INDUCTION OF AUTOTETRAPLOIDY IN GINGER

By

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THESIS

submitted in partial fulfilment of the
requirements for the degree

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Faculty of Agriculture
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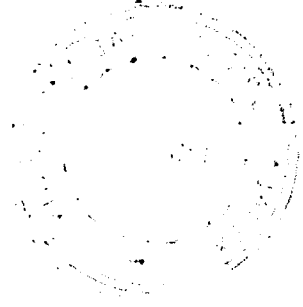
DECLARATION

I hereby declare that the thesis entitled '**Induction of autotetraploidy in ginger**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title, of any other university or society.



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Introduction

INTRODUCTION

Ginger is an important commercial spice esteemed for its aroma, flavour and pungency. India is the largest producer and exporter of ginger contributing to about 42 per cent of the world production and 52 per cent of the export. Kerala share 29 per cent of area and 30 per cent of production of ginger in the country.

The ginger production in India is handicapped by the occurrence of devastating diseases like soft rot and bacterial wilt and high cost of production. In the export front, high fibre content and dull colour of dried rhizomes are factors limiting international competitiveness. Varieties having the advantages of high yield, special quality attributes and resistance to diseases are lacking necessitating urgent efforts in this line so as to keep up the position of India in the world market.

Ginger being propagated exclusively by vegetative means, the number of clones available are limited. Conventional generative breeding to develop resistant and high yielding genotypes is not possible in ginger mainly due to lack of seed set. Sathiabhama (1988) revealed that pollen tube growth in ginger is not sufficient to fertilize the deeply seated ovules, thus leading to lack of seed set. This problem could be overcome through *in vitro* pollination and fertilization obtaining seed set and germination in ginger as reported by Valsala (1994) for the first time. But this technique needs refinement with respect to germination of seeds to suit planned hybridization.

Polyploidy has been very useful in evolution since it is the source of variation. Induced polyploidy is used by plant breeders for improving yield, quality and to impart resistance to pests and diseases. Apart from the stimulatory effect of colchicine which is well documented, it has inhibitory effect on

morphological characters (Atsmon, 1970). At low concentration colchicine act as a mutagent also (Kochhar, 1975). The ginger plant is ideally suited for polyploidy breeding as it has a low chromosome number (2n = 22) and nuclear volume. Furthermore, as the crop is vegetatively propagated, any improvement in yield or quality that may be achieved can readily be maintained.

Improved growth and yield of induced autotetraploids of ginger variety Maran reported by Ramachandran and Nair (1992) suggests its usefulness in creating desired changes in ginger types. The present investigation is an attempt to induce autotetraploidy and to create variability in four commercial varieties of ginger namely Himachal Pradesh, Maran, Nadia and Rio-de-Janeiro using colchicine at varying concentrations following different methods of application.

Review of Literature

REVIEW OF LITERATURE

Introduction

Polyploidy is a condition in which the individuals have more than two sets of chromosomes in their somatic cell. Polyploidy is of two different kinds involving either duplication of the same genome or summation of different genomes referred to as autopolyploidy and allopolyploidy respectively. Autopolyploids can be either natural or artificial. The production of artificial polyploids gained acceptance and momentum with the discovery that the plant alkaloid colchicine extracted from seeds and corms of autumn crocus *Colchicum autumnale* could double the chromosome number. Since the discovery of this alkaloid by Blakeslee and Avery (1937) large number of crop plants of different species have been subjected to colchicine treatment and its effect in different crop plants is briefly reviewed in this chapter.

2.1 Effect of colchicine in inducing polyploidy

Blakeslee and Avery (1937) have stressed the successful use of colchicine as a polyploidising agent over other chemicals. By treating 0.025-0.30 per cent aqueous colchicine, Gupta and Gupta (1975) isolated few tetraploids and mixoploids in *Crotalaria juncea* and *Crotalaria retusa*. Rajendran *et al.* (1977) treated sprouting corms of *Amorphaphalus campanulatus* with 0.20 per cent colchicine and out of 124 treated plants, one was found to be a tetraploid. Bai and Hrishi (1978) obtained tetraploids in a leguminous root crop *Vigna capensis* by treating the seedlings with 0.50 per cent colchicine solution at cotyledonary leaf

stage for 6 h. Goswami and Sarma (1979) noticed that maximum number of tetraploids could be obtained from variety S_t 449 of *Camellia sinensis* compared to S_t 458 and S_t 450, after immersion of the terminal bud in a one per cent colchicine solution for seven days. Kaul *et al.* (1979) reported that colchicine solution at a concentration of 0.40 per cent was the most effective for inducing tetraploids in *Papaver somniferum*. Ramachandran (1982) reported successful induction of tetraploidy in ginger through treating the sprouts with 0.25 per cent aqueous colchicine. Amma *et al.* (1984) induced polyploids in *Hevea brasiliensis* by treating the tender bud sprouts with 0.75 per cent aqueous colchicine. Kulkarni *et al.* (1984) produced tetraploids in *Catharanthus roseus* by immersing apical buds of seven days old seedlings in 0.50 per cent colchicine solution for 19 h. Auto-octoploidy as well as tetraploidy was induced in a diploid *Capsicum annuum* cultivar, cerasiformis by the application of 0.30 per cent colchicine to seedlings at leaf stage (Panda *et al.*, 1984). Zhang and Zhao (1984) induced tetraploids in radish using 0.10 per cent colchicine solution. Tubers of taro treated with 0.50 per cent aqueous colchicine solution for 48 h produced polyploids (Jos *et al.*, 1986). Induction of autotetraploidy has been achieved by Arya *et al.* (1988) in *Trigonella foenum-graecum* by cotton swab method using 0.15 per cent colchicine. Tetraploids were produced by Pushparajan (1988) in *Sida rhombifolia* using 0.30 per cent colchicine solution in seedlings. Dhawan and Tyagi (1989) developed two auto-octoploids (C-43 and C-65) in *Hyoscyamus muticus* by soaking the seeds in 0.50 per cent aqueous solution of colchicine for 72 h. Polyploidy was induced in *Elettaria cardamomum* seeds soaked in 0.50 per cent colchicine for 90 minutes (Sudharshan, 1989). Tetraploids were obtained in *Raphanus sativus* by treating diploid seedlings with 0.30 per cent colchicine solution for 2 h (Kumar and Chaurasia, 1990). Colchicine solution of

0.25 and 0.30 per cent have been reported as optimum concentrations for induction of polyploidy in *Cajanus cajan* variety ICPL 87 and lower concentration (0.10%) appeared to be ineffective whereas higher concentration (0.40%) proved to be toxic but not lethal (Dusane *et al.*, 1991). The treatment of apical buds of *Ageratum conyzoides* with 0.25 per cent colchicine solution from 9 a.m. to 1 p.m. for 3 consecutive days produced autotetraploids (Gaorkar and Torne, 1991). Tetraploidy was induced by Verma and Raina (1991) in *Phlox drummondii* using 0.15 per cent colchicine for 12 h spread equally over 2 days. Biondani *et al.* (1992) induced tetraploids in sugarbeet by treating 0.10 per cent aqueous colchicine solution for 3 or 4 h. Autotetraploids were produced in ginger by treating sprouting buds on the rhizome with 0.25 per cent colchicine solution (Ramachandran and Nair, 1992).

2.2 Cytogenetics in ginger

The chromosome number of 2n = 22 for *Zingiber officinale* has been confirmed by many cytogeneticists (Moringa *et al.*, 1926; Sugiura, 1936; Chakravarthi, 1948; Federov, 1969 and Sathiabhama, 1988), eventhough Takahashi (1931) reported chromosome number of 2n = 24 for the species. Raghavan and Venkatasubhan (1943) reported the cytology of 3 species of Zingiber viz. *Zingiber officinale, Zingiber cassumunar* and *Zingiber zerumbet*, and all of them had a chromosome number of 2n = 22. Autotetraploids (2n = 44) were produced in ginger by colchicine treatment (Ramachandran, 1982; Ramachandran and Nair, 1992).

2.3 Morphological effects of autopolyploidy

2.3.1 Growth habit

Kostoff (1938) found that colchicine treatment in different crop plants have caused highly suppressed germination. According to Stebbins (1971) the increase of cell size, which generally characterises polyploids, does not necessarily lead to increased size of the plant as a whole or even of its individual organs because of the reduction in the number of cell divisions in these plants. In *Zinnia elegans*, polyploids induced by Gupta and Koak (1976) were stunted in growth and was smaller in size when compared with diploids. Bai *et al.* (1976) while observing tetraploids of *Ipomoea obscura* detected the usual gigantism that is associated with polyploidy. Rajendran *et al.* (1977) reported that autotetraploid in *Amorphophalus campanulatus* was more vigorous compared to diploid. Colchicine treated sunflower plants exhibited distinct dwarf characters (Gupta and Roy, 1980). Ramachandran (1982) after treating the sprouting buds of ginger rhizome with 0.25 per cent aqueous colchicine reported that tetraploids were more vigorous than the diploids. Ratnambal and Nair (1982) spotted one exceptionally vigorous plant from among the tetraploid plants. Colchicine induced autotetraploids of *Solanum americanum* were vigorous in growth (Siddiqui, 1983). Induced autotetraploids of *Trigonella foenum-graecum* had luxuriant growth with thick stems and leaves (Anis and Aminuddin, 1985). Jos *et al.* (1986) reported that polyploids in taro were somewhat slower in growth. Bhal and Tyagi (1988) found that initial growth was slow in autotetraploids of *Coleus forskohlii*. Pushparajan (1988) observed increase in the size of plants and leaves in induced tetraploid of *Sida rhombifolia* ssp. *retusa*. In auto-octoploids of *Hyoscyamus muticus* there was an increase in most of the morphological characters such as thickness of the main shoot, size of leaves and floral parts

(Dhawan and Tyagi, 1989). They reported that the increase in all size and decrease in fertility are the two near universal consequences of induced polyploids leading to larger but fewer plant parts. The induced autotetraploids of *Ageratum conyzoides* were dwarf and had a slower growth rate during the early phase of development (Gaonkar and Torne, 1991). Lavania *et al.* (1991) observed that the tetraploids of *Hyoscyamas niger* were more vigorous than diploids. According to Nair and Ravindran (1992) induced polyploid plant in black pepper was vigorous. Srivastava and Raina (1992) observed that initial growth was slow in autotetraploids of *Clitoria*. Sun *et al.* (1994) induced autotetraploids in *Sorghum versicolor* and observed that they were stockier than the original diploids and their stems were thicker and stouter.

2.3.2 Plant height

Verzea *et al.* (1983) reported that autotetraploids in *Datura innoxia* were taller than diploids and more vigorous with greater height at branching. The induced autotetraploids in *Hyoscyamus muticus* were vigorous with increased plant height (Lavania, 1986). Bhal and Tyagi (1988) reported that colchicine induced autotetraploids of *Coleus forskohlii* exhibited increased plant height. According to Dhawan and Tyagi (1989) auto-octoploids of *Hyoscyamus muticus* were accompanied by reduction in plant height. Tetraploids in barley were shorter than the control and had smaller flag leaves (Zhang *et al.*, 1990). In ginger, leafy shoots of autotetraploid attained greater height than the corresponding diploid (Ramachandran and Nair, 1992).

2.3.3 Number of shoots

Sinha and Sinha (1975) reported profuse branching of colchicine treated plants of *Dacus carota* and *Foeniculum vulgare*. According to Dzhurmanski (1980) induced tetraploids in *Glaucium flavum* produced fewer but thicker stems. Number of primary branches were decreased in autotetraploids of *Coleus forskohlii* (Bhal and Tyagi, 1988). Dhawan and Tyagi (1989) observed a reduction in number of branches in colchicine induced autooctoploids of *Hyoscyamus muticus*. Dusane *et al.* (1991) reported that number of branches were reduced in induced tetraploids of *Cajanus cajan* var. ICPL-87. Gaonkar and Torne (1991) detected more number of branches in autotetraploid *Ageratum conyzoides*.

2.3.4 Number and nature of leaves

Johnson and Sass (1944) reported that increase in thickness of leaves observed in the autotetraploid plants is due to the increase in length of palisade and spongy tissues. According to Tandon and Bali (1959), increase in thickness of leaves of autotetraploid plant was due to increase in cell size. In *Ocimum*, polyploids gave nearly double the quantity of leaves in comparison with that of the diploids (Bose and Choudhury, 1962). In *Crotalaria juncea*, leaves of colchiploids were broader while in *Crotalaria retusa*, leaves were smaller than control (Gupta and Gupta, 1975). In *Ipomoea obscura*, polyploids induced by Bai *et al.* (1976) had thicker, broader, coarser and deep green leaves. Studies on *Catharanthus roseus* showed that autotetraploids had broader leaves and lower leaf length by breadth ratios (Kumar, 1982). Ratnambal and Nair (1982) reported that the leaves of colchicine induced autotetraploids of ginger were thick and dark green. Lavania (1986) detected

increase in leaf size and thickness in induced autotetraploids of *Hyoscyamus muticus*. Martinson (1986) observed thicker and darker leaves in colchicine induced tetraploids of *Theobroma cacao*. In *Coleus forskohlii*, leaf area/plant increased and number of leaves/plant decreased in autotetraploids (Bhal and Tyagi, 1988). Pushparajan (1988) observed dark green and thick leaves in the induced tetraploids of *Sida rhombifolia*. Induced tetraploids in cardamom differed from diploids by having thicker leaves with a thick waxy coating on the abaxial surface (Sudharshan, 1989). Gaonkar and Torne (1991) reported smaller and thicker leaves in autotetraploids of *Ageratum conyzoides*. Tetraploids induced by Letchamo *et al.* (1994) in *Chamomilla recutita* possessed thicker, longer and less feathery leaves than diploid plants. In autotetraploids of *Sorghum versicolor*, Sun *et al.* (1994) detected thicker, larger and deep green leaves. Meenakumari *et al.* (1995) recorded large leaf area in induced tetraploids of *Pueraria phaseoloides*.

2.4 Cell size, stomatal count and size

Nair and Ratnambal (1974) reported fewer stomatal cells per unit area in the induced tetraploid of *Areca catechu*. Singh and Roy (1975) detected increased size of stomatal cells in polyploids of *Trichosanthus anguina*. Bai *et al.* (1976) reported a significant increase in length and breadth of stomata, though the number decreased per unit area in autotetraploids of *Ipomoea obscura*. In *Zinnia elegans*, tetraploids had a tendency to show increase in size of cells and organs (Gupta and Koak, 1976). Kaul *et al.* (1979) found larger guard cells in induced tetraploids of *Papaver somniferum*. Gogitidze (1981) revealed that the characters most useful for distinguishing tetraploids from diploids in *Catharanthus roseus* were stoma number per mm^2, stoma length and breadth and number of chloroplasts in the stomatal guard

cells. Jos *et al.* (1986) reported that there was a decrease in the number of stomata as the levels of ploidy increased in taro. Lavania (1986) found larger cells in induced autotetraploids of *Hyoscyamus muticus*. Sudharshan (1989) reported that in induced tetraploids of cardamom, stomata on the abaxial surfaces were larger than in diploids but number of stomata/cm^2 of lamina was lower. Srivastava and Tripathi (1990) observed an increase in cell size in colchicine induced tetraploids of *Atylosia* species. Dusane *et al.* (1991) reported an increase in the diameter of the guard cells and low frequency of stomata on both the sides of the leaf in induced tetraploids of *Cajanus cajan*. Gaonkar and Torne (1991) observed larger stomata in autotetraploid *Ageratum conyzoides*. In *Phlox drummondii*, the number of stomata per unit area in the tetraploid was 40 per cent less and there was a procounced increase in the size of stomata and cell (Verma and Raina, 1991). Tetraploids induced by Srivastava and Raina (1992) in *Clitoria ternatea* showed an increase in cell size and such increase was reflected in pronounced increase in the size of stomata and pollengrains. Letchmo *et al.* (1994) found that the highest number of stomata in the tetraploid plants was 11.7/mm^2 and the lowest number for the diploids was 31.2/mm^2 in *Chamomilla recutita*. They also found that the lowest number of chloroplasts for tetraploid plant was 23 per pair of guard cells, while the highest number of chloroplasts for diploid plants was 22 per pair of guard cells.

2.5 Flowering

Hooker (1894) described ginger as a species with very rare flowering. Holttum (1950) stated that flowers are seldom seen in Malaysia, but are produced in some other countries, thereby it appeared that, flowering in ginger is observed under certain conditions only. Pillai *et al.* (1978) reported that, of the 35 germplasm

collections maintained at CPCRI, Kasaragod all but, six flowered and the flowering started in the last week of October and lasted till early December the peak being in November. Nybe (1978) reported 0.5 to 12 per cent flowering in various ginger types under Vellanikkara conditions. Valsala (1994) reported that biennial plants flowered as early as Ist July and it extended to last week of October whereas normal annual plants flowered only by middle of September and the season extended to 3rd week of November. The seven cultivars studied registered cent per cent flowering when they were maintained as biennials and in annuals, percentage of flowering was only 25.

Flowering was not observed in any of the tetraploid ginger plant by Ratnambal and Nair (1982). But Ramachandran (1982) reported that induced tetraploids of ginger flowered during the second year of planting.

Tetraploid plants of *Atropa belladonna* showed an increase in the size of flowers, berries and seeds (Glazova and Shugaeva, 1970). Rajasekharan (1970) recorded larger flowers in the autotetraploid *Solanum indicum*. Biswas and Battacharya (1971) reported a delay of 8 to 45 days in flowering in the induced polyploid legume *Cyamopsis*. Flowers and pollens of the polyploid of *Trichosanthes* were enlarged as revealed by the studies of Singh and Roy (1975). In a study by Kaul *et al.* (1979) it was noted that induced tetraploids in *Papaver somniferum* were late flowering. Increase in the size of flower, anther and pollen was reported by Singh (1979) in the tetraploids of Portulaca. The delay in flowering in the colchicine treated population of lemongrass was attributed to the prolonged vegetative growth period resulting in later flowering (Meenattoor, 1983). According to Yonglin *et al.* (1984), in rubber, there was no difference between artificial polyploid plants and

natural polyploids in flowering time or flower morphology and size, but both male and female flowers of polyploids were larger than those of the corresponding diploids. In radish, Zhang and Zhao (1984) reported that tetraploids induced with 0.10 per cent colchicine bolted 5-7 days later than the diploids and had larger flowers and petals. The induced tetraploids of *Atylosia* species showed delayed flowering and maturity which could be due to the slower rate of metabolic activities and cell division in the tetraploids (Srivastava and Tripathi, 1990). They also reported that seed setting was much lower as compared to those of diploids, which may be due to high flower shedding in these tetraploids. Verma and Raina (1991) found that flowering was delayed by about 20 days in colchiploid *Phlox drummondii* and the flowers were retained far 4-5 days more than in the diploids giving a better longivity of flowering.

2.6 Pollen fertility

Singh (1976) found that the percentage of pollen sterility was more in the tetraploids of toria than in other diploids. Tetraploids were highly sterile in *Vigna capensis* (Bai and Hrishi, 1978). Lavania (1988) observed that colchicine induced autotetraploids of an improved strain of *Hyoscyamus muticus* showed high pollen fertility. Srivastava and Tripathi (1990) reported that induced tetraploids of *Atylosia* species showed fairly good pollen fertility. Gaonkar and Torne (1991) reported that the frequency of pollen and seed sterility was increased as a result of disturbance in meiosis in 13-20 per cent of PMC's in *Ageratum conyzoides*. According to Ramachandran and Nair (1992) pollen fertility was 85 per cent in the induced autotetraploid of ginger and it was 13 per cent in the diploid. They reported that the high pollen fertility in the artificially induced tetraploid of ginger may be a

consequence of the high frequency of quadrivalents. On the contrary, Sun *et al.* (1994) reported that the low fertility of autotetraploid plants of *Sorghum bicolor* was correlated with number of laggards but not with number of quadrivalents.

2.7 Yield

Two tetraploid varieties of radish Siberia-1 and Siberia-2 were released and they exceeded their diploid parents in yield by 13-30 per cent (Rud, 1967). Reznikova *et al.* (1972) reported that an induced polyploid of peppermint (Krasnodar-2) produced 24.65 kg ha^{-1} menthol compared to 22.04 kg in the diploid. The number and size of tubers were more in autotetraploids as compared to their diploids in *Vigna capensis* a leguminous root crop of India (Bai and Hrishi, 1978). Ratnambal and Nair (1982) reported increase in the size of ginger rhizome in induced autotetraploids. Tuber development was found much restricted in induced tetraploid of taro (Jos *et al.*, 1986). Srivastva and Lavania (1990) revealed that in *Hyoscyamus albus* induced autotetraploids showed a 19.61 per cent lower biomass yield. Ramachandran and Nair (1992) reported larger rhizomes and higher yield in induced autotetraploids of ginger.

2.8 Secondary metabolites

Results of studies by Yankulov and Dzhurmanski (1979) proved that as a result of polyploidization, the rate of atropine synthesis increased to a greater extent than diploids. Autotetraploids in *Datura innoxia* surpassed diploids by upto 40 per cent in total alkaloid yield (Verzea *et al.*, 1983). Wold *et al.* (1983) recorded an increase in thebaine content of *Papaver bracteatum* after polyploidation with colchicine. Ammal and Prasad (1984) observed that in *Costus speciosus*, diosgenin

content is maximum in the diploid followed by triploid and tetraploid. According to Kulkarni *et al.* (1984), diploids and induced autotetraploids of *Catharanthus roseus* did not significantly differ in alkaloid content. Anis and Aminuddin (1985) reported that diosgenin content in the seeds of the tetraploids was 0.6 per cent compared with 0.68 per cent in the diploid in *Trigonella foenum-graecum*. Experiment conducted by Krishnan *et al.* (1985) revealed that autotetraploids of *Catharanthus roseus* were mostly inferior in total root alkaloid content and ajmalicine content. Krishnan (1988) noted that solasodine content was higher in autotetraploids of *Solanum viarum*. Kulkarni and Ravindran (1988) reported that tetraploid lines of *Catharanthus roseus* yielded about 4 and 5 times more leaf and root total alkaloid respectively than the diploid lines. Artificial autotetraploids of *Hyoscyamus niger* yield upto 22.5 per cent more tropane alkaloids than the parental diploids due to a 4.8 per cent increase in drymatter production and a 16.7 per cent increase in alkaloid content (CIMAP, 1990). Srivastava and Lavania (1990) revealed that in *Hyoscyamus albus*, induced autotetraploids showed 18.18 per cent higher alkaloid content than the diploids. The autotetraploid strain of vetiver named as 'Sugandha' exhibited 60 per cent improvement in oil productivity (Lavania, 1991). Tetraploids induced by Lavania and Srivastava (1991) in *Hyoscyamus niger* yielded 22.5 per cent more tropane alkaloid per plant than diploids. Ramachandran and Nair (1992) observed that oil content of rhizomes in ginger was lower in the tetraploid than in the original diploid cultivar.

2.9 Resistance to pest and disease

Verzea *et al.* (1983) reported that autotetraploids of *Datura innoxia* are less resistant to virus diseases. Kulkarni (1984) observed that in *Catharanthus roseus*

autotetraploids were eight times more resistant to dieback disease than diploids. Kulkarni and Ravindra (1988) revealed that tetraploid lines of *Catharanthus roseus* were more resistant to *Pythium aphanidermatum* (which causes die-back and collar rot and root rot) than diploid lines. Some of the tetraploid coffee plants showed increased resistance to *Hemileia vastatrix* compared to their diploids (Mazzafera *et al.*, 1993). Autotetraploids induced by Sun *et al.* (1994) in *Sorghum versicolor* showed more tolerance to certain diseases and insects.

Materials and Methods

MATERIALS AND METHODS

The investigations reported herein were undertaken in the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 1994 to 95 with a view to induce autotetraploids in ginger.

3.1 Varieties of ginger

Commercially popular varieties of ginger suitable for dry ginger production namely Himachal Pradesh, Maran, Nadia and Rio-de-Janeiro for green ginger production, were selected for the induction studies.

3.2 Preparation of rhizome

Rhizomes were treated with fungicide as per package of practices recommendation of KAU (KAU, 1993) and stored in shade. After one month, the rhizomes were spread out in sand beds and they were maintained in a moist condition for sprouting. Rhizome bits of size 15-20 g were selected and except one prominent sprouting bud, all other buds were excised.

3.3 Concentration of chemical

Colchicine was used for the induction of autotetraploidy. Weighed quantity of chemical was first dissolved in 10 ml glycerin and made upto 100 ml using distilled water. Three concentrations of the chemical ie. 0.10, 0.25 and 0.40 per cent were prepared.

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3.4 Methods of application

Two methods of application were followed.

(i) Injection method - Colchicine solution was injected to the activated bud using syringe. After injection, the buds were covered with cotton.

(ii) Hole method - A hole of 3 mm diameter and depth was made close to the sprouting bud using a needle and colchicine solution was applied on the cotton kept inside the hole.

In both the methods, one ml of solution was applied and constant care was taken to see that cotton did not dry up during the course of treatment. Application of colchicine was done between 7 AM - 9 AM and the period of treatment was four hours. After treatment, the rhizomes were washed in running water for one hour and stored in moist sand overnight. The treatment was repeated for two consecutive days after which the rhizomes were planted in plastic mesh trays filled with sand. After 50 days, the surviving plants under treated and control were transplanted individually in polythene bags and grown under identical conditions, as per package of practices recommendation of Kerala Agricultural University (KAU, 1993).

3.5 Treatment combinations

Twenty four combinations were tried in Completely Randomised Design along with one control for each variety. Four varieties, three concentrations of colchicine and two methods of application were tried. Fifteen rhizome bits were treated with colchicine in each treatment.

Varieties	Concentrations of colchicine	Methods of application
V_1 - Himachal Pradesh	C_1 - 0.10%	M_1 - Injection
V_2 - Maran	C_2 - 0.25%	M_2 - Hole
V_3 - Nadia	C_3 - 0.40%	
V_4 - Rio-de-Janeiro		

Treatment combinations tried were

1. $V_1C_1M_1$	7. $V_2C_1M_1$	13. $V_3C_1M_1$	19. $V_4C_1M_1$
2. $V_1C_1M_2$	8. $V_2C_1M_2$	14. $V_3C_1M_2$	20. $V_4C_1M_2$
3. $V_1C_2M_1$	9. $V_2C_2M_1$	15. $V_3C_2M_1$	21. $V_4C_2M_1$
4. $V_1C_2M_2$	10. $V_2C_2M_2$	16. $V_3C_2M_2$	22. $V_4C_2M_2$
5. $V_1C_3M_1$	11. $V_2C_3M_1$	17. $V_3C_3M_1$	23. $V_4C_3M_1$
6. $V_1C_3M_2$	12. $V_2C_3M_2$	18. $V_3C_3M_2$	24. $V_4C_3M_2$

3.6 Observations

The treated and control plants were critically observed to note the following observations.

3.6.1 Survival

The number of plants surviving in each treatment was recorded on the 20th, 40th and 60th day after sowing and the survival percentage was worked out in relation to the control.

3.6.2 Early deformity

The population was critically observed to detect abnormalities if any, at different stages.

3.6.3 Height of plants

Height of each plant was measured at monthly intervals starting from August to December. Plant height was measured from soil surface to the tip of the longest leaf. The mean height of plants in each treatment was estimated.

3.6.4 Number of tillers

The number of tillers in each plant was recorded at monthly intervals starting from August to December. The mean number of tillers per plant in each treatment was calculated.

3.6.5 Number of leaves

The number of leaves in each tiller was recorded at monthly intervals starting from August to December and the total number of leaves in each plant was calculated.

3.6.6 Leaf area

To estimate the leaf area, 100 leaves were collected randomly covering all the treatments. The length and breadth of each leaf was measured and the area was found out in leaf area meter. Three equations were derived from the data based on the leaf length and breadth measurements.

$A = -24 + 3.312\ L$, $A = -41.47 + 37.302\ B$ and $A = 2.86 + 0.84\ LB$, where A is the area, L is the length and B is the breadth of individual leaf. The best fit for prediction of leaf area was found to be $A = -24 + 3.312\ L$.

To record the leaf area, three mature leaves were collected at monthly intervals from each plant. Length and breadth were measured and leaf area was calculated using the equation A = -24 + 3.312 L.

3.6.7 Stomatal count and size

The method described by Jambhale and Nerker (1980) was used for noting the stomatal count and size. The leaves were collected only after exposure to sunlight for two to four hours, preferably at noon. Lower epidermis of leaves were stripped off and placed on a clean glass slide. A drop of two per cent silver nitrate solution was added on the strip for one minute. The strip was then washed thoroughly with distilled water and fixed in few drops of hyposolution (25 g sodium thiosulphate and 0.10 g potassium metabisulphite dissolved in 200 ml distilled water) for few minutes. After washing in distilled water, it was mounted in glycerin and observed under microscope. Number of stomata/mm^2 of leaf was counted in both the control and treated plants. The length and width of stomata were measured from five cells selected at random and stomatal size was compared.

3.6.8 Epidermal cell size

Similar to the stomatal observations, epidermal cell size in both control and treated plants were compared by counting the number of cells/mm^2 of leaf.

3.6.9 Flowering

The plants were critically observed and the plants flowered were noted.

3.6.10 Pollen fertility

The pollen fertility of both control and tetraploid plant were estimated using acetocarmine stain. For the purpose, flower buds were collected at the time of anthesis. The pollen grains were stained and viewed under microscope. All the pollen grains that were well filled and stained were counted as fertile and others as sterile. The pollen grains were counted from 10 microscopic fields and the mean was taken. The fertility percentage was calculated using the formula.

$$\frac{\text{Number of well stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

3.6.11 Yield

Harvesting was done eight months after planting. Yield of each plant in terms of fresh weight of rhizome was recorded. The plants suspected as polyploids based on increased vigour and larger size of stomata were again planted in the next season during April, in separate polythene bags along with control. The observations on the morphological characters and yield was recorded as for the first year.

3.6.12 Rhizome characters

3.6.12.1 Number of primary and secondary fingers

The number of rhizomes originating from the seed rhizome were counted and recorded as the number of primary fingers per plant. The rhizomes originating from the primary fingers were considered as the secondary fingers..ls1

3.6.12.2 Length, breadth, girth and internodal length of primary and secondary fingers

Five primary fingers and secondary fingers were selected at random from each plant for recording the finger characteristics. Length, breadth, girth and internodal length was measured and the mean was found out.

3.6.13 Incidence of pests and diseases

Scoring for incidence of pest and disease was not done since adequate preventive measures against soft rot and stem borer were adopted to save the treated plants.

3.7 Chromosome counting to confirm polyploidy

Plants suspected as polyploids and the control plants were subjected to cytological study and chromosome number was counted.

1. The rhizomes were germinated in plastic trays filled with sand.
2. The roots were collected when they attain 1-2 cm long at 6 AM - 6.15 AM.
3. The roots were pretreated with 0.03 per cent 8-hydroxy-quinoline for 4 h at 4°C.
4. The roots were washed with distilled water and fixed in 1:3 acetic alcohol (Carnoy's A). A drop of ferric acetate was added to the Carnoy's A fluid while fixing.
5. The meristematic tips were squashed in one per cent acetocarmine.
6. The slides were sealed with nail polish and screened for mitotic chromosomes.

Chromosome counting was done from temporary slides only, since excessive staining of cytoplasm reduced the clarity in permanent mounts. Photomicrographs were taken in Leitz Biomed research microscope.

3.8 Statistical Analysis

The statistical analysis was carried out as a Completely Randomised Design with the treatments arranged in a factorial set up. MSTATC package was used to analyse the data.

Results

RESULTS

Induction of autotetraploidy was tried in four varieties of ginger namely, Maran, Himachal Pradesh, Nadia and Rio-de-Janeiro using colchicine at different concentrations and different methods of application on sprouting buds of rhizome. Cytomorphological changes and variation in yield of rhizome brought about by colchicine were studied and the details are presented below.

4.1 Survival percentage

The survival counts of plants were recorded at 20, 40 and 60 days after planting (Table 1). Survival of plants was lower than that of the control in all the varieties at all the stages of observation.

At all stages of observation, survival percentage decreased with increasing concentration irrespective of the varieties. Application of colchicine at 0.10 per cent concentration by injection method recorded the highest survival value in all the four varieties. Hole method of treatment resulted in reduction of survival counts compared to injection.

With advance in time after treatment, a pronounced reduction in the survival of plants was observed and the maximum reduction was recorded at 60 days after planting. The lowest survival percentage recorded was 26.66 per cent for the varieties Himachal Pradesh and Nadia and 33.33 per cent for Maran and Rio-de-Janeiro at 60 days after planting for the colchicine concentration of 0.40 per cent by the hole method of application.

Table 1. Percentage survival of ginger plants at different stages of crop growth as influenced by colchicine treatment

Treatment combinations	20 DAP		40 DAP		60 DAP	
	No.	%	No.	%	No.	%
$V_1C_1M_1$	14	93.00	9	60.00	9	60.00
$V_1C_1M_2$	12	80.00	10	66.66	8	53.33
$V_1C_2M_1$	10	66.66	10	66.66	7	46.66
$V_1C_2M_2$	11	73.33	8	53.33	5	33.33
$V_1C_3M_1$	10	66.66	6	40.00	5	33.33
$V_1C_3M_2$	9	60.00	7	46.66	4	26.66
Control	15	100.00	15	100.00	15	100.00
$V_2C_1M_1$	15	100.00	14	93.00	10	66.66
$V_2C_1M_2$	14	93.00	10	66.66	9	60.30
$V_2C_2M_1$	13	86.66	12	80.00	8	53.33
$V_2C_2M_2$	13	86.66	11	73.33	8	53.33
$V_2C_3M_1$	13	86.66	10	66.66	7	46.66
$V_2C_3M_2$	12	80.00	9	60.00	5	33.33
Control	15	100.00	15	100.00	15	100.00
$V_3C_1M_1$	15	100.00	14	93.00	10	66.66
$V_3C_1M_2$	13	86.66	13	86.66	9	60.00
$V_3C_2M_1$	13	86.66	12	80.00	9	60.00
$V_3C_2M_2$	12	80.00	9	60.00	8	53.33
$V_3C_3M_1$	11	73.33	8	53.33	5	33.33
$V_3C_3M_2$	10	66.66	8	53.33	4	26.66
Control	15	100.00	15	100.00	15	100.00
$V_4C_1M_1$	14	93.00	12	80.00	11	73.33
$V_4C_1M_2$	15	100.00	14	93.00	10	66.66
$V_4C_2M_1$	13	86.66	12	80.00	9	60.00
$V_4C_2M_2$	12	80.00	10	66.66	8	53.33
$V_4C_3M_1$	10	66.66	10	66.66	7	46.66
$V_4C_3M_2$	10	66.66	10	66.66	5	33.33
Control	15	100.00	15	100.00	15	100.00

DAP - Days after planting

4.2 Plant height

Varietal influence on height of plants was found to be significant in all the months observed except October (Table 2). During the first four months, Maran recorded the maximum plant height followed by Himachal Pradesh. Rio-de-Janeiro produced shorter plants during August, September and October, but during later period, Nadia recorded lowest plant height.

A substantial reduction in plant height with increase in concentration of colchicine was noticed (Table 2). Plant height was maximum with 0.10 per cent concentration and minimum with 0.40 per cent concentration.

The difference in plant height depending on method of application was significant only during the initial stages (Table 2). Although, hole method recorded maximum plant height at all stages of observation, significant difference was observed only during August and September.

The interaction effect between concentrations and methods of application on height of the plant was found to be significant in all the months except October (Table 3). The plants treated with 0.10 per cent and 0.25 per cent colchicine by hole method produced taller plants compared to injection method at the same concentration. With increase in concentration of colchicine to 0.40 per cent, the trend was reversed and injection method recorded maximum plant height.

Though, varieties and concentrations had a significant interaction effect during August and December only, Maran treated with 0.25 per cent colchicine produced taller plants during all the months observed (Appendix Ia).

Table 2. Main effect of varieties, concentrations and methods of application of colchicine on plant height

Treatments		Plant height (cm)				
		August	September	October	November	December
Variety	V_1	26.42	48.04	53.17	67.13	64.67
	V_2	30.88	50.42	54.00	67.33	59.71
	V_3	26.79	46.83	53.38	56.00	52.46
	V_4	20.75	39.27	48.13	59.83	57.54
	CD (0.05)	4.87	7.10	NS	6.99	7.48
Concentration	C_1	28.41	50.28	58.50	67.59	62.13
	C_2	27.63	51.66	56.41	66.38	60.97
	C_3	22.59	36.50	41.59	53.75	52.69
	CD (0.05)	4.21	6.15	6.55	6.05	6.48
Method	M_1	24.15	44.44	51.56	61.33	57.08
	M_2	28.27	47.85	52.77	63.81	60.10
	CD (0.05)	3.43	5.02	NS	NS	NS

NS - Non significant

Table 3. Interaction effect between concentrations and methods of application of colchicine on plant height

Treatment combinations		Plant height (cm)				
		August	September	October	November	December
Concentration x method	C_1M_1	24.88	45.56	56.19	62.88	58.25
	C_1M_2	31.94	55.00	60.81	72.31	66.00
	C_2M_1	23.94	47.75	54.00	63.94	56.19
	C_2M_2	31.31	55.56	58.81	68.81	65.75
	C_3M_1	23.63	40.00	44.50	57.19	56.81
	C_3M_2	21.56	33.00	38.69	50.31	48.56
CD (0.05)		5.67	8.28	NS	8.15	8.73

NS - Non significant

The interaction effect between varieties and methods of application on height of the plant was found to be nonsignificant (Appendix Ib).

The interaction effect between the variety Himachal Pradesh, concentrations and methods of application on height of plants was found to be significant only during August and September (Appendix II). Upto October, treated plants had a slower growth and control plants exceeded them in plant height. During November and December, 0.25 and 0.10 per cent colchicine treatment by hole method recorded maximum plant height, respectively.

In Maran, some of the treatment combinations produced more plant height than control though the difference was significant during December only.

In Nadia and Rio-de-Janeiro, no significant difference was noted between control and different treatment combinations with regard to plant height.

4.3 Number of tillers

Initially upto October, there was no significant varietal difference in the number of tillers produced (Table 4). Highest number of tillers was produced by Rio-de-Janeiro followed by Himachal Pradesh at all stages of growth.

The effect of colchicine concentrations on number of tillers was found to be significant except during initial and final stage of growth (Table 4). 0.10 per cent and 0.25 per cent were on par with respect to number of tillers. Significant reduction in number of tillers was observed at 0.40 per cent concentration during September, October and November.

Table 4. Main effect of varieties, concentrations and methods ofapplication of colchicine on number of tillers

Treatments		Total number of tillers/plant				
		August	September	October	November	December
Variety	V_1	1.00	3.50	6.21	10.67	9.58
	V_2	1.00	2.88	5.46	7.25	5.29
	V_3	1.00	3.21	6.67	8.88	7.13
	V_4	1.00	3.13	7.04	11.71	12.50
	CD (0.05)	NS	NS	NS	2.03	2.09
Concentration	C_1	1.00	3.41	7.19	10.97	9.34
	C_2	1.00	3.59	6.56	9.53	7.66
	C_3	1.00	2.53	5.28	8.38	8.88
	CD (0.05)	NS	0.59	1.18	1.76	NS
Method	M_1	1.00	2.94	5.98	9.17	8.38
	M_2	1.00	3.42	6.71	10.08	8.88
	CD (0.05)	NS	0.48	NS	NS	NS

NS - Non significant

Methods of application differed significantly only during September (Table 4). However, hole method was superior to injection with respect to the number of tillers produced.

Concentrations and methods of application showed a significant interaction effect only during November (Table 5). However 0.10 per cent concentration by hole method recorded maximum number of tillers during October, November and December.

The interaction effect of varieties and concentrations was significant only during December (Appendix IIIa). Rio-de-Janeiro treated with 0.10 per cent colchicine recorded maximum number of tillers (15.50).

Varieties and methods of application had a significant interaction effect on number of tillers during October and November (Appendix IIIb)). During October Himachal Pradesh by hole method recorded maximum number of tillers (8.17) and Himachal Pradesh by injection method recorded the minimum (4.25). During November also Himachal Pradesh by hole method recorded maximum number of tillers (13.50) and Maran by hole method recorded the minimum (7.08).

While comparing with control, interaction effect between concentrations, methods of application and varieties was found to be nonsignificant with respect to number of tillers, irrespective of the stage of growth (Appendix IV).

4.4 Number of leaves

Significant varietal difference was noted with respect to number of leaves during September, November and December (Table 6). During September,

Table 5. Interaction effect between concentrations and methods of application of colchicine on number of tillers

	Treatment combinations	Total number of tillers/plant				
		August	September	October	November	December
Concentration x method	C_1M_1	1.00	3.00	6.38	9.00	8.56
	C_1M_2	1.00	3.81	8.00	12.94	10.13
	C_2M_1	1.00	3.13	5.94	8.81	6.81
	C_2M_2	1.00	4.06	7.19	10.25	8.50
	C_3M_1	1.00	2.69	5.63	9.69	9.75
	C_3M_2	1.00	2.38	4.94	7.06	8.00
	CD (0.05)	NS	NS	NS	2.37	NS

NS - Non significant

Table 6. Main effect of varieties, concentrations and methods of application of colchicine on number of leaves

Treatments		Number of leaves/plant				
		August	September	October	November	December
Variety	V_1	3.88	26.33	60.96	114.08	91.33
	V_2	4.13	21.96	51.79	67.91	49.04
	V_3	4.04	24.29	59.29	81.21	71.21
	V_4	3.79	48.54	57.08	91.50	103.25
	CD (0.05)	NS	8.28	NS	20.63	19.88
Concentration	C_1	3.97	39.75	66.47	103.16	89.69
	C_2	3.88	27.75	59.38	90.41	67.56
	C_3	4.03	23.34	46.00	72.47	78.88
	CD (0.05)	NS	7.16	11.85	17.87	17.22
Method	M_1	3.79	28.65	55.79	84.71	76.71
	M_2	4.13	31.92	58.77	92.65	80.71
	CD (0.05)	NS	NS	NS	NS	NS

NS - Non significant

Rio-de-Janeiro recorded maximum number of leaves (48.54) and Maran recorded the minimum (21.96). Himachal Pradesh was on par with Nadia. A progressive increase in number of leaves was observed in all the varieties upto November. In Rio-de-Janeiro, this trend was extended upto December whereas in other varieties a decrease in leaf number was noted. During October and November, Himachal Pradesh recorded highest number of leaves. Maran recorded the lowest number of leaves during all the months observed except August.

Concentrations of colchicine influenced the number of leaves significantly throughout the growth phase of the crop except during August (Table 6). In all the months except August, 0.10 per cent concentration produced maximum number of leaves. During September, 0.10 per cent concentration was significantly superior and produced maximum number of leaves (39.75) whereas no significant difference was noted between 0.25 per cent and 0.40 per cent. During October and November 0.10 per cent and 0.25 per cent were on par and 0.40 per cent recorded the lowest number of leaves. During December, 0.10 per cent was on par with 0.40 per cent which inturn was on par with 0.25 per cent.

Though not significant, hole method was superior to injection in producing maximum number of leaves during all the months observed (Table 6).

The interaction effect between colchicine concentrations and methods of application on number of leaves was significant during all months except August (Table 7). Through out the growth stages, plants treated with 0.10 per cent colchicine by hole method recorded highest number of leaves. As concentration increased to 0.40 per cent, injection method surpassed hole method in the production of leaves in all the months except August. During October, 0.10 per cent by hole

Table 7. Interaction effect between concentrations and methods of application of colchicine on number of leaves

	Treatment combinations	Number of leaves/plant				
		August	September	October	November	December
Concentration x method	C_1M_1	3.56	31.19	60.69	83.13	78.63
	C_1M_2	4.38	48.31	72.25	123.19	100.75
	C_2M_1	3.81	25.19	52.63	81.19	59.38
	C_2M_2	3.94	30.31	66.13	99.63	75.75
	C_3M_1	4.00	29.56	54.06	89.81	92.13
	C_3M_2	4.06	17.13	37.93	55.13	65.63
	CD (0.05)	NS	10.13	16.75	25.26	24.35

NS - Non significant

method produced maximum number of leaves and this was on par with 0.25 per cent by hole method and 0.10 per cent by injection method. During December, 0.10 per cent by hole method produced maximum number of leaves followed by 0.40 per cent injection which was on par with 0.10 per cent injection.

Varieties and concentrations had a significant interaction effect during initial and final stage of growth (Appendix Va). During August, Himachal Pradesh treated with 0.40 per cent colchicine produced maximum number of leaves (4.88) and was significantly superior to Rio-de-Janeiro treated with 0.40 per cent concentration and Himachal Pradesh with 0.25 per cent. During September, Rio-de-Janeiro treated with 0.10 per cent colchicine produced highest number of leaves (74.88) and Maran treated with 0.40 per cent concentration produced the lowest number of leaves (12.00). During December, Rio-de-Janeiro treated with 0.40 per cent colchicine recorded maximum number of leaves (137.75) followed by Rio-de-Janeiro with 0.10 per cent concentration (119.13).

No significant interaction effect was observed between varieties and methods through out the growth phase of the crop (Appendix Vb).

In Himachal Pradesh, with respect to number of leaves, treatments differed significantly from control during August and September (Appendix VI). During August, control recorded highest number of leaves (7.00) followed by plants treated with 0.40 per cent by hole method (5.5). During September, control recorded maximum number (42.00) followed by 0.25 per cent concentration by hole method (40.50). Afterwards, the effect was nonsignificant.

In treatment combinations involving the variety Maran, the treated plants did not differ significantly from control with regard to leaf production upto November. During December, significantly high leaf production was noticed in all treatment combinations when compared to control. No significant difference was noticed in treatment combinations involving Nadia and Rio-de-Janeiro with regard to leaf production throughout the growth phase.

4.5 Leaf area

Varietal influence on leaf area was significant only during later stage of growth (Table 8). Himachal Pradesh recorded the maximum leaf area except during September. During November, Nadia recorded the minimum leaf area and during December, Rio-de-Janeiro was inferior.

Significant variation in leaf area was noticed with varying concentrations of colchicine during all the months except October (Table 8). During September, plants treated with 0.25 per cent colchicine recorded the highest leaf area (32.41 cm^2) whereas during November and December highest leaf area was recorded for plants treated with 0.10 per cent colchicine. During all the months, 0.40 per cent recorded the lowest leaf area.

Methods of application had no significant influence on leaf area throughout the growth phase of the crop (Table 8).

Interaction effect between colchicine concentrations and methods of application showed significant variation in leaf area only during December (Table 9). Application of colchicine at 0.10 per cent by hole method recorded

Table 8. Main effect of varieties, concentrations and methods of application of colchicine on leaf area

Treatments		Leaf area (cm^2)			
		September	October	November	December
Variety	V_1	29.41	31.38	38.81	42.50
	V_2	29.13	27.89	38.78	38.71
	V_3	30.23	27.48	33.81	35.21
	V_4	20.71	24.98	38.71	31.33
	CD (0.05)	NS	NS	4.19	3.88
Concentration	C_1	29.82	29.90	39.87	39.81
	C_2	32.41	28.96	38.18	36.38
	C_3	19.88	24.93	34.54	34.62
	CD (0.05)	6.65	NS	3.63	3.36
Method	M_1	26.72	26.82	37.89	36.20
	M_2	28.03	29.05	37.17	37.67
	CD (0.05)	NS	NS	NS	NS

NS - Non significant

Table 9. Interaction effect between concentrations and methods of application of colchicine on leaf area

	Treatment combinations	Leaf area (cm^2)			
		September	October	November	December
Concentration x method	C_1M_1	26.30	27.64	38.83	36.42
	C_1M_2	33.34	32.17	40.90	43.19
	C_2M_1	31.68	26.19	38.21	33.48
	C_2M_2	33.13	31.74	38.16	39.29
	C_3M_1	22.16	26.63	36.62	38.70
	C_3M_2	17.61	23.23	32.46	30.54
	CD (0.05)	NS	NS	NS	4.75

NS - Non significant

maximum leaf area (43.19 cm^2) whereas 0.40 per cent by hole recorded the minimum (30.54 cm^2).

Variety and concentration interaction was found to be nonsignificant during all the months (Appendix VIIa).

Varieties and methods of application had a significant interaction effect only during November (Appendix VIIb). Himachal Pradesh by hole method recorded the highest leaf area (42.06 cm^2) and Nadia by hole method recorded the lowest (31.04 cm^2). During other months, though not significant, the variety Himachal Pradesh treated by hole method consistently recorded maximum leaf area.

While comparing with control the treatment combination involving Maran and Rio-de-Janeiro alone showed significant difference in leaf area during December and November respectively (Appendix VIII). Maran treated with 0.25 per cent colchicine by injection method recorded maximum leaf area (43.33 cm^2) and was significantly superior over control. In the case of Rio-de-Janeiro, the best treatment combination for realising maximum leaf area (40.14 cm^2) was 0.10 per cent colchicine by the hole method.

4.6 Stomatal observations

Main effect of varieties and colchicine concentrations indicated that there was no significant variation in stomatal length, width and number of stomata/mm^2 depending on the varieties and concentrations of colchicine tried (Table 10).

Methods of application showed a significant influence on stomatal characters and number of stomata/mm^2 (Table 10). Hole method recorded maximum

Table 10. Main effect of varieties, concentrations and methods of application of colchicine on stomatal characters

	Treatments	Stomatal length (μm)	Stomatal width (μm)	Number of stomata/ mm^2
Variety	V_1	149.94	89.55	41.25
	V_2	149.94	89.55	41.55
	V_3	142.99	89.52	44.40
	V_4	149.94	93.02	41.85
	CD (0.05)	NS	NS	NS
Concentration	C_1	149.94	92.15	40.80
	C_2	147.42	90.58	42.15
	C_3	145.25	88.50	44.10
	CD (0.05)	NS	NS	NS
Method	M_1	144.73	87.10	43.80
	M_2	151.68	93.72	40.95
	CD (0.05)	NS	4.86	2.70

NS - Non significant

stomatal length (151.68 μm) and width (93.72 μm) where as number of stomata/mm^2 was maximum in injection method (43.8) denoting a favourable influence of hole method in bringing a polyploidising effect.

All the interaction effects on stomatal length, width and number of stomata/mm^2 were found to be nonsignificant (Table 11, Appendix IXa and IXb).

No significant variation was noticed between treated plants and control in stomatal characters and number of stomata/mm^2 irrespective of the variety (Appendix X).

4.7 Yield

Varieties, concentrations and methods of application of colchicine influenced the yield significantly (Table 12).

Among the four varieties, Himachal Pradesh was significantly superior with respect to yield of rhizome and recorded 118.96 g plant^{-1} followed by Nadia, Maran and Rio-de-Janeiro. The variation between Nadia, Maran and Rio-de-Janeiro was nonsignificant.

Of the different concentrations tried, the lowest concentration of 0.10 per cent colchicine recorded maximum yield (108.75 g plant^{-1}) though it was on par with 0.25 per cent colchicine (105.69 g plant^{-1}). The highest concentration of 0.40 per cent recorded the lowest yield (57.19 g plant^{-1}).

With respect to methods of application, hole method was more effective (101.35 g plant^{-1}) in enhancing the yield compared to injection (79.73 g plant^{-1}).

Table 11. Interaction effect between concentrations and methods of application of colchicine on stomatal characters

	Treatment combinations	Stomatal length (μm)	Stomatal width (μm)	Number of stomata/ mm^2
Concentration x method	C_1M_1	144.73	86.43	43.20
	C_1M_2	155.15	97.88	38.40
	C_2M_1	145.78	88.48	43.20
	C_2M_2	153.06	92.67	41.25
	C_3M_1	143.69	86.40	45.00
	C_3M_2	146.82	90.59	43.20
	CD (0.05)	NS	NS	NS

NS - Non significant

Table 12. Main effect of varieties, concentrations and methods of application of colchicine on rhizome yield

	Treatments	Green yield of rhizome (g plant^{-1})
Variety	V_1	118.96
	V_2	83.13
	V_3	92.50
	V_4	67.58
	CD (0.05)	27.69
Concentration	C_1	108.75
	C_2	105.69
	C_3	57.19
	CD (0.05)	23.98
Method	M_1	79.73
	M_2	101.35
	CD (0.05)	19.58

Concentrations of colchicine and methods of application exhibited a significant interaction effect on yield (Table 13). Yield recorded for 0.10 per cent hole and 0.25 per cent hole were comparable but as concentration increased to 0.40 per cent, hole method resulted in the lowest yield. Other combinations were on par.

The interaction between varieties and concentrations, though not significant, Himachal Pradesh treated with 0.10 per cent colchicine recorded the highest yield (147.50 g plant^{-1}) and Maran treated with 0.40 per cent colchicine recorded the lowest (27.50 g plant^{-1}) (Appendix XIa).

Variety and method of application interaction had no significant influence on yield (Appendix XIb). However, Himachal Pradesh by hole method of application recorded maximum yield (149.17 g plant^{-1}) and Rio-de-Janeiro by injection recorded the minimum (64.33 g plant^{-1}).

No significant difference was noticed between the yield of treated plants and control irrespective of the variety (Appendix XII).

4.8 Screening of plants as suspected polyploids

Plants with larger size of stomata and reduced number per mm^2 were selected as suspected polyploids. The general features observed in the selected plants are presented in Tables 14, 15 and 16.

Plant No.1

The plant was derived from the variety Himachal Pradesh treated with 0.25 per cent colchicine by injection method. During early stages of growth, the plant was slower in growth, but in later stages it exceeded the control in plant

Table 13. Interaction effect between concentrations and methods of application of colchicine on rhizome yield

	Treatment combinations	Green yield of rhizome (g plant^{-1})
Concentration x method	C_1M_1	78.75
	C_1M_2	138.75
	C_2M_1	82.94
	C_2M_2	128.44
	C_3M_1	77.50
	C_3M_2	36.88
	CD (0.05)	33.91

Table 14. Plant height and number of tillers recorded by thirteen suspected polyploids and control varieties during first year

Plant No.	Treatment combinations	Plant height (cm)					Total number of tillers/plant				
		Aug.	Sept.	Oct.	Nov.	Dec.	Aug.	Sept.	Oct.	Nov.	Dec.
1	$V_1C_2M_1$	10	42	52	84	80	1	3	5	9	19
2	$V_1C_1M_2$	44	39	48	65	60	1	3	4	6	9
3	$V_1C_1M_2$	26	35	48	60	62	1	2	4	6	8
4	$V_1C_2M_2$	44	84	90	90	90	1	5	8	13	12
	Control	52	62	65	66	70	1	5	7	8	6
5	$V_2C_3M_1$	24	28	31	60	56	1	1	9	10	6
6	$V_2C_1M_2$	19	25	23	56	52	1	1	3	4	5
7	$V_2C_2M_2$	39	36	21	60	65	1	3	3	5	5
	Control	39	52	58	65	31	1	4	6	8	2
8	$V_3C_2M_2$	20	59	75	80	74	1	4	10	11	10
9	$V_3C_2M_2$	19	49	60	62	68	1	4	8	9	9
	Control	38	53	59	64	43	1	4	4	7	9
10	$V_4C_1M_2$	20	45	46	73	80	1	2	4	9	8
11	$V_4C_1M_2$	25	51	60	80	77	1	4	8	16	14
12	$V_4C_2M_2$	11	24	35	52	60	1	2	3	7	12
13	$V_4C_3M_2$	19	13	16	25	60	1	2	6	8	15
	Control	29	36	44	56	60	1	4	5	7	12

Table 15. Number of leaves and leaf area recorded by thirteen suspected polyploids and control varieties during first year

Plant No.	Treatment combinations	Number of leaves/plant					Leaf area (cm^2)			
		Aug.	Sept.	Oct.	Nov.	Dec.	Sept.	Oct.	Nov.	Dec.
1	$V_1C_2M_1$	2	15	50	117	108	25.68	22.36	57.40	54.16
2	$V_1C_1M_2$	6	12	28	48	63	19.06	25.68	49.39	44.43
3	$V_1C_1M_2$	4	12	44	66	96	5.81	33.40	43.90	42.24
4	$V_1C_2M_2$	5	40	96	182	144	58.80	30.22	43.13	45.55
	Control	7	42	77	106	77	40.58	35.62	37.70	35.05
5	$V_2C_3M_1$	2	7	63	80	54	9.12	30.65	41.58	38.93
6	$V_2C_1M_2$	3	9	21	40	35	12.30	18.34	35.62	30.65
7	$V_2C_2M_2$	4	9	24	50	50	25.68	32.64	34.49	36.15
	Control	4	23	54	89	16	24.02	31.31	39.76	20.71
8	$V_3C_2M_2$	4	32	90	9	100	28.99	37.27	33.96	41.11
9	$V_3C_2M_2$	3	32	88	108	117	35.62	38.10	38.10	36.71
	Control	4	30	27	60	39	43.89	28.89	34.87	38.43
10	$V_4C_1M_2$	2	38	28	72	88	22.37	33.97	58.14	44.43
11	$V_4C_1M_2$	4	116	80	160	140	28.99	40.15	42.44	33.96
12	$V_4C_2M_2$	2	6	18	42	84	12.30	24.38	40.58	40.02
13	$V_4C_2M_2$	3	24	30	32	125	2.40	7.35	13.52	19.06
	Control	4	27	36	80	126	17.40	18.19	14.62	21.37

Table 16. Stomatal characters and yield recorded by thirteen suspected polyploids and control varieties during first year

Plant No.	Treatment combinations	Stomatal length (μm)	Stomatal width (μm)	No. of stomata/ mm^2	Green yield of rhizome (g plant^{-1})
1	$V_1C_2M_1$	183.26	116.62	20	80
2	$V_1C_1M_2$	183.26	116.62	23	60
3	$V_1C_1M_2$	199.92	133.28	18	60
4	$V_1C_2M_2$	183.26	99.96	26	340
	Control	141.61	83.30	44	75
5	$V_2C_3M_1$	183.26	99.96	30	45
6	$V_2C_1M_2$	183.26	99.96	27	20
7	$V_2C_2M_2$	199.92	133.28	16	60
	Control	141.61	91.63	41	46
8	$V_3C_2M_2$	183.26	116.62	26	220
9	$V_3C_2M_2$	183.62	116.62	30	180
	Control	133.28	91.63	46	40
10	$V_4C_1M_2$	216.58	133.28	18	160
11	$V_4C_1M_2$	149.94	83.30	42	100
12	$V_4C_2M_2$	199.92	116.62	19	110
13	$V_4C_3M_2$	183.26	99.96	30	60
	Control	141.61	83.30	45	37

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height. Similar trend was observed with respect to number of tillers, number of leaves and leaf area. Stomata was found to be larger compared to control with reduced number per unit area. Not much difference was noticed in green yield. The plant recorded a green yield of 80 g plan^{-1} whereas control recorded 75 g plant^{-1}.

Plant No.2

The plant identified was from the variety Himachal Pradesh treated with 0.10 per cent colchicine by hole method. The plant was less vigorous than control as measured by plant height, number of tillers and leaves. But there was marked difference in stomatal characters. The plant recorded a stomatal length of 183.26 μm and width of 116.62 μm whereas it was only 141.61 μm in length and 83.30 μm in width in the case of control. The stomatal frequency was found to be lesser than control. Green yield recorded by the plant was 60 g plant^{-1} and that of control was 75 g plant^{-1}.

Plant No.3

The plant was derived from the variety Himachal Pradesh treated with 0.10 per cent colchicine by hole method of treatment. It was less vigorous than control during all the months. In case of tiller and leaf production, the plant was inferior to control except during December. The plant recorded more leaf area than control during November and December. Stomata was longer and wider and number/mm^2 was less compared to control. Green yield recorded was 60 g plant^{-1} as compared to 75 g plant^{-1} in control.

Plant No.4

The plant comes under the variety Himachal Pradesh treated with 0.25 per cent colchicine by hole method. The plant was taller than control except during August. In case of number of leaves, number of tillers and leaf area, it exceeded the control during greater part of the growth phase. Stomata was larger than control and recorded a length of 183.26 μm and width of 99.96 μm. The plant recorded the highest yield of 340 g plant^{-1} as compared to 75 g plant^{-1} in control.

Plant No.5

The plant identified was the variety Maran treated with 0.40 per cent colchicine by injection method. It was shorter than control during all the months except December and it was superior in tiller and leaf production during later stage of growth. During November and December, the plant recorded more leaf area than control. Stomatal length was 183.26 μm and width was 99.96 μm as compared to 141.61 μm and 91.63 μm in control. No difference was noticed in the case of yield.

Plant No.6

The plant was derived from the variety Maran treated with 0.10 per cent colchicine by hole method. The plant was shorter than control during all the months except December. With respect to number of tillers, number of leaves and leaf area, the plant was inferior to control except during December. Stomatal length was 183.26 μm, width 99.96 μm and stomatal frequency was less compared to control. It produced the lowest yield among all the treatments.

Plant No.7

The plant comes under the variety Maran treated with 0.25 per cent colchicine by hole method. With respect to plant height, number of tillers and number of leaves, the plant was inferior to control except during December. But leaf area was higher in all the months except during November. Stomatal length was 199.92 μm and width was 133.28 μm. The plant recorded a yield of 60 g plant^{-1} and control recorded only 46 g plant^{-1}.

Plant No.8

The plant was derived from the variety Nadia treated with 0.25 per cent colchicine by hole method. The plant was taller than control except during August. With respect to tiller and leaf production, it was superior. During October and December, leaf area recorded by the plant was higher than control. It recorded a stomatal length of 183.26 μm and width of 116.62 μm. The plant recorded a better yield of 220 g plant^{-1} and control recorded only 40 g plant^{-1}.

Plant No.9

The plant was derived from the variety Nadia treated with 0.25 per cent colchicine by hole method. The plant was shorter than control during early stage of growth and it exceeded the control in plant height during later stage. Number of tillers and leaves recorded by the plant was higher than that of control. Leaf area was higher during October and November. Stomatal length was found to be 183.20 μm and width 116.62 μm whereas control recorded a length of 133.28 μm and width of 91.63 μm. Yield was much higher than that of control (40 g plant^{-1}).

Plant No.10

The plant was derived from the variety Rio-de-Janeiro treated with 0.10 per cent colchicine by hole method. The plant showed a rapid increase in height where as control showed a gradual increase. The plant produced lesser number of tillers and leaves recorded more leaf area compared with the control. Stomatal frequency was much less compared to control and the size was large.

Plant No.11

The plant was identified in the variety Rio-de-Janeiro treated with 0.10 per cent colchicine by hole method. Except during August, the plant was taller than control. In the case of number of tillers, number of leaves and leaf area, the plant was superior to control. Stomatal length was 149.94 μm and width was 83.30 μm. Yield recorded by the plant was much higher (100.00 g plant^{-1}) than control (37.50 g plant^{-1}).

Plant No.12

The plant was identified in the variety Rio-de-Janeiro treated with 0.25 per cent colchicine by hole method. It was shorter than control during the entire growth stage. With regard to number of tillers and number of leaves, the plant was inferior. But it exceeded the control in leaf area during all the months observed. It recorded a stomatal length of 199.92 μm and width of 116.62 μm. Yield was found to be higher (110.00 g plant^{-1}) than control (37.50 g plant^{-1}).

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Plant No.13

The plant was identified in the variety Rio-de-Janeiro treated with 0.40 per cent colchicine by hole method. The plant was extremely shorter and it recorded more number of tillers than control during later stage of growth. In case of leaf production and leaf area, the plant was inferior. It recorded a stomatal length of 183.26 μm and width of 99.96 μm where as control recorded a length of 141.61 μm and width of 83.30 μm. Raw yield recorded by the plant was 60.00 g plant^{-1} as compared to 37.50 g plant^{-1} in control.

4.9 Cytological confirmation of suspected polyploids

The selected 13 plants and the respective control varieties were subjected to cytological study to confirm the chromosome number. Two of them (Plant No.1 and 10) were found to be autotetraploids as confirmed by the chromosome number (2n = 44) as against 2n = 22 in control. The rest of the plants showed no cytological difference.

4.9.1 Autotetraploid from Himachal Pradesh

The plant was derived from the variety Himachal Pradesh treated with 0.25 per cent colchicine by injection method. It recorded a height of 10, 42, 52, 84 and 80 cm whereas control recorded a height of 52, 62, 65, 66 and 70 cm during August, September, October, November and December, respectively. During early stages of growth, the plant was slower in growth but in later stages it exceeded the control in plant height. Similar trend was observed with respect to number of tillers, number of leaves and leaf area. The number of tillers recorded for the plant was 1, 3, 5, 9 and 9 against 1, 5, 7, 8 and 6 in control from August to December. This recorded 2, 15, 50, 117 and 108 leaves against 7, 42, 77, 106 and 77 in control

during August to December. The leaf area recorded by the plant was 25.68, 22.36, 57.40 and 54.16 cm^2 during September, October, November and December, respectively and control recorded an area of 40.58, 35.62, 37.70 and 35.05 cm^2, respectively from September to December.

The stomatal frequency was found to be less (20/mm^2) compared to control (44/mm^2). But the size was large with a mean length of 183.26 μm and width of 116.62 μm. Stomatal length recorded by control was 141.61 μm and width was 83.30 μm.

An increase in epidermal cell size was noticed in the plant. The number of cells per mm^2 was found to be 30 in the case of tetraploid as against 50 in control.

The plant recorded an yield of 80 g $plant^{-1}$ where as control recorded 75 g $plant^{-1}$ during the first year.

4.9.2 Autotetraploid from Rio-de-Janeiro

The plant was derived from the variety Rio-de-Janeiro treated with 0.10 per cent colchicine by hole method. The height recorded by the plant was 20, 45, 46, 73 and 80 cm during August, September, October, November and December, respectively and control recorded 29, 36, 44, 56 and 60 cm from August to December. The plant showed a rapid increase in height whereas control showed a gradual increase. Number of tillers produced by the plant was 1, 2, 4, 9 and 8, respectively from August to December against 1, 4, 5, 7 and 12 in control. The number of leaves recorded by the plant was 2, 38, 28, 72 and 88, respectively from August to December and in control it was estimated as 4, 27, 36, 80 and 126.

Compared with the control, this plant produced lesser number of tillers and leaves. But leaf area recorded by the plant was much greater than that of control. Leaf area recorded by the plant was 22.37, 33.96, 58.14 and 44.43 cm^2 and that of control was 17.40, 18.19, 14.62 and 21.37 cm^2 during September, October, November and December, respectively.

Stomatal frequency in this plant was much less (18/mm^2) when compared to control (45/mm^2). But the size was large with a mean length of 216.58 μm and width of 133.28 μm. The stomata of control was much smaller with a mean length of 141.61 μm and width of 83.30 μm.

Epidermal cell size was found to be increased. The number of cells per mm^2 was 28 in the plant and it was 50 in case of control.

The plant recorded a yield of 160 g $plant^{-1}$ whereas control recorded only 37 g $plant^{-1}$.

During second year, the plant flowered 6 months after planting. High pollen fertility (64%) was recorded for the autotetraploid than the control (6%). No difference was noticed in pollen size and it was found to be 105 μm, in both autotetraploid and diploid.

4.10 Performance of autotetraploids and selected plants during second year

Morphology and yield

The data on the biometric characters of autotetraploids and selected plants at 4 MAP and 6 MAP and yield of rhizomes during second year are tabulated in Table 17.

Table 17. Biometric characters and yield of rhizomes recorded by autotetraploids and selected plants during second year

Plant No.	Treatment combinations	4 MAP				6 MAP				
		Height (cm)	No. of tillers/ plant	No. of leaves/ plant	Leaf area (cm^2)	Height (cm)	No. of tillers/ plant	No. of leaves/ plant	Leaf area (cm^2)	Green yield of rhizome ($g\ plant^{-1}$)
1	$V_1C_2M_1$ (Autotetraploid)	68	5	49	65.42	96	10	80	58.80	420
2	$V_3C_1M_2$	89	7	50	64.19	84	9	92	37.80	180
3	$V_1C_1M_2$	22	3	8	9.12	20	2	5	38.93	-
4	$V_1C_2M_2$	90	13	130	60.46	95	18	205	62.11	520
	Control	69.5	16.5	137	55.65	66.5	23.5	210	49.41	320
5	$V_2C_3M_1$	26	9	36	15.08	44	10	59	19.06	80
6	$V_2C_1M_2$	58	5	43	35.62	67.0	9	97	32.30	160
7	$V_2C_2M_2$	66	32	296	37.93	86	38	307	25.68	600
	Control	52.5	9	76.5	37.27	49	9.5	79.5	28.43	140
8	$V_3C_2M_2$	108	12	88	67.94	111	17	98	56.25	480
9	$V_3C_2M_2$	90	21	146	56.25	100	26	304	62.11	180
	Control	49	6	31	53.83	51	9	71	47.21	160
10	$V_4C_1M_2$ (Autotetraploid)	39	7	51	54.36	87	11	113	48.86	340
11	$V_4C_1M_2$	90	19	109	52.06	100	24	200	48.86	480
12	$V_4C_2M_2$	50	22	180	42.24	70	16	162	37.34	-
13	$V_4C_3M_2$	30	3	17	15.74	52	4	39	25.68	80
	Control	58	12.5	81	38.1	68.5	15.5	123	39.46	120

MAP - Months after planting

For the tetraploids, the trend in morphlogical characters, slower initial growth which surpassed the diploid control during later stages, reduced number of tillers and leaves with increased leaf area was consistent during the second year. Rhizome yield of 420 g plant^{-1} recorded for tetraploid derived from Himachal Pradesh and 340 g plant^{-1} for tetraploid from Rio-de-Janeiro was consistently high during second year also, when compared to control.

Plant No.4 derived from the variety Himachal Pradesh treated with 0.25 per cent colchicine by hole method was more vigorous in growth habit than the autotetraploid and recorded highest rhizome yield (520 g plant^{-1}) when compared to control.

The highest rhizome yield (600 g plant^{-1} among the selected 13 plants was recorded for the plant No.7 derived from the variety Maran treated with 0.25 per cent colchicine by hole method. The plant was very vigorous in growth habit as indicated by the increased plant height, number of tillers and number of leaves compared to control.

In the variety Nadia, the plant No.8 treated with 0.25 per cent colchicine by hole method was very vigorous as indicated by the biometric characters and recorded highest yield (480 g plant^{-1}) compared to control.

Among the derivatives of the variety Rio-de-Janeiro, plant No.11 treated with 0.10 per cent colchicine by hole method was very vigorous with regard to all the biometric characters and recorded the highest yield (480 g plant^{-1}) compared to control and even exceeded the autotetraploid.

The less vigorous and dwarf plants (Plant No.3, 5 and 13) recorded the lowest yield among the selected plants and it was inferior to control.

4.11 Rhizome characters of autotetraploids and selected plants

The rhizome characters of autotetraploids and selected plants studied during the second year are tabulated in Tables 18 and 19. Among the treated plants of the variety Himachal Pradesh, the plant No.4 treated with 0.25 per cent colchicine by hole method recorded highest number of primary fingers and secondary fingers but reduction in size of rhizome compared to control. The auto-tetraploid derived from Himachal Pradesh, though showed an increase in number of primary and secondary fingers, a reduction in the size was observed.

In Maran, the plant No.7 treated with 0.25 per cent colchicine by hole method which recorded the highest yield showed highest number of primary and secondary fingers. Although a reduction in the size was observed, the rhizomes showed wider internodes.

In Nadia, the plant No.8 treated with 0.25 per cent colchicine by hole method showed an increase in the number and size of rhizomes. Plant No.9 derived from Nadia treated with 0.25 per cent colchicine by hole method of application alone showed an increase in the size of rhizome but the number of secondary fingers was considerably reduced.

Plant No.11 derived from Rio-de-Janeiro recorded highest number of primary and secondary fingers. It also showed an increase in the size of fingers. The autotetraploid derived from Rio-de-Janeiro also showed an increase in the number of primary and secondary fingers compared to untreated control but the size was not increased.

Table 18. Number of primary and secondary fingers of rhizomes in autotetraploids and selected plants

Plant No.	Treatment combinations	No. of primary fingers	No. of secondary fingers
1	$V_1C_2M_1$ (Autotetraploid)	22	29
2	$V_1C_1M_2$	7	10
3	$V_1C_1M_2$	--	--
4	$V_1C_2M_2$	34	33
	Control	17	28
5	$V_2C_3M_1$	7	5
6	$V_2C_1M_2$	13	18
7	$V_2C_2M_2$	42	85
	Control	10	13
8	$V_3C_2M_2$	24	31
9	$V_3C_2M_2$	6	12
	Control	6	22
10	$V_4C_1M_2$ (Autotetraploid)	18	29
11	$V_4C_1M_2$	21	28
12	$V_4C_2M_2$	--	--
13	$V_4C_3M_2$	3	5
	Control	10	28

Table 19. Length, breadth, girth and internodal length of rhizomes in autotetraploids and selected plants

Plant No.	Treatment combinations	Length of rhizome (cm)		Breadth of rhizome (cm)		Girth of rhizome (cm)		Internodal length (cm)	
		Primary finger	Secondary finger	Primary finger	Secondary finger	Primary finger	Secondary finger	Primary finger	Secondary finger
1	$V_1C_2M_1$ (Autotetraploid)	2.67	2.83	2.50	1.75	7.33	6.17	0.45	0.33
2	$V_1C_1M_2$	3.25	3.38	2.13	1.75	7.67	5.75	0.58	0.78
3	$V_1C_1M_2$	-	-	-	-	-	-	-	-
4	$V_1C_2M_2$	3.17	2.50	2.33	1.50	7.17	6.00	0.57	0.57
	Control	3.37	3.25	2.75	1.83	7.88	6.50	0.58	0.63
5	$V_2C_3M_1$	2.83	2.17	1.50	1.17	5.67	4.50	0.57	0.37
6	$V_2C_1M_2$	3.00	2.33	1.83	1.50	5.50	4.83	0.83	0.50
7	$V_2C_2M_2$	3.00	2.83	1.67	1.17	5.17	3.50	0.80	0.63
	Control	3.33	3.00	1.75	1.33	6.67	5.00	0.53	0.62
8	$V_3C_2M_2$	3.17	2.17	2.50	2.00	7.50	5.83	0.60	0.67
9	$V_3C_2M_2$	3.50	2.33	2.00	1.83	6.33	6.83	0.63	0.50
	Control	2.50	2.17	2.00	1.50	7.25	5.00	0.60	0.70
10	$V_4C_1M_2$ (Autotetraploid)	2.67	2.33	1.33	1.17	6.33	5.70	0.40	0.40
11	$V_4C_1M_2$	2.67	3.50	2.17	1.75	6.50	6.00	0.73	0.80
12	$V_4C_2M_2$	-	-	-	-	-	-	-	-
13	$V_4C_3M_2$	2.33	2.33	1.83	1.50	5.83	5.33	0.57	0.50
	Control	3.50	3.33	2.00	1.83	6.00	6.00	0.73	0.80

Discussion

DISCUSSION

In ginger, variability is restricted due to clonal propagation and lack of seedset under natural conditions. Induced polyploidy is used by plant breeders as a means of creating variability. The present study is an attempt to induce autotetraploidy and create variability, using colchicine, in four commercial varieties of ginger and to study it's consequent changes in morphology, yield and quality.

5.1 Survival percentage

The survival of plants in comparison to the control was lower in the treated plants. Similar results were reported in Nicotiana (Smith, 1939), in cotton (Stephens, 1940) and in *Ageratum conyzoides* (Gaonkar and Torne, 1991). Survival percentage on 60th day in most of the treatment combinations were notably low from the initial indicating that the survival data upto 60th day need to be watched to get a reliable index of lethality, consequent to colchicine treatment in ginger. Similar mortality of seedlings at different stages of growth was reported in Zinnia by Bose and Panigrahi (1969) and Srivastava (1965). The survival percentage in all the treatments was found to decrease as the concentration increased. Similar results were obtained by Sen and Chheda (1958) in blackgram. Hole method of application resulted in low survival compared to injection perhaps due to the prolonged retention and better penetration of colchicine solution resulting in more lethality to cells. Reduction in survival of plants could be considered as an indication of the toxic effect of colchicine. The toxic effect was more pronounced with increasing concentration of colchicine which brought out a drastic reduction in the survival of plants.

5.2 Morphological characters

5.2.1 Height of plant

Height of treated plant was not significantly influenced by colchicine treatment. During initial stages of growth, the height of colchicine treated plants was much lower than that of control. But during later stage, most of the treated plants exceeded the control in plant height. Initial slow rate of growth may be due to the reduced rate of cell division, lower amount of growth hormone or lower rate of metabolic activities. Stunted growth was reported in colchicine treated seedlings of *Atylosia sp.* (Srivastava and Tripathi, 1990) and in Okra (Rajasekharan and Ganesan, 1968).

With increase in concentration of colchicine, a reduction was noticed in the plant height. Out of the whole population of treated plants, one plant of Rio-de-Janeiro treated with 0.40 per cent colchicine by hole method was found to be extremely shorter with reduced leaf size. Dusane *et al.* (1991) observed that plants treated with 0.40 per cent colchicine in *Cajanus cajan* were dwarf, bushy and with smaller leaves.

Hole method showed a favourable influence in increasing the plant height at all stages.

5.2.2 Number of tillers

In the case of number of tillers, there was no significant difference between control and treated plants. With increase in concentration, a reduction was noticed in the number of tillers produced.

Highest number of tillers was produced by Rio-de-Janeiro followed by Himachal Pradesh at all stages of growth. Hole method was superior to injection in enhancing the tiller production.

5.2.3 Number of leaves and leaf area

With respect to number of leaves and leaf area, plants treated with lower concentration of colchicine recorded maximum values. No consistent varietal difference was noticed with respect to number of leaves where as Himachal Pradesh recorded maximum leaf area throughout the growth stages except September. Hole method was superior to injection in producing more number of leaves and leaf area though the difference was not significant.

5.3 Stomatal observations

There was no significant variation in stomatal length, width and number of stomata/mm^2 depending on the varieties and concentrations of colchicine tried. With respect to methods of application, hole method recorded maximum stomatal length and width and fewer stomata indicating its favourable influence in bringing polyploidising effect.

There was no significant variation in stomatal length, width and mean number of stomata/mm^2 between control and treated plants. But there were individual plants with reduced number and increased size of stomata.

5.4 Yield

With respect to yield, Himachal Pradesh recorded the highest yield

among the four varieties. Increase in concentration of colchicine brought out a reduction in yield. Hole method of treatment was superior to injection in enhancing the yield.

5.5 Screening of plants as suspected polyploids

Plants with reduced number and increase in size of stomata were selected as suspected polyploids, since reduction in the number and increased size of stomata is pointed out as an indication of polyploid nature of plants by several workers (Dhawan and Tyagi, 1989; Verma and Raina, 1991 and Letchamo *et al.*, 1994).

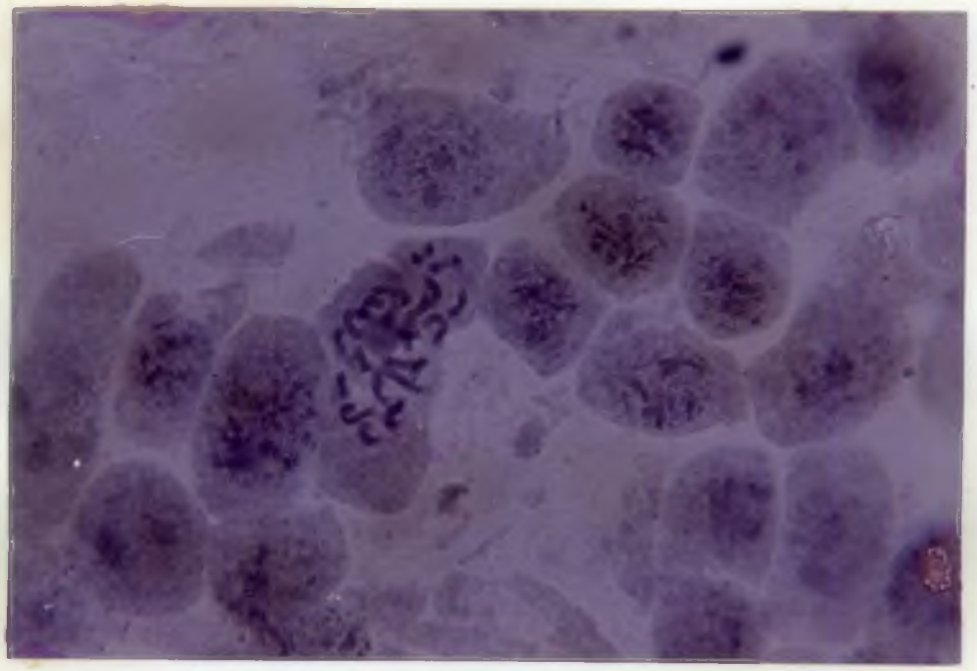
5.6 Cytological confirmation of suspected polyploids

The thirteen suspected polyploids and the control plants were subjected to cytological study. Control plants showed a chromosome number of 2n = 22 indicating their diploid nature (Plates 1 a & 1 b). The chromosome number of 2n = 22 for *Zingiber officinale* was confirmed by many cytogeneticists (Moringa *et al.*, 1926; Sugiura, 1936; Chakravarthi, 1948; Federov, 1969 and Sathiabhama, 1988).

Out of the thirteen suspected polyploids, two plants (Plant No.1 and 10) showed 2n = 44 and were confirmed as autotetraploids (Plates 2 a & 2 b) while no cytological difference was observed in other plants. This indicates that reduction in the number and increase in size of stomata alone could not be taken as a criterion for identifying polyploids and chromosome counting is very essential to confirm polyploidy. Induced autotetraploids with 2n = 44 were already reported in ginger (Ramachandran, 1982; Ratnambal and Nair, 1982; Ramachandran and Nair, 1992). The tetraploids obtained in this study were derived from the variety Himachal

Plate 1a. Mitotic chromosomes in diploid ginger variety Himachal Pradesh (2n = 22) (x 3500)

Plate 1 b. Mitotic chromosomes in diploid ginger variety Rio-de-Janeiro (2n = 22) (x 3600)



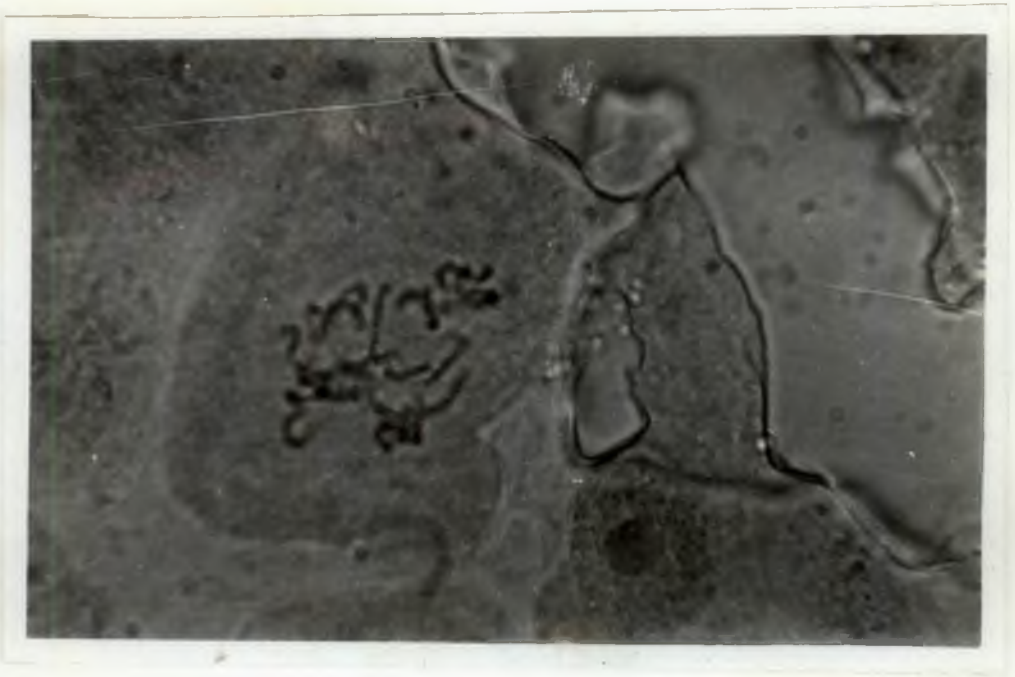
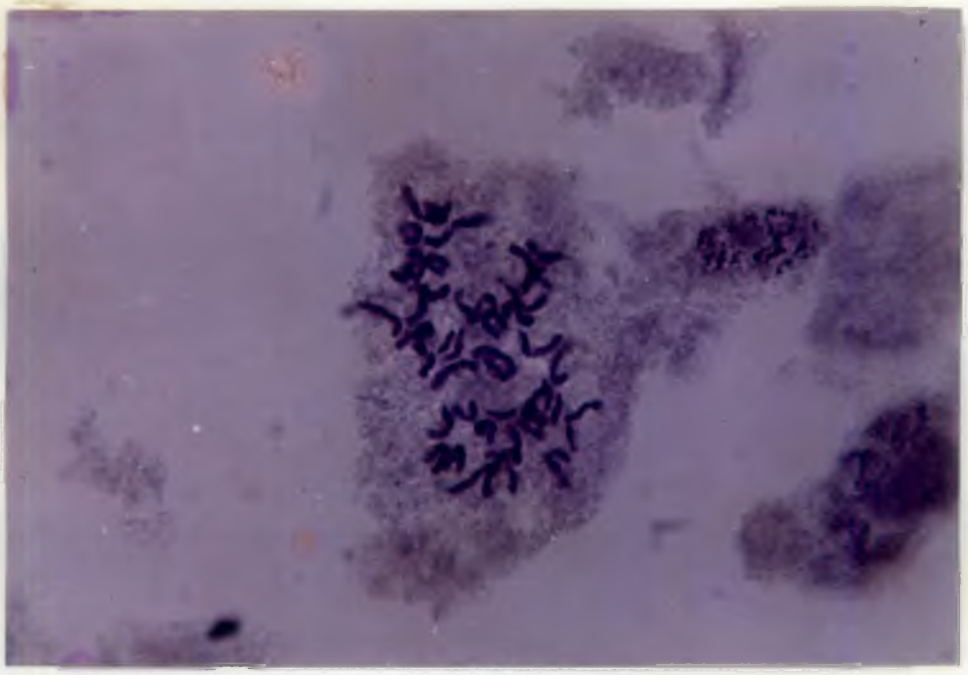
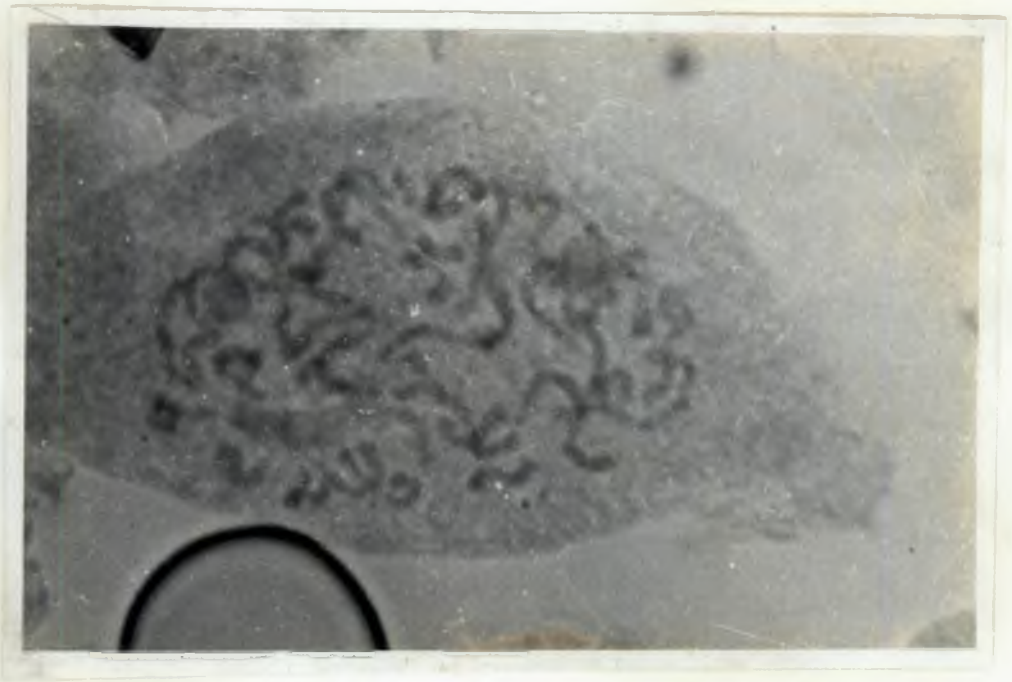


Plate 2 a. Mitotic chromosomes in autotetraploid ginger derived from the variety Himachal Pradesh (2n = 44) (x 3500)

Plate 2b. Mitotic chromosomes in autotetraploid ginger derived from the variety Rio-de-Janeiro (2n = 44) (x 3600)





Pradesh treated with 0.25 per cent colchicine by injection method and variety Rio-de-Janeiro treated with 0.10 per cent colchicine by hole method. Differential varietal response to varying concentrations of colchicine and method of treatment in ginger was reported. Ramachandran and Nair (1992) induced tetraploids in the variety Maran using 0.25 per cent colchicine by hole method of treatment. Ratnambal and Nair (1982) produced tetraploids in Rio-de-Janeiro through colchicine treatment by wetting the growing point by means of cotton.

5.6.1 Morphology of autotetraploids

The two autotetraploids and the eleven selected plants were grown for two seasons and the plants were critically observed. Variations in morphological and stomatal characters and yield potential were noted.

5.6.1.1 Plant height

The two autotetraploids showed slow initial growth which surpassed the diploid control during later stages (Plates 3 & 4). Initial slow growth was recorded in the autotetraploids of *Glycine* by Amarsingh (1968), in sorghum tetraploids by Magoon and Tayyab (1968) and in *Coleus forskohlii* by Bhal and Tyagi (1988). Initial slow rate of growth may be due to the reduced rate of cell division, lower amount of growth hormone or lower rate of metabolic activities.

Ratnambal and Nair (1982) noticed stunted growth in tetraploid ginger during early stages. At full growth the tetraploid plants were taller than diploids as observed by Ramachandran and Nair (1992) in ginger.

Plate 3. Diploid and autotetraploid plants of ginger variety Himachal Pradesh

Plate 4. Diploid and autotetraploid plants of ginger variety Rio-de-Janeiro


HIMACHAL PRADESH
0.25% COLCHICINE
INJECTION
HIMACHAL PRADESH
CONTROL

5.6.1.2 Number of tillers

The isolated autotetraploids produced fewer but thicker tillers. Similar results were obtained in *Glaucium flavum* by Dzhurmanski (1980). Number of branches decreased in induced autotetraploids of *Coleus forskohlii* (Bhal and Tyagi, 1988) and *Cajanus cajan* (Dusane *et al.*, 1991).

Ramachandran and Nair (1992) reported thicker leafy shoots in autotetraploids of ginger. The increase in cell size of tetraploids may be a reason for the thicker but fewer number of tillers (Dhawan and Tyagi, 1989).

5.6.1.3 Number of leaves and leaf area

During major part of the growth stage, tetraploids recorded lesser number of leaves compared to control. But leaf area was found to be much greater than that of control.

Increased leaf area and reduced number of leaves were reported in induced autotetraploids of *Coleus forskohlii* (Bhal and Tyagi, 1988), increased leaf area in induced autotetraploids of ginger (Ramachandran and Nair, 1992) and induced tetraploids of *Pueraria phaseoloides* (Meenakumari, 1995). This increase in size of the leaf can be attributed to increase in cell size in the tetraploids (Gadonkar and Torne, 1991).

5.6.2 Flowering and pollen fertility

The autotetraploid derived from the variety Rio-de-Janeiro flowered during August in the second year of planting. This is in agreement with the report of Ramachandran and Nair (1992) who observed flowering in autotetraploid ginger.

Increased pollen fertility (64%) was observed in the autotetraploid as against 6 per cent in the corresponding diploid (Plates 5 & 6). Increased pollen fertility is taken a criterion for identifying polyploids (Singh, 1992). Colchicine induced autotetraploids showed high pollen fertility in *Hyoscyamus muticus* (Lavania, 1988) and *Atylosia* sp. (Srivastava and Tripathi, 1990). Similar observations were made by Ramachandran and Nair (1992) in ginger who reported 85 per cent pollen fertility in the induced autotetraploid as against 13 per cent in the diploid. The increased pollen fertility in the tetraploid may be a consequence of the high frequency of quadrivalent formation (Ramachandran and Nair, 1992).

5.6.3 Stomatal characters

The autotetraploids showed larger stomata and reduced number per unit area (Plates 7a & 7b and 8a & 8b). Pronounced increase in the size of stomata was observed in autotetraploids of *Ipomoea obscura* (Bai *et al.*, 1976), *Coleus forskohlii* (Bhal and Tyagi, 1988), *Ageratum conyzoides* (Gaonkar and Torne, 1991) and *Phlox drummondii* (Verma and Raina, 1991). Low frequency of stomata was observed in induced autotetraploids of *Areca catechu* (Nair and Ratnambal, 1974), *Ipomoea obscura* (Bai *et al.*, 1976) and *Cajanus cajan* (Dusane *et al.*, 1991).

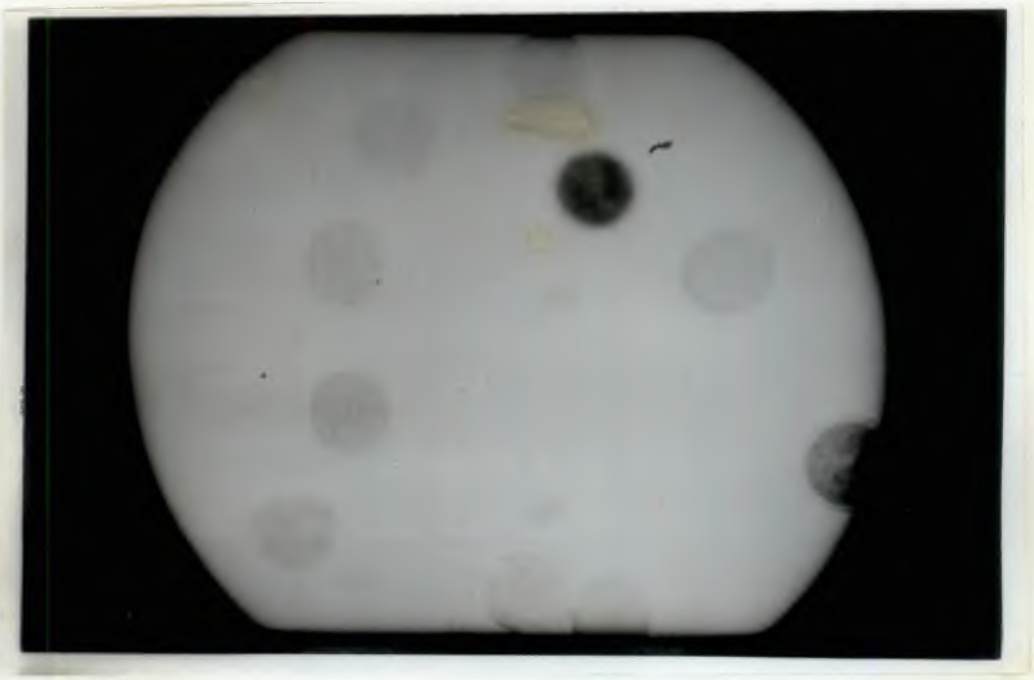
The doubling of chromosome number is associated with increase in cell size and such increase may be reflected in pronounced increase in the size of stomata (Srivastava and Raina, 1992).

5.6.4 Epidermal cell size

Epidermal cell size was found to be increased by 56-60 per cent in

Plate 5. Pollen grains of diploid ginger variety Rio-de-Janeiro (x 375)

Plate 6. Pollen grains of autotetraploid ginger derived from the variety Rio-de-Janeiro (x 375)



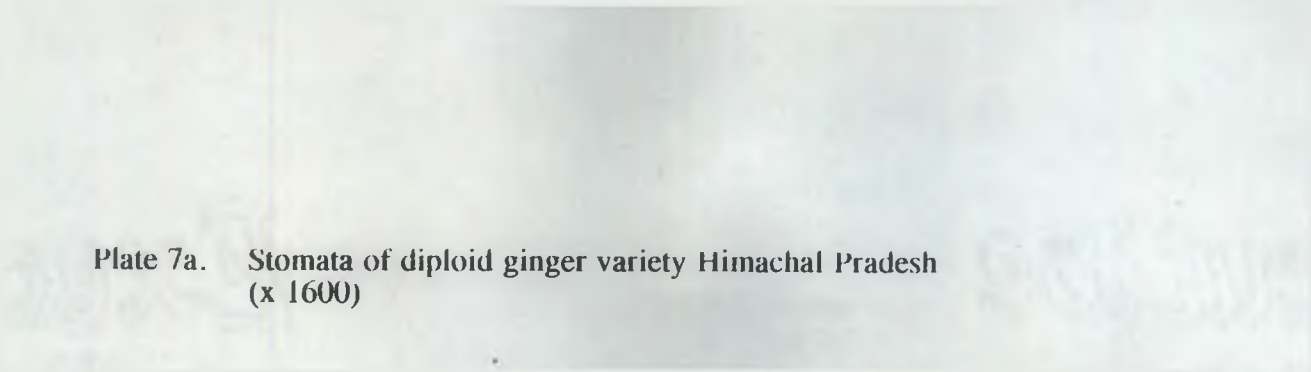


Plate 7a. Stomata of diploid ginger variety Himachal Pradesh (x 1600)

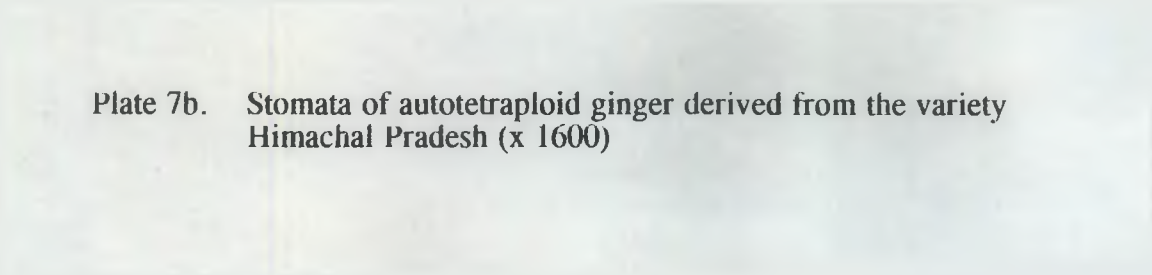
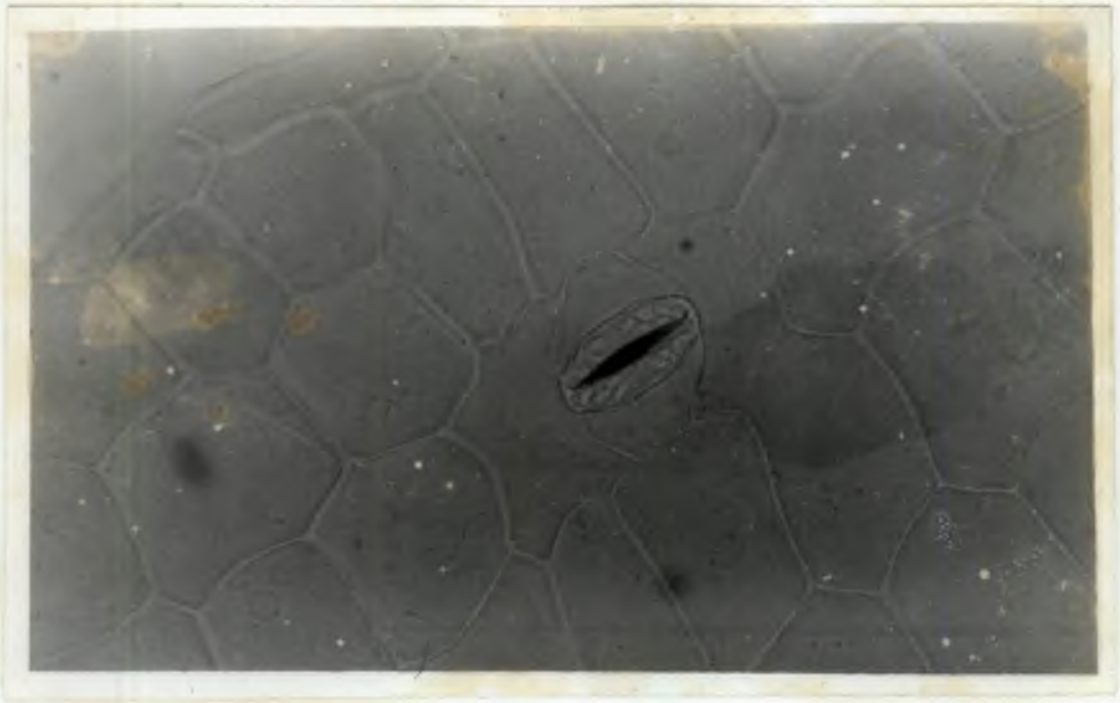


Plate 7b. Stomata of autotetraploid ginger derived from the variety Himachal Pradesh (x 1600)



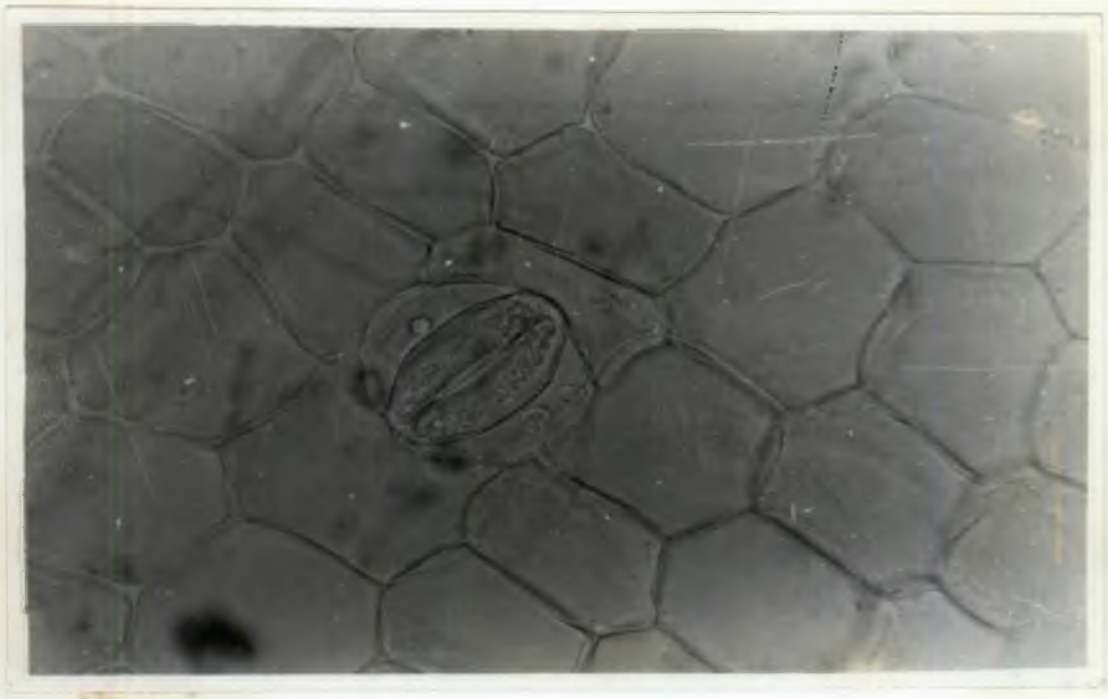
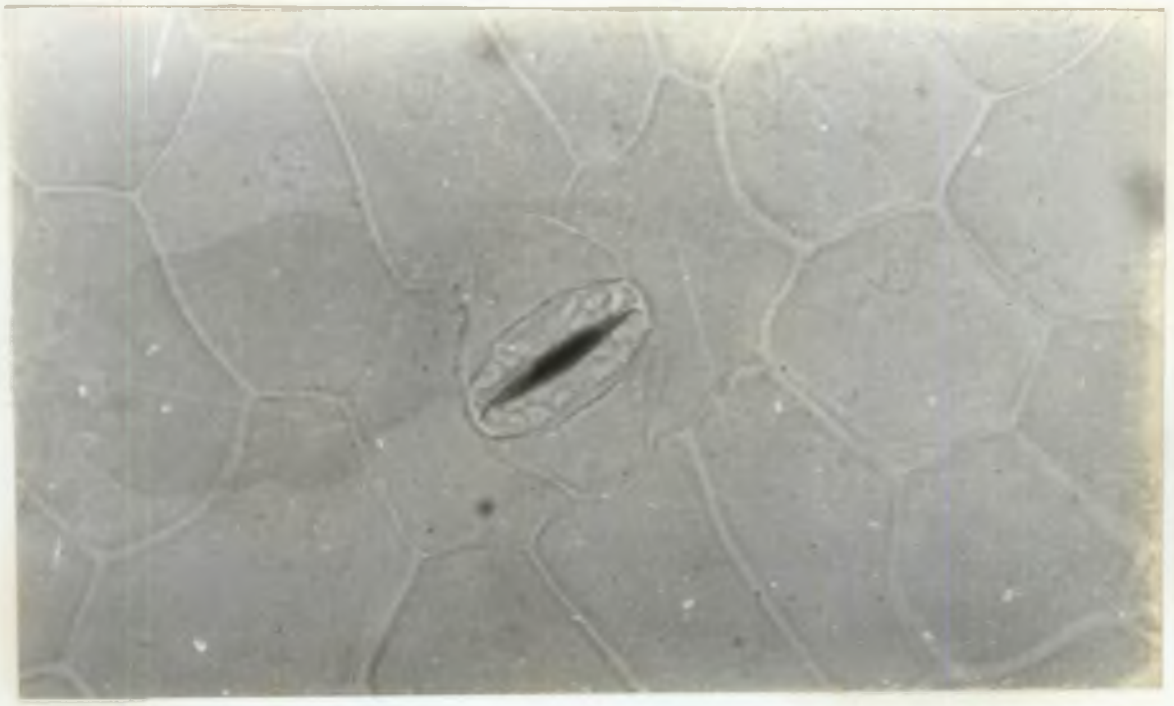
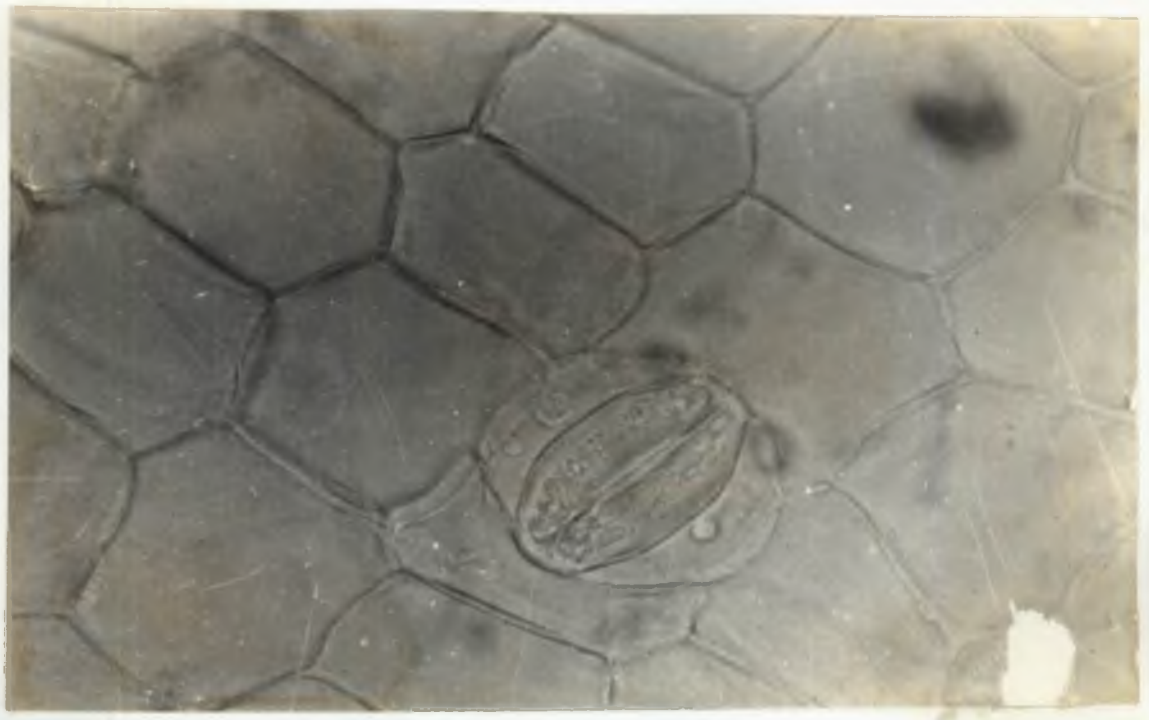


Plate 8a. Stomata of diploid ginger variety Rio-de-Janeiro (x 1600)

Plate 8b. Stomata of autotetraploid ginger derived from the variety Rio-de-Janeiro (x 1600)





autotetraploids compared to control. Similar results were reported by Gupta and Koak (1976) in *Zinnia elegans* and Srivastava and Raina (1992) in *Clitoria ternatea*.

5.6.5 Yield and rhizome characters

Fresh rhizome yield of the autotetraploids were consistently high during the two seasons, when compared to the diploids. Ramachandran and Nair (1992) reported an increase in fresh weight of rhizome in induced autotetraploid ginger compared to control.

The rhizomes of autotetraploids showed an increase in number of primary and secondary fingers but a reduction in the size (Plates 9 & 10). This is contradictory to the report of Ratnambal and Nair (1982) and Ramachandran and Nair (1992), who observed increase in the size of rhizome and reduced branching tendency in induced autotetraploids of ginger. The high yield observed in the autotetraploids and selected plants may be a consequence of increased number of fingers. In confirmity with the present finding, Nybe (1978) reported a positive correlation between number of fingers and rhizome yield.

5.6.6 Selection of morphological variants

Out of the eleven plants studied for two seasons, four variants (Plant No.4, 7, 8 and 11) with vigorous growth habit and high yield potential which exceeded the control and even the autotetraploid were selected for further evaluation (Table 20). Plant No.4, 7, and 8 were derived from the variety Himachal Pradesh, Maran and Nadia respectively treated with 0.25 per cent colchicine by the hole method of application. Plant No.11 was a deerivative of the variety Rio-de-Janeiro treated with 0.1 per cent colchicine by the hole method of application. Thus the

Plate 9. Rhizomes of diploid, autotetraploid and morphological variant of variety Himachal Pradesh

Plate 10. Rhizomes of diploid, autotetraploid and morphological variant of variety Rio-de-Janeiro


HIMACHAL PRADESH
0·25/HOLE
HIMACHAL PRADESH
CONTROL
HIMACHAL PRADESH
0·25% INJECTION
TETRAPLOID


RIO-DE-JANEIRO
0·1% HOLE
RIO-DE-JANEIRO
CONTROL
RIO-DE-JANEIRO
0·1/HOLE
TETRAPLOID

Table 20. Performance of autotetraploids and four selected morphological variants compared to control varieties

Treatment combinations	Plant height (cm)		Number of tillers/plant		Number of leaves/plant		Leaf area (cm^2)		Raw yield of rhizome ($g\ plant^{-1}$)	
	4 MAP	6 MAP	4 MAP	6 MAP	4 MAP	6 MAP	5 MAP	6 MAP	I year	II year
$V_1C_2M_1$ (Autotetraploid)	68.0	96.0	5.0	10.0	49.0	80.0	65.42	58.80	80	420
$V_1C_2M_2$	90.0	95.0	13.0	18.0	130.0	205.0	60.46	62.11	340	520
Control	69.5	66.5	16.5	23.5	137.0	210.0	55.65	49.41	75	320
$V_2C_2M_2$	66.0	86.0	32.0	38.0	296.0	307.0	37.93	25.68	60	600
Control	52.5	49.0	9.0	9.5	76.5	79.5	37.27	28.43	46	140
$V_3C_2M_2$	108.0	111.0	12.0	17.0	88.0	98.0	67.94	56.25	220	480
Control	49.0	51.0	6.0	9.0	31.0	71.0	53.83	47.21	40	160
$V_4C_1M_2$ (Autotetraploid)	39.0	87.0	7.0	11.0	51.0	113.0	54.36	48.86	160	340
$V_4C_1M_2$	90.0	100.0	19.0	24.0	109.0	200.0	52.06	48.86	100	480
Control	58.0	68.5	12.5	15.5	81.0	123.0	38.10	39.46	37.5	120

MAP - Months after planting

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morphological changes in treated but nondoubled plants may be due to the mutagenic effect of colchicine at low concentrations (Sun *et al.*, 1994).

To sum up, the investigations resulted in the identification of two autotetraploids derived from the variety Himachal Pradesh treated with 0.25 per cent colchicine by injection method and Rio-de-Janeiro treated with 0.1 per cent colchicine by hold method. The autotetraploids were characterised by slower initial growth which surpassed the diploid control during later stages, reduced number of tillers and leaves with increased leaf area and larger epidermal cells and stomata with reduced number/mm^2 and increased pollen fertility. The autotetraploid derived from Rio-de-Janeiro flowered during second year of planting. The pollen fertility of the autotetraploid was very much higher than the diploid. The autotetraploids were more productive than the corresponding diploids which were consistent during the second year also. The study was also successful in isolating four variants with distinct morphological characters and high yield potential which even surpassed the autotetraploids. Further evaluation of the autotetraploids and selected variants is warranted to observe the stability in rhizome yield and to understand its status with respect to quality attributes, dry yield and resistance to pests and diseases.

Summary

SUMMARY

The present study was conducted at the College of Horticulture, Vellanikkara during the year 1994-95 with a view to induce autotetraploids and to create variability in ginger. Induction of polyploidy was tried in four commercial varieties of ginger namely Himachal Pradesh, Maran, Nadia and Rio-de-Janeiro using three concentrations of colchicine (0.10, 0.25 and 0.40%) and two methods of application (hole and injection).

Survival of plants as influenced by colchicine treatment and the cyto-morphological changes brought out were studied. The results obtained at different stages of growth of the treated plants were compared with the control and conclusions drawn are summarised below.

Survival of treated plants was reduced with increase in concentration of colchicine. Hole method of application resulted in reduction of survival counts compared to injection. At all stages of observation, the survival of treated plants was lower than that of control in all treatments.

The colchicine treated plants showed slower initial growth which exceeded the control during later stage of growth, though the difference was non-significant. Number of tillers, number of leaves and leaf area were not significantly influenced by colchicine treatment. Increase in concentration of colchicine brought out a substantial reduction in all the biometric characters studied indicating the use of colchicine at low concentration for stimulatory effect. Hole method of treatment was found to be superior to injection method in having a positive influence on the biometric characters studied.

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With respect to yield, varietal difference was evident and Himachal Pradesh was significantly superior. Colchicine at 0.10 per cent concentration applied by hole method was more effective in enhancing the yield.

The length and width of stomata and mean number per mm^2 of leaf were not significantly influenced by the varieties and concentrations of colchicine. Hole method showed a favourable effect in inducing polyploidy as denoted by the larger stomatal size and fewer number.

Individual plants with fewer stomata and larger size were isolated as suspected polyploid. Thirteen suspected polyploids and four control varieties were subjected to cytological study to confirm their chromosome number. Out of the 13 plants, two plants (Plant No.1 and 10) with $2n = 44$ against $2n = 22$ in control were confirmed as autotetraploids while others showed no cytological difference. The autotetraploids were derived from the variety Himachal Pradesh treated with 0.25 per cent colchicine by injection method and Rio-de-Janeiro treated with 0.10 per cent colchicine by hole method.

The tetraploids were characterised by slower initial growth which surpassed the diploid control during later stages, reduced number of tillers and leaves with increased leaf area and larger epidermal cells and stomata with reduced number/mm^2 of leaf. The autotetraploid derived from Rio-de-Janeiro flowered during second year of planting. The pollen fertility of the autotraploid was very much higher than the diploid. The autotetraploids recorded higher rhizome yield than the corresponding diploids which was consistent during the second year also.

Four morphological variants with distinct vigorous growth and higher yield potential than the autotetraploids were also screened out for further evaluation.

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* Originals not seen

Appendices

Appendix Ia. Interaction effect between varieties and concentrations of colchicine on plant height

	Treatment combinations	Plant height (cm)				
		August	September	October	November	December
Variety x concentration	V_1C_1	.50	52.00	60.38	68.75	69.38
	V_1C_2	50	49.25	51.00	68.25	64.00
	V_1C_3	26.25	42.88	48.13	64.38	60.63
	V_2C_1	28.63	58.00	63.75	73.50	57.25
	V_2C_2	40.88	60.63	63.50	75.25	71.00
	V_2C_3	23.13	32.63	34.75	53.25	50.88
	V_3C_1	32.63	50.38	55.50	59.63	56.13
	V_3C_2	23.38	49.88	60.88	63.38	59.25
	V_3C_3	24.38	40.25	43.75	45.00	42.00
	V_4C_1	21.88	40.75	54.38	68.50	65.75
	V_4C_2	23.75	46.88	50.25	58.63	49.63
	V_4C_3	16.63	30.25	39.75	52.38	57.25
	CD (0.05)	8.02	NS	NS	NS	12.34

Appendix Ib. Interaction effect between varieties and methods of application of colchicine on plant height

	Treatment combinations	Plant height (cm)				
		August	September	October	November	December
Variety x method	V_1M_1	21.58	43.25	48.50	63.83	60.58
	V_1M_2	31.25	52.83	57.83	70.42	68.75
	V_2M_1	29.25	47.42	52.25	63.67	59.66
	V_2M_2	32.50	53.42	55.75	71.00	59.75
	V_3M_1	25.33	46.58	54.50	57.25	54.33
	V_3M_2	28.25	47.08	52.25	54.75	50.58
	V_4M_1	20.42	40.50	51.00	60.58	53.75
	V_4M_2	21.08	38.08	45.25	59.08	61.33
	CD (0.05)	NS	NS	NS	NS	NS

NS - Non significant

Appendix II. Interaction effect between varieties, concentrations and methods of application of colchicine on plant height

Treatment combinations	Plant height (cm)				
	August	September	October	November	December
$V_1C_1M_1$	24.75	43.00	56.25	63.25	64.75
$V_1C_1M_2$	36.25	61.00	64.50	74.25	74.00
$V_1C_2M_1$	9.50	37.25	38.00	62.00	56.00
$V_1C_2M_2$	35.50	61.25	64.00	74.50	72.00
$V_1C_3M_1$	30.50	49.50	51.25	66.25	61.00
$V_1C_3M_2$	22.00	36.25	45.00	62.50	60.25
Control	52.00	62.00	65.50	66.00	70.00
CD (0.05)	11.95	11.25	NS	NS	NS
$V_2C_1M_1$	26.25	54.50	60.75	69.00	56.00
$V_2C_1M_2$	31.00	61.50	66.75	78.00	58.00
$V_2C_2M_1$	42.25	57.00	64.00	71.00	66.25
$V_2C_2M_2$	39.50	64.25	63.00	79.00	75.75
$V_2C_3M_1$	19.25	30.75	32.00	50.00	56.75
$V_2C_3M_2$	27.00	34.50	37.50	56.00	45.00
Control	39.50	52.50	58.50	65.00	31.50
CD (0.05)	NS	NS	NS	NS	19.89
$V_3C_1M_1$	28.00	48.50	56.75	57.75	57.00
$V_3C_1M_2$	37.25	52.25	54.25	61.50	55.25
$V_3C_2M_1$	21.75	47.00	63.00	63.50	57.75
$V_3C_2M_2$	25.00	52.75	58.75	63.25	60.75
$V_3C_3M_1$	26.25	44.25	43.75	50.50	48.25
$V_3C_3M_2$	22.50	36.25	43.75	39.50	35.75
Control	38.50	53.50	59.50	64.00	43.50
CD (0.05)	NS	NS	NS	NS	NS
$V_4C_1M_1$	20.50	36.25	51.00	61.50	55.25
$V_4C_1M_2$	23.25	45.25	57.75	75.50	76.25
$V_4C_2M_1$	22.25	49.75	51.00	58.75	44.75
$V_4C_2M_2$	25.25	44.00	49.50	58.50	54.50
$V_4C_3M_1$	18.50	35.50	51.00	61.50	61.25
$V_4C_3M_2$	14.75	25.00	28.50	43.25	53.25
Control	29.00	36.00	44.50	56.50	60.50
CD (0.05)	NS	NS	NS	NS	NS

NS - Non significant

Appendix IIIa. Interaction effect between varieties and concentrations of colchicine on number of tillers

	Treatment combinations	Number of tillers/plant				
		August	September	October	November	December
Variety x concentration	V_1C_1	1.00	3.88	7.13	10.88	9.25
	V_1C_2	1.00	3.75	7.13	11.38	11.88
	V_1C_3	1.00	2.88	4.38	9.75	7.63
	V_2C_1	1.00	3.00	6.25	8.75	4.75
	V_2C_2	1.00	3.75	6.38	7.88	4.63
	V_2C_3	1.00	1.88	3.75	5.13	6.50
	V_3C_1	1.00	3.75	7.75	10.63	7.88
	V_3C_2	1.00	3.13	6.63	9.25	6.85
	V_3C_3	1.00	2.75	5.63	6.75	6.63
	V_4C_1	1.00	3.00	7.63	13.63	15.50
	V_4C_2	1.00	3.75	6.13	9.63	7.25
	V_4C_3	1.00	2.63	7.38	11.88	14.75
	CD (0.05)	NS	NS	NS	NS	3.45

Appendix IIIb. Interaction effect between varieties and methods of application of colchicine on number of tillers

	Treatment combinations	Number of tillers/plant				
		August	September	October	November	December
Variety x method	V_1M_1	1.00	3.00	4.25	7.83	7.92
	V_1M_2	1.00	4.00	8.17	13.50	11.25
	V_2M_1	1.00	2.42	5.92	7.42	4.83
	V_2M_2	1.00	3.33	5.00	7.08	5.75
	V_3M_1	1.00	3.33	6.50	9.67	7.58
	V_3M_2	1.00	3.08	6.83	8.08	6.67
	V_4M_1	1.00	3.00	7.25	11.75	13.17
	V_4M_2	1.00	3.25	6.83	11.67	11.83
	CD (0.05)	NS	NS	1.84	2.74	NS

NS - Non significant

Appendix IV. Interaction effect between varieties, concentrations and methods of application of colchicine on number of tillers

Treatment combinations	Number of tillers/plant				
	August	September	October	November	December
$V_1C_1M_1$	1.00	3.50	5.50	7.00	6.75
$V_1C_1M_2$	1.00	4.25	8.75	14.75	11.75
$V_1C_2M_1$	1.00	2.50	4.25	7.00	10.00
$V_1C_2M_2$	1.00	5.00	10.00	15.75	13.75
$V_1C_3M_1$	1.00	3.00	3.00	9.50	7.00
$V_1C_3M_2$	1.00	2.75	5.75	10.00	8.25
Control	1.00	5.00	7.00	8.50	6.50
CD (0.05)	NS	NS	NS	NS	NS
$V_2C_1M_1$	1.00	2.25	5.50	7.25	4.00
$V_2C_1M_2$	1.00	3.75	7.00	10.25	5.50
$V_2C_2M_1$	1.00	3.50	7.50	9.25	4.25
$V_2C_2M_2$	1.00	4.00	5.25	6.50	5.00
$V_2C_3M_1$	1.00	1.50	4.75	5.75	6.25
$V_2C_3M_2$	1.00	2.25	2.75	4.50	6.75
Control	1.00	4.00	6.50	8.50	2.50
CD (0.05)	NS	NS	NS	NS	NS
$V_3C_1M_1$	1.00	3.75	7.50	11.25	7.75
$V_3C_1M_2$	1.00	3.75	8.00	10.00	8.00
$V_3C_2M_1$	1.00	3.00	6.00	9.50	6.50
$V_3C_2M_2$	1.00	3.25	7.25	9.00	7.25
$V_3C_3M_1$	1.00	3.25	6.00	8.25	8.50
$V_3C_3M_2$	1.00	2.25	5.25	5.25	4.75
Control	1.00	4.00	4.50	7.50	9.00
CD (0.05)	NS	NS	NS	NS	NS
$V_4C_1M_1$	1.00	2.50	7.00	10.50	15.75
$V_4C_1M_2$	1.00	3.50	8.25	16.75	15.25
$V_4C_2M_1$	1.00	3.50	6.00	9.50	6.50
$V_4C_2M_2$	1.00	4.00	6.25	9.75	8.00
$V_4C_3M_1$	1.00	3.00	8.75	15.25	17.25
$V_4C_3M_2$	1.00	2.25	6.00	8.50	12.25
Control	1.00	4.50	5.00	7.50	12.00
CD (0.05)	NS	NS	NS	NS	NS

NS - Non significant

Appendix Va. Interaction effect between varieties and concentrations of colchicine on number of leaves

	Treatments combinations	Number of leaves/plant				
		August	September	October	November	December
Variety x concentration	V_1C_1	3.88	28.00	72.00	123.38	96.13
	V_1C_2	2.88	28.50	57.63	117.75	103.00
	V_1C_3	4.88	22.50	53.25	101.13	74.88
	V_2C_1	3.38	25.50	62.88	77.00	53.88
	V_2C_2	4.63	28.38	64.38	84.13	48.00
	V_2C_3	4.38	12.00	28.13	42.63	45.25
	V_3C_1	4.75	30.63	70.88	101.63	89.63
	V_3C_2	3.25	25.88	61.13	85.00	66.38
	V_3C_3	4.13	16.38	45.88	57.00	57.63
	V_4C_1	3.88	74.88	60.13	110.63	119.13
	V_4C_2	4.75	28.25	54.38	74.75	52.88
	V_4C_3	2.75	42.50	56.75	89.13	137.75
	CD (0.05)	1.46	14.33	NS	NS	34.44

Appendix Vb. Interaction effect between varieties and methods of application of colchicine on number of leaves

	Treatment combinations	Number of leaves/plant				
		August	September	October	November	December
Variety x method	V_1M_1	3.33	22.08	48.25	92.25	78.67
	V_1M_2	4.42	30.58	73.67	135.92	104.00
	V_2M_1	3.83	21.00	56.17	69.50	42.17
	V_2M_2	4.42	22.92	47.42	66.33	55.92
	V_3M_1	4.08	25.08	59.67	82.83	83.83
	V_3M_2	4.00	23.50	58.92	79.58	58.58
	V_4M_1	3.92	46.42	59.08	94.25	102.17
	V_4M_2	3.67	50.67	55.08	88.75	104.33
	CD (0.05)	NS	NS	NS	NS	NS

NS - Non significant

Appendix VI. Interaction effect between varieties, concentrations and methods of application of colchicine on number of leaves

Treatment combinations	Number of leaves/plant				
	August	September	October	November	December
$V_1C_1M_1$	3.50	23.00	54.75	84.00	73.25
$V_1C_1M_2$	4.25	33.00	89.25	162.75	119.00
$V_1C_2M_1$	2.25	16.50	32.50	78.50	92.25
$V_1C_2M_2$	3.50	40.50	82.75	157.00	113.75
$V_1C_3M_1$	4.25	26.75	57.50	114.25	70.50
$V_1C_3M_2$	5.50	18.25	49.00	88.00	79.25
Control	7.00	42.00	77.00	106.50	77.00
CD(0.05)	2.41	11.39	NS	NS	NS
$V_2C_1M_1$	2.75	22.25	63.00	69.00	35.50
$V_2C_1M_2$	4.00	28.75	62.75	85.00	72.25
$V_2C_2M_1$	4.75	30.50	72.50	93.75	42.00
$V_2C_2M_2$	4.50	26.25	56.25	74.50	54.00
$V_2C_3M_1$	4.00	10.25	33.00	45.75	49.00
$V_2C_3M_2$	4.75	13.75	23.25	39.50	41.50
Control	4.00	23.50	54.50	89.50	16.00
CD(0.05)	NS	NS	NS	NS	29.25
$V_3C_1M_1$	4.00	31.25	73.50	95.00	107.00
$V_3C_1M_2$	5.50	30.00	60.25	100.25	72.25
$V_3C_2M_1$	3.25	24.25	52.25	76.00	63.50
$V_3C_2M_2$	3.25	27.50	70.00	94.00	69.25
$V_3C_3M_1$	5.00	19.75	53.25	77.50	81.00
$V_3C_3M_2$	3.25	13.00	38.50	36.50	34.25
Control	4.00	30.50	27.00	60.00	39.00
CD(0.05)	NS	NS	NS	NS	NS
$V_4C_1M_1$	4.00	48.25	51.50	84.50	98.75
$V_4C_1M_2$	3.75	101.50	68.75	136.75	139.50
$V_4C_2M_1$	5.00	29.50	53.25	76.50	39.75
$V_4C_2M_2$	4.50	27.00	55.50	73.00	66.00
$V_4C_3M_1$	2.75	61.50	72.50	121.75	168.00
$V_4C_3M_2$	2.75	23.50	41.00	56.50	107.50
Control	4.50	27.00	36.00	60.50	126.00
CD(0.05)	NS	NS	NS	NS	NS

NS - Non significant

Appendix VIIa. Interaction effect between varieties and concentrations of colchicine on leaf area

	Treatment combinations	Leaf area (cm^2)			
		September	October	November	December
Variety x concentration	V_1C_1	32.72	35.14	37.70	45.29
	V_1C_2	31.89	29.29	41.18	41.18
	V_1C_3	23.61	29.70	37.56	41.04
	V_2C_1	32.72	29.30	41.29	39.63
	V_2C_2	38.93	32.46	41.04	42.01
	V_2C_3	15.74	21.92	34.01	34.49
	V_3C_1	35.20	26.06	34.99	37.40
	V_3C_2	29.82	29.34	34.69	35.09
	V_3C_3	25.68	27.03	31.75	33.12
	V_4C_1	18.64	29.11	45.47	36.90
	V_4C_2	28.99	24.77	35.82	27.25
	V_4C_3	14.50	21.06	34.85	29.85
	CD (0.05)	NS	NS	NS	NS

Appendix VIIb. Interaction effect between varieties and methods of application of colchicine on leaf area

	Treatment combinations	Leaf area (cm^2)			
		September	October	November	December
Variety x method	V_1M_1	27.61	27.52	35.57	40.24
	V_1M_2	31.20	35.23	42.06	44.76
	V_2M_1	27.34	28.01	40.65	39.94
	V_2M_2	30.92	27.78	36.90	37.49
	V_3M_1	29.82	26.51	36.59	34.45
	V_3M_2	30.65	28.44	31.04	35.96
	V_4M_1	22.09	25.23	38.74	30.17
	V_4M_2	19.33	24.73	38.69	32.49
	CD (0.05)	NS	NS	5.93	NS

NS - Non significant

Appendix VIII. Interaction effect between varieties, concentrations and methods of application of colchicine on leaf area

Treatment combinations	Leaf area (cm^2)			
	September	October	November	December
$V_1C_1M_1$	27.34	29.82	32.62	37.98
$V_1C_1M_2$	38.10	40.46	42.79	52.59
$V_1C_2M_1$	26.51	22.12	40.76	35.29
$V_1C_2M_2$	37.27	36.47	41.60	47.07
$V_1C_3M_1$	28.99	30.62	33.32	47.46
$V_1C_3M_2$	18.23	28.77	41.79	34.62
Control	40.58	35.62	37.70	35.05
CD (0.05)	NS	NS	NS	NS
$V_2C_1M_1$	29.82	31.27	41.66	37.85
$V_2C_1M_2$	35.62	27.34	40.92	41.41
$V_2C_2M_1$	42.24	31.64	42.65	43.33
$V_2C_2M_2$	35.62	33.28	39.43	40.68
$V_2C_3M_1$	9.95	21.13	37.65	38.62
$V_2C_3M_2$	21.54	22.71	30.37	30.36
Control	24.02	31.31	39.76	20.71
CD(0.05)	NS	NS	NS	10.64
$V_3C_1M_1$	34.79	25.80	38.27	36.20
$V_3C_1M_2$	35.62	26.32	31.72	38.61
$V_3C_2M_1$	27.34	26.04	36.13	30.86
$V_3C_2M_2$	32.30	32.64	33.26	39.33
$V_3C_3M_1$	27.34	27.70	35.36	36.30
$V_3C_3M_2$	24.02	26.37	28.14	29.93
Control	43.89	28.89	34.87	38.43
CD (0.05)	NS	NS	NS	NS
$V_4C_1M_1$	13.26	23.66	42.75	33.65
$V_4C_1M_2$	24.02	34.56	48.19	40.14
$V_4C_2M_1$	30.65	24.94	33.30	24.44
$V_4C_2M_2$	27.34	24.59	38.35	30.07
$V_4C_3M_1$	22.37	27.07	40.15	32.44
$V_4C_3M_2$	6.64	15.06	29.54	27.25
Control	17.40	18.19	14.62	21.37
CD (0.05)	NS	NS	11.63	NS

NS - Non significant

Appendix IXa. Interaction effect between varieties and concentrations of colchicine on stomatal characters

	Treatment combinations	Stomatal length (μm)	Stomatal width (μm)	Number of stomata/ mm^2
Variety x	V_1C_1	158.27	95.80	39.45
concentration	V_1C_2	147.86	89.55	41.25
	V_1C_3	143.69	83.30	43.20
	V_2C_1	152.02	91.63	39.45
	V_2C_2	152.03	89.55	41.25
	V_2C_3	145.78	87.47	45.00
	V_3C_1	139.53	87.47	45.00
	V_3C_2	147.86	91.59	43.20
	V_3C_3	141.61	89.52	45.00
	V_4C_1	149.94	93.73	39.45
	V_4C_2	149.94	91.63	43.20
	V_4C_3	149.94	93.72	43.20
	CD (0.05)	NS	NS	NS

Appendix IXb. Interaction effect between varieties and methods of application of colchicine on stomatal characters

	Treatment combinations	Stomatal length (μm)	Stomatal width (μm)	Number of stomata/ mm^2
Variety x method	V_1M_1	148.55	87.47	42.45
	V_1M_2	151.33	91.63	41.25
	V_2M_1	147.16	86.08	42.45
	V_2M_2	152.72	93.02	40.05
	V_3M_1	141.61	87.41	45.00
	V_3M_2	144.39	91.64	43.80
	V_4M_1	141.61	87.47	45.00
	V_4M_2	158.27	98.58	38.75
	CD (0.05)	NS	NS	NS

NS - Non significant

Appendix X. Interaction effect between varieties, concentrations and methods of application of colchicine on stomatal characters

Treatment combinations	Stomatal length (μm)	Stomatal width (μm)	Number of stomata/ mm^2
$V_1C_1M_1$	145.76	87.47	45.00
$V_1C_1M_2$	170.77	104.13	33.75
$V_1C_2M_1$	154.11	91.63	37.50
$V_1C_2M_2$	141.61	87.47	45.00
$V_1C_3M_1$	145.78	83.30	45.00
$V_1C_3M_2$	141.61	83.30	41.25
Control	141.61	83.30	44.00
CD (0.05)	NS	NS	NS
$V_2C_1M_1$	154.11	87.47	37.50
$V_2C_1M_2$	149.94	95.80	41.25
$V_2C_2M_1$	145.78	87.47	45.00
$V_2C_2M_2$	158.27	91.63	37.50
$V_2C_3M_1$	141.61	83.30	43.00
$V_2C_3M_2$	149.94	91.63	42.00
Control	141.61	91.63	45.00
CD (0.05)	NS	NS	NS
$V_3C_1M_1$	137.45	83.30	48.00
$V_3C_1M_2$	141.61	91.63	45.00
$V_3C_2M_1$	141.61	87.38	46.00
$V_3C_2M_2$	154.11	95.80	39.00
$V_3C_3M_1$	145.78	91.54	42.00
$V_3C_3M_2$	137.45	87.50	47.00
Control	133.28	91.63	46.00
CD (0.05)	NS	NS	NS
$V_4C_1M_1$	141.61	87.48	43.00
$V_4C_1M_2$	158.27	99.98	40.00
$V_4C_2M_1$	141.61	87.47	44.00
$V_4C_2M_2$	158.27	95.80	38.00
$V_4C_3M_1$	141.61	87.47	45.00
$V_4C_3M_2$	158.27	99.96	36.00
Control	141.61	83.30	45.00
CD (0.05)	NS	NS	NS

NS - Non significant

Appendix XIa. Interaction effect between varieties and concentrations of colchicine on rhizome yield

	Treatment combinations	Raw yield of rhizome (g plant^{-1})
Variety x concentration	V_1C_1	147.50
	V_1C_2	123.75
	V_1C_3	85.63
	V_2C_1	99.38
	V_2C_2	122.50
	V_2C_3	27.50
	V_3C_1	104.38
	V_3C_2	112.50
	V_3C_3	60.63
	V_4C_1	83.75
	V_4C_2	64.00
	V_4C_3	55.00
	CD (0.05)	NS

Appendix XIb. Interaction effect between varieties and methods of application of colchicine on rhizome yield

	Treatment combinations	Raw yield of rhizome (g plant^{-1})
Variety x Method	V_1M_1	88.75
	V_1M_2	149.17
	V_2M_1	67.92
	V_2M_2	98.33
	V_3M_1	97.92
	V_3M_2	87.08
	V_4M_1	64.33
	V_4M_2	70.83
	CD (0.05)	NS

NS - Non significant

Appendix XII. Interaction effect between varieties, concentrations and methods of application of colchicine on rhizome yield

Treatment combinations	Raw yield of rhizome (g plant^{-1})
$V_1C_1M_1$	82.50
$V_1C_1M_2$	212.50
$V_1C_2M_1$	68.75
$V_1C_2M_2$	178.75
$V_1C_3M_1$	115.00
$V_1C_3M_2$	56.25
Control	87.50
CD (0.05)	NS
$V_2C_1M_1$	63.75
$V_2C_1M_2$	135.00
$V_2C_2M_1$	106.25
$V_2C_2M_2$	138.75
$V_2C_3M_1$	33.75
$V_2C_3M_2$	21.25
Control	46.00
CD (0.05)	NS
$V_3C_1M_1$	115.00
$V_3C_1M_2$	93.75
$V_3C_2M_1$	95.00
$V_3C_2M_2$	130.00
$V_3C_3M_1$	83.00
$V_3C_3M_2$	37.50
Control	40.00
CD (0.05)	NS
$V_4C_1M_1$	53.75
$V_4C_1M_2$	113.75
$V_4C_2M_1$	61.75
$V_4C_2M_2$	66.25
$V_4C_3M_1$	77.50
$V_4C_3M_2$	32.50
Control	37.50
CD (0.05)	NS

NS - Non significant

INDUCTION OF AUTOTETRAPLOIDY IN GINGER

By

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ABSTRACT OF A THESIS

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ABSTRACT

A study was conducted at the College of Horticulture, Vellanikkara during the year 1994-95 with a view to induce autotetraploids and to create variability in ginger. Induction of autotetraploidy was tried in four commercial varieties of ginger namely Himachal Pradesh, Maran, Nadia and Rio-de-Janeiro using three concentrations of colchicine (0.10, 0.25 and 0.40%) and two methods of application (injection and hole). The effect of colchicine on survival of plants and cyto-morphological characters were recorded.

Colchicine treatment by the hole method reduced the survival of plants which decreased with increase in concentration.

The colchicine treated plants showed slower initial growth which exceeded the control during later stage of growth. Number of tillers, number of leaves and leaf area were not significantly influenced by colchicine treatment. Application of colchicine at low concentration is advocated in ginger to have a stimulatory effect on biometric characters. Hole method of treatment was preferential to injection in having a positive influence on biometric characters.

With respect to yield, colchicine at the lowest concentration of 0.10 per cent applied by hole method recorded the highest yield.

Stomatal size and frequency were not significantly influenced by varieties, concentrations and methods of application.

Individual plants with fewer stomata and larger size were isolated as suspected autotetraploids. Out of the thirteen plants, plants with 2n = 44 against 2n = 22 in control were confirmed as autotetraploids. Two autotetraploids were derived from the variety Himachal Pradesh treated with 0.25 per cent colchicine by injection method and Rio-de-Janeiro treated with 0.10 per cent colchicine by hole method.

The autotetraploids were characterised by slower initial growth which surpassed the diploid control during later stages, reduced number of tillers and leaves with increased leaf area and larger epidermal cells and stomata with reduced number/mm^2 of leaf. The autotetraploid derived from Rio-de-Janeiro showed increased pollen fertility than the diploid. The autotetraploids recorded higher rhizome yield than the corresponding diploids consistently during the second year also.

Morphological variants with higher yield potential than the autotetraploids were also screened out for further evaluation.