ARTIFICIAL INDUCTION OF POLYPLOIDY IN *Cucumis sativus* L.

By

K.G. GIRISH KUMAR

THESIS

submitted in partial fulfilment of the requirement for the Degree of

MASTER OF SCIENCE IN AGRICULTURE

FACULTY OF AGRICULTURE
KERALA AGRICULTURAL UNIVERSITY

Department of Agricultural Botany
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM

1993

DECLARATION

I hereby declare that this thesis entitled "**Artificial induction of polyploidy in Cucumis sativus L.**" is a bonafied 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, associateship, fellowship or other similar title of any University or Society.

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CERTIFICATE

Certified that this thesis entitled "**Artificial induction of polyploidy in Cucumis sativus L.**" is a record of research work done independently by Sri. **K.G.Girish kumar** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Chandramony.D

Dr.(Mrs.) **D.CHANDRAMONY**
Chairman
Advisory Committee

Vellayani
8th July 1993.

APPROVED BY :

CHAIRMAN :

Dr.(Mrs.) D.CHANDRAMONY

MEMBERS :

1. Dr.R.GOPIMONI
2. Dr.(Mrs.) S.T.MERCY
3. Dr.C.S.JAYACHANDRAN NAIR

EXTERNAL EXAMINER

19.11.93

ACKNOWLEDGEMENT

The author wishes to place on record his deep sense of gratitude and indebtedness to ;

Dr.(Mrs.) D.Chandramony, Associate Professor, Department of Agricultural Botany, College of Agriculture, Vellayani and Chairman of the Advisory Committee, for her valuable guidance, constant inspiration, constructive criticisms and unfailing interest which helped a lot to the completion of the study.

Dr.R.Gopimony, Professor and Head, Department of Agricultural Botany, College of Agriculture Vellayani, and member of Advisory Committee for going through the manuscript and making very useful corrections.

Dr.(Mrs.) S.T.Mercy, Professor, Department of Agricultural Botany, College of Agriculture Vellayani and member of Advisory Committee for her valuable corrections and suggestions through out the course of work.

Dr. C.S.Jayachandran Nair, Associate Professor, Department of Horticulture College of Agriculture, Vellayani for his untiring help at all stages of the work.

Dr.N.Krishnan Nair and Prof.K.Gopakumar former Heads of the Department, Department of Agricultural Botany for their very valuable help given during the early phase of study.

Dean, College of Agriculture, Vellayani and Associate Dean, College of Horticulture, Vellanikkara for permiting me to carry out the in vitro studies at College of Horticulture, Vellanikkara with out which the work could not have been completed.

Dr.Rajeevan, Associate Professor and staff of Department of Pomology, College of Horticulture, Vellanikkara for providing all guidance and help to carry out the in vitro studies at Tissue culture lab attached to their Department.

Sri.C.E.Ajith Kumar, Computer Programmer, College of Agriculture, Vellayani for his help in analysing the data.

teaching and non teaching staff of Department of Agriculture Botany especially to Smt. Lekha Rani, Assistant Professor for her help in carrying out cytological studies and post graduate scholars for their support extended throughout the course of the investigation.

his wife Smt.Ragini,R for her untiring help in copying the manuscript and valuable criticisms which helped a lot in preparing the thesis.

M/s N.C.Computers, Jagathy, Thiruvananthapuram for their sincere services in neatly typing the thesis.

The Kerala Agricultural University for awarding a fellowship for the Post Graduate Programme.

Vellayani,
July 1993.

(K.G.GIRISH KUMAR)

CONTENTS

LIST OF TABLES

10. Interaction effect of colchicine concentration and mode of treatment on survival and various morphological characters in Delila.

11. Interaction effect of colchicine concentration and period of treatment on survival and various morphological characters in Delila.

12. Interaction effect of mode of treatment and period of treatment on survival and various morphological characters in Delila.

13. Effect of colchicine concentration, mode of treatment and period of treatment on various morphological characters in Delila.

14. Interaction effect of colchicine concentration and mode of treatment on various morphological characters in Delila.

15. Interaction effect of colchicine concentration and period of treatment on various morphological characters in Delila.

16. Interaction effect of mode of treatment and period of treatment on various morphological characters in Delila.

17. Effect of colchicine concentration, type of embryo and day of treatment on survival and various plant characters in Seethal.

18. Interaction effect of colchicine concentration and type of embryo on survival and various plant characters in Seethal.

19. Interaction effect of colchicine concentration and day of treatment on survival and various plant characters in Seethal.

20. Interaction effect of type of embryo and day of treatment on survival and various plant characters in Seethal.

21. Effect of colchicine concentration, type of embryo and day of treatment on survival and various plant characters in Delila.

22. Interaction effect of colchicine concentration and type of embryo on survival and various plant characters in Delila.

23. Interaction effect of colchicine concentration and day of treatment on survival and various plant characters in Delila.

24. Interaction effect of type of embryo and day of treatment on survival and various plant characters in Delila.

LIST OF ILLUSTRATIONS

Figure

LIST OF PLATES

13. Variations noticed on plantlet growth in culture medium in variety Delila.

14. Flowering noticed in culture medium.

15. Shoot development without leaf formation.

16. Callus development with out organogenesis.

17. Growth of plantlets in green house - comparison of control with colchicine treated one in Seethal.

18. Growth of plantlets in green house - comparison of control with colchicine treated one in variety Delila.

LIST OF APPENDICES

Appendix

LIST OF ABBREVATIONS

mm = millimeter

cm = centimeter

g = gram

°C = degree celsius

DAP = days after planting

% = per cent

Fig. = figure

N = normal

No. = number

IAA = Indole Acetic Acid

mg/L = milligrams per litre

KAU = Kerala Agricultural University

INTRODUCTION

INTRODUCTION

Chromosome doubling and its manifestation on cytomorphology have been well documented in a wide range of plants by plant breeders. The phenomenon of polyploidy has been recognised as one of the most outstanding methods that can be adopted for crop improvement in many crops. Eventhough induction of polyploidy has been debated as a promising method of crop improvement in cucurbits (Shiffriss, 1942; Singh and Yadav, 1984) it has been less exploited in Cucumis sativus.

Cucumis sativus the species used in the present study is popularly used as salad cucumber. Autopolyploids, if they are vigorous and better yielding can be used as new varities or can be used for processing with normal deploids to produce seedless triploids as in the case of watermelon, apple, pear etc.

Induction of polyploidy necessitates selection of a suitable polyploidising agent, its effective concentration and a suitable method for its application. Colchicine, an alkaloid of plant orgin, and the most widely

accepted and commonly used polyploidising agent was selected for inducing polyploidy in the present investigation.

Taking into account of all these factors, the present investigation was undertaken to analyse the effect of colchicine for inducing polyploidy in Cucumis sativus under in - vivo and in in - vitro conditions. The main objectives were :-

1. To find out the effect of colchicine for the induction of polyploidy in seed, seedling and apical bud treatment in Cucumis sativus under in- vivo conditions.

2. To standardise a suitable medium for embryo culture in Cucumis sativus.

3. To study the effect of colchicine under in - vitro treatments.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Literature available on the various aspects related to the present investigation on the induction of polyploidy by seed, apical bud and seedling treatment and by *in vitro* treatments in cucurbits have been briefly reviewed here. Similar works on *Cucumis sativus* are found to be meagre.

Polyploidy in cucurbitaceae

The role of polyploidy in cucurbitaceous plants had been studied by many research workers and had been reviewed both from the cytogenetic and evolutionary point of view. Until 1947, role of chromosomal changes in cucurbitaceae was less debated. Bhaduri and Bose (1947) reported that chromosomal alterations were mainly responsible for the speciation in different genera of this family. Ayyangar (1949) and Stebbins (1950)) were of the opinion that chromosomal alterations were not the only reason responsible for speciation in the whole family cucurbitaceae. Whitaker (1950) agreed with this opinion and suggested that polyploidy might have played an important

role in some genera of cucurbitaceae based on his studies in Echinocystis macrocarpa.

In cucurbits amphiploidy was found to be successful. When amphiploids raised from the hybrids of five cultivated species of cucurbita, recombinants with better baking qualities were produced especially from the cross C. maxima X C.moschata (Pearson et al. 1951; Whitaker and Bohn, 1950). But according to Bemis (1950) such efforts were unsuccessful with wild species of Cucurbita.

Polyploid forms were identified in a number of genera like Trichosanthes, Melothria, Momordica, Citrullus and Cucumis (Batra, 1952 ; Shimotsuma, 1965 ; Roy et al. 1966; Singh, 1974; Agrawal and Roy, 1976; Singh and Roy, 1979; Dane and Tsuchiya, 1979). Roy and Ghosh (1971) and Singh (1975) reported that in genera Luffa and Cucumis polyploidy induction showed positive response on its fruit characters. In Citrullus vulgaris (Kihara, 1951; Andrus et al, 1971) reported that the polyploids were superior to the diploids.

Many natural polyploids were reported in cucurbitaceae by many research workers which include Trichosanthes cucumeroids (Yamaha and Suematsu, 1936), Echinocystis macrocarpa (Whitaker, 1950); Melothria. purpusilla (Kumar and Vishveshwariah, 1951); Coccinia indica

(Kumar and Vishveswariah, 1952); and Corallocarpus welwitschil (Miege, 1962); Cucumis ficifolius, Cucumis hepladactylus (Shimotsuma, 1965); Momordica dioica (Roy et al. 1966); Gomphogyne cissiformis (Roy and Trivedi, 1966); Trichosanthes palmata (Verghese, 1972); T. bracteata (Thakur, 1973); Momordica assamica (Singh, 1979); Cucumis acuteatus and C.figarei (Dane and Tsuchiya, 1976).

Singh and Yadav (1984) induced polyploidy in Cucumis melo var. momordica and C. prophetarum and compared their characters with C. pustulatus, C.membranifolius C.meeusei and C.heptadactylus and concluded that C. meeusei and C. heptadoctylus might be allopolyploids whereas C.pustulatus and C. membranifolius might be segmental allopolyploids. Induced polyploids attained diploid behaviour and showed possibility of producing polyploids by selection.

Gene transfer system with recessive "ms" was reported to be useful in the improvement of tetraploid cultivars in Citrullus lanatus and in the production of seedless cultivars of triploid water melons (Love et al. 1986). In another report, tetraploids of Cucumis zeyheri were classified in two races as alloploids and segmental autoploids, the former possessing six trivalents (Anon. 1986).

Effect of colchicine

Colchicine, an alkaloid extracted from Colchicum autumnale has been widely used as a polyploidising agent. Early discovery by Blakeslee and Avery (1937) and Nebel and Ruttle (1938) that polyploid strains of plants can be produced through the application of this chemical paved a new way in crop improvement.

Colchicine mainly affects the mitosis. Suppression of anaphase and lengthening the duration of metaphase resulted in doubling of chromosomes (Halberstoedler and Beck, 1943 ; Van't Hof, 1965; Davidson and MacLeod; 1966; MacLeod and Davidson, 1968). Mechanism of its action was sometimes thought to alter viscosity of cytoplasm through gel-sol dynamic equilibrium (Malawista, 1965; Chakraborty and Biswas, 1965; Affonso et al. 1967).

Krishnan et al. (1970) ascribed pairing failure induced by colchicine, as observed at late prophase and metaphase I of meiotic stages as the cause of asynapsis or desynapsis.

Tubulin (microtubular protein) the sub unit protein present in the membraneous system in the cell was identified as a colchicine binding protein by Weisenberg et al. (1968). Artvinli (1987) confirmed this finding and

explained this as responsible for the failure of chromsomal separation into daugther nuclei and thereby causing chromosomal doubling. Singh (1983) reported that pure colchicine is chemically with the formula $C_{22}H_{25}O_6N$ which can block the spindle formation and inhibit the movement of sister chromatids to opposite poles. So the resulting restitution nucleus contain double the number of chromosomes.

Seed treatment

Treatment of seeds with colchicine at different durations has been found to be effective in inducing chromosome doubling.

Singh and Yadav (1984) soaked seeds of Cucumis melo var. momordica and C.prophetarum in moist filter paper for 12 hours and immersed in colchicine solution for different periods and produced successfully the tetraploid derivatives. Yadava et al. (1986) treated the F_1 hybrid seeds of Cucumis dipsaceus X C anguria var. anguria and C. dipsaceus X C. anguria var. longipus with 0.15 and 0.25 per cent colchicine solution and developed amphidiploids. Dzevaltovskii (1982) also developed induced polyploids by seed treatment with colchicine solutions in Melo sativus, Cucumis sativus and Citrullus vulgaris.

Apical bud treatment

Soaking the apical buds of the plant is yet another method to induce polyploidy. Usually cotton soaked with colchicine is kept on the apical buds of young seedling for desired duration. Singh (1975) developed colchiploids of Cucumis melo by apical bud treatment. This method was also found to be an effective method in Cucumis melo var. momordica and Cucumis prophetarum (Singh and Yadav, 1984). Dzevaltovskii (1982) also treated apical buds of Melo sativus Sag., Cucumis sativus and Citrullus vulgaris for producing colchiploids.

Seedling treatment

Treating the whole seedling with colchicine is also described as an effective method to induce polyploidy. Treating seedling with different concentrations of colchicine solution for different duration was reported to be effective in Luffa (Roy and Ghosh, 1971) and Cucumis (Singh ,1975). Mackiewicz (1989) also induced chromosomal alterations by seedling treatment with colchicine in Cucumis sativus.

Morphological variations

Many workers have raised induced polyploids with morphological variations in different genera like Cucumis, Luffa, Trichosanthes, and Momordica (Shifriss, 1942; Hartmair, 1943; Batra, 1952; Roy 1970; Singh , 1975; Roy and Ghosh, 1971).

According to Singh (1979) induction of polyploidy in cucurbitaceae plants resulted in initial stunted growth but finally resulted in gigantism of the plants. Amphidiploids of Cucumis sp. exhibited initial retarded growth and subsequently increased vigour (Singh, 1986). According to Singh and Yadav (1984) colchiploids of Cucumis melo. var. momordica and Cucumis prophetarum showed increased thickness of stem, length of petiole, length and breadth of leaves, length of calyx tube, size of guard cells, length of stomatal aperture, pollen size etc. Yadava et al. (1986) observed significant increase in vegetative parameters like length and breadth of leaves and calyx length in amphidiploids of Cucumis sp. over their diploid counterparts.

Singh (1979) observed delayed flowering particularly in the case of female flowers and reduction in number of flowers in the polypolids of cucurbitaceae plants.

Singh and Yadav (1984) reported that polyploid derivatives of Cucumis sp. produced large female flowers. Yadava et al. (1986) recorded significant increase in size of male and female flowers in the polyploids of Cucumis sp.

Pollen characters

Colchiploids of Cucumis melo var. momordica and C. prophetarum possessed high pollen fertility (76% and 77% respectively) as reported by Singh and Yadav (1984). On the contrary, Singh (1975) reported reduced pollen fertility in cucurbits due to unstable meiotic cycle. Singh (1979) noted appreciably high pollen fertility in the autotetraploids and almost complete sterility in the triploids of cucurbits. In amphidiploid derivatives of Cucumis anguria increased pollen fertility was observed by Yadava et al., 1986. Singh (1975) reported that in polyploids of cucurbits, the initial large size of the ovary did not keep pace with the further development of the plant and finally resulted in comparatively smaller fruits or even deformed ones. But Singh and Yadav (1984) reported that the polyploid cucurbits produced comparatively larger fruits and seeds eventhough there is reduction in the number of fruits per plant and number of seeds per fruit. Mackiewicz (1989) reported that by colchicine effect there was reduction in fruit size in cucumber.

Cytological effects

The action of colchicine on mitosis has been found to be greatly efficient, highly specific and totally selective. Observations by Nebel and Ruttle (1938) and Levan (1938) showed that colchicine inhibited the spindle formation of the dividing cells with out affecting the division of chromosomes.

Singh (1979) reported that in majority of polyploid forms of cucurbits the total chromatin length was much less than the actual multiplied chromatin length of their related diploid counter parts. In polyploids of cucurbits, chromosomes undergo an overall diminution in size, though there was exact multiplication of DNA content (Darlington, 1965; Sharma, 1975). According to Turkov et al. (1974) the root tips of Cucumis sativus had to be pretreated in saturated solution of α-bromonaphthalene at +5°C for three hours and then to be fixed in 1:1 acetic alcohol mixture for cytological observation. Before staining with ferric acetocarmine the root tips are to be treated in 3N HCl at room temperature for 40 minutes. Additional cold treatment was required one to get differential constriction in the chromosomes. According to Dane and Tsuchiya (1976) for root tip cytological analysis a pre-treatment of 3 hours in 0.002M 8-hydroxy quinoline at

temperature or 21 hours in water at 0°C was required for proper chromosome condensation and microtubule depolymerisation in cucurbits. Root tips have to be fixed for 48 hours at room temperature in 3:1 mixture of 95% ethanol and glacial acetic acid and stained for two or more days at room temperature in 0.7 per cent acetocaramine.

Induced polyploids in cucurbits showed comparatively low frequency of multivalents irrespective of the size of the chromosome (Singh, 1975), thus indicating that the presence of more than one pair of homologous chromosomes was not the only requisite for multivalent association and that probably the pairing was genetically controlled on account of which only bivalent association was observed in some pollen mother cells.

Feulgen stained chromosome, did not differ by their banding pattern from acetocarmine stained chromosome, but the former ones were less intensively stained (Turkov et al. 1974). They also reported that by comparing the pattern of differential staining of chromosomes all the seven pairs of chromosomes could be identified in Cucumis sativus.

Singh and Yadava (1984) observed 24 bivalents in Cucumis pustulatus and C.membranifolius whereas a few

quadrivalents were recorded in C.meeusei and C.heptadactylus which pointed out their allo or segmental allopolyploid nature. At metaphase-I colchiploids derived from Cucumis melo var. momordica and C. prophetarum showed univalents and multivalents along with normal bivalents. The mean chiasma frequency was almost identical in natural polyploids. Ramachandran and Narayanan (1985) found that the total nuclear DNA content of chromosomes in Cucumis varied from 1.373 to 2.483 picogram in diploids and 2.846 to 3.886 picogram in tetraploids. Yadava et al. (1986) reported that chromosome association in the amphidiploids of cucurbits showed a number of multivalent association besides bivalents and univalents.

In-vitro techniques

Experiments done by White (1934, 1939), Gautheret (1938), Nobecourt (1939), Reinert (1958), Steward et al. (1958) and Movel (1960) were often referred to as land marks in the history of in vitro techniques in crop improvement. First major completely defined medium for in vitro culture was developed by Murashige and Skoog (1962).

The technique of artificial culturing of embryos had been employed in growing interspecific hybrids, intergeneric hybrids, monoploids and polyploids (Heinz, et al. 1977). Embryo culture technique had also been

reported as a way to produce viable mutants in several crop plants (Ghosh, 1982).

In Cucumis sativus plant regeneration was reported from axillary bud explants by Handley and Chambliss (1979). Callus differentiation from cotyledons, hypocotyl explants and root segments were also found to be successful in Cucumis sativus (Sekioka and Tanaka, 1981; Trulson and Shahin, 1986).

Several factors are found to be affecting the developmental pattern of embryoids in-vitro, such as osmolarity, carbon source, reduced nitrogen, growth regulators and culture conditions. (Ammirato, 1983).

Protoplast culture in Cucumis sativus was also found to be successful but plant regeneration was reported to be at very low frequency (Orczyk and Malepszy, 1985; and Trulson and Shahin, 1986).

According to Wehner and Locy (1981) the differential abilities of cucumber in the culture media depend on the genotype. Plant regeneration from leaf callus (Malepszy and Orczyk, 1983); differentiation of meristemoids and embryos from anther culture (Lazarte and Sassar, 1982), and hypocotyl callus (Rajasekharan et al., 1963; Ziv and

Gadasi, 1986) were also reported. Ziv and Gadasi (1986) also reported that eventhough plants were produced successfully, abnormal somatic embryos and shoots often resulted in the medium Wehner and Locy (1981) showed a low yield of shoots (12% of explants produced 2 shoots) accompanied by callus production from cotyledon explants.

According to Kim et al. (1988) callus growth occurred on the peripheral zone of expanding cotyledon fragments placed in MS medium supplemented with various concentrations of auxin (2,4-D and NAA) and cytokinin (BAP and kinetin). The growth rates of different cultivars of cucumber were found to be different.

Gambley and Dodd (1990) used seeds of Cucumis sativus, after surface sterlisation with sodium hypochlorite solution, for cotyledon culture in MS medium modified with different concentrations of cytokinins, 6-benzyl aminopurine (BAP), kinetin and N^6-(2-isopentyl) adenine (2iP), and auxins α-naphthalene acetic acid (NAA) or Indol Acetic Acid (IAA). When cotyledons are grown on the kinetin medium for 16 days as pre treatment before dissection, the basal region yielded a mean of 51 shoots after 7 days. The plantlets obtained using this technique was successfully grown in pots in the glass house.

MATERIALS AND METHODS

MATERIALS AND METHODS

The investigation was carried out with a view to standardise an effective technique for the induction of polyploidy in Cucumis sativus under in vivo and in vitro conditions. The study was designed as two experiments. Experiment I (in - vivo studies) was carried out with a objective of studying the effect of colchicine for inducing polyploidy in seed, seedling and apical bud of Cucumis sativus. Experiment II (in - vitro studies) was carried out for standardising the medium for embryo culture of Cucumis sativus. The study also envisaged the observations on the effect of colchicine on proembryos, embryo of mature seed and embryo of dry seeds in in - vitro treatments.

Materials

Varieties

The varieties used for the investigation were 'Seethal' and 'Delila'. The seeds of both varieties were received from Department of Agricultural Botany, College of Agriculture, Vellayani.

Materials used for colchicine treatment

In Experiment I seeds, seedling (just sprouted seeds) and apical bud (apical buds of the seedlings at two leaf stage) were subjected to colchicine treatment.

In Experiment II embryos from premature seeds, mature seeds and dry seeds were subjected to colchicine treatment and used for inoculation for culture.

Colchicine

Colchicine, an alkaloid derived from the plant Colchicum autumnale was used as a polyploidising agent for both the experiments.

In Experiment I the concentrations of colchicine used were 0.2, 0.3 and 0.4 per cent. It was reduced to 1/10th strength for in - vitro treatments in Experiment II as 0.02, 0.03 and 0.04.

Medium

Experiment II, the in - vitro study was conducted using modified Murashige and Skoog (MS) medium the composition of which is given in Appendix-I.

Surface sterilising agent

In Experiment II, the seeds were surface sterilised with 0.1 per cent mercuric chloride solution for 12 minutes before excising the embryos.

Glass wares

Steam sterilised 150 ml, 100 ml and 50 ml Erlenmeyer conical flasks and 50 ml and 100 ml tubes were used for culturing the embryos.

Methods

Experiment I was mainly a field study laid out in Randomised Block Design (RBD) for both the varieties.

Experiment I

Design = R B D

No. of treatments = 27

No. of replication = 2

Treatments

In Experiment I treatments of colchicine concentration, mode of treatment and period of treatment were fixed as follows.

Colchicine concentration

c_1 = 0.2 %

c_2 = 0.3 %

c_3 = 0.4 %

Mode of treatment

m_1 = seed treatment

m_2 = seedling treatment

m_3 = apical bud treatment

Period of treatment

t_1 = 2 hours

t_2 = 4 hours

t_3 = 6 hours

Treatment combinations

$c_1 m_1 t_1$	$c_2 m_1 t_1$	$c_3 m_1 t_1$
$c_1 m_1 t_2$	$c_2 m_1 t_2$	$c_3 m_1 t_2$
$c_1 m_1 t_3$	$c_2 m_1 t_3$	$c_3 m_1 t_3$
$c_1 m_2 t_1$	$c_2 m_2 t_1$	$c_3 m_2 t_1$

$C_1 M_2 T_2$	$C_2 M_2 T_2$	$C_3 M_2 T_2$
$C_1 M_2 T_3$	$C_2 M_2 T_3$	$C_3 M_2 T_3$
$C_1 M_3 T_1$	$C_2 M_3 T_1$	$C_3 M_3 T_1$
$C_1 M_3 T_2$	$C_2 M_3 T_2$	$C_3 M_3 T_2$
$C_1 M_3 T_3$	$C_2 M_3 T_3$	$C_3 M_3 T_3$

The treatments were same for both varieties viz. Seethal and Delila.

Pre soaking of seeds

Fully matured undamaged seeds of both varieties were taken, cleaned and then rinsed with distilled water. These seeds were then presoaked in distilled water for ten hours before colchicine treatment.

Seed treatment

Solutions of 0.2, 0.3 and 0.4 per cent were prepared by dissolving required quantities of colchicine in 5 to 10 drops of ethyl alcohol and then made up to the required volume by adding distilled water. The presoaked seeds were then soaked in these solutions seperately for durations two, four and six hours.

After the treatment, seeds were washed thoroughly in distilled water and then sown in polybags filled with 1:1:1 (soil:sand:cowdung) potting mixture. Three seeds were sown in each polybag.

Seedling treatment

Untreated seeds were spread in petri dishes containing moistened cotton and kept for germination. When the radicle came out and attained a length of about one centimeter, the entire material was covered with cotton and then soaked with solutions of colchicine at different concentrations with two replications for different durations as fixed.

Apical bud treatment

Untreated seeds were allowed to grow up to two leaf stage in polybags filled with 1:1:1 potting mixture. The apical buds of these seedlings were then covered with small cotton and then soaked with colchicine solutions. The period of treatment and concentrations of colchicine solutions were as per the treatments fixed.

After treatments these plant materials were thoroughly rinsed in distilled water and planted carefully in polybags with out causing any damage to growing points.

Experiment II

This experiment consisted of an in - vitro laboratory study in Completely Randomised Design (CRD) for both varieties.

Design = C R D

No. of treatments = 27

No. of replications = 3

Different treatments fixed were

Colchicine concentration

c_1 = 0.02 %

c_2 = 0.03 %

c_3 = 0.04 %

Type of embryo

e_1 = pro embryo

e_2 = embryo of mature seed

e_3 = embryo of dried seed

Day of treatment

d_1 = treatment on the same day of inoculation

d_2 = treatment on the second day of inoculation

d_3 = treatment on the third day of inoculation

Treatment combinations

$c_1 e_1 d_1$	$c_2 e_1 d_1$	$c_3 e_1 d_1$
$c_1 e_1 d_2$	$c_2 e_1 d_2$	$c_3 e_1 d_2$
$c_1 e_1 d_3$	$c_2 e_1 d_3$	$c_3 e_1 d_3$
$c_1 e_2 d_1$	$c_2 e_2 d_1$	$c_3 e_2 d_1$
$c_1 e_2 d_2$	$c_2 e_2 d_2$	$c_3 e_2 d_2$
$c_1 e_2 d_3$	$c_2 e_2 d_3$	$c_3 e_2 d_3$
$c_1 e_3 d_1$	$c_2 e_3 d_1$	$c_3 e_3 d_1$
$c_1 e_3 d_2$	$c_2 e_3 d_2$	$c_3 e_3 d_2$
$c_1 e_3 d_3$	$c_2 e_3 d_3$	$c_3 e_3 d_3$

Ten culture tubes were used for each treatment so as to get sufficient number of tubes after possible contaminations.

Pro-embryos

Immature fruits were collected from plants in the field about one week before its maturity. From these fruits, the seeds were seperated out, cleaned and washed thoroughly in running water. These seeds were again washed thoroughly in distilled water and then surface sterilised with 0.1 per cent mercuric chloride solution. Embryos from these seeds were excised out and used as proembryo.

Mature embryo

Seeds were seperated out from fully mature fruits, cleaned and washed thoroughly. These seeds were again washed well in distilled water and surface sterilised by soaking in 0.1 per cent mercuric chloride solution for 12 minutes and washed thoroughly in sterile water. Embryos from these seeds were used as mature embryos.

Embryo of dry seed

Seeds seperated out from fully matured fruits were dried under sun for two to three days. The seeds were surface sterilised by 0.1 per cent merpcuric chloride solution and embryos from these seeds were used as embryos of dry seed.

The surface sterilised seeds were kept inside laminar air flow chamber and subjected to UV irradiation for 20 minutes before culture.

Extraction of embryos

The sterilised seeds kept inside the laminar air flow chamber were split open by sterile scalpel and forceps and the embryos were seperated out along with a small rim of endosperm. Same method of extraction was followed for proembryos, mature embryos and embryos of dried seeds.

Culture medium

Standardisation of culture medium

MS medium was selected as the basal medium for embryo culture. To standardise the best combination the basal medium was supplemented with different concentrations of Indol Acetic Acid (IAA) and Kinetin as follows.

IAA = 0.05, 0.1 and 0.2 mg/L

Kinetin = 0.05, 0.1 and 0.2 mg/L

Medium supplemented with 0.1 mg/L of IAA was selected as the medium for the present study (see Appendix - I).

Inoculation of embryo

The excised embryos were used for inoculation in tubes/flasks containing 15 to 25 ml of medium in laminar air flow chamber in a sterile condition. Immediately after inoculation the cultured tubes/flasks were arranged in racks under flourescent lamps at 1000 lux.

Colchicine treatment

Colchicine treatment was carried out at three levels.

1. **On the same day of inoculation**

The pro-embryos, mature embryos and embryos of dried seeds were treated with the polyploidising agent in specified concentrations in conical flasks. The materials were soaked in colchicine solutions for 30 minutes with intermittent shaking. The treated materials were taken out, washed thoroughly in double distilled steam sterilised water and then inoculated on the medium.

2. **Treatment two days after inoculation**

The excised embryos were cultured properly in the standardised medium as presented earlier and allowed for incubation. After two days of culturing, the explants were

taken out from the culture media and washed thoroughly in sterile distilled water to remove traces of media. These explants were treated with respective concentrations of colchicine solutions for 30 minutes as described above and then planted in fresh media after thorough washing with double distilled sterile water.

3. Treatments three days after inoculation

The cultured plant materials were allowed to grow for four days in the media and then taken out, washed thoroughly with sterile distilled water and treated with colchicine for 30 minutes as done earlier. After colchicine treatment explants were washed with double distilled water, replanted in a fresh medium and allowed to grow.

Hardening of embryo culture plants

The explants were allowed to grow in the medium up to four leaf stage. The plants were then taken out, washed thoroughly with double distilled sterile water, 0.1 per cent Bavistin solution and transfered to polybags filled with sterile potting mixture. These plants were kept inside the lab for 7 to 10 days for hardening.

Planting in the main field

In Experiment I, seedlings were allowed to grow in the polybags up to four leaf stage and then transferred to the experimental plot laid out according to the design. Two plants were allowed to grow per pit. Two seedlings with out any treatment were planted in a pit in each replication as control.

For Experiment II the polybagged plants after proper hardening were transferred to green house and the regeneration percentage was recorded.

Observations

Detailed observations on the following characters were taken to evaluate the effect of different treatments in both experiments.

Experiment I

Observations were taken from both plants retained in each pit. Their mean value was calculated and recorded as the treatment value for tabulation.

Survival percentage at four leaf stage

After colchicine treatment the seedlings were allowed tp grow in polybags up to four leaf stage.

Observations were then taken to record the number of seedlings that survived with out any damage and percentage calculated.

Length of vine

The length of vine of plants was measured from the base to the growing tip of longest vine in each treatment at 30 and 60 days after planting.

Number of branches per plant

At 30 and 60 days after planting total number of branches in the two plants in a pit was recorded and number of branches per plant recorded.

Number of leaves per plant

The number of leaves per plant was recorded at 30 and 60 days after planting.

Number of days taken for first male flower opening

Observations on number of days taken for first male flower opening were made for each plant in the field.

Number of days taken for first female flower opening

Observations on number of days taken for the first female flower opening were recorded for each plant.

Number of male flowers per plant

Number of male flowers opened in each plant was recorded daily and the total number of male flowers produced per plant was calculated.

Number of female flowers per plant

Number of female flowers opened in each plant was recorded daily and the total female flowers produced per plant was calculated.

Stomatal length

The length of ten randomly selected stomata was measured using a standard ocular micrometer and the mean was recorded.

Number of fruits per plant

The total number of fruits in each plant was recorded.

Length of fruit

The length of each fruit from each plant was taken in centimeter and mean value was estimated.

Girth of fruit

The girth in centimeter of each fruit from each plant was measured and mean value was estimated.

Weight of fruit

Each fruit from each plant was seperately weighed and the mean weight of fruit was estimated for each treatment.

Pollen size

The size of pollen was determined by measuring the diameter using standard ocular micrometer. Diameter of twenty fertile pollen grains per treatment was measured and recorded as mean value.

Pollen sterility

Anthers from flower buds were collected from plants in each treatment and tapped in a drop of acetocarameine stain on a clean slide. The pollen grains were examined under low power. Pollen sterility was

estimated by counting the fertile and sterile pollen grains seperately from twenty microscopic fields per treatment. The sterility was expressed as percentage.

Cytological observations

Root tips of colchicine treated seeds were collected for cytological studies. Root tips were excised at 9.15 AM and fixed in carnoy's I fluid and then preserved in 70 per cent ethyl alcohol. The fixed materials were hydrolysed in 0.1 N HCl for 30 minutes at 60°c, put in Feulgen stain for 30 to 40 minutes and then in 2% acetocaramine for 10 to 12 minutes for proper staining, squash preparations were made and slides observed under high power (40x) of the microscope for cytological studies.

Experiment II

Number of days taken for embryogenesis

Embryos were inoculated in three stages of growth and observations were made on number of days taken to start embryogenesis.

Regeneration percentage

Observations were taken on total number of embryos showing regeneration in each treatment and percentage calculated.

Number of leaves produced

After 45 days of inoculation number of leaves produced by each plantlet was recorded.

Length of plantlets 45 days after treatment

After 45 days of treatment length of each plantlet in the culture tubes/flasks were measured and recorded.

Other morphological features

The morphological features shown by the developing calli like flowering in the culture tubes, abnormal growth behaviour etc. were recorded.

Survival percentage under green house condition

The embryo cultured plants were transferred to green house on attaining four leaf stage. After subjecting it to proper hardening observations were taken on the number of plants survived in each treatment and expressed as percentage.

RESULTS

RESULTS

Experiment I

The results of the *in-vivo* studies conducted on *Cucumis sativus* with an objective of analysing the effect of colchicine on survival and morphological characters of the plants are presented below.

Survival percentage

The data on percentage of survival of plants in the variety 'Seethal' at two weeks after colchicine treatment are presented in Table-1 and Fig.1.

Compared to control, treated plants showed significantly low survival percentage. Colchicine concentration, mode of treatment and duration of treatment exerted significant influence on the survival of plants. The survival percentage decreased progressively from 82.78 per cent to 55.97 per cent as colchicine concentration increased from 0.2 (C_1) to 0.4 (C_2) per cent. Among different modes of treatment seed treatment (m_1) recorded significantly high survival percentage (71.75) over seedling treatment (m_2) and apical bud treatment (m_3). Survival of

plants also decreased progressively with increase in the period of colchicine treatment. There was significant reduction in the survival of plants from 69.22 to 60.11 as the period of treatment increased from two to six hours. Effect of four hours treatment was found to be on par with the effect of two hours treatment.

Data on interaction effect of colchicine concentration and mode of treatment with respect to variety Seethal are given in Table-2. Interaction effect of colchicine concentration and mode of treatment was found to be significant. Highest value (83.00) and lowest value (43.58) were recorded for seed treatment of colchicine at 0.2 per cent ($c_1 m_1$) and apical bud treatment of colchicine at 0.4 per cent concentration ($c_3 m_3$) respectively.

Interaction effects of colchicine concentration with period of treatment and mode of treatment with period of treatment on the variety Seethal are presented in Table-3 and 4 respectively. The effects were not significant.

Data on survival percentage in the three factor interaction of treatments in the variety Seethal are given in Appendix II.

Among the different interaction of treatments the highest value (90.50) for survival percentage was recorded

with 0.2 per cent colchicine concentration given as seedling treatment for two hours period ($c_1 m_2 t_1$) and lowest value (39.00) for survival percentage was recorded in the treatment of 0.4 per cent colchicine given as apical bud treatment for six hours ($c_3 m_3 t_3$).

The data on survival percentage of seedlings two weeks after colchicine treatment in Delila are presented in Table-9 and Fig.1.

Untreated control plants showed significantly higher survival percentage (99.50) compared to the treated plants.

As colchicine concentration increased from 0.2 per cent (c_1) to 0.4 per cent (c_3) significant reduction in survival percentage, from 85.53 to 59.56, was noticed. Among different modes of treatment, seed treatment (m_1) recorded highest value (74.03) which was on par with seedling treatment (m_2). Effect of apical bud treatment (m_3) was significantly low (68.92). With increasing period of treatment from two hours (t_1) to six hours (t_3) significant reduction from 76.67 to 66.92 per cent was noticed in the survival of plants in Delila.

Data depicted in Table-10 revealed that interaction effect of colchicine concentration and mode

Compared to control, treated plants showed significantly reduced length of vine at 30 days of growth stage.

Significant variation was observed for the treatments only at 30 days of growth stage. There was a progressive reduction in the length of vine at 30 days stage of growth associated with progressive increase in colchicine concentration. At 0.2 per cent colchicine concentration the vine length at 30 days growth was maximum (106.16), which was significantly higher than the values recorded for 0.3 and 0.4 per cent concentration. Highest value (102.28) for length of vine was recorded in seed treatment (m_1) which was significantly higher than the values recorded for seedling and apical bud treatments. Among the periods of treatment, two hours treatment of colchicine recorded the maximum vine length (100.54) which was significantly higher than that of six hours' colchicine treatment. At 60 days stage there was no significant difference among the treatments for vine length.

Data on interaction effect of colchicine and mode of treatment on the variety Seethal are given in Table-2. At 30 days growth stage in Seethal highest value (112.33) for length of vine was recorded for 0.2 per cent colchicine concentration given as seed treatment ($c_1 m_1$) and the lowest

value (82.92) was recorded for 0.4 per cent colchicine concentration given as apical bud treatment ($c_2 m_3$). At 60 days stage there was no significant difference for vine length among the treatments.

The interaction of colchicine concentration and period of treatment produced significant effect only at 30 days stage as indicated in the data given in Table-3. Treatment of 0.2 per cent colchicine for four hours ($c_1 t_2$) produced maximum vine length (112.41) at 30 days stage of growth. Vine length was reduced to minimum (87.37) in the treatment of colchicine, 0.4 per cent given for six hours duration ($c_2 t_3$).

Data depicted on Table-4 showed the interaction effect of mode of treatment with period of treatment, on length of vine in the variety Seethal. At 30 days of growth stage maximum length of vine (108.83) was recorded for seed treatment was given for four hours. Vine length was minimum (84.73) when apical bud treatment was given for six hours. At 60 days stage the treatments did not show any significant difference for vine length.

Among the treatment combinations as presented in Appendix II seed treatment of 0.2 per cent colchicine for four hours ($c_1 m_1 t_2$) recorded the highest vine length (119.00)

and apical bud treatment of colchicine at 0.4 per cent level for four hours ($c_3 m_3 t_2$) recorded lowest vine length (65.25) at 30 days stage of growth. At 60 days growth stage the three factor interaction effects were not significant.

The data on effect of treatments on length of vine in Delila are depicted in Table-9 and Fig.2.

At 30 days of growth stage treated plants produced significantly reduced vine length compared to the control plants. But at 60 days of growth there was not significant difference between the treated and control plants.

With increasing concentration of colchicine from 0.2 to 0.4 per cent, vine length reduced significantly from 94.88 to 54.88 cm. Among modes of treatment, seedling treatment (m_2) recorded lowest value (69.37) which was on par with apical bud treatment (m_3) and highest value (78.33) was recorded by seed treatment. When period of treatment was increased from two to six hours progressive decrease in vine length was noticed from 77.86 to 68.30 cm. At 60 days of growth stage there was no significant difference among the treatments.

Interaction effects of colchicine concentration with mode of treatment were presented as in Table-10. The effects were significant at 30 days growth stage but were

not significant at 60 days growth stage. Seed treatment with 0.2 per cent colchicine ($c_1 m_1$) recorded highest length of vine (105.33 cm) and lowest value (50.12 cm) was recorded by seedling treatment at 0.4 per cent colchicine concentration ($c_3 m_2$). At 60 days of growth stage the effects were not significant.

Data presented on Table-11 and 12 revealed that interaction effect of colchicine concentration with period of treatment and mode of treatment with period of treatment was not significant with respect to variety Delila in 30 and 60 days of growth stages.

Three factor interaction of treatments (Appendix IV) was also significant in Delila at 30 days of growth stage. Colchicine at 0.2 per cent given for two hours by seed treatment ($c_1 m_1 t_1$) produced highest (116.00 cm) and 0.4 per cent colchicine concentration by seedling treatment for four hours produced lowest (40.50 cm) vine length. Here also at 60 days of growth stage the interaction effects were insignificant.

Number of branches

The data on number of branches in the variety Seethal at 30 and 60 days of growth stages are presented in Table-1.

Compared to control, colchicine treated plants produced significantly lower number of branches at 30 days growth stage. At 60 days stage there was no significant difference between the treated and control plants.

As colchicine concentration increased from 0.2 to 0.4 per cent the number of branches per plant reduced progressively and significantly from 10.78 to 6.95. Seed treatment produced the highest number (9.64) of branches as compared to apical bud treatment which recorded the lowest value (7.51). Four hours of colchicine treatment (t_2) produced maximum number of branches (9.38) which was on par with two hours of treatment. Six hours of colchicine treatment produced minimum number of branches (7.86). The effect of treatments was found to be insignificant at 60 days of growth stage (see Fig.3).

Interaction effect of colchicine concentration with mode of treatment on this parameter in Seethal showed significant variation only during 30 days of growth stage as shown in Table-2. Colchicine at 0.2 per cent given as seedling treatment ($c_1 m_2$) produced highest number (11.40) of branches and 0.4 per cent colchicine given as apical bud treatment ($c_3 m_3$) produced lowest (4.40) number of branches

at 30 days growth stage. At 60 days growth stage the interaction effect was insignificant.

Data depicted on Table-3 revealed that interaction of colchicine concentration with period of treatment was not significant with respect to number of branches produced per plant at both the growth stages.

Interaction of mode of treatment with period of treatment as shown in Table-4 showed significant effect at 30 days of growth stage. Seedling treatment for 4 hours ($m_2 t_2$) produced highest number of branches (10.68) and was on par with seed treatment for two hours ($m_1 t_1$). Lowest value was recorded by apical bud treatment for six hours. At 60 days stage the interaction effect was not significant.

Data on the effect of various treatments on number of branches per plant in the variety Delila are presented in Table-9.

At 30 days of growth stage, compared to control, the treated plants produced significantly lower number of branches per plant (see Fig.3.). At 60 days stage the treatment effects were not significant.

Increase in the concentration of colchicine from 0.2 to 0.4 per cent produced significant fall in the number

of branches from 5.57 to 3.41 at 30 days of growth stage. Among the modes of treatment, seed treatment (m_1) recorded the highest value (5.17) and the apical bud treatment recorded the lowest value (4.34). Increase in the period of treatment from two to six hours produced significant decrease in this parameter from 5.06 to 3.99. No significant effect was noticed at 60 days of growth stage.

Interaction effect of colchicine concentration with mode of treatment given in Table-10. The effects were significant only at 30 days of growth stage. Seed treatment of colchicine at 0.2 per cent recorded highest (6.56) and seed treatment of colchicine at 0.4 per cent recorded lowest (2.88) value.

Data on interaction effects of colchicine concentration with period of treatment are given in Table-11. Colchicine at 0.2 per cent applied for two hours recorded the maximum value (6.40) and colchicine 0.4 per cent applied for a period of six hours recorded the minimum value (2.57). At 60 days of growth stage the effect was insignificant.

Interaction of mode of treatment and period of treatment are presented in Table-12. The interaction effects were significant. Seedling treatment for two hours ($m_2 t_1$)

recorded maximum number of branches (5.87) which was on par with seed treatment for two hours ($m_1 t_1$). Lowest number of branches was produced by seedling treatment for six hours ($m_2 t_3$) and was on par with apical bud treatment for six hours ($m_3 t_3$).

Three factor interaction of various treatments on number of branches in Delila are given in Appendix-IV. The interaction effect was found to be significant only at 30 days of growth stage. Seed treatment of colchicine 0.2 per cent for a period of two hours recorded maximum (7.30) and seedling treatment of colchicine at 0.4 per cent for four hours recorded the minimum (2.40) values for number of branches per plant.

Number of leaves per plant

The data on number of leaves per plant in the variety Seethal at the two growth stages are given in Table-1 (see Fig.4).

In Seethal, the different treatments exerted significant effect on the production of leaves per plant at 30 days growth stage. Treated plants showed significantly lower number of leaves compared to control. Colchicine concentration, mode of treatment and period of treatment produced significant influence. Mean number of leaves per

plant reduced considerably from 62.71 to 38.52 as colchicine concentration increased from 0.2 (c_1) to 0.4 per cent (c_3). Seedling treatment (m_2) produced maximum number of leaves per plant (57.54) and it was significantly superior to apical bud treatment (m_3). Period of treatment also exerted significant influence by reducing the number of leaves produced per plant. Number of leaves from 60.14 to 42.57 as the period of treatment increased from two to six hours. At 60 days of growth stage various treatments did not exert any significant influence on this parameter.

Data on Table-2 revealed that interaction of colchicine concentration and mode of treatment had significant influence during 30 days growth stage in Seethal. At 30 days of growth stage seedling treatment with 0.2 percent colchicine ($c_1 m_2$) recorded highest value (76.37) whereas apical bud treatment with 0.4 percent colchicine produced lowest value (31.08) with respect to this parameter.

Data on interaction effects of colchicine concentration with period of treatment in respect of this parameter in Seethal are depicted in Table-3. Here also only at 30 days of growth stage, the effect produced was significant. As colchicine concentration and period of treatment were increased the number of leaves produced per

plant was significantly reduced. Treatment of colchicine 0.2 per cent for two hours ($c_1 t_1$) recorded the highest (71.42) and colchicine 0.4 per cent treatment for six hours ($c_3 t_3$) recorded the lowest (28.82) values.

Interaction of mode of treatment with period of treatment did not exert any significant influence at both the growth stages (Table-4).

Among the treatment interactions (Appendix-II) also significant variation was observed at 30 days of growth stage (Fig.4). Highest (88.50) and lowest values (27.25) were recorded at $c_1 m_2 t_1$ and $c_3 m_3 t_3$ respectively.

Data on number of leaves produced per plant in Delila are presented in Table-9. Compared to control treated plants produced significantly lower number of leaves per plant at 30 days growth stage. At 60 days of growth stage the treatment effect were not significant.

The number of leaves per plant was decreased with increased levels of colchicine concentration. As colchicine level increased from 0.2 to 0.4 per cent, the number of leaves decreased from 67.79 to 42.83. Apical bud treatment (m_3) recorded the lowest value of 56.27 followed by seed treatment (m_1) and seedling treatment (m_2). As period of treatment increased from two to four hours, number of leaves

per plant significantly decreased from 72.24 to 48.93. But different treatments did not exert any significant influence on this parameter at 60 days of growth stage.

Data on interaction effect of colchicine concentation with mode of treatment in Delila are presented in Table-10. Colchicine at 0.4 per cent level given as apical bud recorded the lowest number (39.92) of leaves and seedling treatment of colchicine 0.2 per cent produced the highest number (73.05) of leaves. No significant response was noticed at 60 days of growth stage.

Interaction effects of colchicine concentration with period of treatment are presented in Table-11. The treatments were significant only at 30 days stage. Colchicine 0.2 per cent treated for two hours recorded maximum number of leaves (85.66) and colchicine 0.4 per cent treated for six hours recorded minimum number of leaves (40.11).

Interaction of mode of treatment and period of treatment was also significant as presented in Table-12. At the 30 days growth stage the effect was significant. Apical bud treatment with 0.4 per cent colchicine produced lowest number of leaves (45.00) and seed treatment of colchicine 0.2 per cent produced highest number of leaves (74.25). At 60 days of growth stage no significant effect was noticed.

Data presented on Appendix-IV revealed the significant effect of three factor interactions at 30 days stage of growth. In Delila, the apical bud treatment of colchicine 0.4 per cent for six hours recorded the lowest value of 16.00 and seed treatment of colchicine 0.2 per cent concentration for two hours recorded the highest value of 88.25.

Number of days taken for first flower opening

The data on number of days taken for first flower opening in the variety Seethal are depicted in Table-1.

Compared to control, the first flower opening was significantly delayed in the treated plants (see Fig.5).

The data revealed that both male and female flower opening was delayed significantly as colchicine concentration increased from 0.2 to 0.4 per cent. Mode of treatment did not influence the opening of male flowers but influenced the opening of female flowers. Female flower opening was delayed to the maximum (36.89 days) in the apical bud treatment and the earliest flower opening (34.78 days) was recorded in the seed treatment. Period of treatment also exerted significant influence on the first flower opening. Maximum number of days (36.94) was recorded

in the six hours treatment and minimum number of days (34.78) was recorded in the two hours treatment.

Data on interaction effect of colchicine concentration with mode of treatment on this parameter are given in Table-2. Here both male and female flower openning was delayed to a maximum (26.50 and 40.33 respectively) in the apical bud method treatment of colchicine at 0.4 per cent. Among treated plants minimum number of days were taken for male and female flower opening (23.17 and 32.33 days) in the seed treatment of colchicine at 0.2 per cent.

Interaction effect of colchicine concentration with period of treatment (Table-3) and mode of treatment with period of treatment (Table-4) were not significant with respect to the period taken for first male and female flower opening in the variety Seethal.

Among the interaction of all the treatments as shown in Appendix-II, apical bud treatment of colchicine 0.4 per cent for six hours took maximum days and seedling treatment of colchicine for four hours took minimum days for first male flower opening. For female flower opening in Seethal seedling treatment of colchicine 0.4 per cent for six hours took maximum days and apical bud treatment of colchicine 0.2 per cent for two hours took minimum days.

The data on mean effect of treatments on number of days taken for male and female flower opening in Delila are presented in Table-9 (see Fig.5).

Male and female flower opening was delayed significantly in colchicine treated plants when compared to untreated plants.

As colchicine concentration increased from 0.2 to 0.4 per cent, the male and female flower opening was delayed progressively from 20.81 to 25.67 days for the male flowers and 25.33 to 31.89 days for the female flowers. Mode of treatment had no significant influence with respect to this parameter. With the increase in the period of treatment from two to six hours, the number of days taken for male flower opening was also increased from 19.94 to 26.31 and the number of days taken for female flower opening was increased from 26.00 to 28.31 days.

Interaction effect of colchicine concentration with mode of treatment are depicted in Table-10. Apical bud treatment with 0.4 per cent colchicine concentration produced maximum delay in male and female flower opening whereas seed treatment with 0.2 per cent colchicine took minimum number of days for flower opening.

Interaction effect of colchicine concentration with period of treatment (Table-11) was also found to be significant on both male and female flowers. As colchicine concentration and period of treatment were increased both male and female flower opening were significantly delayed. Data on interaction effect of mode of treatment with period of treatment are presented in Table-12. Treating apical buds with 0.4 per cent colchicine produced maximum delay in both male and female flower opening (28.42 and 29.17 respectively). Whereas seed treatment for two hours recorded earliness for male and female flower opening (19.33 and 25.83 days respectively).

Three factor interactions of treatments (Appendix-IV) were also significant. Seedling treatment of 0.4 per cent colchicine for six hours ($c_3 m_2 t_3$) and apical bud treatment of 0.2 per cent colchicine for two hours ($c_1 m_3 t_1$) recorded maximum and minimum values respectvely with respect to male flower opening. For female flower opening, seedling treatment of 0.4 per cent colchicine for two hours ($c_3 m_1 t_1$) and apical bud treatment of 0.4 per cent colchicine for six hours ($c_3 m_3 t_3$) recorded lowest and highest values respectively.

Number of flowers produced per plant

The data on the total number of male and female flowers produced per plant in Seethal are depicted in Table-5.

Compared to control plants, colchicine treated plants produced lesser number of flowers per plant.

Significant decrease of both male and female flower number was noticed in Seethal with increasing levels of colchicine concentration from 0.2 to 0.4 per cent. Number of both the male and female flower decreased from 579.78 to 538.00 and 27.17 to 23.56 respectively. Mode of treatment did not exert any significant effect on this parameter. Least number of male and female flowers (511.44 and 23.06 respectively) were produced by six hour (t_3) colchicine treatment. For male flowers four hour treatment produced maximum number which was on par with the two hour treatment.

Data on interaction effect of colchicine concentration with mode of treatment on number of flowers in Seethal are given in Table-6 Minimum number of male flower (449.00) were producd when 0.4 per cent colchicine was given as seedling treatment (c_3m_2) and maximum number of male flowers (610.67)were produced when colchicine was given as

by 0.2 per cent level applied as seedling treatment ($c_1 m_2$). In the case of female flowers, 0.2 per cent seed treatment and seedling treatment ($c_1 m_1$ and $c_1 m_2$) recorded the highest (27.66) and 0.3 per cent and 0.4 per cent apical bud treatments ($c_2 m_3$ and $c_3 m_3$) recorded lowest (23.00) values.

Interaction of colchicine concentration with period of treatment (Table-7) was not significant for both male and female flowers per plant in Seethal. Number of flowers was not significantly influenced by the interaction of mode of treatment with period of treatment (Table-8).

The data on number of male and female flower produced per plant in Delila are presented in Table-13.

Compared to control plants, colchicine treated plants showed significant decrease in male and female flower production.

As colchicine concentration increased from 0.2 to 0.4 per cent, number of male flowers decreased from 450.67 to 328.75 and number of female flowers decreased from 16.78 to 14.06. Mode of treatment and period of treatment did not exert any significant influence on this parameter in Delila.

Data on interaction effect of colchicine concentration with mode of treatment are presented in

Table-14. Colchicine at 0.2 per cent level applied by seed treatment prodduced significantly higher number of both male and female flowers (507.50 and 20.00 respectively). Lowest number of male and female flowers (287.67 and 12.83 respectively) were noticed in 0.4 per cent colchicine as apical bud treatment ($c_1 m_3$) and seedling treatment ($c_3 m_1$).

Interaction of colchicine treatment with period of treatment showed significance only on number of male flowers (Table-15). Maximum male flower production (470.33) were noticed in 0.2 per cent seed treatment ($c_1 t_1$) and minimum (351.77) was noticed in 0.4 per cent apical bud treatment ($c_3 t_3$).

Data presented on Table-16 revealed that interaction of mode of treatment with period of treatment did not exert any significant influence on the production of male and female flowers in Delila.

Three factor interaction of different treatment on number of male and female flower production in Delila are given in Appendix-V. Maximum value (577.5) for number of male flowers per plant was recorded in $c_1 m_1 t_1$ and minimum (217.50) in $c_3 m_2 t_3$ level. In the case of female flowers per plant the treatments seed treatment of 0.2 per cent

colchicine for six hours ($c_1 m_1 t_3$) and seedling treatment of 0.4 per cent colchicine for six hours ($c_3 m_2 t_3$) recorded maximum (22) and minimum (11) respectively.

Stomatal length

Data on stomatal length in Seethal are given in Table-5 and Fig.8.

Colchicine treated plants produced comparatively large sized stomata than the control plants.

With increase in colchicine concentration from 0.2 to 0.4 per cent stomatal length increased from 0.106 to 0.127 mm. Mode of treatment showed no significant response. With increase in period of treatment from two to six hours, stomatal length increased from 0.119 to 0.126 mm.

Interaction effect of colchicine concentration with mode of treatment (Table 6) was also significant in Seethal. Highest stomatal length (0.129 mm) was noticed when 0.4 per cent colchicine concentration was given as seedling treatment ($c_3 m_2$) and it was on par with the apical bud treatment of colchicine 0.4 per cent. ($c_3 m_3$). Lowest value (0.097 mm) was recorded in the seed treatment of colchicine 0.2 per cent ($c_1 m_1$).

Data on interaction effect of colchicine concentration and period of treatment are given in Table-7. With progressive increase in colchicine concentration and period of treatment from c_1t_1 level to c_3t_3 level stomatal length progressively increased from 0.102 to 0.135 mm.

Interaction of mode of treatment and period of treatment in Seethal (Table-8) was not signifcant with respect to the stomatal length.

Data on the effect of treatments on stomatal length in Delila are given in Table-13 and Fig.8. Colchicine treated plants showed signficantly higher length of stomata compared to control plants.

As colchicine concentration increased from 0.2 to 0.4 per cent, stomatal length increased significantly from 0.103 to 0.130 mm. Mode of treatment showed no signficance on stomatal length in Delila. With progressive increase in period of treatment from two to six hours, stomatal length increased from 0.111. to 0.124 mm.

Interaction of colchicine concentration with mode of treatment (Table-14) showed signficant effect on stomatal length in Delila. Lowest value (0.088 mm) was noticed in seed treatment of 0.2 per cent colchicine (c_1m_1) and highest

value (0.133 mm) at apical bud treatment of 0.4 per cent colchicine ($c_3 m_3$).

With increasing colchicine concentration with period of treatment from $c_1 t_1$ to $c_3 t_3$ progressive increase in stomatal length from 0.092 to 0.134 mm was noticed in Delila (Table-15).

Data on interaction effect of mode of treatment with period of treatment are presented in Table-16. Seedling treatment for two hours ($m_2 t_1$) recorded lowest (0.104 mm) and apical bud treatment for six hours ($m_3 t_3$) recorded highest (0.132 mm) stomatal length.

Among the three interactions of all treatments (Appendix-V) seed treatment of 0.2 per cent colchicine for two hours ($c_1 m_1 t_2$) and apical bud treatment of 0.4 per cent colchicine for six hours ($c_3 m_3 t_3$) recorded lowest (0.090) and highest (0.165) values respectively for stomatal length.

Fruit characters

Total number of fruits produced per plant, length of fruit, girth of fruit and weight of fruit were studied in present investigation as fruit characters. Data on fruit characters in Seethal are presented in Table-5. Data on interaction effect of colchicine concentration with mode of treatment (Table-6), colchicine concentration with period of

treatment (Table-7) mode of treatment with period of treatment (Table-8) and three factor interaction of treatment (Appendix-III) are also given. It was noticed that all the treatment effects including main and interaction effect did not exert any significant influence on various fruit characters in Seethal.

Main and interaction efects of treatments on fruit characters in Delila are given Tables 13, 14, 15 ,16 and Appendix-V. It was noticed that in Delila also main and interaction effect of treatments did not exert any significant influence on various fruit characters.

Pollen characters

The data on pollen characters viz., pollen size and pollen sterility percentage on Seethal are presented in Table 5 (see Fig.6 and 7).

Control plants showed significant variation from treated plants with respect to pollen characters in Seethal. Siginificantly bigger pollen grains with high sterility were produced by treated plants compared to untreated control plants.

With progressive increase in colchicine concentration from 0.2 to 0.4 per cent pollen size increased

from 0.058 to 0.069 mm in Seethal. At the same time, pollen sterility percentage increased from 28.74 to 49.19. Apical bud treatment gave higher pollen size (0.066 mm) compared to other two modes of treatment. Mode of treatment did not exert any influence on pollen sterility. With increasing period of treatment, size and sterility also exhibited increase.

Data on interaction of colchicine concentration and mode of treatment in Seethal are given in Table 6. The interaction exerted significant influence only in pollen size and not in pollen sterility. Apical bud treatment of 0.4 per cent colchicine (c_3m_3) recorded maximum pollen size (0.072 mm) and seed treatment of 0.2 per cent colchicine (c_1m_1) recorded the minimum value (0.054 mm).

Interaction of colchicine concentration with period of treatment (Table-7) exerted significant influence in both pollen size and sterility characters. Minimum values for pollen size and pollen sterility percentage (0.051 mm and 22.32%) were noticed in colchicine treatment at 0.2 per cent for a period of two hours (c_1t_1) and it increased progressively to 0.073 mm pollen size and 59.85 sterility percentage in the treatment combination of 0.4 per cent colchicine given for six hours (c_3t_3).

seedling treatment for two hours (m_2t_1) and highest value (54.47%) was recorded in the apical bud treatment for six hours (m_3t_3).

Among interaction of all treatments (Appendix-V) 0.2 per cent seed treatment for two hours ($c_1m_1t_1$) and 0.4 per cent apical bud treatment for six hours ($c_3m_3t_3$) recorded lowest (0.030 mm) and highest (0.053 mm) values respectively with respect to pollen size. Lowest pollen sterility (11.5%) and highest pollen sterility (68.15%) were noticed at apical bud treatment of 0.2 per cent colchicine for two hours ($c_1m_3t_1$) and 0.4 per cent colchicine given as apical bud for six hours ($c_3m_3t_3$).

Cytological observation

Cytological studies were carried out in different seed treatments. Presoaked seeds were treated with colchicine concentration of 0.2, 0.3 and 0.4 per cent for two, four and six hours. Swelling of root tips was observed in all treatment. Root tips of about 0.5 cm were cut from the sprouted seeds of different treatments fixed in carnoy's I fluid and preserved in 70 per cent alcohol were hydrolysed using 1N HCl for 30 minutes at 60° C. Root tips were put in Feulgen stain for 30 to 40 minutes and then in 2 per cent acetocaramine for 10 to 12 minutes. Squash preparations were made and slides were observed under the microscope. Much enlarged cells were observed along with the normal

Table 1 Effect of colchicine concentration, mode of treatment and period of treatment on survival and morphological characters upto flower opening in Seethal

Treatments	Survival percentage	Length of vine (cm)		No of branches per plant		No of leaves per plant		Days taken to male flower opening	Days taken to female flower opening
		30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP		
c_1	82.78	106.16	134.22	10.78	18.84	62.71	262.39	24.30	34.50
c_2	57.44	94.84	136.25	8.61	17.99	51.37	260.82	25.56	34.61
c_3	55.97	88.72	133.39	6.95	17.16	38.52	260.49	28.28	38.44
CD	6.10	3.98	NS	0.75	NS	2.29	NS	1.20	1.15
m_1	71.75	102.28	135.75	9.64	17.03	55.57	257.60	25.06	34.78
m_2	61.86	96.74	133.61	9.81	18.63	57.54	260.51	25.50	35.89
m_3	62.58	90.79	136.49	7.51	16.32	39.08	259.19	24.28	36.89
CD	6.10	3.98	NS	0.75	NS	2.29	NS	NS	1.15
t_1	69.22	100.54	134.94	9.09	17.60	60.14	259.93	24.33	34.78
t_2	66.86	98.41	136.82	9.38	18.22	49.89	257.33	24.33	35.83
t_3	60.11	90.77	134.09	7.86	18.17	42.57	26.44	26.17	36.94
CD	6.10	3.98	NS	0.75	NS	2.29	NS	1.20	1.15
Treatment mean	66.61	95.41	135.93	9.00	18.19	52.86	261.31	24.79	35.63
Control	99.25	120.00	139.49	15.00	20.11	86.95	263.90	20.50	29.50
CD	9.14	5.97	NS	1.12	NS	3.44	NS	1.80	1.73

CD - At 5% level NS - Not Significant

Table 3 Interaction effect of colchicine concentration and period of treatment on survival and morphological characters upto flower opening in Seethal

Treatment interactions	Survival percentage	Length of vine (cm)		No of branches per plant		No of leaves per plant		Days taken to male flower opening	Days taken to female flower opening
		30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP		
$c_1 t_1$	86.67	103.70	134.92	11.42	19.60	71.42	259.81	24.16	34.00
$c_1 t_2$	83.50	112.41	135.05	11.20	19.01	60.67	262.11	23.83	34.17
$c_1 t_3$	78.17	102.35	132.68	9.72	17.92	56.03	265.25	24.60	35.33
$c_2 t_1$	58.58	93.25	135.00	8.78	17.16	61.00	261.58	24.00	34.83
$c_2 t_2$	58.08	90.33	140.17	9.02	19.17	50.25	258.00	25.33	34.50
$c_2 t_3$	55.67	32.58	139.58	6.02	17.62	42.87	269.06	27.00	34.50
$c_3 t_1$	62.41	104.67	134.91	7.08	18.08	48.00	265.38	24.00	35.50
$c_3 t_2$	59.00	92.48	135.25	7.93	18.47	38.75	251.68	23.83	38.83
$c_3 t_3$	46.50	87.37	130.00	5.83	18.98	28.82	265.00	27.50	41.00
CD	NS	6.90	NS	NS	NS	3.96	NS	NS	NS

CD - At 5% level NS - Not significant

Table 5 Effect of colchicine concentration, mode of treatment and period of treatment on morphological characters from number of flowers to pollen characters in Seethal

Treatment	No. of male flowers per plant	No. of female flowers per plant	Stomatal length (mm)	No. of fruits per plant	Length of fruit (cm)	Girth of fruit (cm)	Weight of fruit (g)	Pollen size (mm)	Pollen sterility (%)
c_1	579.78	27.17	0.106	3.94	40.94	27.93	1384.72	0.058	28.74
c_2	571.61	23.89	0.117	3.11	40.69	27.42	1408.61	0.066	42.83
c_3	538.00	23.56	0.127	3.88	41.82	28.53	1366.11	0.069	49.19
CD	30.35	1.31	0.004	NS	NS	NS	NS	0.002	3.02
m_1	544.83	25.28	0.117	4.22	43.12	28.34	1291.67	0.062	41.08
m_2	575.61	25.28	0.118	3.44	42.27	29.13	1313.33	0.063	38.42
m_3	568.94	24.06	0.121	3.28	38.06	29.42	1334.35	0.066	41.26
CD	NS	NS	NS	NS	NS	NS	NS	0.002	NS
t_1	581.33	27.00	0.119	4.17	42.67	29.26	1256.78	0.061	38.01
t_2	596.61	24.56	0.119	3.28	41.44	29.31	1251.11	0.064	35.83
t_3	511.44	23.06	0.126	3.50	39.94	30.31	1315.56	0.068	46.92
CD	30.35	1.31	0.004	NS	NS	NS	NS	0.002	3.02
Treatment mean	569.96	25.16	0.120	3.77	41.08	28.69	1315.45	0.062	39.28
Control	652.15	33.00	0.089	4.00	39.25	30.11	1238.25	0.042	11.95
CD	45.53	1.96	0.006	NS	NS	NS	NS	0.003	4.53

CD - At 5% level NS - Not significant

Table 6 Interaction effect of colchicine concentration and mode of treatment on various morphological characters in Seethal (number of flowers to pollen characters)

Treatment	No. of male flowers per plant	No. of female flowers per plant	Stomatal length (mm)	No. of fruits per plant	Length of fruit (cm)	Girth of fruit (cm)	Weight of fruit (g)	Pollen size (mm)	Pollen sterility (%)
$c_1 m_1$	594.83	27.66	0.097	4.66	46.00	33.13	1415.00	0.054	38.32
$c_1 m_2$	610.67	27.66	0.101	3.66	40.07	29.75	1392.50	0.055	37.18
$c_1 m_3$	559.33	26.16	0.116	3.50	36.75	32.11	1446.67	0.063	30.72
$c_2 m_1$	592.00	24.17	0.124	3.67	44.75	33.12	1414.17	0.068	41.38
$c_2 m_2$	566.33	24.50	0.129	3.68	44.75	34.13	1357.50	0.065	43.33
$c_2 m_3$	581.00	23.00	0.128	3.91	35.88	28.33	1454.17	0.062	43.76
$c_3 m_1$	497.00	24.00	0.124	3.66	38.63	29.07	1361.68	0.064	53.55
$c_3 m_2$	449.00	23.67	0.129	3.83	42.00	35.50	1420.11	0.069	44.75
$c_3 m_3$	556.50	23.00	0.122	4.17	44.83	32.00	1376.28	0.072	49.28
CD	52.57	2.26	0.007	NS	NS	NS	NS	0.004	NS

CD - At 5% level NS - Not significant

Table 7 Interaction effect of colchicine concentration and period of treatment on various morphological characters in Seethal (number of flowers to pollen characters)

Treatment interactions	No. of male flowers per plant	No. of female flowers per plant	Stomatal length (mm)	No. of fruits per plant	Length of fruit (cm)	Girth of fruit (cm)	Weight of fruit (g)	Pollen size (mm)	Pollen sterility (%)
$c_1 t_1$	579.83	30.50	0.102	5.5	46.4	27.0	1216.67	0.051	22.32
$c_1 t_2$	604.16	25.83	0.104	2.8	42.2	27.7	1249.17	0.058	29.03
$c_1 t_3$	530.83	25.16	0.109	3.5	34.1	29.1	1288.33	0.063	34.83
$c_2 t_1$	595.16	25.67	0.128	3.3	40.5	26.6	1492.50	0.064	43.37
$c_2 t_2$	622.00	23.33	0.130	3.7	44.9	28.5	1375.50	0.067	39.03
$c_2 t_3$	522.17	22.67	0.118	2.8	36.8	27.1	1249.50	0.067	46.08
$c_3 t_1$	569.00	24.83	0.127	3.7	39.5	28.2	1398.33	0.068	48.35
$c_3 t_2$	563.67	24.50	0.119	3.8	37.1	31.7	1325.25	0.065	39.38
$c_3 t_3$	481.33	21.33	0.135	4.1	49.0	34.8	1311.55	0.073	59.85
CD	NS	NS	0.007	NS	NS	NS	NS	0.004	5.23

CD - At 5% level NS - Not significant

Table 7 Interaction effect of colchicine concentration and period of treatment on various morphological characters in Seethal (number of flowers to pollen characters)

Treatment interactions	No. of male flowers per plant	No. of female flowers per plant	Stomatal length (mm)	No. of fruits per plant	Length of fruit (cm)	Girth of fruit (cm)	Weight of fruit (g)	Pollen size (mm)	Pollen sterility (%)
$c_1 t_1$	579.83	30.50	0.102	5.5	46.4	27.0	1216.67	0.051	22.32
$c_1 t_2$	604.16	25.83	0.104	2.8	42.2	27.7	1249.17	0.058	29.03
$c_1 t_3$	530.83	25.16	0.109	3.5	34.1	29.1	1288.33	0.063	34.83
$c_2 t_1$	595.16	25.67	0.128	3.3	40.5	26.6	1492.50	0.064	43.37
$c_2 t_2$	622.00	23.33	0.130	3.7	44.9	28.5	1375.50	0.067	39.03
$c_2 t_3$	522.17	22.67	0.118	2.8	36.8	27.1	1249.50	0.067	46.08
$c_3 t_1$	569.00	24.83	0.127	3.7	39.5	28.2	1398.33	0.068	48.35
$c_3 t_2$	563.67	24.50	0.119	3.8	37.1	31.7	1325.25	0.065	39.38
$c_3 t_3$	481.33	21.33	0.135	4.1	49.0	34.8	1311.55	0.073	59.85
CD	NS	NS	0.007	NS	NS	NS	NS	0.004	5.23

CD - At 5% level NS - Not significant

Table 8 Interaction effect of mode of treatment and period of treatment on various morphological characters in Seethal (number of flowers to pollen characters)

Treatment interactions	No. of male flowers per plant	No. of female flowers per plant	Stomatal length (mm)	No. of fruits per plant	Length of fruit (cm)	Girth of fruit (cm)	Weight of fruit (g)	Pollen size (mm)	Pollen sterility (%)
$m_1 t_1$	550.67	26.23	0.117	4.50	49.30	29.95	1244.17	0.059	39.60
$m_1 t_2$	583.83	25.67	0.117	4.17	43.25	30.60	1236.67	0.060	36.85
$m_1 t_3$	542.00	25.83	0.118	4.00	36.83	27.46	1404.17	0.067	46.80
$m_2 t_1$	589.16	27.83	0.119	4.00	39.07	27.00	1215.83	0.058	36.33
$m_2 t_2$	586.33	24.83	0.116	2.50	40.83	27.83	1246.67	0.067	32.12
$m_2 t_3$	551.33	23.16	0.120	3.83	46.92	32.55	1477.50	0.064	46.12
$m_3 t_1$	604.16	26.83	0.120	3.17	37.83	27.83	1398.33	0.065	37.60
$m_3 t_2$	626.66	23.16	0.123	3.87	40.25	29.50	1370.11	0.067	38.22
$m_3 t_3$	561.00	22.16	0.124	2.72	36.08	30.91	1401.12	0.067	47.50
CD	NS	NS	NS	NS	NS	NS	NS	NS	NS

CD - At 5% level NS - Not Significant

Table 9 Effect of colchicine concentration, mode of treatment and period of treatment on survival and morphological characters upto flower opening in Delila

Treatment interactions	Survival percentage	Length of vine (cm)		No of branches per plant		No of leaves per plant		Days taken to male flower opening	Days taken to female flower opening
		30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP		
c_1	85.53	94.88	123.27	5.57	14.63	67.79	219.67	20.81	25.33
c_2	71.64	67.99	124.89	4.90	14.27	65.99	218.93	22.06	23.69
c_3	59.56	54.88	120.15	3.41	14.18	42.83	216.11	25.67	31.89
CD	3.19	4.66	NS	0.58	NS	3.41	NS	0.82	1.28
m_1	74.03	78.33	129.33	5.17	14.67	57.89	226.71	22.89	27.72
m_2	73.78	69.37	126.94	4.37	14.63	62.56	222.89	23.11	26.53
m_3	68.92	70.06	125.87	4.34	14.32	56.27	223.11	23.53	26.67
CD	3.19	4.66	NS	0.58	NS	3.41	NS	NS	NS
t_1	76.67	77.86	127.31	5.06	14.18	72.24	223.77	19.94	26.00
t_2	73.17	71.81	128.31	4.83	14.84	55.24	218.33	22.28	26.61
t_3	66.92	68.30	126.54	3.99	14.60	48.93	222.61	26.31	28.31
CD	3.19	4.66	NS	0.58	NS	3.41	NS	0.82	1.28
Treatment mean	73.22	74.28	127.93	4.86	14.61	60.44	222.03	22.63	26.01
Control	99.50	120.00	131.15	11.00	15.32	81.33	229.12	17.00	28.00
CD	4.78	6.99	NS	0.86	NS	5.11	NS	1.21	1.92

CD - At 5% level NS - Not significant

Table 10 Interaction effect of colchicine concentration and mode of treatment on survival and morphological characters upto flower opening in Delila

Treatment interactions	Survival percentage	Length of vine (cm)		No of branches per plant		No of leaves per plant		Days taken to male flower opening	Days taken to female flower opening
		30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP		
c_1m_1	87.73	105.33	129.00	6.56	15.58	68.83	228.67	19.42	22.16
c_1m_2	87.83	90.90	133.58	3.92	15.08	73.05	211.50	21.50	25.66
c_1m_3	81.00	98.42	127.22	6.23	14.83	61.50	218.83	21.50	23.17
c_2m_1	71.16	59.73	134.33	6.05	15.35	58.92	235.30	22.17	23.50
c_2m_2	73.50	66.63	131.50	5.30	13.71	71.67	220.33	22.00	22.25
c_2m_3	70.25	77.33	123.83	3.35	13.75	67.38	231.17	22.00	25.23
c_3m_1	63.00	65.83	134.66	2.88	13.08	43.92	222.17	25.00	30.50
c_3m_2	60.00	50.12	125.75	3.90	15.08	42.67	206.83	25.83	31.50
c_3m_3	55.00	68.50	136.58	3.45	14.33	39.92	219.33	26.17	31.67
CD	NS	8.07	NS	0.99	NS	5.09	NS	1.42	2.22

CD - At 5% level NS - Not significant

Table 12 Interaction effect of mode of treatment and period of treatment on survival and morphological characters upto flower opening in Delila

Treatment interactions	Survival percentage	Length of vine (cm)		No. of branches per plant		No. of leaves per plant		Days taken to male flower opening	Days taken to female flower opening
		30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP		
m_1t_1	79.33	80.60	121.50	4.90	14.47	74.25	226.83	19.33	25.83
m_1t_2	74.00	68.63	134.00	5.48	14.75	55.08	228.33	22.17	28.33
m_1t_3	68.75	62.17	135.50	5.08	14.80	48.33	231.00	27.17	29.00
m_2t_1	75.25	71.67	128.92	5.87	14.17	72.25	223.67	19.83	26.00
m_2t_2	70.83	68.73	121.50	4.15	15.05	57.66	220.11	23.17	26.83
m_2t_3	75.41	67.73	130.42	3.10	14.67	57.66	227.30	26.33	26.75
m_3t_1	70.16	81.92	131.50	4.37	14.91	70.22	220.83	20.67	26.16
m_3t_2	61.16	77.25	129.42	4.86	14.72	53.58	223.13	21.50	26.67
m_3t_3	61.71	75.00	126.72	3.80	14.33	45.00	220.11	28.42	29.17
CD	NS	NS	NS	0.99	NS	5.09	NS	1.42	2.22

CD - At 5% level NS - Not significant

Table 13 Effect of colchicine concentration, mode of treatment and period of treatment on various morphological characters in Delila (number of flowers to pollen characters)

Treatments	No. of Male flowers per plant	No. of female flowers per plant	Stomatal length (mm)	No. of fruits	Length of fruits (cm)	Girth of fruits (cm)	Weight of fruit (g)	Pollen size (mm)	Pollen sterility (%)
c_1	450.67	16.78	0.103	5.56	47.47	29.91	1385.56	0.038	30.21
c_2	336.94	15.39	0.122	5.00	49.56	47.42	1310.28	0.040	30.96
c_3	328.75	14.06	0.130	5.17	52.14	29.42	1259.45	0.044	51.44
CD	21.91	1.44	0.007	NS	NS	NS	NS	0.001	2.54
m_1	360.69	15.94	0.113	5.83	49.44	29.11	1235.00	0.039	35.68
m_2	371.94	14.89	0.119	5.78	51.44	29.36	1279.00	0.040	36.86
m_3	357.72	15.39	0.121	5.11	48.31	30.13	1340.83	0.043	39.97
CD	NS	NS	NS	NS	NS	NS	NS	0.001	2.54
t_1	388.19	16.17	0.111	6.06	48.17	285.79	1298.81	0.040	27.96
t_2	389.78	15.00	0.119	5.61	49.75	28.77	1271.11	0.041	30.71
t_3	392.39	15.06	0.124	5.06	30.31	31.03	1285.56	0.041	53.94
CD	NS	NS	0.007	NS	NS	NS	NS	NS	2.54
Treatment mean	397.61	15.69	0.120	5.68	49.28	29.33	1281.40	0.040	36.32
Control	602.50	19.55	0.082	5.91	43.44	27.11	1191.25	0.030	3.90
CD	32.86	2.15	0.003	NS	NS	NS	NS	0.002	3.50

CD - At 5% level NS - Not significant

Table 14 Interaction effect of colchicine concentration and mode of treatment on various morphological characters in Delila (number of flowers to pollen characters)

Treatment interactions	No. of male flowers per plant	No. of female flowers per plant	Stomatal length (mm)	No. of fruits	Length of fruits (cm)	Girth of fruits (cm)	Weight of fruit (g)	Pollen size (mm)	Pollen sterility (%)
c_1m_1	507.50	20.00	0.088	6.83	47.33	24.50	1230.88	0.033	25.28
c_1m_2	422.00	15.17	0.113	7.83	46.50	26.08	1292.50	0.038	32.22
c_1m_3	422.50	15.17	0.106	7.13	48.50	30.50	1303.33	0.041	33.12
c_2m_1	396.73	15.00	0.122	5.8	46.00	30.75	1299.17	0.041	36.50
c_2m_2	388.50	15.33	0.117	5.33	45.33	29.92	1325.15	0.038	26.05
c_2m_3	363.00	15.83	0.125	5.17	47.41	33.17	1203.33	0.042	30.03
c_3m_1	417.83	12.83	0.126	4.83	49.11	28.50	1175.00	0.043	45.25
c_3m_2	305.34	14.17	0.127	5.22	48.91	30.33	1417.50	0.043	52.32
c_3m_3	287.67	15.17	0.133	5.17	50.12	29.42	1185.83	0.047	56.77
CD	37.95	2.48	0.012	NS	NS	NS	NS	0.002	4.39

CD - At 5% level NS - Not significant

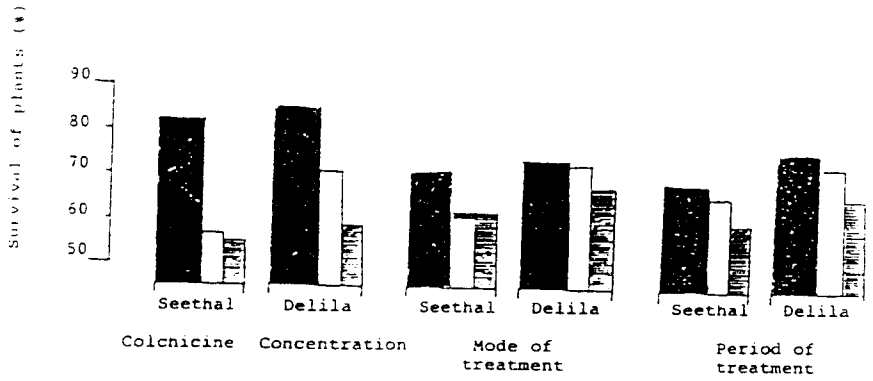

Fig.1: Effect of treatment on survival percentage in both varieties

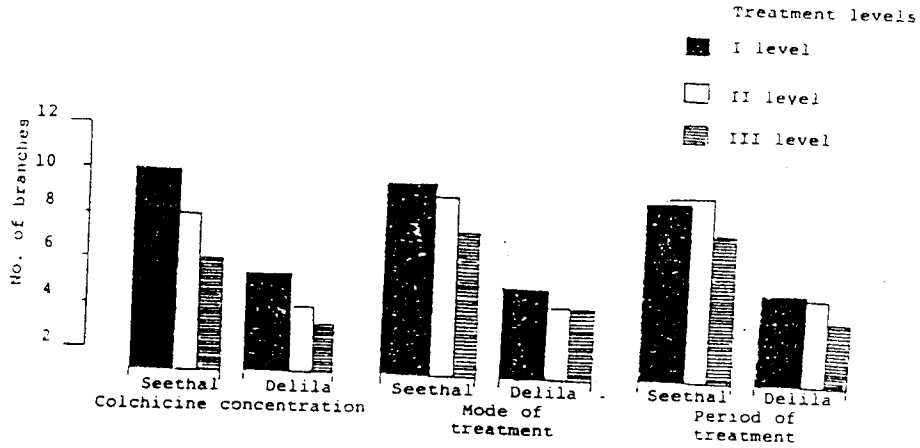

Fig.3: Effect of treatments on number of branches at 30 days of growth stage in both varieties.

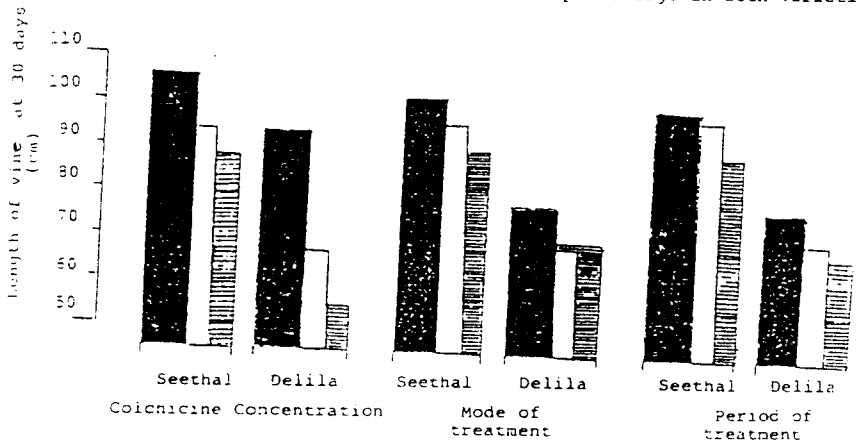

Fig.2: Effect of treatments on length of vine at 30 days of growth stage in both varieties.

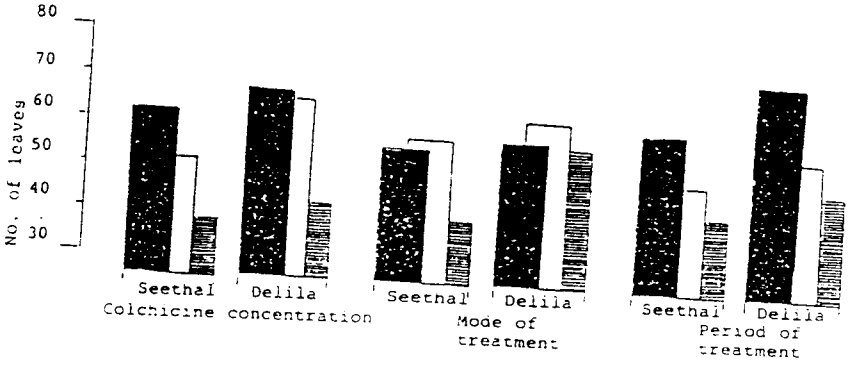

Fig.4: Effect of treatments on number of leaves at 30 days of growth stage in both varieties.

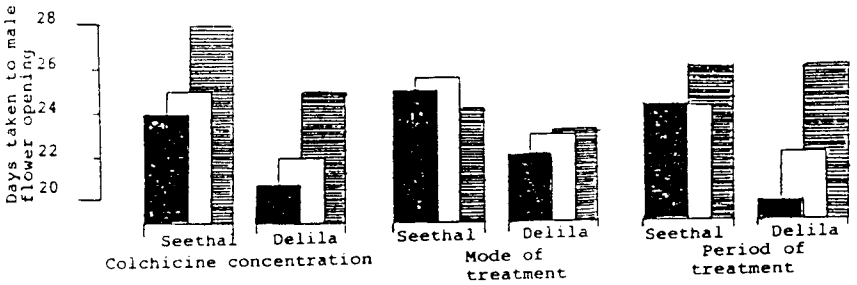

Fig.5: Effect of treatments on number of days of taken for male flower opening in both varieties.

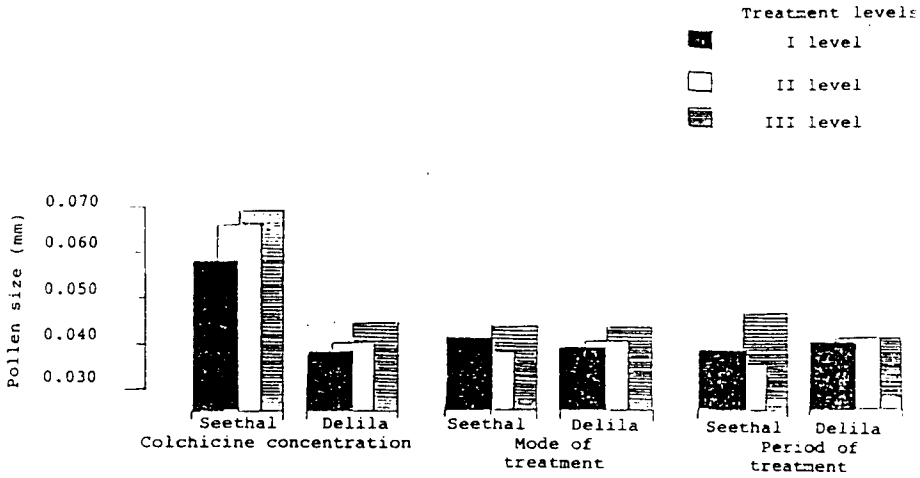

Fig.6: Effect of treatments on pollen size in both varieties.

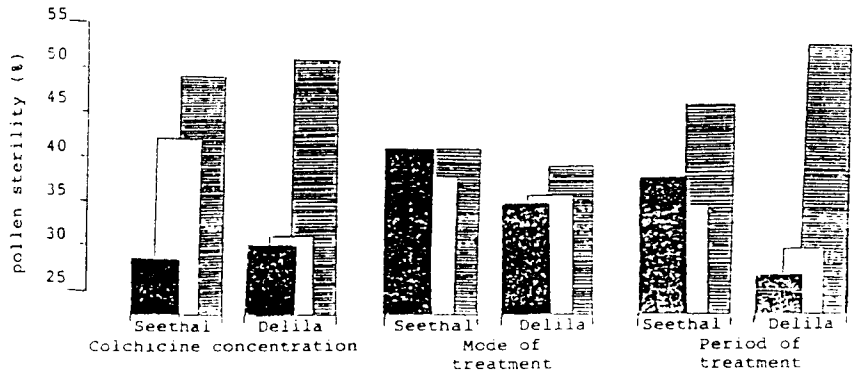

Fig.7: Effect of treatments on pollen sterility (%) in both varieties

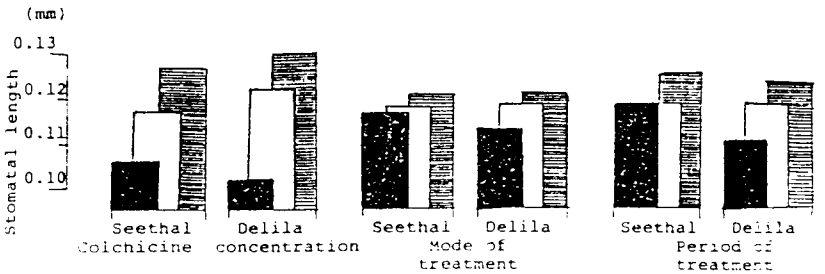

Fig. 8: Effect of treatments on stomatal length in both varieties.

diploid cells. Mitotic division stages were observed in the normal diploid cells. Metaphase and anaphase stages were seen frequently as shown in plate - 6. Large sized cells could be seen in the case of colchicine treated root tips (plate - 7). But the chromosomes were not well stained in them and could not be seen clearly. Highly stained artifacts were also seen in excess in colchicine induced cells.

Experiment II

Standardisation of suitable medium for embryo culture

To standardise the best combination of medium for embryo culture, MS medium was selected as the basal medium and supplemented with different concentrations of IAA and Kinetin as follows.

IAA = 0.05,0.1 and 0.2 mg/L

Kinetin = 0.05,0.1 and 0.2 mg/L

Callus development was noticed in all the six variations of the medium tried. Shoot differentiation was found to be high in medium supplemented with IAA. Among the three concentrations of IAA tried, maximum callus growth rate was observed with the medium supplemented with 0.1 mg/L IAA. Early embryo differentiation was also noticed in this

combination. High callus induction rate and fast callus growth response were noticed with this medium. MS medium supplemented with 0.05 and 0.2 mg/L of IAA also gave early callus induction. But delay in shoot differentiation was noted in these media compared to 0.1 mg/L of IAA combination.

Eventhough callus induction was noticed in media supplemented with kinetin, the rate of callus induction and callus growth was very slow. Shoot differentiation was also very poor in these media. Hence MS medium supplemented with 0.1 mg/L of IAA was standardised as suitable medium for embryo culture of Cucumis sativus.

The results on the effect of colchicine concentration, type of embryo used for culturing and day of treatment on callus induction and callus growth rate are presented below.

Number of days taken for embryogenesis

The data on number of days taken for embryogenesis in the variety 'Seethal' are given in Table 17.

Colchicine concentration had significant influence on embryogenesis. In the culture medium embryogenesis was significantly and progressively delayed along with the

increase in colchicine concentration. At the highest colchicine concentration of 0.04% maximum period of 23.85 days was taken for embryogenesis as against the minimum period of 14.67 days taken for embryogenesis at colchicine concentration 0.02%. The time taken for embryogenesis showed significant difference in the three types of embryos with pro-embryos recording maximum period for embryogenesis (27.93 days) and embryos of dry seeds recording the minimum period for embryogenesis (11.6 days).

Days of treatment did not exert any significant influence on the time taken for embryogenesis.

The interaction effect of colchicine concentration with type of embryos is presented in Table 18. Significant difference were recorded for the time taken for embryogenesis in the different treatment combination. Maximum period (35.44 days) was taken for embryogenesis by the pro-embryos treated with 0.04%. Colchicine(c_3e_1). The time taken for embryogenesis was minimum (8.00 days) when the embryos of dry seeds were treated with the lowest colchicine concentration of 0.02% (c_1e_3).

The interaction effects of colchicine concentration with day of treatment and the interaction effect of different types of embryos with day of treatment were not significant.

The three factor interaction of treatments (Appendix VI) was also significant. Maximum value was recorded at 0.4 per cent colchicine applied on pro-embryo on the same day of inoculation ($c_3e_1d_1$) and minimum was obtained when dry seed embryos were treated with 0.02 per cent colchicine on third day of inoculation ($c_1e_3d_3$).

An appraisal of the data presented on Table 21 indicated significant influence of colchicine concentration and type of embryo in Delila with respect to days taken for embryogenesis.

Untreated proembryos, mature embryos and dry seed embryos showed early embryogenesis compared to colchicine treated ones. With increasing colchicine concentration embryogenesis was delayed significantly recording a maximum of 30.11 days at 0.04 per cent level. Among types of embryos maximum delay (29.04 days) was noticed with proembryos and minimum (14.78 days) with dry seed embryos. Days of treatment did not exert any significant influence.

Data on interaction of colchicine concentration with type of embryo are given in Table 22. With proembryos at 0.04 per cent concentration (c_3e_1) maximum delay (40.78 days) was noticed for embryogenesis. Fastest embryogenesis at 11.00 days was observed for the c_1e_3 combination.

Interaction of colchicine concentration with days of inoculation and types of embryo with days of inoculation was found to be insignificant.

Among interaction values of all treatments (Appendix VII) minimum (9.60) was recorded with dry seed embryos treated by 0.03 per cent colchicine on the same day of inoculation ($c_2e_3d_1$) and maximum value was recorded with pro-embryos when treated by 0.04 per cent colchicine on same day of incoculation ($c_3e_1d_1$).

Regeneration percentage

The data on regeneration percentage of calli in culture tubes in respect of variety Seethal are depicted in Table 17.

Treatments exerted significant effect on regeneration treated embryos were compared to untreated control embryos.

As colchicine concentration increased from 0.02 to 0.04 per cent regeneration percentage decreased significantly from 33.42 to 9.90. With proembryos lowest regeneration (0.83%) was noticed and maximum (43.38%) with embryos of dry seeds. Day of treatment did not exert any significant influence on regeneration.

Data on interaction effect of colchicine concentration with type of embryo are presented in Table 18. Maximum regeneration obtained was recorded with c_1e_3 combination (53.47%) and minimum value (0.37%) for c_3e_1. Interaction of colchicine concentration with day of treatment and type of embryo with day of treatment had no significance on regeneration percentage in Seethal.

Among interactions of all treatments in Seethal (Appendix VI), dry seed embryo when treated with 0.02 per cent colchicine on the third day of inoculation ($c_1e_3d_3$) recorded maximum (68.62) and pro-embryo treated by 0.04 per cent colchicine on the same day of inoculation ($c_3e_1d_1$) recorded minimum (0.04) regeneration percentage.

Data presented on Table 21 revealed significant influence of colchicine concentration and type of embryo on regeneration percentage in Delila. A significant fall in regeneration percentage from 17.47 to 8.79 was noticed with increase in level of colchicine concentration from 0.02 to 0.04 per cent. Pro-embryo(e_1) and dry seed embryo (e_3) recorded lowest (0.42) and highest (24.48) regeneration percentage respectively. Day of treatment had no significance on regeneration percentage in Delila.

Two factor interaction of treatments did not exert any significant variation in Delila with respect to regeneration percentage.

Three factor interaction of treatments was significant with respect to regeneration percentage in Delila. Maximum value (35.11) and minimum value (0.13) were recorded at dry seed embryos treated by 0.02 per cent colchicine on the same day of inoculation ($c_1e_2d_1$) and pro-embryos treated by 0.04 per cent colchicine on the the same day of inoculation ($c_2e_1d_1$) respectively.

Number of leaves produced 45 days after treatment

Data on number of leaves produced in tubes 45 days after treatment are presented in Table 17.

Untreated dry seed embryo produced significantly higher number of leaves than the mean efffect of treated ones. Whereas untreated pro-embryos showed decreased number of leaves.

With increasing colchicine concentration a progressive reduction in number of leaves from 2.51 to 1.62 was noticed. Dry seed embryo and pro-embryos recorded maximum (2.55) and minimum (0.21) number of leaves respectively. Day of treatment showed no significant effect on this parameter.

Interaction effect of colchicine concentration with type of embryo showed significnat effect (Table 17). The combination c_1e_3 recorded maximum (3.25) and c_3e_1 minimum (0.37) values. Interaction of colchicine concentration with day of treatment and type of embryo with day of treatment was not significant in Seethal.

The interaction of effect of treatments are given in Appendix VI. Dry seed embryos treated with colchicine 0.02 per cent on the same day of inoculation ($c_1e_3d_1$) and pro-embryos treated with 0.04 per cent colchicine on the same day of inoculation ($c_3e_1d_1$) recorded maximum and minimum values respectively.

The data on number of leaves produced in the culture tubes after 45 days in Delila are depicted in Table 21.

Untreated dry seed embryo produced significantly more number of leaves than mean effect of treated embryos. Values observed in case of mature embryos was on par with mean effects. Control proembryos produced a lower value than the mean of treatment effect.

With increasing colchicine concentration from 0.02 to 0.04 per cent a significant fall in number of leaves produced in tubes was noticed from 2.33 to 1.49. Pro-embryos

recorded lowest (1.58) and dry seed embryo the highest (2.34) values. No significant effect was exerted by day of colchicine treatment.

Data depicted on Table 22 revealed significant influence of interaction of colchicine concentration with type of embryo. Here $c_1 e_3$ recorded maximum number of leaves while the minimum number was recorded by $c_3 e_1$. Interaction effect of colchicine concentration with day of treatment and type of embryo with day of treatment were insignificant.

Among interaction of all factors (Appendix VII) significant variations were noticed, with dry seed embryos treated with 0.02 per cent colchicine on the same day of inoculation ($c_1 e_3 d_1$). Proembryos treated with 0.04 per cent colchicine on both second and third day of inoculation ($c_3 e_1 d_2 / c_3 e_1 d_3$) recording maximum (8.66) and minimum (0.29) values respectively.

Length of plantlet 45 days after treatment

Data on length of plantlet at 45 days after treatment in Seethal are given in Table 17.

Untreated proembryos and mature embryos showed significantly lower plant length than the mean effect of all the treated embryos. Control dry seed embryo produced

significantly higher length compared to mean effect of treated ones.

Length of plantlet was reduced progressively and significantly with increase in colchicine concentration from 0.02 to 0.04 per cent. Type of embryo also exerted significant effect. Pro-embryos recorded lowest (0.50) and dry seed embryos showed maximum (2.10) length of plantlet in Seethal. Day of treatment had no significance.

Data on interaction effect of colchicine concentration with type of embryo on length of plantlet in Seethal are given in Table 18. Maximum and minimum values were recorded by c_1e_3 and c_3e_1 respectively. Interaction of colchicine concentration with day of treatment and type of embryo with day of treatment were not significant.

Three factor interaction between treatments (Appendix VI) showed significant influence on Seethal. The combination of 0.03 per cent colchicine when treated on pro embyos on the second day of inoculation recorded minimum (2.11) and dry seed embryo treatment with 0.03 per cent colchicine on the second day of inoculation maximum (8.32) plantlet length.

Data presented on Table 21 showed significant effect of treatment on length plantlets at 45 days stage in Delila.

Control pro-embryos and mature embryos produced significantly lower length of plantlet than mean effect of the treated ones. Dry seed embryo produceed significantly higher plantlet length than mean effect of treated ones.

A significant decrease in plantlet length from 9.11 to 6.39 was noticed with increasing colchicine concentration from 0.02 to 0.04 per cent. Dry seed embryo (e_3) recorded maximum plantlet length of 8.55 followed by mature embryo (4.92) and pro-embryo (3.16). No significant variation was noticed with day of treatment.

All two factor interactions were insignificant with respect to this parameter in Delila.

Among interaction of all treatments, colchicine treatment at 0.02 per cent on dry seed embryos at the same day of inoculation ($c_1e_3d_1$) and colchicine at 0.04 per cent on the second day of inoculation of pro-embryos ($c_3e_1d_2$) recorded maximum (8.52) and minimum (1.32) values respectively.

Survival percentage under green house condition

Data on survival percentage of plants in green house for the variety Seethal are presented in Table 17.

Untreated pro-embryos showed lesser survival than mean effect of treated ones but both treatments were on par with each other. Untreated mature embryos and dry seed embryos showed higher survival percentage than the mean effect of treated ones.

The survival of embryo cultured plants in the green house was very low in Seethal. With increasing colchicine concentration from 0.02 to 0.04, a fall in survival from 1.61 to 1.31 per cent was noticed. With pro-embryos the survival recorded was 0.82 which increased to 1.69 where dry seed embryos were used. Day of inoculation showed no significant influence.

All of the two factor interaction were not significant with respect to survival of plants in the green house in Seethal.

Among three factor interactions (Appendix VI) dry seed embryos treated with 0.02 per cent colchicine on the same day of inoculation ($c_1 e_3 d_1$) recorded maximum value of 3.32 and the value was significantly lowered to 0.29 at colchicine treatment of 0.04 per cent on pro-embryos on the same and second day of inoculation ($c_3 e_1 d_1$ and $c_3 e_1 d_2$).

Data on survival percentage of plants in Delila are given in Table 21.

Untreated pro-embryos showed lower survival percentage than mean effect of treated embryos but was on par. Untreated mature embryos showed higher survival but was also on par with mean effect of treated ones. Control dry seed embryo showed significantly higher survival precentage than the mean effect of treated ones.

As colchicine level increased from 0.02 tO 0.04 per cent, decrease in survival percentage from 1.58 to 1.24 was recorded. Pro embryo recorded lowest survival percentage (1.01) and corresponding highest value (1.53) was shown by mature seed embryo. Day of treatment exerted no siginificant effect.

All two factor interactions were insignificant with respect to this parameter in Delila.

Among three factor interactions (Appendix VII) colchicine treatment of 0.02 per cent on dry seed embryos on same day of inoculation ($c_1e_3d_3$) recorded maximum and colchicine treatment of 0.04 per cent on pro-embryos on the second day of inoculation ($c_3e_1d_2$) recorded minimum value in Delila.

Morphological variations noticed

Some morphological variations were noticed on developing plantlets in the culture medium. The

common feature noticed in colchicine treated embryos was the stunted growth. Failure in callus differentiation was also noticed with higher levels of colchicine treatment. Very poor callus induction and callus differentiation were noticed with the pro-embryo in the culture medium.

Immature flowering was noticed in the culture tubes when dry seed embryos were treated with 0.03 per cent colchicine on the second day of inoculation ($c_2e_3d_2$; plate - 14). In one tube (see plate - 15) shoot development without leaf formation was observed. In some tubes (see plates 8 to 13) stunted growth was noticed on colchicine treatments. Only a callus mass with out organ differentiation was developed in some tubes (plate - 16).

The plantlets after hardening were transferred to green house. These plantlets showed very poor development and low survival percentage. The plantlets remained without any further development in green house for 10 to 15 days and majority of them gradually died eventhough they were cared properly.

Table 17 Effect of colchicine concentration, type of embryo and day of treatment on survival and various plant characters in Seethal

Treatments	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced in tubes 45 days after treatment	Length of plantlet 45 days after treatment (cm)	Survival of plants under green house condition (%)
c_1	14.67	33.42	2.51	8.31	1.61
c_2	18.93	23.74	2.10	5.12	1.52
c_3	23.85	9.90	1.62	4.33	1.31
CD	1.62	8.36	0.21	1.12	NS
e_1	27.93	0.83	1.64	0.50	0.82
e_2	17.85	22.85	2.04	0.70	1.53
e_3	11.67	43.38	2.55	2.10	1.69
CD	1.62	8.36	0.21	1.12	0.17
d_1	19.48	19.62	2.11	6.61	1.57
d_2	19.04	21.39	2.00	5.88	1.43
d_3	18.93	26.06	2.03	6.72	1.44
CD	NS	NS	NS	NS	NS
Treatment mean	18.17	24.84	2.14	6.01	1.50
Control (proembryo)	13.17	1.57	1.63	0.43	1.14
Control (mature embryo)	7.67	61.37	2.58	3.51	1.82
Control (embryo of dry seed)	5.33	76.67	3.64	6.91	2.14
CD	4.87	25.07	0.61	0.89	0.52

CD - At 5% level NS - Not significant

Table 18 Interaction effect of colchicine concentration and type of embryo on survival and various plant characters in Seethal

Treatment interactions	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced in tubes 45 days after treatment	Length of plantlet 45 days after treatment (cm)	Survival of plants under green house condition (%)
c_1e_1	22.56	1.38	1.82	3.12	1.31
c_1e_2	13.44	35.40	2.47	7.22	1.64
c_1e_3	8.00	53.47	3.25	8.31	1.87
c_2e_1	25.77	0.74	1.73	2.12	1.26
c_2e_2	18.44	23.30	2.07	5.71	1.51
c_2e_3	12.56	47.18	2.49	5.93	1.78
c_3e_1	35.44	0.37	1.36	0.63	1.09
c_3e_2	21.67	9.85	1.59	1.16	1.43
c_3e_3	14.44	19.48	1.91	2.71	1.40
CD	2.81	14.47	0.36	1.61	NS

CD - At 5% level NS - Not significant

Table 19 Effect of colchicine concentration and day of treatment on survival and various plant characters in Seethal

Treatment interactions	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced in tubes 45 days after treatment	Length of plantlet 45 days after treatment (cm)	Survival of plants under green house condition (%)
c_1d_1	14.78	27.31	2.59	7.33	1.77
c_1d_2	14.22	32.46	2.47	7.12	1.49
c_1d_3	15.00	40.48	2.47	6.91	1.56
c_2d_1	18.22	21.78	2.31	6.56	1.56
c_2d_2	19.88	22.18	2.11	6.91	1.49
c_2d_3	18.67	27.27	2.08	6.33	1.50
c_3d_1	25.44	9.77	2.10	5.88	1.37
c_3d_2	23.00	9.52	2.00	6.37	1.30
c_3d_3	23.11	10.43	1.99	6.32	1.25
CD	NS	NS	NS	NS	NS

CD - At 5% level NS - Not significant

Table 20 Interaction effect of type of embryo and day of treatment on survival and various plant characters in Seethal

Treatment interactions	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced in tubes 45 days after treatment	Length of plantlet 45 days after treatment (cm)	Survival of plants under green house condition (%)
e_1d_1	28.67	0.55	1.68	5.12	1.18
e_1d_2	27.33	1.07	1.61	5.61	1.22
e_1d_3	27.77	0.86	1.61	5.59	1.65
e_2d_1	17.67	14.77	2.09	6.12	1.26
e_2d_2	17.89	17.33	2.04	6.23	1.43
e_2d_3	18.00	22.11	2.61	6.19	1.49
e_3d_1	12.11	43.54	2.49	6.55	1.86
e_3d_2	11.89	40.25	2.31	6.39	1.64
e_3d_3	11.00	46.34	2.49	6.49	1.55
CD	NS	NS	NS	NS	NS

CD - At 5% level NS - Not significant

Table 21 Effect of colchicine concentration, type of embryo and day of inoculation on survival and various plant characters in Delila

Treatments	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced in tubes 45 days after treatment	Length of plantlet 45 days after treatment (cm)	Survival of plants under green house condition (%)
c_1	15.19	17.47	2.33	9.11	1.58
c_2	17.52	13.83	1.89	8.59	1.43
c_3	30.11	8.79	1.49	6.39	1.24
CD	1.51	5.51	0.18	1.32	0.14
e_1	29.04	0.42	1.58	3.16	1.01
e_2	19.00	15.19	1.80	4.92	1.53
e_3	14.78	24.48	2.34	8.55	1.52
CD	1.51	5.51	0.18	1.32	0.14
d_1	21.96	14.43	1.92	4.22	1.52
d_2	20.26	12.57	1.93	4.39	1.44
d_3	21.59	13.09	1.86	5.32	1.40
CD	NS	NS	NS	NS	NS
Treatment mean	17.74	15.67	3.29	5.11	1.42
Control (proembryo)	14.00	1.90	2.33	2.13	1.00
Control (mature embryo)	7.00	46.33	2.67	3.99	1.52
Control (embryo of dry seed)	5.23	61.17	11.33	6.93	1.93
CD	4.54	16.53	2.29	1.10	0.43

CD - At 5% level NS - Not significant

Table 22 Interaction effect of colchicine concentration and type of embryo on survival and various plant characters in Delila

Treatment interactions	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced in tubes 45 days after treatment	Length of plantlet 45 days after treatment (cm)	Survival treatment under green house condition (%)
c_1e_1	19.98	0.58	1.82	5.39	1.35
c_1e_2	14.78	5.22	2.17	6.33	1.71
c_1e_3	11.00	9.91	2.90	7.31	1.69
c_2e_1	26.56	0.51	1.72	6.30	1.23
c_2e_2	14.56	7.72	1.78	7.30	1.56
c_2e_3	11.44	7.88	2.17	7.32	1.51
c_3e_1	40.78	8.44	1.18	5.11	1.04
c_3e_2	27.67	8.12	1.43	5.21	1.31
c_3e_3	21.88	1.10	1.85	5.22	1.36
CD	2.61	NS	0.35	NS	NS

CD - At 5% level NS - Not significant

Table 23 Interaction effect of colchicine concentration and day of treatment on survival and various plant characters in Delila

Treatment interactions	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced in tubes 45 days after treatment	Length of plantlet 45 days after treatment (cm)	Survival treatment under green house condition (%)
c_1d_1	14.78	18.55	2.30	7.38	1.80
c_1d_2	14.00	16.20	2.38	7.42	1.34
c_1d_3	16.78	17.11	2.29	6.59	1.61
c_2d_1	17.22	18.10	1.95	6.32	1.52
c_2d_2	17.00	18.21	1.90	6.19	1.39
c_2d_3	18.33	17.33	1.90	7.00	1.38
c_3d_1	15.11	16.52	1.50	6.80	1.22
c_3d_2	17.33	16.38	1.53	5.90	1.28
c_3d_3	16.11	18.11	1.45	6.01	1.22
CD	NS	NS	NS	NS	NS

CD - At 5% level NS - Not significant

Table 24 Interaction effect of type of embryo and day of treatment on survival and various plant characters in Delila

Treatment interactions	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced in tubes 45 days after treatment	Length of plantlet 45 days after treatment (cm)	Survival treatment under green house condition (%)
e_1d_1	30.11	10.35	1.57	8.11	1.13
e_1d_2	28.22	0.48	1.56	7.82	1.24
e_1d_3	28.77	0.42	1.59	7.35	1.33
e_2d_1	27.11	0.89	1.79	7.22	1.39
e_2d_2	28.34	8.11	1.88	7.11	1.42
e_2d_3	25.12	8.28	1.72	7.12	1.41
e_3d_1	26.78	9.71	2.38	7.23	1.43
e_3d_2	28.11	9.99	2.36	7.60	1.42
e_3d_3	28.13	9.32	2.26	7.01	1.19
CD	NS	NS	NS	NS	NS

CD - At 5% level NS - Not significant

DISCUSSION

DISCUSSION

Polyploids are produced artificially by treatment with some chemicals of which colchicine is the most successful and widely used. Colchicine interferes with the spindle development in the dividing meristematic cells. Once a polyploid cell is formed, its normal division will give rise to a polyploid tissue, branch, flowers, fruits, seeds etc and a polyploid plant is established. But due to the cytogenetic instability caused by the numerical alteration in chromosomes the nature and usefulness of the autopolyploids are not predictable. The autopolyploids, if they are vigorous and better yielding, can be used as new varieties or for crossing with normal diploids to produce seed sterile triploids as in the case of water melon, apple and pear which are commercially important.

Cucumis sativus the species used in the present study is popularly used as salad cucumber. So autopolyploids if produced can either be used as new varieties or can be used for the production of sterile seedless triploids for commerical use. The present investigation was done with the objective of inducing

polyploids in Cucumis sativus through in-vivo treatment of seeds, seedlings and apical buds and also through in-vitro treatments of embryos of seeds. In the in-vivo experiment 0.2, 0.3 and 0.4 per cent of colchicine were applied on seeds apical bud and seedlings for a period of two hours, four hours and six hours. In the in-vitro experiment 0.02, 0.03 and 0.04 per cent of colchicine were applied on pro-embryos, embryos from mature seed and embryos from dry seed in culture medium for a period of two hours, four hours and six hours. The results obtained from the study are discussed below.

Experiment I (In-vivo)

Survival of plants

Colchicine is a poisonous plant alkaloid which causes many lethal effects on plants at higher concentrations. With increasing concentration it can cause low survival of plants and hence a study on the survival of colchicine treated plants is important to find out the critical level of colchicine for inducing poyploidy with out much lethal effects.

In the present investigation, of the three levels of colchicine concentration and period of treatments tried, the lowest level of both gave the highest survival

percentage in both the varieties Seethal and Delila. Mode of treatment also had significant effect recording highest survival in the seed treatment in both varieties. Lowest survival was recorded in both the varieties when apical bud treatment was resorted to. Survival was lowered significantly at the highest colchicine concentration (0.4%) when applied by apical bud treatment in both varieties. In seed treatment of colchicine, 0.2 per cent concentration for a period of two hours recorded maximum survival of plants in both the varieties. It can be concluded that, lower level of colchicine concentration by seed treatment is desirable for best survival.

Colchicine treated cells showed disturbances in mitotic division stages. Colchicine being a poisonous substance, often has some sublethal effects which affect the cellular activity. This may be the reason for the low survival percentage noticed with high concentrations of colchicine and also with longer period of treatments.

Many workers earlier have noticed this sublethal effects of colchicine which caused low survival percentage. Reports of Singh (1979) in Melothria; Singh and Yadav (1984) and Yadava et al. (1986), in Cucumis; Singh and Roy (1988) in Petunia; are in confirmity with these observations.

Morphological variations

For the morphological characters viz. length of vine, number of branches and number of leaves per plant significant variations were observed in the colchicine treated plants during the early stages ie. 30 days of growth stage.

Maximum reduction in vine length, number of branches per plant and number of leaves per plant were noticed at the highest colchicine level (0.4%) in both varieties. Period of treatment also showed similar pattern of variations. Among modes of treatment, apical bud treatment resulted in maximum reduction of vine length and number of branches and leaves per plant in both varieties. The initial stunted growth expressed as reduced vine length and number of branches and leaves may be attributed to the sublethal effect of colchicine.

Ramanujam and Joshi (1941) in Cicer arie tinum; Andrus et al. in water melon (1971); Singh (1975) in Cucumis melo; and in Cucumis sp. by Singh and Yadav (1984) and Yadava et al. (1986) noticed similar observations. Significant variations observed with respect to these parameters in colchicine treated plants, suggested occurrence of polyploid derivatives among the treated

population as the stunted growth nature was reported as an indication of polyploidy.

At 60 days of growth stage the treatment effects were not found significant for vine length, number of branches and number of leaves per plant. The drug colchicine supresses anaphase and causes lengthening of duration of metaphase. Probably colchicine induced cells may not keep pace with surrounding normal diploid cells during cell division and multiplication. So the colchicine affected cells might have been suppressed by activ ely dividing normal diploid cells as the plant grows. This may be the reason for the insignificant effect of treatments at 60 days of growth stage. Similar results were reported by Shifriss (1942) and Davidson (1979) in Cucumis. Amin (1940) had also reported similar initial stunted growth of colchicine treated plants only during early growth stages. Janaki Ammal and Bezbaruach (1962) in Catharanthus roseus also noticed similar observations. Singh (1979) and Yadava et al. (1984) reported initial stunted growth pattern in colchicine treated Cucumis sp.

A delay in flowering by two to eight days was noticed among the colchicine treated plants compared to control plants in both varieties. The delay was recorded in both male and female first flower opening. With increase in

colchicine concentration from 0.2 to 0.4 per cent and also period of treatment from two to six hours, delay in the opening of both male and female flowers increased progressively. Seedling treatment and apical bud treatments showed much delay for first flowering in Seethal compared to seed treatment. Delayed flowering was reported as a common feature exhibited by colchiploids in many crop specieis. Randolph (1932) in maize; Kumar and Abraham (1942) in Phaseolus; Tandon and Chinoy (1950) in Amaranthus bilitum; Srivastava (1950) in sesame; Singh and Yadav (1984) and Singh and Roy (1988) in Cucumis all observed delayed flowering associated with polyploid forms. The observations of present investigation suggested that colchicine treatments were effective in producing colchiploids in Cucumis sativus. The initial stunted growth in colchiploids as reported may be the cause of delayed flowering in the colchicine treated plants in the present study.

In respect ot the number of male and female flowers produced per plant the treated plants showed significant reduction compared to control plants in both varieties. Flower production was significantly and progressively reduced along with increase in colchicine concentration in both varieties. In the variety Delila reduction in flower production increased along with the

increase in the period of treatment also. Many reports are there in confirmation with these observations. Singh (1979) and Singh and Yadav (1984) noticed reduction in number of flowers in artificially induced polyploid forms in Cucumis sp. Roy and Ghosh (1971) in Luffa and Roy et al. (1960) in Momordica also recorded similar observations. The initial stunted vegetative growth rate of the polyploids can be attributed to the reduction in number of flowers produced per plant.

In respect of stomatal length, significant variations were observed on the colchicine treated plants compared to control in both varieties. With increasing colchicine concentrations and period of treatment there was progressive increase in stomatal length. Many earlier findings confirm the above observations Lathika (1968) reported that polyploidy is accompanied by gigas characters as expressed by increased size of pollen and stomata. Reports by Singh (1979) in Cucumis sp; Singh and Yadav (1984) and Yadava et al. (1986) in Cucumis sativus reported increased stomatal size associated with polyploid derivatives in Cucumis sp. Variations noticed in stomatal length, a reliable morphological index to identify polyploid forms, suggest the existence of colchiploids among treated population.

With respect to the fruit characters taken for study viz. number of fruits per plant, length of fruit, girth of fruit and weight of fruit, the different treatments did not exert any significant influence in both varities. Stebbins (1950) in cucurbits, reported that the common gigantism was not an universal feature especially in relation to fruit characters. According to earlier reports polypolid derivatives in Cucurbitaceae produce small fruits compared to their diploid counterparts (Singh, 1979; Singh and Yadav, 1984).

Shifriss (1942) reported the occurence of smaller fruits as against the general expectation of larger fruits in Cucumis sp. He indicated that initial increase in size of ovary do not keep pace with further development of plant, thus resulted in the development of smaller fruits. Singh (1979) reported that in Cucurubitaceae polyploidy brings about gigantism in all the vegetative features while producing smaller fruits than expected. Findings of Singh (1975) in Cucumis melo also agree with the present obervation.

Considering the different morphological characters in general colchicine exerted significant influence on all characters at the early growth stages which indicate the possible occurence of polyploids among the treated plants.

Treatment effect prevailed even at flowering stage which started at 23 to 25th day of planting and extended upto 30 to 35th day. But the characters did not agree with the usual gigantic characters of polyploids at later stages. A probable reason that may be attributed for this is the fading out of polyploid cells due to the overgrowth of the surrounding actively dividing diploid cells as the plants grow. This may be the reason for the absence of polyploid characterstics at the later stage of growth.

Pollen size and sterility

Increased pollen size with high percentage of sterility was often described as an associated feature with polyploid forms in many of the crop species. Increase in pollen size and sterility were noticed with increasing colchicine concentrations and also with increasing periods of treatment, uniformly in both varities. Highest values for pollen size and pollen sterility were noticed from apical bud treatment.Similar observations were reported by Turkov et al (1974) and Yadava et al (1984) in Cucumis sp., Luongdinhoua (1950) in Oryza sp; Amstrong and Robertson (1960) in Trifolium hybridum and Mercykutty and Kumar (1983) in pea. Many mitoic abnormalities were reported in the meiotic division of polyploids which may be the probable reasons for high pollen sterility.

Except the fruit characters, all other morphologicl characters, pollen size and sterility generally showed a positive response with increasing colchicine concentrations and periods of treatment in both varities. Similarly apical bud treatment was found to be causing more detrimental effects compared to other modes of treatment. Survival of plants decreased drastically by increasing treatment levels. It can safely be concluded that seed treatment of colchicine at low concentration (0.2%) for a minimum period of two hours is desirable for inducing polyploidy since the higher concentrations of colchicine produced pronounced deleterious effects.

Cytological studies

Cytological studies were conducted in the root tips collected from germinating colchicine treated seeds. The roots showed swelling as shown in plates - 1 to 3. Mitotic division stages could be observed in the root tip in the normal diploid cells as shown in plate - 6. The chromosomes are very small in size. Even though colchicine affected cells showed a much enlarged size, the chromosomes were not clearly seen (see plate - 7). An important feature reported in cucurbits is that in majority of polyploids the chromatin length is much less than the actual chromatin length of their related diploid counterparts (Singh, 1979).

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The chromosomes undergo an oveall diminition in size, though there is exact mulitiplication of DNA contents (Darlington 1965 and Sharma 1974, 1975).

Dane and Tsuchiya (1976) reported that while using root tips of cucurbits as a material for cytological studies, a pretreatment under deep freezing temperature with 0.002M 8-hydroxyquinoline was required for proper chromosome condensation and microtubule depolymerisation. Ramachandran and Narayanan (1985) have confirmed these findings. The difficulty noticed in cytological observations of colchicine treated root tip cells in the present investigation can be attributed to the minute chromosome size, low chromosome condensation, decreased chromatin length in polyploid form, and poor stain uptake shown by the chromosomes of this species.

Experiment II (In-vitro)

The experiment was undertaken to standardise a suitable culture medium for embryo culture of Cucumis sativus and also to study the effect of different levels of colchicine, type of embryo and day of treatment on callus growth and plant development. The results obtained in the present investigation are discussed below.

Standardisation of culture medium for embryo culture

In the present investigation MS medium was used as the basal medium. This medium was supplemented with three levels each of IAA and Kinetin ie. at 0.05, 0.1 and 0.2 mg/L. Comparing the callus initiation and callus growth rate on the media supplemented with IAA and Kinetin, IAA supplemented medium was found to be better. With the addition of either IAA or Kinetin, callus growth was observed. But callus initiation rate was more in the modified MS medium. MS medium supplemented with IAA at 0.1mg/L gave a better embryo take, early tissue differentiation and early callus growth. Hence this was selected as the standardised medium for embryo culture in the present study. Standardisation trial was conducted using embryo from dry seeds. An early uptake by using modified MS media was reported Cucumis sativus by Rajasekharan et al (1963); Sekioka and Tanaka (1981); Ziv and Gadasi (1986) and Kim et al. (1988) in Cucumis sativus.

Number of days taken for embryogenesis

Delay in embryogenesis was noticed in both varieties with increasing colchicine concentration. Maximum delay was noticed when pro-embryos were used for culturing and minimum delay was recorded when dry seed embryos were

used for culturing. Day of colchicine treatment showed no significant effect in both varieties.

These results agree with earlier reports by Martin and Sandhal (1984), Ziv and Gadasi (1986) and Fassuliotis and Nelson (1988) . They suggested that the success of in-vitro techniques varies with the plant material used for culturing. Also many earlier reports suggested the critical requirement of chemicals for plant regeneration in artificial medium. Handley and Chambliss (1979); Fassuliotis and Nelson (1986, 1988); Kim et al. (1988) and Gambley and Dodd (1990) reported the importance of various chemicals in the medium controlling plant regeneration in Cucumis sativus.

The lethal effect of colchicine in the medium and the maturity level of embryo are the deciding factors for embryogenesis in the present study. Immature embryos especially pro-embryos are more liable to damage than mature embryos during the process of culturing. The inferior performance of pro-embryos and delay in embrogenesis with increasing colchicine concentration may be attributed to these factors and their high sensitivity to colchicine.

Growth characters

Reduction in growth characters viz. number of leaves produced and length of plantlet 45 days after

treatment with increasing colchicine concentration was noticed in both varities. Plantlet length and leaf production were maximum when dry seed embryo was used and minimum with pro-embryos. Day of treatment did not exert any significant influence.

The growth rate of cultured material in culture tubes was influenced by different doses of colchicine and different types of embryos used. The remarkable variations showed by cultured embryos indicated the sublethal effect of colchicine and innate ability of dry seed embryos to survive in the medium.

Morphological variations

Some morphological abnormalities such as flowering in the culture tubes, stunted growth of plantlets. failure in callus differentiation and organogenesis etc. were noticed in the colchicine treated calli. Sublethal effect and biochemical variations caused by the alkaloid colchicine in the medium may be the probable reason which hinders the further development and immature flowering in the medium. John Staba (1990) reported that callus development was affected with variation in the chemical composition of the medium.

Survival of plants

Two parameters viz. regeneration percentage of calli in the medium and survival percentage of plants under green house were studied in the colchicine treated embryos of Cucumis sativus.

Drastic fall in the regeneration percentage of calli was noticed with increasing colchicine concentrations in both varities. With increasing colchicine concentrations, reduction in survival of plants under green house was noticed in Delila. In Seethal, the reduction was significant. Pro-embryos reduced the minimum regeneration percentage of calli and survival in the green house, in both varities. Day of treatment exerted an insignificant effect only.

It is worthwhile to note that the stage of embryo development determined the level of colchicine sensitivity. Regeneration percentage of the treated pro-embryos was poor. But the regeneration percentage was good for the mature and dry seed embryos. This indicated that the maturity factor played a major role in the regeneration of callus. Among the controls also the pro-embryos recorded comparatively low regeneration percentage. Reduction in regeneration in colchicine treated embryos may be due to biochemical changes

induced by colchicine during early stages of development of embryos and also due to the physiological immaturity of the embryos to develop in the medium.

Significant reduction in survival was noticed in the embryo culture plants in the green house in all treatments. Even the dry seed embryos which showed maximum callus growth and regeneration in the culture medium recorded poor survival in the green house.

Arya (1987) reported production of excess quantities of phenolics by dry seed embryos upon development in the medium which is deleterious for further development. John Staba (1990) reported increasing level of phenolics content in medium along with callus development which hinders further growth and development. Poor callus growth shown by pro-embryos and mature embryos in culture tubes from the initial stage onwards may be one probable reason for the very low survival shown by plants in the green house. During growth and development of plantlet from dry seed embryos, production of excess quantities of phenolic compounds by the plantlets may occur which hinders further plant development. The transferring of cultured plants to green house may produce severe transfer shock and may result in the low survival of plants. Many reasons had been attributed for the poor survival of tissue cultured plants

under green house conditions. Leaves with poor or no development of cuticular wax on leaf surfaces, poor development of epicuticle and pronounced mesophyll air spaces may be one probable reason (Grout and Aston, 1978; Donnelly and Vidaver, 1984). Impaired stomatal mechanism with non-closing stomata has also been cited as a reason by Brainerd and Fuchigami (1982) and Capellades *et al*. (1990). Availability of surplus sugar in the medium makes the *in-vitro* plants mixo-or heterotrophic and not fully phototrophic. So they have only poor photosynthetic ability (Grout and Aston, 1977 and Lee *et al*. 1985). Lack of stomata, epicuticle, healthy root system etc. in the embryo cultured plants may result in poor survival. Here the change from heterotropic to autotropic condition in soil condition may not be gentle always. Lack of good root system, stomatal openings, epicuticle etc. may cause loss of plantlets in the green house.

It can be concluded that dry seed embryos of *Cucumis sativus* are ideal for *in-vitro* treatment with colchicine. Eventhough higher concentrations of colchicine produced maximum variations, its lethal hazards suggested that its lower concentrations are more desirable for inducing polyploidy under *in-vitro* conditions.

SUMMARY

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The investigation was carried out as two separate experiments. Experiment I (*in-vivo* studies) was undertaken in the Department of Agricultural Botany, College of Agriculture, Vellayani and Experiment II (*in-vitro* studies) was conducted at the Tissue Culture Laboratory attached to the Department of Pomology, College of Horticulture, Vellanikkara. The investigation was conducted with a view to study the effect of colchicine on *Cucumis sativus* under *in-vivo* and *in-vitro* conditions. The study materials included two varieties of *Cucumis sativus* viz., 'Seethal' and 'Delila'. The main objectives of the study were to study the effect of colchicine applied in different concentrations (0.2, 0.3 and 0.4%) for different periods of treatment (2, 4 and 6 hours) on seeds, seedlings and apical buds of *Cucumis sativus* plants ; to standardise a medium for embryo culture under *in-vitro* conditions and to study the effect of colchicine applied in different concentrations (0.02, 0.03 and 0.04 %) for different periods of treatment (2, 4 and 6 hours) on pro-embryos, mature embryos and embryos of dry seeds. In the first experiment observations were recorded on the survival of plants, growth characters,

fruit characters, stomatal length, pollen size and pollen sterility. In the second experiment observations were recorded on regeneration of callus, embryogenesis, survival and growth characters in greenhouse conditions. Based on the results eminated following conclusions were made.

Experiment I

1. Colchicine concentration of 0.4 per cent and period of treatment of six hours resulted in minimum survival of plants in both varieties. Survival was maximum when 0.2 per cent concentration was resorted to and when the treatment was given for two hours. Seed treatment gave maximum survival.

2. At 30 days of growth stage vine length, number of branches and leaves/plant reduced significantly in all colchicine treated plants compared to control. At higher levels of treatments, the variations increased significantly.

3. A progressive delay in number of male and female flower opening was noticed with increasing colchicine concentration and periods of treatment in both varieties. Mode of treatment did not exert any significant influence in both varieties. All the colchicine treatments effected significant delay in flowering when compared to control.

4. A significant reduction in number of male and female flowers per plant was recorded in the colchicine treated plants when compared with control. At higher levels of colchicine concentration and periods of treatment the reduction was much higher.

5. A significant increase in stomatal length was recorded with increasing concentrations of colchicine in both varieties. Mode of treatment did not produce any significant influence.

6. Fruit characters viz., number of fruits per plant, length of fruit, girth of fruit and weight of fruit were not significantly influenced by various colchicine treatments in both varieties.

7. There was a progressive increase in pollen size and pollen sterility percentage in both varieties with increasing colchicine concentration levels. Apical bud treatment given to period of six hours produced largest pollen grains and produced highest sterility in the variety Seethal. But in the variety Delila the above treatment produced only maximum sterility. The pollen size was not significantly changed.

Progressive increase in variation was noticed for all characters along with the increase in colchicine

concentration and period of treatment. Apical bud treatment found to be most damaging. All treatments showed significant variations for the different characters studied compared to control plants. Survival of plants was lowered significantly in higher levels of colchicine concentrations and periods of treatment is not desirable. Hence the study indicated that seed treatment of 0.2 per cent colchicine given for a period of two hours can be considered as ideal to be used for the induction of polyploidy in Cucumis sativus.

Experiment II

1. Delayed embryogenesis was resulted in colchicine treatments. The delay showed progressive increase in response to the progressive increase in colchicine concentration from 0.02 to 0.04 percent in both the varieties. Considerable delay in embryogenesis was noted when pro-embryos were used for culturing. Day of treatment did not influence significantly the number of days taken for embryogenesis.

2. Progressive and significant reduction in regeneration percentage of callus was noticed with increasing colchicine concentration in both the varieties. Pro-embryos recorded significantly low regeneration

percentage. No significant variation was produced by day of treatment.

3. With respect to number of leaves produced per plant in the culture tubes at 45 days after treatment significantly low values were recorded by colchicine at 0.04 per cent concentration in both Seethal and Delila. Leaf production was significantly low when pro-embryos were used. Day of treatment did not exert significant effect.

4. Length of plantlet at 45 days after treatment was studied as an index of growth rate of plantlets in culture medium. It was decreasing with increasing levels of colchicine concentration in both the varieties. Plantlets from dry seed embryos showed maximum growth rate compared to those from mature and pro-embryo.

5. Survival of plants under green house condition was also found to be affected significantly, by colchicine concentration at 0.04 per cent level. Plants from pro-embryos showed least survival under green house condition. Day of treatment had no significant influence on this parameter.

6. Some abnormal growth behaviours were noticed in the plantlets in culture tubes like failure in callus development, lack of leaf formation in medium, premature flowering etc. in the colchicine treated embryos.

7. Under in-vitro study also, eventhough higher concentrations of colchicine produced maximum variations on callus growth and plantlet development, they recorded low survival of plants. Lowest level of colchicine 0.02 per cent produced significant variation from the control for all characters studied. Hence this can be considered as ideal to be used for inducing polyploidy under in-vitro with less lethal effects. With respect to planting material used for inoculation dry seed embryos were found to be best suited for culturing.

REFERENCES

REFERENCES

Affonso, O.R., E.Mitidiert and L.P.Ribeiro.1967. Comparative effects of carbon tetra chloride and colchicine on Xanthine dehydrogenase. Experientia 23 :291 - 293.

Agrawal, P.K and R.P.Roy. 1976. Natural polyploids in Cucurbitaceae I. Cytological studies in triploids Momordica dioica Roxb. Caryologia 29 : 7 - 13.

Amin, K.C. 1940. A preliminary note on interspecific hybridisation and use of colchicine in cotton. Curr. Sci. 9 : 74 - 75.

Ammirato, P.V. 1983. Embryogenesis In: Evens, D.A., W.R. Sharp, P.V. Ammirato and Y.Yamada. eds. The Hand book of plant cell culture, Techniques for Propagation and Breeding, I. Mac Millan Publishing Co. New York. pp. 82.

Andrus, C.F., V.S. Shisadri and P.C. Grimball. 1971. Production of seedless watermelon. Tech. Bull. No. 1425. U.S.D.A. 1 - 12.

Anonymous, 1986. Netherlands Institute for Horticultural Plant Breeding. Cytogenetics in Annual report.

Armstrong, J.M and R.W. Robertson., 1960. Studies on colchicine induced tetraploids of Trifolium hybridum. Comparison of characters of tetraploid and diploid. Canadian J. Genet.Cyt. 52:371 - 378.

Artvinli, S. 1987. Cytoskeliton, Microtubules, Tublin and Colchicine, a review. Cytologia 52 : 189 - 197.

Arya, K. 1987. In - vitro techniques in relation to induced mutations in ground nut. M.sc(Ag) thesis submitted to K.A.U : pp. 45 - 94.

Ayyangar.K.R. 1949. Proc. 36th Ind.Sci.Congress. Pt. 3: 137.

Batra, S. 1952. Induced tetraploid in musk melon. J. Hered 113 : 141 - 148.

Bemis, W.P. 1950. Polyploid hybrids from the crosses Cucurbita moschata Lam. Poir X C. factidissima J. AM. Soc. Hort. Sci. 95 : 529.

Bhaduri, P.N and P.C. Bose. 1947. Cytological investigation in some common cucurbits with special reference to fragmentation of chromosomes as physical basis of speciation. J. Genet. 48 : 237 - 256.

Darlington, C.D. 1965. Recent advances in Cytology. 2nd Ed. J and A, Churchill Ltd, London.

Davidson, D and R.D. MacLeod . 1966. Changes in mitotic indices in roots of Vicia faba I. Antagonistic effects of colchicine and IAA. Chromosoma 18 : 421 - 437.

Donnelly, D.J and W.E. Vidaver. 1984. Leaf anatomy of red raspberry transferred from culture to soil. J. Amer. Soc. Hort. Sci. 109 : 172 - 176.

*Dzevaltovskii, A.K. 1982. Some features of the formation of the male and female gametophytes in induced polyploids of Melo sativus Sag., Cucumis sativus.L and Citrullus vulgaris.Schrad. Okhrana izuch i obogashch rastit mira. resp. mezhduved. nauch. sb 9 : 115 - 119.

Fassuliotis, G and B.V.Nelson. 1986. Conversion in-vitro of Cucumis metuliferus and C. metaliferus & C. anguria embryos. In : Withers & PG Alderson (Eds.) Plant Tissue Culture and Agricultural application, Butterworths, London. pp. 419 - 426.

Fassuliotis, G and B.V. Nelson. (1988) Interspecific hybrids of Cucumis metaliferus X C. anguina obtained through embryo culture and somatic embryogenesis.

Gambley, R.L and W.A. Dodd. 1990. An in-vitro technique for the production de novo of multiple shoots in cotyledon explants of Cucumber(Cucumis sativus L). Plant Cell,Tissue and Organ culture. 20:177 - 183.

Ghosh, P.D. 1982. Induced mutagenesis and chromosome instability in tissue culture of certain crop plants Sci. Acad. Medals for Young Scientist. Lectures, 24 - 42.

*Gautheret, R.J 1938. Sur le repiquage des cultures tissue cambial de, Salix Capraea l. r hebd Scane. Acad. Sci. Paris. 206 125 - 127. Grout,B.W.W and M.J. Aston. 1977. Modified leaf anatomy of cauliflower plantlets regenerated from meristem culture. Ann. Bot. 42 : 993 - 995.

Grout, B.W.W and M.J. Aston. 1978. Transplanting of cauliflower plants regenerated from meristem culture II. Carbon dioxide fixation and the development of photosynthetic ability. Hor. Res. 17 : 65 - 71.

Halberstoedler, L and A. Beck. 1943. Influence of colchicine alone and combined with X-rays on Paramecium. Nature. 152: 275 - 276.

Handley, L.W and O.L. Chambliss. 1979. In-vitro propagation of Cucumis sativus L. Hort. Science. 14 : 22 - 23.

*Hartmair, V. 1943. Colchicine induzierte polyploidy bei Garten. Zuchter. 15 : 13 - 16.

Heinz, D.J., M.Krishnamurthi., L.G.Nickell and A.Maretzki. 1977. Cell, Tissue and Organ culture in sugarcane improvement. In : Reinert, J and Y.P.S. Bajaj eds: Applied and fundamental aspects of Plant cell, Tissue and Organ culture. Springer - Verlag, New York. I Ed. pp 3 - 17.

Janaki Ammal, E.K and Bezbaruach. 1962. Induced tetraploidy in Catharanthus roseus. Proc. Indian Aca. Sci. Sec. B. 7(6) : 339 - 342.

Kihara, H. 1951. Triploid water melon. Proc. Amer. Soc. Hort. Sci. 58 : 217 - 230.

Kim, S.G., J.R. Chang, H.C. Cha and K.W.Lee. 1988 Callus growth and plant regeneration in diverse cultivars of cucumber (Cucumis sativus.L) Plant cell, Tissue and Organ culture 12 : 75 - 80.

Krishnan, R., M.L. Magoon and R.V.Bai. 1970. Desynapsis in Colocasia intiquorum Scott. Genetica. 41 : 170 - 178.

Kumar, L.S.S and A. Abraham. 1942. A study of colchicine induced polyploidy in Phaseolus radiatus L. Jour. Uni.Bombay. 11 : part III.

Kumar, L.S.S and S.Vishveshwariah. 1951. Chromosome number of some wild cucurbits Curr. Sci. 20 : 211.

Kumar, L.S.S and S.Vishveshwariah. 1952. Sex mechanism in Coccinia. Wight and Arn. Nature 17 : 330.

Lathika, M. 1968. Studies on the C_2 generation of the colchicine induced polyploids of chillies. Msc. (Ag) thesis submitted to K.A.U.

Lazarte, J.E and C.C. Sasser. 1982. Asexual embryogenesis and plantlet development in anther culture of Cucumis sativus L. Hort. Science. 17 : 88.

Lee, N., H.Y. Westzstein and H.E. Sommer. 1985. Effects of quantum flux density on photosynthetic and chloroplasts ultra structure in tissue cultured plantlets and seedling of Liquidambar styraciflua.L. towards improved acclamatisation and fixed survival. Plant physiol. 78 : 637 - 641.

*Levan, A. 1938. The effect of colchicine on root mitosis in Allium. Hereditae. 24: 471 - 486.

Love, S.L., B.B. Rhodes and P.E.Nugeut. 1986. Controlled pollination transfer of a nuclear male sterile gene from a diploid to a tetraploid watermelon line. Euphytica. 35 (2): 633 - 638.

*Luongdinhoua. 1950. Artificial polyploidy in oryzas. Sciken Jiho (Biological reports) 4 : 43 - 53.

Macleod, R.D and D.Davidson 1968. Changes in mitotic indices in roots of Vicia faba III. Effects of colchicine on cell cycle times. Exp. Cell. Res. 52 : 541 - 544.

*Mackiewicz, H.O. 1989. Gene mutations from colchicine treatment in cucumber and head cabbage. Biuletyn Warzywniczy supplement : 59 - 42.

Malawista, S.E. 1965. On the action of colchicine : The melonocyte model. J. Exp. Med. 122 : 361 - 384.

Martin, I.S and M.R. Sondhal. 1984. Early stages of somatic embryo differntiation from callus cells of beans (Phaseolus vulgaris L.) grown in liquid medium. J. Plant. Physiol. 117 : 97 - 103.

*Malepszy, S and A.N. Orczyk. 1983. In-vitro culture of Cucumis sativus L. I. Regeneration of plantlet from callus formed by leaf explants Z. pflanzen physiol. 111: 273 - 276.

Mercykutty, V.C and H. Kumar. 1983. Studies on induced tetraploids in four diverse cultivars of pea. Cytologia 48 : 51 - 58.

*Miege, J. 1962. Quatrieme liste de nomoers chromosomiques d'especes d' Afrique Occidentale. Rev. cytol. et Biol. Veg. 24 : 149 - 164.

Movel, G. 1960. Producing virus free cymbidium. Am. Orchid. Soc. Bull. 29: 495 - 497.

Murashige, T and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol.Plant. 15 : 473 - 497.

*Nebel, R.R and M.L. Ruttle. 1938. The cytological and genetical significance of colchicine. J. Hered. 28 : 3 - 9.

*Nobecourt, P. 1939. Sur la parennite et l' augmentation de volume des cultures de tissues vegetaux. C.R. S'eane Soc. Biol. 130 : 1270 - 1271.

*Malepszy, S and A.N. Orczyk. 1983. In-vitro culture of Cucumis sativus L. I. Regeneration of plantlet from callus formed by leaf explants Z. pflanzen physiol. **111**: 273 - 276.

Mercykutty, V.C and H. Kumar. 1983. Studies on induced tetraploids in four diverse cultivars of pea. Cytologia **48** : 51 - 58.

*Miege, J. 1962. Quatrieme liste de nomoers chromosomiques d'especes d' Afrique Occidentale. Rev. cytol. et Biol. Veg. **24** : 149 - 164.

Movel, G. 1960. Producing virus free cymbidium. Am. Orchid. Soc. Bull. 29: 495 - 497.

Murashige, T and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol.Plant. **15** : 473 - 497.

*Nebel, R.R and M.L. Ruttle. 1938. The cytological and genetical significance of colchicine. J. Hered. **28** : 3 - 9.

*Nobecourt, P. 1939. Sur la parennite et l' augmentation de volume des cultures de tissues vegetaux. C.R. S'eane Soc. Biol. **130** : 1270 - 1271.

Orczyk, W. and S. Malepszy. 1985. In- vitro culture of Cucumis sativus L. V. Stabilising effect of glycine on leaf protoplasts. Plant Cell Reports. 4 : 269 - 273.

Pearson, O.H., R. Hopp and G.W. Bohn. 1951. Notes on species crosses in Cucurbita. Proc.Am. Soc. Hort. Sci. 57: 310.

Rajasekharan, K., M.C. Mullius and Y.Nair. 1963. Flower formation in-vitro by hypocotyl explants of cucumber (Cucumis sativus L.) Ann.Bot. 52 : 417 - 420.

Ramanujam, S and A.B.Joshi. 1941. Colchicine induced polyploidy in crop plants. I. Gram (Cicer arietinum L.) Indian Agri. Sci. 11: 835 - 849.

Ramachandran, C and R.K.J. Narayanan. 1985. Chromosomal variation in Cucumis. Theoretical and applied Genetics. 69 (5/6): 497 - 502.

*Randolph, L.F. 1932. Some effects of high temperature on polyploidy and other variations in maize. Proc. Nat. Acad. Sci. U.S.18 : 222 - 229.

*Reinert,J. 1958. Morphogenase and ihre knotrolle an gewe be kul furen an karotten. Naturwissenschaften 45 : 344 - 345.

Roy, R.P., V.Thakur and R.N. Trivedi. 1966. Cytogenetic studies in the genus Momordica. J. Cytol. Genet. 1 : 30 - 40.

Roy, R.P and R.N. Trivedi. 1966. Cytology of Gomphogyne cissiformis. Curr Sci. 35 : 420 - 421.

Roy, R.P and J.Ghosh. 1971. Experimental polyploids of Luffa echinata Roxb. Nucleus. 14 : 111 - 118.

Sekioka, T.T and J.S. Tanaka . 1981. Differentiation in callus cultures of cucumber Cucumis sativus L.Hortscience. 16 : 45.

Singh, A.K. 1974. Cytological studies in Melothira. Linn. Ann. of Arid zone Res. 13 : 266 - 268.

Singh, A.K and R.P. Roy., 1975. Cytomorphological studies in polyploids of Trichosanthes anguina L. Cytologia. 40 : 13 - 20.

Singh, A.K. 1975. Studies on the colchiploids of Cucumis melo L. Necleus 18 : 38 - 43.

Singh, A.K. 1979. Cucurbitaceae and polyploidy. Cytologia 44 : 897 - 905.

Singh, A.K and R.P.Roy 1979. Cytological studies in Trichosanthus. J. Cytol. Genet. 14 : 50 - 51.

Singh, A.K and K.S. Yadav. 1984. Cytogenetics of Cucumis L. IV. Comparative study on natural and induced polyploids. Cytologia. 49 : 183 - 194.

Singh, B.D. 1983. Plant Breeding Principles and Methods. Kalyani publishers. New Delhi. pp. 453 - 454.

Singh, R.N and S.K.Roy. 1988. Induced colchiploidy in Petunia hybrida. Cytologia. 53 : 580 - 584.

Sharma, A.k. 1974. Plant cytogenetics. The Cell Nucleus 2 : 263 - 291.

Sharma, A.K. 1975. The concept of chromosome dynamism - control differentiation, reproduction and metabloism in Eukaryota .Nucleus. 18 : 93 - 97.

*Shimotsuma, M. 1965. Chromosome studies of some Cucumis sp. Seiken Ziho Rept. Kihara. Inst. Biol. Res. 17 : 11 - 16.

*Shifriss, O. 1942. Polyploids in Genus Cucumis L.J. Hered. 33 : 144 - 152.

Srivastava, R.N. 1950. Production of fertile autotetraploids in sesame and their breeding behaviour. J. Hered. 47 : 241 - 244.

Stebbins, G.L. Jr. 1950. Variation and Evolution in plants. Columbia Univ. press, Newyork. pp. 38 - 78.

Steward, F.C., M.O. Mapes and K. Mears. 1958. Growth and organised development of cultured cells. II. Organisation in cultures growth from freely suspended cells. Am. J. Bot. **46** : 705 - 708.

Tandon, S.S and J.J. Chinoy. 1950. Colchicine induced polyploidy in Amaranthus bilitum. Sci. and Cult. **15** : 398.

Thakur, G.K. 1973. A natural tetraploid in genus Trichosanthes from Bihar. Proc. 69th Ind. Sc. Cong. Pt. 3 : 334.

Trulson, A.J and E.A. Shahin. 1986. In-vitro plant regeneration in the genus Cucumis. Plant Science. **47** : 35 - 43.

Turkov, N.D., G.A. Shelepina and M.B. Pushnov. 1974. The identification of individual chromosomes in the caryotype of the cucumber (Cucumis sativus L.) as the base of their linear differentiation. Cytologia **40** : 31 - 34.

Van't Hof. 1965. Relationships between mitotic cycle durations, period duration and the average rate of DNA synthesis in the root meristem of several plants. Exp. Cell. Res. 39 : 48 - 58.

Verghese, B.M. 1972. Cytology and origin of tetraploid Trichosanthes palmata Roxb. Genetica. 43:292 - 307.

Wehner, T.C and R.D. Locy. 1981. In-vitro adventions shoot and root formation of cultivars and lines of Cucumis sativus L. Hort. Science. 16 : 759 - 760.

Weisenberg, R.C., G.G. Borisy and E.W. Taylor. 1968. The colchicine binding protein of mammalian brain and its relation to microtubules. Biochemistry 7 : 4466 - 4479.

Whitaker, T.W. 1950. Polyploidy in Echnocystis. Madrona 5 : 209.

Whitaker, T.W and G.W. Bohn. 1950. The taxonomy, genetics, production and uses of cultivated species of Cucurbita. Econ. Bot. 4 : 52.

*White, P.R. 1934. Potential unlimited growth of excised tomato root tips in a liquid medium. Plant physiol. Lancaser. 9 : 585 - 600.

*White, P.R. 1939. Potentially unlimited growth of excised plant callus in an artificial medium. Am. J. Bot. 26 : 59 -64.

Yadava, K.S., A.K. Singh and H.C.Arya.1984.Cytogenetic investigation in Cucumis 1. Cytologia 49 : 1 - 9.

Yadava , K.S., A.K.Singh., R.P Roy and U.C. Jha. 1986. Cytogenetics in Cucumis L.VI. Synthetic amphidiploids. Nucleus. 29 (1,2) : 58 - 62.

Yamaha, G and S.Suematsu. 1936. Sci. Rep. Tokyo University Ser. 3 :21.

Ziv, M and G. Gadazi. 1986 Enhanced embryogenesis and plant regeneration from Cucumber (Cucumis sativus) callus by activated charcoal in solid / liquid double layer cultures . Plant Science. 47 : 115 - 122.

APPENDICES

Appendix I

Composition of modified MS medium used for embryo culture

Constituent	g/l
1. NH_4NO_3	165.0
2. KNO_3	190.0
3. $MgSO_4.7H_2O$	37.0
4. $CaCl_2.2H_2O$	44.0
5. KH_2PO_4	17.0
6. $FeSO_4.7H_2O$	27.84
7. Na_2EDTA	37.24
8. $MnSO_4.7H_2O$	16.9
9. $ZnSO_4.7H_2O$	8.6
10. H_3BO_3	6.2
11. KI	0.83
12. $CoCl_2.6H_2O$	0.12
13. $CuSO_4.5H_2O$	0.12
14. $Na_2MoO_4.2H_2O$	0.125
15. Glycine	0.20
16. Nicotinic acid	0.50
17. Pyridoxine	0.05
18. Thiamine HCl	0.05
19. IAA	0.01
20. Sucrose	30 gm
21. Agar	6 gm
pH	5.5

Appendix II

Effect of interaction of treatments on survival and various morphological characters in Seethal

Treatment interactions	Survival percentage	Length of vine (cm)		No of branches per plant		No of leaves per plant		Days taken to male flower opening	Days taken to female flower opening
		30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP		
$c_1 m_1 t_1$	85.5	114.5	140.2	13.6	19.5	77.8	251.5	24.5	35.5
$c_1 m_1 t_2$	87.0	119.0	141.3	10.9	18.7	73.0	256.5	26.0	34.5
$c_1 m_1 t_3$	76.5	103.5	133.7	7.9	17.5	64.5	269.9	25.0	36.0
$c_1 m_2 t_1$	90.5	103.5	130.5	8.9	19.8	88.5	261.9	25.5	35.0
$c_1 m_2 t_2$	83.0	104.4	135.3	12.3	19.4	71.2	261.8	23.5	34.5
$c_1 m_2 t_3$	73.5	91.8	131.8	18.0	20.3	69.5	266.2	25.0	35.5
$c_1 m_3 t_1$	83.8	93.1	134.0	11.8	18.8	48.0	266.0	22.5	32.0
$c_1 m_3 t_2$	80.5	113.8	128.0	10.3	17.5	37.5	266.7	22.0	33.5
$c_1 m_3 t_3$	84.5	111.8	132.6	8.3	18.5	34.5	260.0	22.0	35.0
$c_2 m_1 t_1$	56.0	99.0	136.8	8.7	18.6	63.5	239.9	25.0	34.0
$c_2 m_1 t_2$	56.8	93.0	141.2	8.4	19.3	51.0	256.5	25.0	34.0
$c_2 m_1 t_3$	65.5	80.0	134.8	10.5	20.7	42.5	266.0	27.0	32.5
$c_2 m_2 t_1$	50.5	98.3	130.2	11.4	19.9	63.5	276.5	26.0	34.5
$c_2 m_2 t_2$	56.8	91.5	139.5	10.2	18.3	53.3	273.8	27.0	32.0
$c_2 m_2 t_3$	47.8	88.0	143.0	4.4	18.5	51.1	273.6	29.0	33.5
$c_2 m_3 t_1$	69.5	32.3	138.0	6.3	18.65	57.0	247.9	24.0	35.5
$c_2 m_3 t_2$	60.8	79.8	139.8	8.5	18.1	46.5	244.9	24.0	36.0
$c_2 m_3 t_3$	53.8	90.5	141.0	9.2	17.8	35.0	267.4	25.0	37.5
$c_3 m_1 t_1$	82.5	114.5	139.3	9.1	19.6	56.0	272.6	26.0	32.5
$c_3 m_1 t_2$	73.5	106.5	120.0	9.2	19.5	44.2	237.7	23.5	34.5
$c_3 m_1 t_3$	62.5	111.7	123.3	8.6	20.9	28.2	262.0	25.5	39.5
$c_3 m_2 t_1$	60.0	88.5	132.0	7.5	20.6	52.5	270.5	24.0	35.5
$c_3 m_2 t_2$	56.5	92.9	137.0	9.5	19.3	41.0	265.7	23.3	41.5
$c_3 m_2 t_3$	38.0	111.7	142.0	5.5	19.4	31.0	266.0	26.0	42.0
$c_3 m_3 t_1$	44.8	74.4	139.5	4.7	18.5	35.5	283.0	23.5	39.0
$c_3 m_3 t_2$	47.0	74.5	133.0	5.2	19.6	30.5	249.7	24.5	40.5
$c_3 m_3 t_3$	39.0	62.6	132.0	3.4	19.5	27.2	267.0	31.5	41.5
$c_0 m_0 t_0$	97.3	120.0	148.4	15.0	23.5	106.7	263.9	23.5	29.5
CD	9.1	5.9	NS	1.1	NS	3.4	NS	1.2	1.2

CD - At 5% level NS - Not significant

Appendix III

Effect of interaction of treatment on various morphological characters in Seethal

Treatment interactions	No. of male flowers per plant	No. of female flowers per plant	Stomatal length (mm)	No. of fruits per plant	Length of fruits (cm)	Girth of fruits (cm)	Weight of fruit (g)	Pollen size (mm)	Pollen sterility (%)
$c_1 m_1 t_1$	545.5	30.5	0.101	6.0	61.0	21.5	697.5	0.050	21.5
$c_1 m_1 t_2$	679.0	27.5	0.090	3.5	38.8	24.0	1080.0	0.051	27.6
$c_1 m_1 t_3$	480.0	25.0	0.102	4.5	38.3	23.1	1267.5	0.061	35.9
$c_1 m_2 t_1$	589.5	31.5	0.111	5.5	35.2	26.5	1295.0	0.052	19.0
$c_1 m_2 t_2$	608.0	25.5	0.093	2.0	43.5	27.5	1322.0	0.060	28.5
$c_1 m_2 t_3$	634.5	26.0	0.111	3.5	41.5	29.5	1260.0	0.060	34.0
$c_1 m_3 t_1$	604.5	29.5	0.112	5.0	43.0	33.0	1350.7	0.060	26.5
$c_1 m_3 t_2$	595.5	24.5	0.131	3.0	44.5	38.0	1045.0	0.071	31.1
$c_1 m_3 t_3$	478.5	24.5	0.112	2.5	22.8	34.0	1337.0	0.061	34.6
$c_2 m_1 t_1$	619.0	25.0	0.143	4.0	49.5	34.0	1465.0	0.071	42.0
$c_2 m_1 t_2$	580.0	22.5	0.133	4.0	51.5	38.0	1418.0	0.072	38.4
$c_2 m_1 t_3$	577.0	25.0	0.128	3.0	33.0	32.0	1297.5	0.071	43.2
$c_2 m_2 t_1$	580.0	25.5	0.128	3.0	44.3	23.0	1350.0	0.060	45.6
$c_2 m_2 t_2$	615.5	24.5	0.132	2.5	47.5	23.0	1347.5	0.072	37.2
$c_2 m_2 t_3$	507.5	23.5	0.122	2.5	42.5	25.0	1375.0	0.062	41.8
$c_2 m_3 t_1$	586.5	26.5	0.133	3.0	27.8	22.0	1662.5	0.062	41.6
$c_2 m_3 t_2$	570.3	23.0	0.143	3.0	35.2	24.0	1300.0	0.063	47.9
$c_2 m_3 t_3$	485.5	22.5	0.119	2.5	34.8	24.0	1400.0	0.063	54.7
$c_3 m_1 t_1$	487.5	23.5	0.129	4.0	37.5	25.0	1217.0	0.063	44.6
$c_3 m_1 t_2$	556.5	27.0	0.129	4.0	39.2	29.0	1150.0	0.062	61.3
$c_3 m_1 t_3$	449.5	21.5	0.133	3.0	39.5	27.05	1647.5	0.072	45.3
$c_3 m_2 t_1$	598.0	26.5	0.147	3.0	37.5	31.0	1002.5	0.062	31.5
$c_3 m_2 t_2$	437.0	24.5	0.133	2.5	31.2	32.5	1070.5	0.073	57.5
$c_3 m_2 t_3$	378.5	12.5	0.150	2.5	37.0	43.5	1797.5	0.08	45.1
$c_3 m_3 t_1$	421.5	24.5	0.130	3.0	57.5	28.5	1175.0	0.08	42.0
$c_3 m_3 t_2$	498.5	22.0	0.113	3.0	32.5	33.5	1465.5	0.073	62.5
$c_3 m_3 t_3$	400.5	22.5	0.153	3.0	39.7	34.0	1770.0	0.074	67.5
$c_0 m_0 t_0$	754.5	33.0	0.090	7.0	39.5	34.0	1197.0	0.04	11.9
CD	33.34	1.31	0.004	NS	NS	NS	NS	0.002	3.0

CD - At 5% level NS - Not significant

Appendix IV

Effect of interaction of treatments on survival and various morphological characters in Delila

Treatment interactions	Survival percentage	Length of vine (cm)		No of branches per plant		No of leaves per plant		Days taken to male flower opening	Days taken to female flower opening
		30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP		
$c_1 m_1 t_1$	95.00	116.00	113.5	7.3	14.5	88.25	223.5	19.5	24.50
$c_1 m_1 t_2$	84.00	103.5	117.5	7.2	16.8	64.75	235.5	21.0	28.5
$c_1 m_1 t_3$	84.25	96.5	126.0	5.2	15.5	53.5	227.0	24.0	28.5
$c_1 m_2 t_1$	88.5	98.8	124.7	4.8	15.3	83.8	222.5	18.5	26.0
$c_1 m_2 t_2$	87.5	87.7	131.0	4.5	16.8	46.5	205.0	23.5	26.0
$c_1 m_2 t_3$	87.5	86.3	145.0	5.5	13.3	38.9	207.0	22.5	25.0
$c_1 m_3 t_1$	91.0	96.3	128.5	5.7	14.0	85.0	213.5	17.5	25.0
$c_1 m_3 t_2$	72.0	87.0	118.2	7.5	15.0	43.0	219.5	19.5	23.0
$c_1 m_3 t_3$	80.0	82.0	124.0	5.5	15.0	56.5	223.5	21.3	21.5
$c_2 m_1 t_1$	74.5	61.5	143.5	4.8	16.4	79.5	235.4	19.0	25.0
$c_2 m_1 t_2$	73.5	62.0	135.5	6.0	15.3	48.5	236.0	21.5	23.5
$c_2 m_1 t_3$	65.5	56.0	133.0	7.4	14.4	48.5	234.5	26.0	24.5
$c_2 m_2 t_1$	73.5	66.5	135.0	8.3	13.4	76.5	232.0	19.5	22.5
$c_2 m_2 t_2$	77.5	65.0	126.5	3.2	14.5	71.0	211.5	22.5	22.5
$c_2 m_2 t_3$	69.5	68.5	124.5	4.4	14.3	67.5	217.5	24.0	22.5
$c_2 m_3 t_1$	79.3	79.5	124.0	3.4	13.5	72.9	233.5	19.5	21.8
$c_2 m_3 t_2$	82.0	61.5	122.0	3.4	13.5	66.7	232.5	21.0	24.5
$c_2 m_3 t_3$	49.5	62.5	127.0	3.2	12.5	62.5	227.5	25.5	25.0
$c_3 m_1 t_1$	68.5	41.0	141.0	2.7	12.3	54.7	221.5	19.5	20.5
$c_3 m_1 t_2$	64.5	34.0	136.0	3.2	14.5	52.0	214.0	24.0	27.5
$c_3 m_1 t_3$	56.5	49.8	129.0	2.6	13.8	31.0	231.0	21.5	32.0
$c_3 m_2 t_1$	63.7	53.5	118.5	4.6	15.0	56.5	216.5	21.5	36.0
$c_3 m_2 t_2$	60.8	40.5	119.8	4.7	16.5	55.5	218.5	23.5	29.5
$c_3 m_2 t_3$	55.0	58.0	122.3	2.4	13.5	16.0	216.5	32.5	32.0
$c_3 m_3 t_1$	56.0	65.3	137.7	4.1	15.7	52.7	215.5	25.0	33.5
$c_3 m_3 t_2$	49.6	65.3	129.5	3.6	14.0	51.0	224.0	24.0	31.0
$c_3 m_3 t_3$	48.5	81.5	128.2	2.6	14.1	16.0	218.5	28.5	36.5
$c_0 m_0 t_0$	99.5	120.0	134.6	11.0	16.5	02.8	234.5	17.0	28.0
CD	4.78	6.99	NS	0.86	NS	5.11	NS	0.82	1.28

CD - At 5% level NS - Not significant

Appendix V

Effect of interaction of treatments on various morphological characters in Delila

Treatment interaction	No. of male flowers per plant	No. of female flowers per plant	Stomatal length (mm)	No. of fruits per plant	Length of fruits (cm)	Girth of fruits (cm)	Weight of fruit (g)	Pollen size (mm)	Pollen sterility (%)
$c_1 m_1 t_1$	577.5	20.5	0.090	8.5	43.7	24.0	1150.0	0.030	13.0
$c_1 m_1 t_2$	549.0	17.5	0.089	6.5	45.5	22.0	1273.5	0.031	18.3
$c_1 m_1 t_3$	396.0	22.0	0.091	5.5	52.8	27.0	1305.0	0.041	44.5
$c_1 m_2 t_1$	416.0	18.0	0.092	7.5	51.5	28.5	1307.5	0.041	26.7
$c_1 m_2 t_2$	401.5	14.5	0.121	7.0	36.5	28.5	1310.5	0.041	31.0
$c_1 m_2 t_3$	448.5	13.0	0.131	7.5	60.0	32.5	1260.0	0.042	40.0
$c_1 m_3 t_1$	417.5	13.0	0.097	7.5	45.8	28.1	1350.0	0.041	11.5
$c_1 m_3 t_2$	415.5	15.5	0.113	5.0	40.0	27.0	1395.0	0.042	32.4
$c_1 m_3 t_3$	434.5	17.0	0.123	4.0	37.0	32.5	1247.5	0.041	41.8
$c_2 m_1 t_1$	420.5	18.0	0.122	5.5	42.0	32.5	1315.0	0.042	38.0
$c_2 m_1 t_2$	359.5	13.0	0.132	4.5	69.0	27.5	1247.5	0.042	22.0
$c_2 m_1 t_3$	411.3	14.0	0.121	6.0	62.5	38.5	1335.0	0.042	49.0
$c_2 m_2 t_1$	389.5	15.0	0.103	4.0	57.5	29.0	1272.5	0.041	25.2
$c_2 m_2 t_2$	387.0	17.5	0.123	4.5	57.5	29.5	1352.5	0.042	13.2
$c_2 m_2 t_3$	389.3	13.5	0.133	6.0	44.0	31.0	1360.0	0.033	53.5
$c_2 m_3 t_1$	365.0	18.0	0.122	5.5	48.0	30.8	1150.0	0.041	16.5
$c_2 m_3 t_2$	355.5	14.5	0.122	5.0	50.3	32.8	1160.0	0.043	20.2
$c_2 m_3 t_3$	368.5	15.0	0.122	4.5	55.5	30.0	1300.0	0.043	53.5
$c_3 m_1 t_1$	401.0	14.5	0.133	5.5	63.5	22.0	1050.0	0.042	32.3
$c_3 m_1 t_2$	409.0	14.0	0.119	4.5	56.0	30.5	1150.0	0.043	31.9
$c_3 m_1 t_3$	443.5	12.0	0.119	6.0	47.5	31.5	1325.0	0.051	71.5
$c_3 m_2 t_1$	290.0	14.5	0.123	4.0	60.5	27.5	1590.0	0.042	38.4
$c_3 m_2 t_2$	305.0	14.0	0.133	4.5	49.0	33.0	1425.0	0.042	55.8
$c_3 m_2 t_3$	217.5	11.0	0.142	6.0	52.0	24.8	1237.5	0.051	62.7
$c_3 m_3 t_1$	321.5	16.0	0.143	5.5	43.5	30.0	1195.0	0.052	50.6
$c_3 m_3 t_2$	326.5	14.5	0.144	5.0	41.8	27.5	1162.5	0.043	51.6
$c_3 m_3 t_3$	319.5	15.0	0.165	4.0	60.8	32.0	1200.0	0.053	68.1
$c_0 m_0 t_0$	602.5	23.5	0.050	9.5	36.9	34.0	1182.5	0.028	3.90
CD	21.5	1.4	0.007	NS	NS	NS	NS	0.001	2.54

CD - At 5% level NS - Not significant

Appendix VI

Effect of interaction of treatments on survival and various plant characters in Seethal

Treatment interactions	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced 45 days after treatment	Survival percentage	Length of plantlet 45 days after treatment (cm)
$c_1e_1d_1$	22.36	1.08	2.31	0.99	4.32
$c_1e_1d_2$	20.54	1.09	2.31	0.29	3.11
$c_1e_1d_3$	24.65	1.79	2.31	0.91	2.92
$c_1e_2d_1$	13.30	17.90	4.97	2.31	3.19
$c_1e_2d_2$	13.98	34.17	5.32	1.64	3.22
$c_1e_2d_3$	12.95	47.72	4.97	1.21	3.29
$c_1e_3d_1$	8.64	62.32	11.30	3.32	4.92
$c_1e_3d_2$	7.98	58.69	8.59	2.00	6.99
$c_1e_3d_3$	7.28	68.62	8.95	2.31	6.22
$c_2e_1d_1$	25.63	0.52	2.31	0.29	2.13
$c_2e_1d_2$	28.08	1.03	2.00	0.91	2.11
$c_2e_1d_3$	23.31	0.66	1.64	0.62	2.61
$c_2e_2d_1$	17.64	17.25	2.95	1.64	7.39
$c_2e_2d_2$	17.66	21.48	3.65	0.62	8.11
$c_2e_2d_3$	19.99	31.06	3.32	1.64	6.79
$c_2e_3d_1$	11.31	47.38	5.65	2.65	8.12
$c_2e_3d_2$	13.66	43.87	4.65	2.31	8.32
$c_2e_3d_3$	12.61	50.09	5.32	1.59	8.11
$c_3e_1d_1$	37.78	0.04	0.99	0.00	2.12
$c_3e_1d_2$	32.96	1.01	0.62	0.29	2.07
$c_3e_1d_3$	35.31	0.05	0.91	0.29	2.18
$c_3e_2d_1$	21.97	9.08	1.31	1.31	6.44
$c_3e_2d_2$	21.90	9.39	1.64	0.91	5.49
$c_3e_2d_3$	20.99	11.02	1.64	0.91	5.76
$c_3e_3d_1$	16.33	20.15	2.65	1.48	6.78
$c_3e_3d_2$	13.98	17.99	3.00	0.91	6.37
$c_3e_3d_3$	12.91	20.10	2.31	0.94	6.92
Control-e_1	14.92	1.48	1.64	0.29	2.12
Control-e_2	7.66	60.96	7.98	2.31	4.01
Control-e_3	5.32	78.65	12.25	3.57	7.13
CD	4.25	4.38	2.14	1.50	1.23

CD - At 5% level NS - Not significant

Appendix VII

Effect of interaction of treatments on survival and various plant characters in Delila

Treatment interactions	No. of days taken for embryogenesis	Regeneration percentage	No. of leaves produced 45 days after treatment	Survival percentage	Length of plantlet 45 days after treatment (cm)
$c_1e_1d_1$	20.99	0.48	2.00	1.31	3.31
$c_1e_1d_2$	16.99	0.76	2.65	0.29	3.62
$c_1e_1d_3$	21.31	0.50	2.31	0.91	2.99
$c_1e_2d_1$	13.26	19.96	3.32	2.31	4.21
$c_1e_2d_2$	13.96	18.96	4.32	1.21	4.12
$c_1e_2d_3$	16.99	21.76	3.62	2.31	4.32
$c_1e_3d_1$	9.95	35.11	8.66	3.32	8.52
$c_1e_3d_2$	10.98	28.81	7.66	0.91	6.61
$c_1e_3d_3$	11.98	30.60	7.32	1.64	8.39
$c_2e_1d_1$	26.59	0.43	1.94	0.99	2.61
$c_2e_1d_2$	26.23	0.43	1.64	0.29	2.42
$c_2e_1d_3$	26.65	0.39	2.31	0.29	2.33
$c_2e_2d_1$	15.23	16.99	2.61	1.31	6.32
$c_2e_2d_2$	13.63	15.50	2.31	1.64	6.19
$c_2e_2d_3$	14.58	12.98	1.64	1.31	5.32
$c_2e_3d_1$	9.60	27.95	3.96	1.64	7.11
$c_2e_3d_2$	11.00	25.12	3.96	0.99	7.32
$c_2e_3d_3$	13.57	24.30	3.32	1.16	6.92
$c_3e_1d_1$	42.64	0.13	0.62	0.00	2.11
$c_3e_1d_2$	41.33	0.26	0.29	0.00	2.11
$c_3e_1d_3$	38.20	0.36	0.29	0.19	1.91
$c_3e_2d_1$	29.96	8.96	0.99	1.31	3.92
$c_3e_2d_2$	25.66	8.59	1.31	0.29	4.56
$c_3e_2d_3$	27.29	12.49	0.91	0.62	4.72
$c_3e_3d_1$	19.99	19.07	2.31	0.29	5.19
$c_3e_3d_2$	22.28	14.22	2.65	1.94	5.99
$c_3e_3d_3$	23.15	14.08	2.31	0.54	5.82
Control-e_1	13.98	1.87	2.61	0.15	1.11
Control-e_2	7.62	46.21	6.61	1.31	3.18
Control-e_3	5.32	61.16	11.30	1.64	8.32
CD	4.54	3.59	1.99	1.42	1.62

CD - At 5% level NS - Not significant

W = $C_2m_1t_3$

C = Control

Z = $C_3m_1t_3$

C = Control

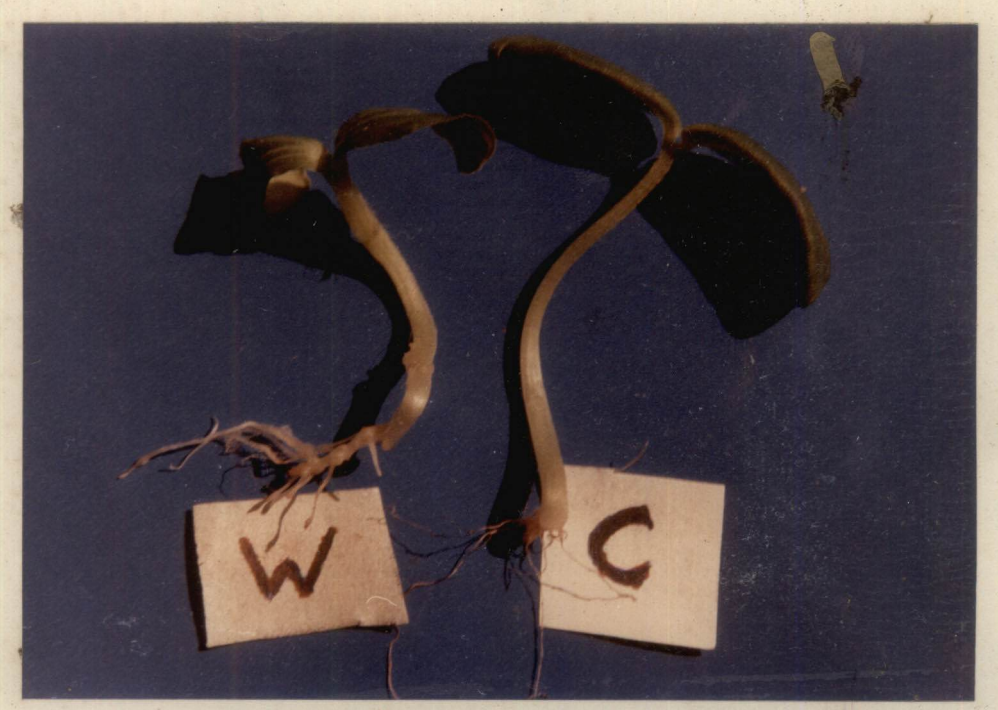


Plate 1. Variations noticed on germination of colchicine treated and control seeds after five days of treatment in variety seethal.

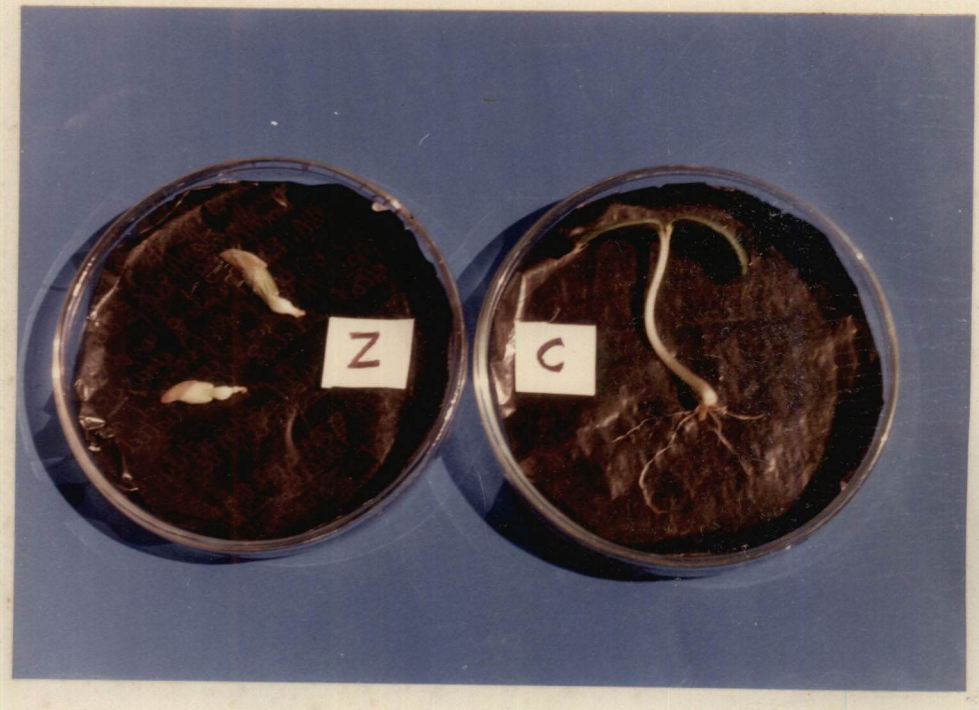


Plate 2. Variations noticed on germination of colchicine treated and control seeds after five days of treatment in variety seethal.

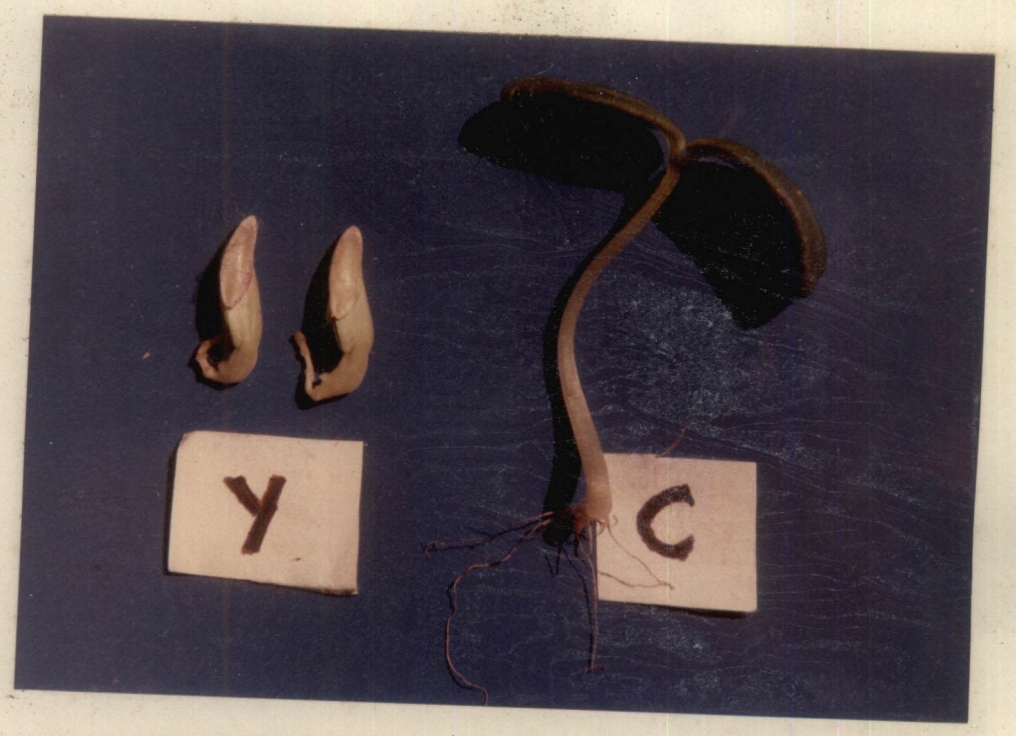


Plate 3. Variations noticed on germination of colchicine trea and control seeds after five days of treatment in variety Delila.

Plate 4. Variations noticed on seedling growth after ten days of colchicine treatment in variety seethal.

Plate 5. Variations noticed on seedling growth after ten days of colchicine treatment in variety Delila.

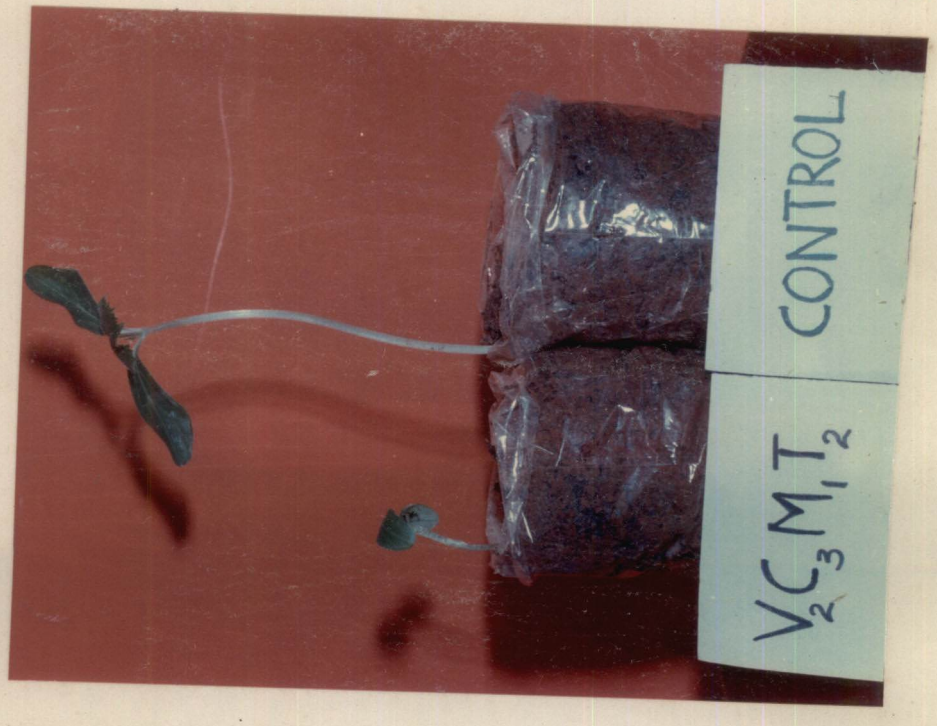
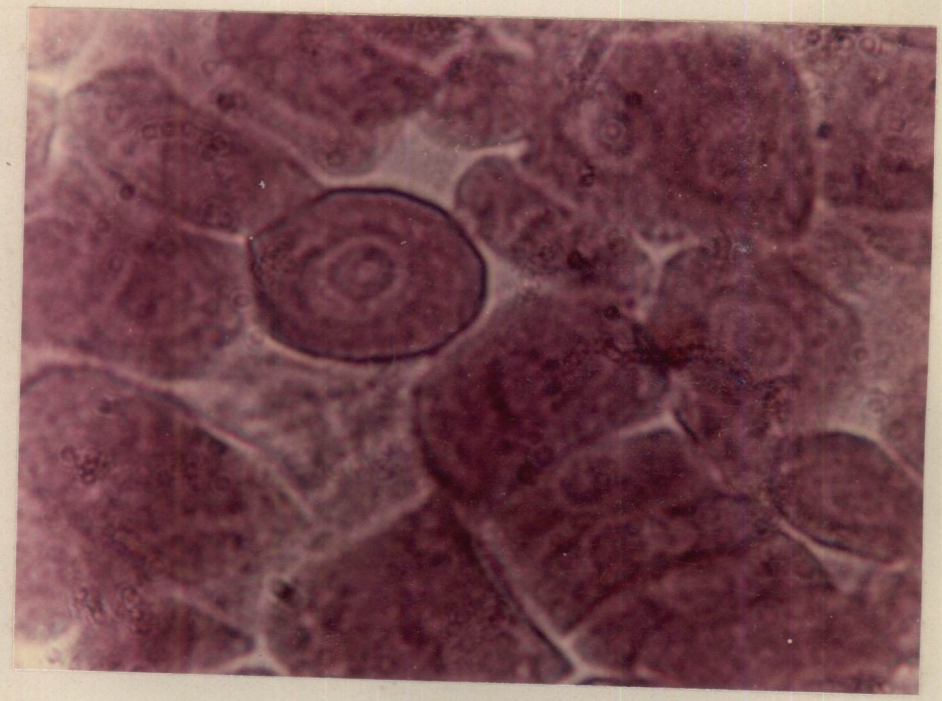


Plate 6. Diploid cells - metaphase and anaphase stages.



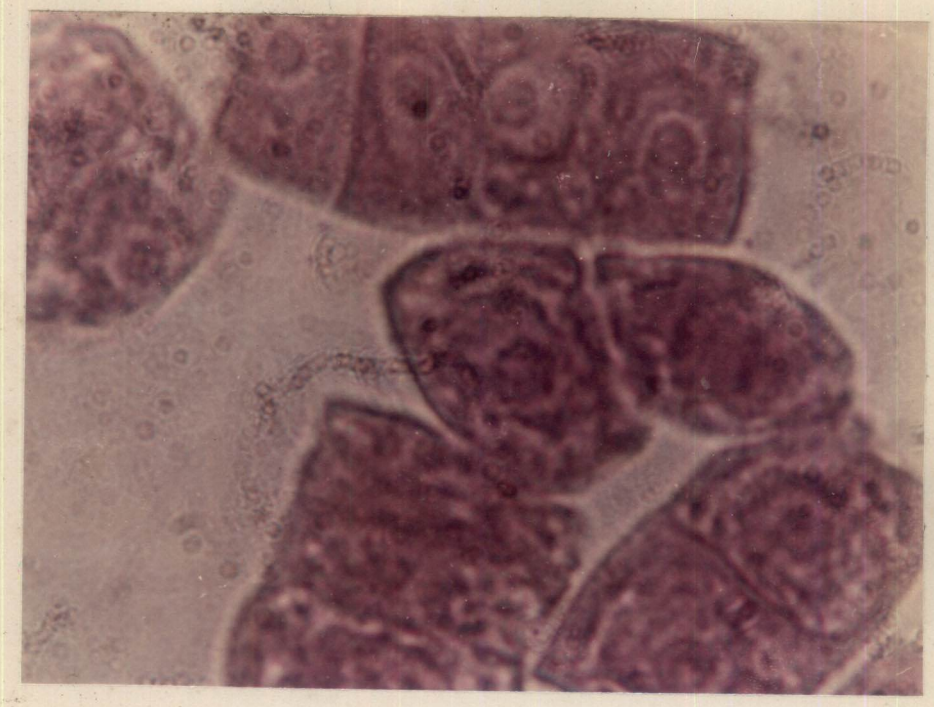


Plate 7. Colchicine treated cells.

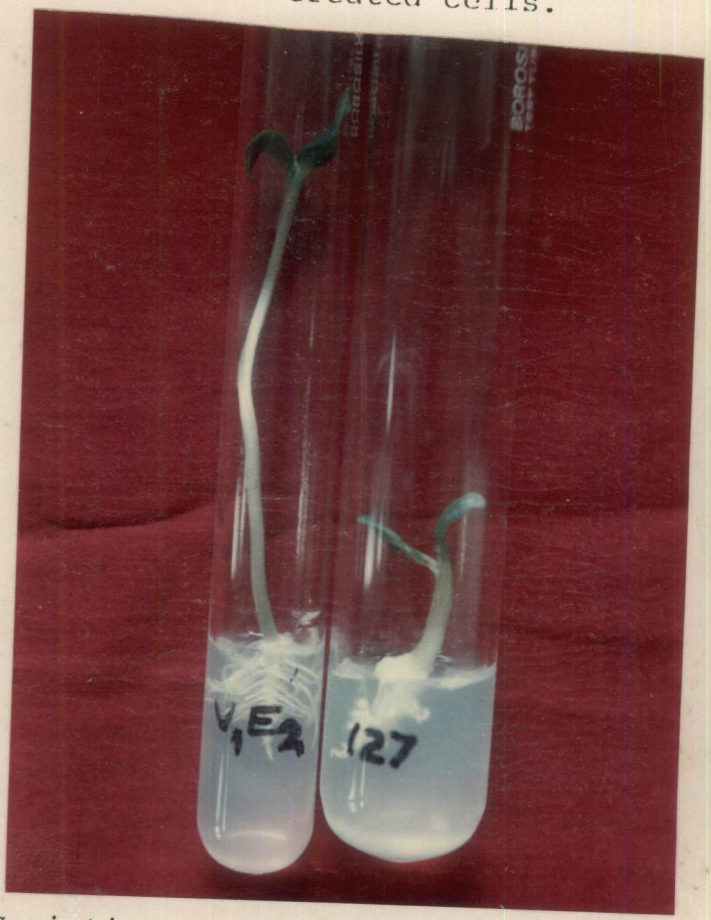


Plate 8. Variations noticed on plantlet growth in culture medium in variety seethal.

$127 = c_3e_2d_1$

Plate 14. Flowering noticed in culture medium

452 = $C_2e_3d_2$ Variety - Delila

Plate 13. Variations noticed on plantlet growth in culture medium in variety Delila.

485 = $C_2e_3d_1$

Plate 16. Callus development without organogenesis.

Plate 15. Shoot development without leaf formation.

Plate 17. Crowth of plantlets in greenhouse - comparison of control with colchicine treated one in seethal.

Plate 18. Growth of plantlets in greenhouse - comparison of control with colchicine treated one in variety Delila.

ARTIFICIAL INDUCTION OF POLYPLOIDY IN *Cucumis sativus* L.

By

K.G. GIRISH KUMAR

ABSTRACT OF A THESIS

submitted in partial fulfilment of the requirement for the Degree of

MASTER OF SCIENCE IN AGRICULTURE

FACULTY OF AGRICULTURE
KERALA AGRICULTURAL UNIVERSITY

Department of Agricultural Botany
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM

1993

ABSTRACT

The present investigation entitled "Artifical induction of polyploidy in Cucumis sativus L. was carried out as two separate experiments. Experiment I, was(in-vivo study) was carried out at the College of Agriculture, Vellayani during the period September 1991 to February 1991. Experiment II (in-vitro study) was carried out at the Tissue culture laboratory attached to Department of Pomolgy, College of Horticulture, Vellanikkara during the period January 1991 to July 1991. The main objective of Experiment I was to study the effect of colchicine for inducing polyploidy in seed, seedling and apical bud treatments. Objectives of Experiment II were to standardise a suitable medium for embryo culture and to study the effect of colchicine on proembryos, mature embryos and dry seed embryos under in -vitro conditions. Experiment I was laid out in RBD with two replications. Experiment II was carried out in CRD with three replications. The two varieties of Cucumis sativus used for the present study were Seethal and Delila. The abstract of results is given below.

Experiment I

Survival of plants in both Seethal and Delila was significantly affected by increasing colchicine concentration 0.2 to 0.4 per cent and with increasing period of treatment from two to six hours. Survival was significantly low in apical bud treatment. Maximum survival was noticed in seed treatment of colchicine 0.2 per cent for a period of four hours. At early growth stage significant reduction was noticed in length of vine, number of branches per plant and number of leaves per plant along with the increase in colchicine concentrations, from 0.2 to 0.4 per cent, and period of treatment, from two to six hours. Seed treatment gave maximum value for these parameters in both varieties except number of leaves in Delila. These variations seen during early growth stages were found to be diminishing at later growth stage (60 days growth stage). Delay in both male and female flower opening along with significant reduction in number of male and female flowers was noticed in higher colchicine concentrations and in lower period of treatments. Mode of treatment did not exert any significant influence on number of days taken for flower opening and total number of flowers produced per plant in both varieties except on number of days taken for female flower opening in Seethal in which by apical bud treatment

maximum delay was noticed. With increasing colchicine concentration from 0.2 to 0.4 per cent and period of treatment from two to six hours significant increase in stomatal length was noticed in both varieties. Mode of treatment exerted no significant influence on stomatal length. All the fruit characters ie. number of fruits per plant, length of fruit, girth of fruit and weight of fruit studied, were not significantly influenced by the treatments tried. In both varieties pollen size and sterility increased considerably with increasing colchicine concentration. Apical bud treatment gave significantly high values for pollen size and pollen sterility in Delila. Seed treatment recorded minimum pollen size and pollen sterility. Cytological studies were conducted in the root tips of colchicine treated seeds and metaphase and anaphase stages were obtained in the normal diploid cells. But the enlarged colchicine affected cells showed very poor stainability.

Experiment II

Standardisation of a suitable medium was carried out by using MS medium as the basal medium. MS medium supplemented with 0.1 mg/L of IAA was found suitable for embryo culture. Three types of embryo viz., pro-embryo, mature embryo and dry seed embryo were used for embryo culturing.

Embryogenesis was delayed significantly with increase in colchicine concentration from 0.02 to 0.04 per cent in both varieties. When pro-embryos were used for inoculation significant delay was noticed for embryogenesis in both varieties. Regeneration of calli was reduced significantly with increase in colchicine concentration. Pro-embryos gave lowest and dry seed embryo gave highest regeneration percentage in both varieties. Length of plantlet and number of leaves produced per plantlet in culture tubes were reduced significantly in the higher levels of colchicine concentration. Pro-embryos gave lowest and dry seed embryos gave highest values with respect to these parameters. Plantlets from pro-embryos showed lowest survival under green house conditions in both varieties. Colchicine concentration exerted no significant influence in Seethal. But in Delila with increasing colchicine concentration from 0.02 to 0.04 per cent, survival of plants in green house reduced significantly. Day of treatment had no significant influence in all the parameters studied.

On the basis of present study it can be concluded that different concentrations of colchicine, different periods of treatment and different modes of colchicine treatment can induce significant changes in the survival of plants, cytomorphological characters of the plants and

pollen sterility. With increasing colchicine conentration and period of treatment the variations increased progressively. But considering the lethal effects as reflected on the survival of plants, 0.2 per cent colchicine application for two hours by seed treatment is desirable under in-vivo condition. Under in-vitro condition use of dry seed embryo is best for embryo culture which can be successfully carried out by using MS medium modified with 0.01 mg/L of IAA. Colchicine 0.02 per cent can be used for the induction of polyploidy under in -vitro conditions. Since it is effective in producing variations with minimum deleterious effects.