results showed that the most effective method for inducing tetraploidy in cluster beans by colchicine was by treating the apical bud of the seedlings with its aqueous solution. Practically all the doses gave two or more polyploids in all the three concentrations, viz. 0.1%, 0.25% and 0.5% .

Comparative effectiveness of seedling treatment over seed treatment in inducing polyploidy with colchicine has been previously reported by Kumar and Abraham (1942) in Phaseolus radiatus, Evans (1955) in Trifolium pratense , Armstrong and Robertson (1956) in Trifolium hybridum and Sen and Bhowel (1959) in Vigna sinensis . But production of a higher percentage of tetraploids by seed treatments have also been reported by Ramanujam and Joshi (1951) in Cicer arietinum.L. and Bragodo (1955) in Trifolium pratense.

Sen and Bhowel (1959) observed that in Vigna sinensis, the main reason for the failure of seed treatment seemed to be the arrested root growth of the affected plants which could not be overcome even with the best cultural conditions provided.

In the present work also it is assumed that the production of lesser number of polyploids by seed treatment may be either due to the failure of germination of some of the affected seeds or due to arrested root growth found in some of the affected plants.

3. Rate of growth and height of plants.

In the present investigation, the tetraploids were slow growing in the early stages as seen by their reduced height, but they surpassed the diploids in height towards the concluding stages. A decreased growth rate in tetraploids was reported by Kumar and Abraham (1942) in Phaseolus radiatus , Saito (1957) in American water melons and Janaki Ammal and Bezbarauh (1962) in Catharanthus roseus. According to Kumar and Abraham an increase in cell size and nuclear size lead to various alterations in the physiological needs of the cell. The effect of this was manifested in a decreased growth rate in the tetraploid which consequently took longer time to reach maturity and flowering stage than the diploids.

The tetraploids of cluster beans showed an increase

in height, having stouter stem with long internodes. Increased shoot elongation by colchicine treatment had been reported by Sharma and Datta (1957) in corriander (Coriandrum sativum) . But there is difference of opinion regarding the effect of colchicine on shoot elongation. Sen and Chedda (1958) reported a decrease in height in Phaseolus mungo which is supported by the findings of Zimmermann (1958) in forage grasses and Sen and Bhowel (1959) in Vigna sinensis.

In the present study it was found that the number of nodes in the tetraploid plants was also found to increase along with the increase in the internodel length. Therefore the increase in height of the plant may partly be due to the increase in number of nodes.

(4) Leaf characters.

In the present study, the tetraploid plants showed larger and broader leaves than the diploids. They were thicker and dark green in colour. Similar observations were recorded previously by Armstrong and Robertson (1960) in alsike clovers and Thombre and Desai (1960) in Agave cantala . However,Sen and Chedda (1958) reported smaller leaves in tetraploid blackgram than in the diploids.

The increase in thickness of the leaves of tetraploids is in agreement with the findings of many workers. An increase in the thickness of leaves was previously reported by Kumar and Abraham (1942) in

Phaseolus radiatus , Sen and Chedda (1958) in black gram and Sen and Vidyabhushan (1960) in horse gram. According to Sen and Vidyabhushan (1960) the increase in thickness is due to the increase in size of the cells of all the three regions - epidermis, palisade and spongy parenchyma.

In the present investigation, the tetraploids showed larger stomata, but fewer per unit area. The cell size was also larger. This is in agreement with the findings of Kumar and Abraham (1942) in Phaseolus radiatus, Armstrong and Robertson (1960) in alsike clovers, and Sen and Bhowel (1960) in Vigna sinensis. However, Hertzsch (1951) observed that the measurements of stomata in the tetraploid Vicia villosa differed in no way from the diploids.

In cluster beans, the increase in size and thickness of the leaves of tetraploids may be due to an increase in the size of the cells of epidermis, palisade and spongy parenchyma. The larger cell size consequently lead to the production of larger stomata.

(5) Flowering.

Delayed flowering was observed in the tetraploid plants of cluster beans. They had a much longer flowering period, and in general the growth period was prolonged. All the floral parts were larger in tetraploids than in the diploids. Larger flowers in induced tetraploids were previously reported by Kluge (1959) in Fragaria vesca

Sen and Vidyabhushan (1960) in horse gram, Jain et al (1962) in Antirrhimum majus and Ammal and Bezbaruah (1962) in Catharenthus roseus.

Sen and Vidyabhushan (1960) attributed the delayed flowering of tetraploids-like other physiological phenomena - to slower rate of metabolic activity and further suggested that the continued flowering of the plants was probably due to lesser exhaustion of the plants through decreased fruit setting.

In cluster beans, the tetraploids produced larger number of flowers than the diploids. Armstrong and Robertson (1960) reported an increase in the number of flowers in alsike clovers. However,Kluge (1959) recorded fewer flowers in induced tetraploids or Fragaria vesca . Increased production of flowers can be explained as a natural outcome from the vigorous growth of the plants leading to the production of larger inflorescences and thereby to larger number of flowers.

(6) Sterility and Size of pollen .

The percentage of sterility of pollen grains was found to be more in induced tetraploids than in the diploids. An increase in pollen sterility of tetraploids was reported by Vig (1962) in Cyamopsis psoralioides, Luongdinhcua (1950) in Oryza sativa , Sen and Chedda (1958) in Phaseolus mungo, and Islam (1960) in Anona squamosa . Sen and Vidyabhushan (1960) reported that

80 to 90% of the pollen grains were stainable in the auto-tetraploids of horse gram. In the present study the highest percentage of pollen sterility was noticed in the octoploid plants. This may be due to some irregularities in the meiotic divisions.

The tetraploids of cluster beans showed larger pollen grains compared to that of the diploids. Similar observations on the increased size of pollen were made by many workers. Illies (1956) in birch, Osone (1958) in tea plant, and Armstrong and Robertson (1960) in alsike clovers, reported giant pollen grains. While, Hertzsch (1951) reported that in Vicia villosa the pollen grains of the polyploid plants differed in no way from the normal. Giant pollen grains obtained in the polyploids of cluster beans may be due to larger size of the pollen mother cells.

7. Meiosis.

The tetraploids of cluster beans showed 14 bivalents egenthough some of the cells showed only 13 bivalents. Vig (1962) reported tetraploid guar plants with 2 n = 26 and 2 n = 28 chromosomes (2 n = 14 from the normal) Wada (1940, 49, 50) proved that in Tradescantia, spindle fibres did not develop at prophase with concentration of o.05 % to 0.1% colchicine. Carpentier (1954) by treating roots and root-lets of groundnut with 0.005% colchicine solution produced C-mitotic cells. Smith and Hiner (1960) observed typical colchicine mitotic effects in Allium cepa.

by treating the root tissue with concentrations of 0.025 - 0.8% solution.

In the present study four octoploid plants with 28 bivalents at diakinensis were also observed. These octoploids were produced by the treatment of 0.5% solution for a period of 6 hrs and 9 hrs durations. Kumar and Abraham (1942) noticed octoploid cells in one portion of a tetraploid root of Phaseolus radiatus.L. by colchicine treatment. Therefore it is assumed that higher concentration for a longer period led to further colchicine mitosis of the tetraploid cells.

(8) Fruit setting and yield.

In the present enquiry, the tetraploids showed a slight decrease in the percentage of fruit setting compared to the diploids. This is in agreement with the findings of Singh (1955) in Carica papaya, Kluge (1959) in Fragaria vesca , Sen and Bhowel(1959) in Vigna sinensis , and Sen and Vidyabhushan (1960) in horse gram.

Probabilities of gene-conditioned reduced fertility have been suggested by Randolph (1941) in maize. Stebbins (1947) is of opinion that sterility is mainly due to genetically controlled factors of an unknown nature. Parthasarathy and Rajan (1953) suggested that

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Warmke, H.E.	1945	Experimental polyploidy and rubber content in Taraxacum kok-saghyz . Bot. Gaz. 106 (3) 316-324.
* Yokoyama, S. and Matusui, H.	1957.	On the induction of Polyploidy in tea plant. Tea, Res. Jour. No.9. 8-11.
* Zimmermann, K.F.	1958	Polyploidy breeding in forage crops. P.B. absts. 31. No. 1. 45.

* Originals not seen.

PLATE I. Two week old seedlings of Control plants.

PLATE II Two week old plants developed from seeds which were treated with 0.25% solution for 6 hrs.

PLATE 1

PLATE 2

PLATE III Two week old plants developed from seeds which were treated with 0.5% solution for 3 hrs.

PLATE IV Two week old Plants developed from seeds which were treated with 0.5% solution for 9 hrs.

PLATE 3

PLATE 4

PLATE V. Size and distribution of stomata in diploid plants.

PLATE VI Size and distribution of stomata in tetraploid plants.

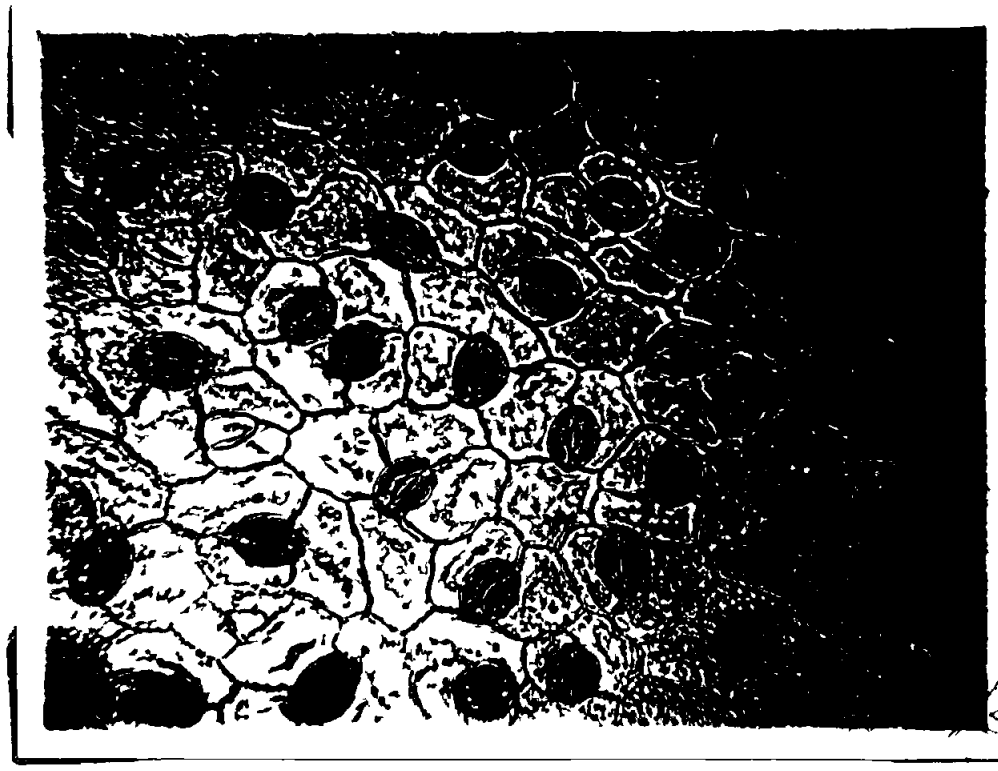

PLATE 5

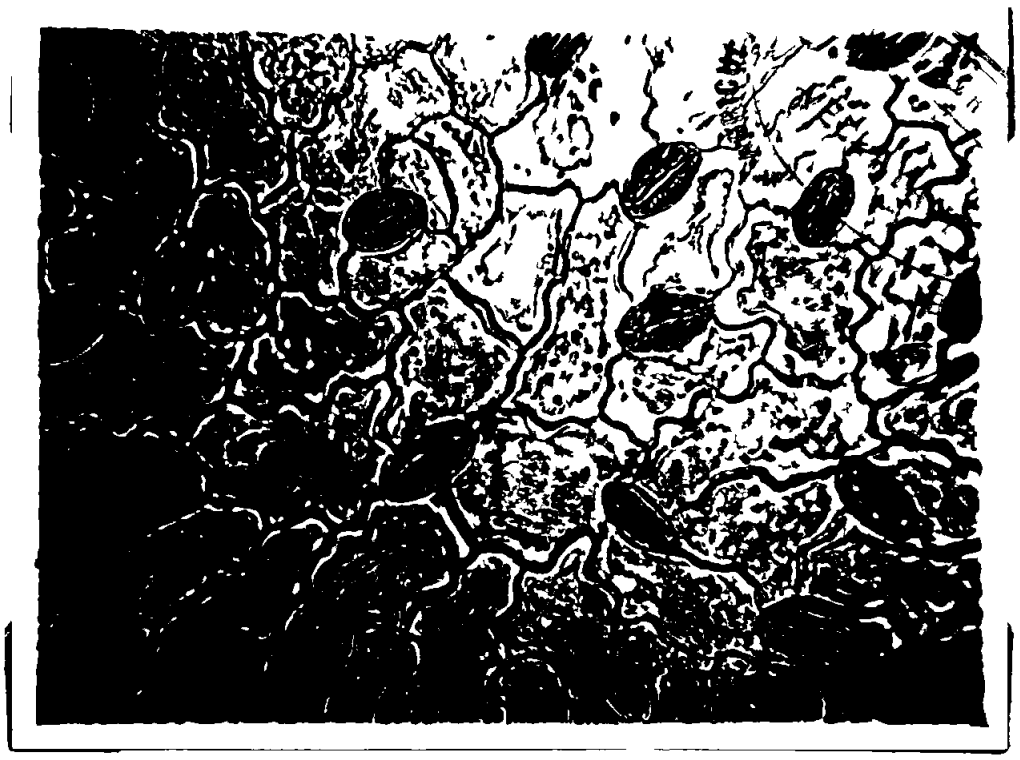

PLATE 6

PLATE VII Leaf thickness of the diploid plants.

PLATE VIII Leaf thickness of the tetraploid plants.

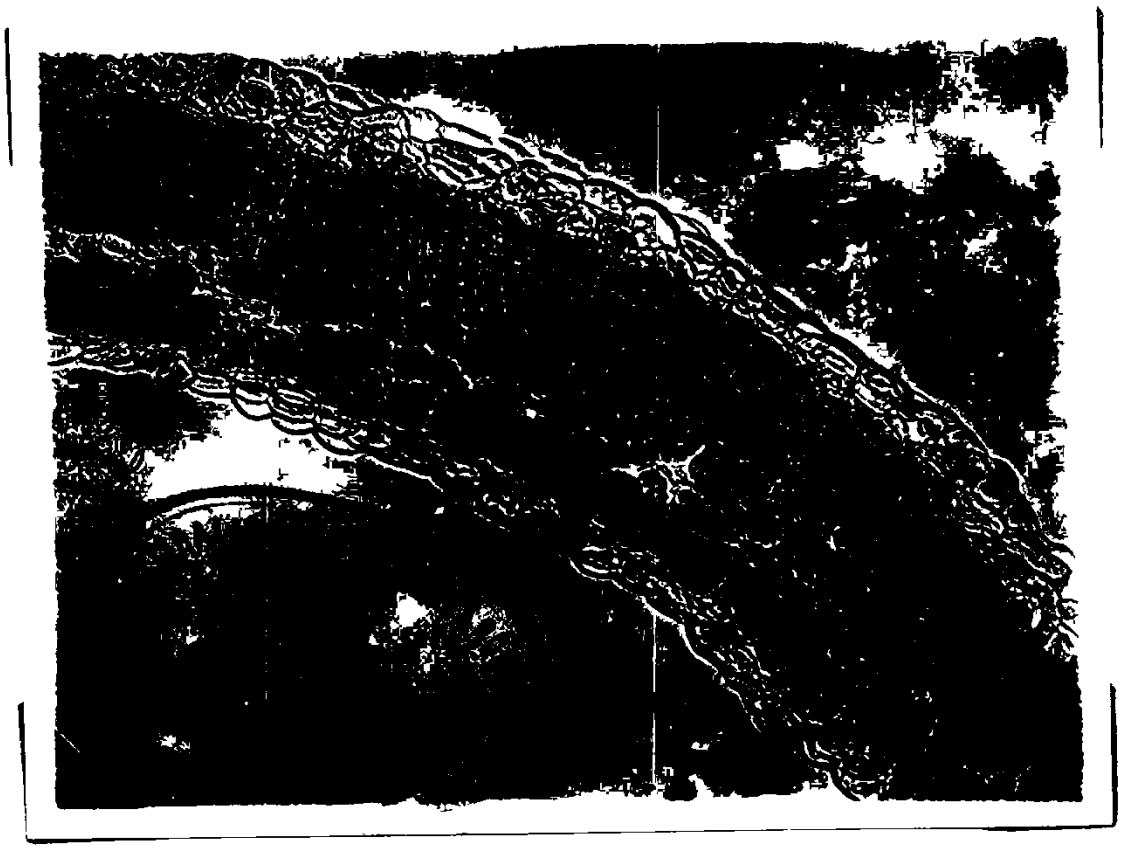

PLATE 7

PLATE 8

PLATE IX First meiotic metaphase of the drploid plant showing seven bivalents.

PLATE X First meiotic anaphase of the tetraploid plant showing fourteen chromosomes in each pole.

PLATE 9

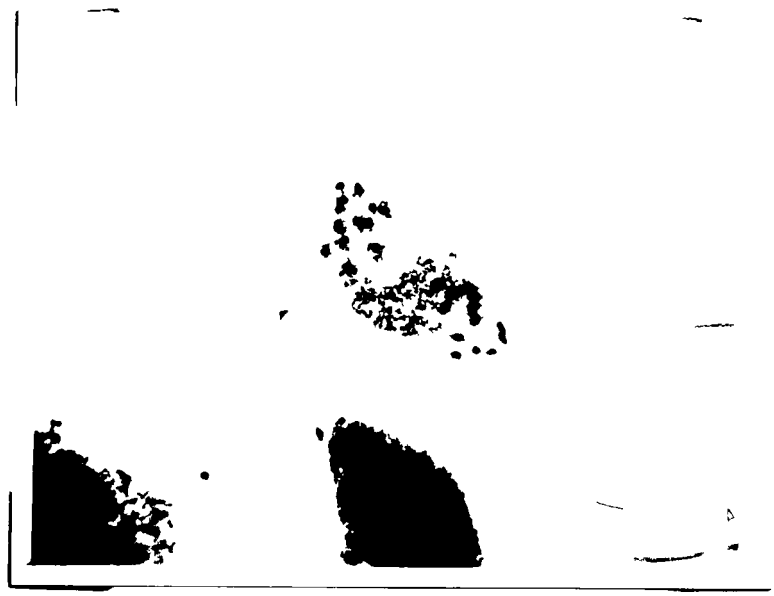

PLATE 10

PLATE XI Diploid and tetraploid plants showing differences in the external characters.

PLATE XII An octoploid plant showing suppression of growth of the terminal bud.

PLATE 11

PLATE 12