RESPONSE OF GLADIOLUS TO RAPID CLONING THROUGH *IN VITRO* TECHNIQUES

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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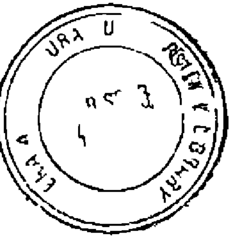

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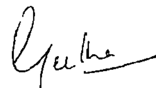

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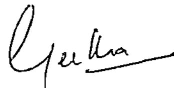

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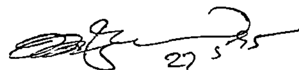

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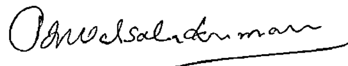

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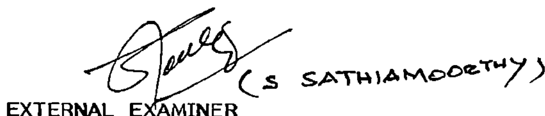

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EXTERNAL EXAMINER

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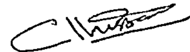

C T SAKKEER HUSSAIN

So glory to Him
In whose Hands is
The dominion of all things
And to Him will ye
Be all brought back

To my Parents ....

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ABBREVIATIONS

AC	Activated charcoal
BAP	6 Benzyl aminopurine
CW	coconut water
2 4 D	2 4-dichlorophenoxy acetic acid
2 4 5 T	2 4 5 trichlorophenoxy acetic acid
2ip	N^6 [2 Isopentenyl] adenine
EDTA	Ethylene diamine tetra acetate
Kinetin	6-Furfuryl amino purine
MS	Murashige and Skoog (1962) medium
SH	Shenk and Hilderbrandt (1972) medium
W/m^2	Watts per meter square

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Introduction

INTRODUCTION

Gladiolus one of the most important bulbous ornamental plants belongs to the family Iridaceae and is grown for the attractive spike of varying colour and length It ranks fourth in the international cut flower trade

Recently it has become a popular cut flower in our State but majority of the spikes come from outside the State especially from Bangalore Considering the increasing demand for the cut flowers adaptability trials and standardisation of agro techniques were initiated in the All India Co ordinated Floriculture Improve ment Project Department of Pomology and Floriculture College of Horticulture Vellanikkara

The major problem faced in the cultivation of gladiolus is the non avail ability of quality planting material at a reasonable price By using conventional methods the number of daughter corms (cormels) produced by one mother corm in one year is very little and it takes 3 4 seasons to produce a corm which is large enough for commercial purpose as the spike size of gladiolus is influenced by the corm size Another problem faced by the industry is due to the dormancy of the corms This actually limits the rapid production of the planting material through conventional methods

In many ornamental plants tissue culture as an alternative method for rapid multiplication has been practiced Rapid multiplication through *in vitro* tech niques is desirable for bye passing dormancy and for bulking up new cultivars or disease free stocks to provide sufficient material for field planting In gladiolus also

advantages of tissue culture propagation compared to the conventional methods were reported by Ziv *et al* (1970) Thereafter various workers like Simonson and Hilderbrandt (1971) Hussey (1977) and Bajaj *et al* (1983) have worked in the field of gladiolus tissue culture and protocols were developed Still certain problems like low rate of multiple shoot production low rate of survival etc are associated with gladiolus tissue culture

The present studies were undertaken to examine the response of various explants and to identify the most suitable explant and the media combination for *in vitro* cloning of gladiolus Studies were also aimed to increase the size of *in vitro* produced corms

Review of Literature

REVIEW OF LITERATURE

Literature on aspects pertaining to the *in vitro* propagation of gladiolus is briefly reviewed hereunder In certain aspects in which not much work has been done in gladiolus relevant studies in other bulbous ornamentals are also included

2 1 *In vitro* propagule multiplication

According to Murashige (1974) there are three possible routes available for *in vitro* propagule multiplication

i) Enhanced release of axillary buds

ii) Production of adventitious buds through organogenesis

iii) Somatic embryogenesis

For the enhanced release of axillary buds primary meristems like shoot tips and axillary buds are mainly used as explants Shoot tip culture ensures genetic uniformity while somatic organogenesis through a callus phase may be useful for recovery of useful variant lines Somatic embryogenesis though limited to a few plant species is the most rapid mode of plant regeneration (Evans *et al* 1981)

2 2 *In vitro* studies in gladiolus

2 2 1 Explants

Success of *in vitro* propagation always depends on the proper selection of explants The response varies accordingly to the type stage and physiological age of the explants

Zıv *et al* (1970) selected explants from ınflorescence axıs of gladıolus for *ın vıtro* studıes That taken from the ınflorescence axıs before the emergence of the spıke responded better than the explants collected after emergence Sımonson and Hılderbrandt (1971) ınduced callus from corm and stem tıps of gladıolus Hussey (1977) reported axıllary buds as the most ıdeal explant for *ın vıtro* propaga tıon of gladıolus Accordıng to Zıv (1979) the axıllary buds of gladıolus prolıferated shoots The buds excısed from the cormels of gladıolus were also used as explants by Konoshıma (1980)

In a trıal conducted by Takatsu (1982) to generate vırus free plants of gladıolus apıcal buds of gladıolus corms of sıze 0 3 mm cube were found to be optımum Callus cultures were establıshed from excısed segments of the ınflores cence flower stalks denuded flower and bracts of gladıolus by Bajaj *et al* (1983) Dıckens *et al* (1986) stımulated axıllary bud growth on corm explants of gladıolus Later ın 1987 Lılıen Kıpnıs and Kochba used the lateral and apıcal buds of gladı olus corms and cormels as explants Lı and Wang (1989) reported the productıon of callus from gladıolus corms Kamo *et al* (1990) also could develop callus from ınflorescence stalks taken from green house grown plants of the cultıvars Blue Isle and Huntıng Song Accordıng to Arora and Grewal (1990) better shoot prolıferatıon was obtaıned only from nodal segments of ınflorescence excısed from field grown plants just before headıng stage

Zakutsakaya and Murın (1990) could ıdentıfy corm bud halves as best explant by testıng varıous parts of corm buds Many other scıentısts (Rumynın *et al* 1990 Zıv 1990 Steımtz *et al* 1991 De Bruyn and Ferreıra 1992) have also opıned that bud from corms of gladıolus produce hıghest frequency of shoots

Callus could also be regenerated from cormel shoot tips of cultivar Beauty spot Jo Wagner Vink s Glory and Wild Rose (Rao *et al* 1991)

2 2 2 Surface sterilants

The explants collected from the field harbour numerous microorganisms which when inoculated onto the nutrient medium contaminate the entire *in vitro* system Hence surface sterilisation is resorted prior to inoculation of explants The aim of surface sterilisation is to remove all the microorganisms present on the ex plant with a minimum of damage to the plant or part to be cultured The efficiency of the sterilants used are found to vary depending upon the type of chemical concen tration and time of exposure etc

Ziv *et al* (1970) disinfected the inflorescence stalk (hidden within the leaves) in 90 per cent ethanol after removing the outer leaves Then the stalk was exposed m a sterile room and a cut was given below the first floret The pieces were then dipped in $CaHClO_4$ solution (90 g/l) for 15 minutes Discs of about 3 4 mm size were made leaving terminal 50 mm below the florets The discs were made on a sterile filter paper soaked in one per cent ascorbic acid to prevent tissue browning Hussey (1976a) used soft tissue paper soaked in 90 per cent ethanol to wipe off the contaminants over the surrounding leaf bases of the newly formed corms Sufficient ethanol was used to wet the surface of the leaf but not to penetrate the spaces around the surface of axillary buds

Ziv (1979) further modified the surface sterilization technique in order to ensure complete asepsis The buds excised from the corms as described were steri lised in 5 per cent Ca $HClO_4$ for 10 minutes and rinsed three times in sterile water

Bajaj *et al* (1983) used freshly prepared chlorine water for surface sterilising the segments of spike rachis perianth leaf bract axillary buds and small cormels of gladiolus The duration of treatment varied from 6 to 8 minutes

De Bruyn and Ferreira (1992) followed another method They soaked the corms in water for one day after which they were heat treated and fungicide treated for 30 minutes in a solution of benomyl (2 g l^{1}) and folpet (4 g l^{1}) at 45 °C The larger corms were then sterilised for 40 minutes in a 1 5 per cent sodium hypochlor ite solution while a 1 2 per cent solution was used for 30 minutes for the smaller corms

2 2 3 Culture media

Composition of media will influence the growth and morphogenesis of plant tissues A proper medium should contain not only adequate quantity of major plant nutrients like salts of nitrogen phosphorous potassium magnesium and sulphur and micronutrients like salts of iron manganese zinc boron copper molybdenum cobalt but also carbohydrates usually sucrose less weight organic compounds like vitamins aminoacids and plant growth regulators Murashige and Skoog s (1962) medium developed for tobacco has been used for *in vitro* culture of plant species (Bonga 1980) The MS medium has the highest salt content and some workers found it beneficial to reduce the strength by half (Skirvin 1980 and Griffis *et al* 1981)

According to Ziv *et al* (1970) modified MS medium was ideal for in florescence segments for callusing Simonson and Hilderbrandt (1971) also induced callus in modified MS medium from corm and stem tips

Hussey (1975 and 1976b) reported that MS medium was ideal for the *in vitro* propagation of the members of the family Iridaceae Liliaceae and Amaryllidaceae if supplemented with growth factors

Half strength MS medium in which iron is added as ferrous enthylene diamine sulphate (25 mg l ^{1}) was used by Hussey (1977) for *in vitro* release of axillary buds from corm explants Ziv (1979) reported MS medium and half strength MS medium as ideal basal medium for gladiolus Bajaj *et al* (1983) also tried different media for gladiolus tissue culture he could obtain callus from inflorescence stalk axillary buds and cormels in MS medium supplemented with growth regulators

MS medium as the ideal medium for *in vitro* propagation of gladiolus was also reported by various workers (Dickens *et al* 1986 Lilien Kipnis and Kochba 1987 Dantu and Bhojawani 1987 Kim *et al* 1988 Ziv 1990 Kamo *et al* 1990 Arora and Grewal 1990 Steinitz *et al* 1991 and De Bruyn and Ferreira 1992)

2 2 4 Growth regulators

According to Krikorian (1982) the success of an *in vitro* system is directly influenced by the correct growth regulator used and its optimum concentration Skoog and Miller (1957) showed in tobacco cultures that the type of morphogenesis could be influenced by concentration of auxin and cytokinin in the medium Shoot induction is promoted when cytokinin level is higher than the auxin Root induction can be achieved by increasing auxin level relatively over cytokinin level Intermediate concentrations of these will tend to produce unorganised tissue

Murashige (1974) reported cytokinin as ideal growth regulator for the axillary shoot proliferation by overcoming the apical dominance Hu and Wang (1983) made a study of the growth regulators used for meristem and shoot tip culture and found that among the cytokinin containing culture establishment medium sixty eight per cent formed with BAP 23 per cent with kinetin and 9 per cent with 2iP The effectiveness of the cytokinin varies with the plant species Lo *et al* (1980) reported that high cytokinin content was deleterious to the initiation and elongation of roots of the plants The deleterious effect of residual cytokinin in stage 2 culture in suppressing the root formation was reported by Ancora *et al* (1981) The role of exogenous auxin in culture proliferation medium is to nullify the suppressive effect of high cytokinin concentrations on axillary shoot elongation thereby restoring normal shoot growth (Lundergan and Janick 1980) Hasegawa (1980) reported that the high concentration of auxin may induce callus formation

The use of auxins (NAA IAA and 2 4 D) and cytokinins (Kinetin and BAP) have been reported in gladiolus for *in vitro* culture (Ziv *et al* 1970 Hussey 1976a and b Ziv 1979 Konoshima 1980 Bajaj *et al* 1983 Lilien Kipnis and Kochba 1987 Dickens *et al* 1986 Kim *et al* 1988 Kamo *et al* 1990 Zakuts kaya and Murin 1990 Steinitz *et al* 1991 and De Bruyn and Ferreira 1992) The influence of GA on shoot elongation has been also noticed by Arora and Grewal (1990) The effect of growth retardants on shoot proliferation and morphogenesis in liquid cultured gladiolus plants have also been reported (Ziv 1990 and 1991)

2 2 5 Medium supplements

Medium supplements are certain complex organic additives which in fluence the establishment and growth of *in vitro* cultures Apart from the inorganic constituents of the media which give consistent results the organic medium supple ments often does not give any definite results Adenine Adenine sulphate caseine hydrolysate yeast extract peptones coconut water tomato juice banana homo genate activated charcoal etc are some of the complex substances added to the media

In gladiolus use of activated charcoal for *in vitro* rooting (Ziv 1979 Lilien Kipnis and Kochba 1987 and Rumynin *et al* 1990) and coconut water for anther culture (Bajaj *et al* 1983) have been reported

2 2 6 Culture environment

Murashige (1974 1977) observed that light intensity quality and dura tion affect the growth of *in vitro* culture He found that the optimum light intensity for shoot formation in a large number of herbaceous species to be around 1000 lux The optimum day light period was considered to be 16 hours for a wide range of plants Yeoma (1986) reported that the environmental temperature at the original habitat of a particular species should be taken into consideration while fixing the culture temperature under *in vitro* condition

Ziv *et al* (1970) could develop callus from the inflorescence explants when cultured in dark and the light influenced the regeneration of various organs

INTRODUCTION

Gladiolus one of the most important bulbous ornamental plants belongs to the family Iridaceae and is grown for the attractive spike of varying colour and length It ranks fourth in the international cut flower trade

Recently it has become a popular cut flower in our State but majority of the spikes come from outside the State especially from Bangalore Considering the increasing demand for the cut flowers adaptability trials and standardisation of agro techniques were initiated in the All India Co ordinated Floriculture Improve ment Project Department of Pomology and Floriculture College of Horticulture Vellanikkara

The major problem faced in the cultivation of gladiolus is the non avail ability of quality planting material at a reasonable price By using conventional methods the number of daughter corms (cormels) produced by one mother corm in one year is very little and it takes 3 4 seasons to produce a corm which is large enough for commercial purpose as the spike size of gladiolus is influenced by the corm size Another problem faced by the industry is due to the dormancy of the corms This actually limits the rapid production of the planting material through conventional methods

In many ornamental plants tissue culture as an alternative method for rapid multiplication has been practiced Rapid multiplication through *in vitro* tech niques is desirable for bye passing dormancy and for bulking up new cultivars or disease free stocks to provide sufficient material for field planting In gladiolus also

advantages of tissue culture propagation compared to the conventional methods were reported by Ziv *et al* (1970) Thereafter various workers like Simonson and Hilderbrandt (1971) Hussey (1977) and Bajaj *et al* (1983) have worked in the field of gladiolus tissue culture and protocols were developed Still certain problems like low rate of multiple shoot production low rate of survival etc are associated with gladiolus tissue culture

The present studies were undertaken to examine the response of various explants and to identify the most suitable explant and the media combination for *in vitro* cloning of gladiolus Studies were also aimed to increase the size of *in vitro* produced corms

Review of Literature

REVIEW OF LITERATURE

Literature on aspects pertaining to the *in vitro* propagation of gladiolus is briefly reviewed hereunder In certain aspects in which not much work has been done in gladiolus relevant studies in other bulbous ornamentals are also included

2 1 *In vitro* propagule multiplication

According to Murashige (1974) there are three possible routes available for *in vitro* propagule multiplication

i) Enhanced release of axillary buds

ii) Production of adventitious buds through organogenesis

iii) Somatic embryogenesis

For the enhanced release of axillary buds primary meristems like shoot tips and axillary buds are mainly used as explants Shoot tip culture ensures genetic uniformity while somatic organogenesis through a callus phase may be useful for recovery of useful variant lines Somatic embryogenesis though limited to a few plant species is the most rapid mode of plant regeneration (Evans *et al* 1981)

2 2 *In vitro* studies in gladiolus

2 2 1 Explants

Success of *in vitro* propagation always depends on the proper selection of explants The response varies accordingly to the type stage and physiological age of the explants

Zıv *et al* (1970) selected explants from ınflorescence axıs of gladıolus for *ın vıtro* studıes That taken from the ınflorescence axıs before the emergence of the spıke responded better than the explants collected after emergence Sımonson and Hılderbrandt (1971) ınduced callus from corm and stem tıps of gladıolus Hussey (1977) reported axıllary buds as the most ıdeal explant for *ın vıtro* propaga tıon of gladıolus Accordıng to Zıv (1979) the axıllary buds of gladıolus prolıferated shoots The buds excısed from the cormels of gladıolus were also used as explants by Konoshıma (1980)

In a trıal conducted by Takatsu (1982) to generate vırus free plants of gladıolus apıcal buds of gladıolus corms of sıze 0 3 mm cube were found to be optımum Callus cultures were establıshed from excısed segments of the ınflores cence flower stalks denuded flower and bracts of gladıolus by Bajaj *et al* (1983) Dıckens *et al* (1986) stımulated axıllary bud growth on corm explants of gladıolus Later ın 1987 Lılıen Kıpnıs and Kochba used the lateral and apıcal buds of gladı olus corms and cormels as explants Lı and Wang (1989) reported the productıon of callus from gladıolus corms Kamo *et al* (1990) also could develop callus from ınflorescence stalks taken from green house grown plants of the cultıvars Blue Isle and Huntıng Song Accordıng to Arora and Grewal (1990) better shoot prolıferatıon was obtaıned only from nodal segments of ınflorescence excısed from field grown plants just before headıng stage

Zakutsakaya and Murın (1990) could ıdentıfy corm bud halves as best explant by testıng varıous parts of corm buds Many other scıentısts (Rumynın *et al* 1990 Zıv 1990 Steımtz *et al* 1991 De Bruyn and Ferreıra 1992) have also opıned that bud from corms of gladıolus produce hıghest frequency of shoots

Callus could also be regenerated from cormel shoot tips of cultivar Beauty spot Jo Wagner Vink s Glory and Wild Rose (Rao *et al* 1991)

2 2 2 Surface sterilants

The explants collected from the field harbour numerous microorganisms which when inoculated onto the nutrient medium contaminate the entire *in vitro* system Hence surface sterilisation is resorted prior to inoculation of explants The aim of surface sterilisation is to remove all the microorganisms present on the ex plant with a minimum of damage to the plant or part to be cultured The efficiency of the sterilants used are found to vary depending upon the type of chemical concen tration and time of exposure etc

Ziv *et al* (1970) disinfected the inflorescence stalk (hidden within the leaves) in 90 per cent ethanol after removing the outer leaves Then the stalk was exposed in a sterile room and a cut was given below the first floret The pieces were then dipped in $CaHClO_4$ solution (90 g/l) for 15 minutes Discs of about 3 4 mm size were made leaving terminal 50 mm below the florets The discs were made on a sterile filter paper soaked in one per cent ascorbic acid to prevent tissue browning Hussey (1976a) used soft tissue paper soaked in 90 per cent ethanol to wipe off the contaminants over the surrounding leaf bases of the newly formed corms Sufficient ethanol was used to wet the surface of the leaf but not to penetrate the spaces around the surface of axillary buds

Ziv (1979) further modified the surface sterilization technique in order to ensure complete asepsis The buds excised from the corms as described were steri lised in 5 per cent Ca $HClO_4$ for 10 minutes and rinsed three times in sterile water

Bajaj *et al* (1983) used freshly prepared chlorine water for surface sterilising the segments of spike rachis perianth leaf bract axillary buds and small cormels of gladiolus The duration of treatment varied from 6 to 8 minutes

De Bruyn and Ferreira (1992) followed another method They soaked the corms in water for one day after which they were heat treated and fungicide treated for 30 minutes in a solution of benomyl (2 g l^{1}) and folpet (4 g l^{1}) at 45°C The larger corms were then sterilised for 40 minutes in a 1 5 per cent sodium hypochlor ite solution while a 1 2 per cent solution was used for 30 minutes for the smaller corms

2 2 3 Culture media

Composition of media will influence the growth and morphogenesis of plant tissues A proper medium should contain not only adequate quantity of major plant nutrients like salts of nitrogen phosphorous potassium magnesium and sulphur and micronutrients like salts of iron manganese zinc boron copper molybdenum cobalt but also carbohydrates usually sucrose less weight organic compounds like vitamins aminoacids and plant growth regulators Murashige and Skoog s (1962) medium developed for tobacco has been used for *in vitro* culture of plant species (Bonga 1980) The MS medium has the highest salt content and some workers found it beneficial to reduce the strength by half (Skirvin 1980 and Griffis *et al* 1981)

According to Ziv *et al* (1970) modified MS medium was ideal for in florescence segments for callusing Simonson and Hilderbrandt (1971) also induced callus in modified MS medium from corm and stem tips

Hussey (1975 and 1976b) reported that MS medium was ideal for the *in vitro* propagation of the members of the family Iridaceae Liliaceae and Amaryllida ceae if supplemented with growth factors

Half strength MS medium in which iron is added as ferrous enthylene diamine sulphate (25 mg l ^{1}) was used by Hussey (1977) for *in vitro* release of axi llary buds from corm explants Ziv (1979) reported MS medium and half strength MS medium as ideal basal medium for gladiolus Bajaj *et al* (1983) also tried dif ferent media for gladiolus tissue culture he could obtain callus from inflorescence stalk axillary buds and cormels in MS medium supplemented with growth regula tors

MS medium as the ideal medium for *in vitro* propagation of gladiolus was also reported by various workers (Dickens *et al* 1986 Lilien Kipnis and Kochba 1987 Dantu and Bhojawani 1987 Kim *et al* 1988 Ziv 1990 Kamo *et al* 1990 Arora and Grewal 1990 Steinitz *et al* 1991 and De Bruyn and Ferreira 1992)

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Ziv *et al* (1970) could develop callus from the inflorescence explants when cultured in dark and the light influenced the regeneration of various organs

De Bruyn and Ferreira (1992) studied the effect of different BAP levels and sucrose concentrations as well as different temperatures on *in vitro* corm production of gladiolus The best corm production was observed in a medium with 6 to 9 per cent sucrose kept at 15 C

Materials and Methods

MATERIALS AND METHODS

The investigations on the response of gladiolus to rapid cloning through *in vitro* techniques were carried out in the Department of Pomology and Floriculture and the Plant Tissue Culture Laboratory attached to the All India Co ordinated Research Project on Floriculture College of Horticulture Vellanikkara during 1992 94 The details regarding the methodology adopted and the analytical techniques are presented in this chapter

In the present study the response of various explants of gladiolus to rapid cloning was attempted through enhanced release of axillary buds somatic organogenesis and somatic embryogenesis (Murashige 1974)

3 1 The explants

The details of different types of explants used for the study are given in Table 1

3 1 1 Collection and preparation of explants

The explants were collected from the gladiolus plants raised in the field of Department of Pomology and Floriculture Planting of gladiolus was done in a staggered manner to make the explants available throughout the season

Leaf explants were collected at different stages of the plant growth viz soon after sprouting two leaf stage five leaf stage and also from the boot leaf

Table 1 Explants used for *in vitro* culture studies of gladiolus

Route	Explant
* Enhanced release of axillary buds	Corm axillary buds Buds from cormel tips Inflorescence nodal segments
* Somatic organogenesis	
1) Direct	Inflorescence internodal segments
2) Callus mediated	Inflorescence segments Flower buds Flower bud bracts Corm internodal pieces Corm axillary buds and Cormel tips Leaf segments Root segments
* Somatic embryogenesis	Leaf segments

Leaves collected were first wiped with tissue paper dipped in 90 per cent ethyl alcohol and moved to the sterile room Pieces of size 5 cm were made out of the leaves

Inforescence explants were collected soon after the emergence of the inflorescence and also from plants with 5 to 6 fully developed leaves the stage when the spike is completely hidden within the leaves Plants were severed at stem base outer leaves were removed to expose the stalk hard base portion discarded and then stalks were cut into pieces of 5 cm and kept on sterilised filter paper young flower buds and flower bud bracts collected from the inflorescence were kept separately

Roots collected from the field grown plants were washed in running tap water and then washed with teepol water followed by sterile distilled water Using bloting paper the materials were made free of water and then wiped with cotton dipped in 70 per cent alcohol and then transferred to sterile room

Corms and cormels were lifted from the soil and washed free of soil and dirt particles and dried under shade They were then treated with 0 2 per cent car bandazim for 30 minutes and again dried under shade Corms are swollen stem bases which comprise several nodes each of which has a superficial axillary bud Most cultivars of gladiolus have 3 to 4 nodes per corm delineated by leaf bases which completely sheath the corm Each leaf base encloses the axillary bud and many younger leaf bases and their buds When the leaves and inflorescence die down after flowering the overlapping leaf bases covering the newly formed corm dry out and soon become thin and membraneous The axillary buds were exposed after peeling of all the scale leaves and then cut out together with a piece of corm tissue The axillary buds collected were washed thoroughly and kept in distilled water after

adding 3 to 4 drops of Extran and kept for half an hour Internodal pieces of the corm were also prepared in the same way

Cormels are miniature corms which are adventitious to the main corm Cormels were peeled off the scales and kept as such in Extran water after thorough washing

3 1 2 Standardisation of surface sterilisation methods

Surface sterilisation of the explants were done in order to make the explants free of contaminations and microorganisms Details of the chemicals used for the surface sterilisation of the explants are given in Table 2 Observations were made on the percentage of contamination and survival on twenty cultures each after every week For this purpose MS medium with optimum levels of growth regulators was used

The explants after surface sterilisation were rinsed four times with sterile distilled water and dried by carefully transferring them onto sterile filter paper placed over a sterile petri plate The leaf pieces were then reduced to size of 1 0 cm The end portions from both sides of the inflorescence segments were removed and made to sizes of 50 0 mm discs Root segments were reduced to sizes of 1 0 cm to 2 0 cm Axillary buds along with the corm tissues taken from the corms and cormel tips after surface sterilisation were reduced in size by removing the tissue from the exposed sides before inoculation into the media

Table 2 Dıfferent chemıcals used for surface sterılısatıon of gladıolus explants

Sl No	Sterılant	Concentratıon (%)	Duratıon of treatment (mınutes)	Explant
1	Mercurıc chlorıde	0 1	1 2 3	Infloresence and leaf explants
2	Mercurıc chlorıde	0 1	10 15 20 25	Corm cormel root explants
3	Mercurıc chlorıde	0 2	10 15 20 25	
4	Ethyl alcohol followed Mercurıc chlorıde	50 0 0 1	3 10 15 20 25	
5	Sodıum hypochlorıte	40 0	10 15 20 25	

3 2 Culture media

The culture media used for the study were MS (Murashige and Skoog 1962) modified MS SH (Shenk and Hilderbrandt 1972) and White s (White 1943) The chemical composition of the media is given in Appendix I

The chemicals used for preparing the culture media were of analytical grade from British Drug House (BDH) Sisco Research Laboratories (SRL) Merck or Sigma

Standard procedures (Gamborg and Shyluk 1981) were followed for the preparation of MS medium Stock solutions of major and minor nutrients were prepared first by dissolving the required quantity of chemicals in double distilled water and were stored under refrigerated conditions in amber coloured bottles The stock solutions of nutrients were prepared fresh once in two months and those of vitamins aminoacids and growth regulators were prepared once in a month

Specific quantities of the stock solutions of chemicals and growth regulators were pipetted out into 1000 ml beaker Sucrose and inositol were added fresh and dissolved The volume was then made upto about 1000 ml by adding double glass distilled water The pH of the solution was adjusted between 5 5 and 5 8 Agar was then added to the medium and stirred thoroughly

SH and Whites media were prepared by weighing out required quantities of major and minor nutrients and were dissolved in double distilled water Growth regulators sucrose and inositol were added fresh and the volume was made up to 1000 ml

The solutions were then melted by keeping in a water bath maintained at a temperature of 90 95 C until the media became clear About 15 ml of the medium was poured hot to oven sterilised culture vessels which were previously rinsed twice with double distilled water The containers with the medium were then tightly plugged with non adsorbant cotton wool plugs Borosilicate test tubes of size 15 0 x 2 5 cm and 10 0 x 2 5 cm were used as the containers

In order to ensure aseptic condition of the medium the containers plugged with cotton plugs were autoclaved for 15 20 minutes at 15 psi pressure and 121 °C temperature (Dodds and Roberts 1985) After sterilisation the culture vessels were immediately transferred to the culture room

3 3 Inoculation of explants

All the inoculation operations were carried out under perfect aseptic conditions m a Klenzaid s laminar air flow cabinet

To inoculate the explants on the culture medium the cotton wool plug of the culture vessel was removed and the vessel neck was first flammed over a gas burner kept in the chamber The sterile explants were quickly transferred into the medium using sterile forceps The neck of the culture vessel was once again flammed and the cotton wool plug was replaced quickly

The culture vessels were then transferred to a culture room where they were incubated at a temperature of 27 ± 2 C Artificial illumination was provided using cool white fluorescent lamps The light intensity was maintained at 2000 lux Photoperiod was fixed as 16 h per day which was regulated by a diurnal timer

3 4 Season of explant collection on *in vitro* survival of explants

The establishment of corm and cormel explants collected from field grown gladiolus varied with season of their collection An experiment was therefore conducted to standardise the best season for corm and cormel explant collection and inoculation in which culture establishment was more and contamination rate the minimum Explants were collected and inoculated for this purpose from January to December The medium used was MS containing optimum amounts of nutrients and growth regulators Observations on the percentage of cultures survived were recorded after three weeks of culturing

3 5 The routes

3 5 1 Enhanced release of axillary buds

3 5 1 1 Explant choice

The explants used for the enhanced release of axillary buds are corm axillary buds cormel tips and nodal segments of inflorescence axis A trial was conducted initially for screening of different explants of gladiolus for enhanced release of axillary buds in MS medium Observations were recorded on the response from 10 cultures Based on these further studies were conducted on axillary buds from corms and cormel tips only

3 5 1 2 Culture establishment (Stage 1)

The culture establishment trials were carried out using axillary buds of corm and cormel tips in MS medium supplemented with cytokinins viz BAP kinetin and 2ip (1 0 mg l^{-1} to 4 0 mg l^{-1}) alone and in combination with NAA (0 5

mg l ^{1} and 1 0 mg l ^{1}) Observations on the number of days taken for shoot bud emergence number of shoots and number of days taken for shoot elongation were recorded after three weeks of culturing

3 5 1 2 1 Influence of stages of development of corm and cormels on culture establishment

The corms and cormels were collected at different stages of development as given below

Stage I	Soon after drying of inflorescence
Stage II	30 days after drying of inflorescence
Stage III	60 days after drying of inflorescence
Stage IV	90 days after drying of inflorescence (Ready for field planting in in the next season)

The purpose of this was to know the effect of growth regulators on breaking the dormancy at different stages of corm and cormel development

3 5 1 2 2 Effect of media on culture establishment

The best level of growth regulators identified in the above trial was compared in both White s medium and SH medium

3 5 1 3 Shoot proliferation (Stage 2)

The elongated buds in the Stage 1 from corms and cormels were separated carefully under aseptic condition and cultured to induce shoot proliferation Trials were conducted in MS medium supplemented with cytokinins (BAP kinetin and 2iP each at 1 0 2 0 3 0 and 4 0 mg l ^{1} alone and in combination with auxin

(NAA 0 5 and 1 0 mg l ^{1}) Nature of response of the elongated buds from corm axillary buds and cormel tips as influenced by cytokinins and auxin were recorded on the formation of multiple axillary buds callus growth shoot formation and root formation

3 5 1 3 1 Influence of frequent subculturing on multiple axillary bud formation

This trial was conducted for four generation on MS medium supplemented with BAP (2 0 and 3 0 mg l ^{1})and NAA (0 0 0 5 and 1 0 mg l ^{1})

3 5 1 3 2 Elongation of multiple axillary bud aggregates

Elongation of multiple axillary buds were tried on MS medium both with full concentration of inorganic salts (MS_a) and half the concentration of inorganic salts (MS_b) supplemented with BAP and NAA (0 0 and 1 0 mg l ^{1}) and responses were recorded Observations on the number of days taken for shoot elongation length of shoot etc were recorded

3 5 1 3 3 Effect of media on shoot elongation for multiple axillary buds

The different media viz full strength MS medium (MSa) half strength MS medium (MS_b) SH and white s were tried and obseverations were recorded

3 5 1 3 4 Standardisation of medium supplements

Effect of medium supplements viz coconut water (0 0 1 0 2 0 5 0 10 0 and 15 0%) and activated charcoal (0 0 0 1 0 2 0 3 and 0 4%) were tried for the elongation of bud aggregates in MS basal medium Observations on the number of days taken for shoot elongation number of days taken for root initiation

number of shoots and roots produced and nature of roots were recorded

3 5 1 4 *In vitro* rooting (Stage 3)

Studies on *in vitro* rooting were carried out on elongated shoots from Stage 2 in basal medium containing half and full strength of inorganic salts (both liquid and semi solid) and SH basal medium Trials were also conducted to study the effect of different auxins (IBA and NAA each at 0 5 1 0 and 2 0 mg l ^{1} incombina tion with different levels of sucrose (1 0 2 0 and 3 0%) In order to study the effect of light on rooting experiments were conducted with MS medium supplemented with IBA at 1 0 and 2 0 mg l ^{1} with and without light Observations on days taken for root initiation number and length of roots and nature of roots were recorded

3 5 1 4 1 Effect of activated charcoal on rooting

Trials were also conducted to study the influence of different levels of activated charcoal on rooting of elongated shoots of gladiolus MS medium was supplemented with activated charcoal at different levels (0 1 0 2 0 3 and 0 4 per cent) and observations were recorded

3 5 1 5 Planting out (Stage 4)

The following observations were recorded on the growth parameters of gladiolus at the time of planting out

Plant height

Length from the collar region to the tip of the plantlets was measured and expressed in centimeter

Number of leaves per plantlet

The total number of leaves borne by a plantlet were counted and record ed

Average length of the roots

Length of the roots was measured from the collar region to the tip and the mean length was expressed in centimeters

3 5 1 5 1 Planting out and acclimatization

The cotton plug of the culture vessels were removed sterile water added to the vessels and kept as such for 10 to 15 minutes Then rooted plantlets were taken out from culture vessels with the help of forceps The agar adhering to the roots were completely removed by thorough washing with running tap water

In order to study the effect of media on the growth of plantlets the following media were tried

1 Fine sand

2 Coarse sand

3 Potting mixture

4 Coco peat

Sterilization was done to make the media free of contaminants and then drenched with Bavistin (0 1%) solution

The plantlets were also treated with Bavistin (0 1%) solution for five minutes before planting out

3 5 1 5 2 Standardisation of hardening treatments

In order to acclimatise the plantlets produced *in vitro* a trial to standard ise the hardening treatment was also carried out The plantlets were subjected to the following post transfer treatments

1 Keeping in open

2 Covering the plantlets with polythene cover with holes for two weeks

3 Covering the plantlets with microscope cover for two weeks

4 Keeping the plantlets in mist chamber (improvised)

5 Spraying 20 0 mg l^{-1} triadimefon solution (1 (4 chlorophenoxy) 3 3 dimethyl 1 (1H 1 2 4 triazol 1 yl) 2 butanone) a week on plantlets kept in open

6 Spraying 20 0 mg l^{-1} triadimefon solution twice a week on plantlets kept under mist chamber

The plants were gradually exposed to sunlight Water was sprayed fre quently to prevent the plantlets from desiccation MS solution of 1/10 strength was also given on alternate days

Observations were made on percentage of plantlet survival after four weeks and percentage of plantlets that have produced corms after six weeks of cul turing

3 5 2 Somatic organogenesis

3 5 2 1 Explant choice

The explants used for both direct organogenesis and callus mediated organogenesis are given in Table 2

3 5 2 2 Direct organogenesis

Internodal pieces of inflorescence inoculated to the modified MS medium (Appendix I) supplemented with cytokinins (BAP kinetin at concentrations of 0 0 0 5 1 0 2 0 and 3 00 mg l ^{1}) in combination with auxin (NAA at 5 0 10 0 15 0 and 20 0 mg l ^{1}) and adenine sulphate 10 0 mg l ^{1} The observations like number of cultures showing direct organogenesis days taken for organogenesis and number of shoots and roots produced etc were recorded

3 5 2 3 Callus mediated organogenesis

3 5 2 3 1 Callus initiation

Corm internodal pieces leaves inflorescence discs flower buds and flower bracts were inoculated into MS media containing different levels of 2 4-D and 2 4 5 T (1 2 3 and 4 mg l ^{1}) The observations were recorded on the percent age of cultures initiating callus and number of days taken for callus initiation

Modified MS medium supplemented with Adenine sulphate 10 0 mg l ^{1} and auxin (NAA at 5 0 10 0 15 0 20 0 mg l ^{1}) alone and in combination with cytokinins (BAP kinetin at 0 5 1 0 2 0 and 3 0 mg l ^{1}) were also used for callus initiation trials for leaf and inflorescence explants The cultures were incubated under light as well as dark conditions The observations like percentage of cultures callusing days taken for callusing growth score nature of callus etc were record ed

Root explants collected from both *in vivo* and *in vitro* gladiolus were inoculated to MS medium supplemented with various concentrations of BAP (0 0 1 0 and 2 0 mg l ^{1}) and NAA (0 0 1 0 and 2 0 mg l ^{1}) for the production of callus

and ıncubated at 25°C ± 2 wıth a 16 h photoperıod provıded by whıte flurescent tubes Observatıons on number of cultures producıng callus and days taken for callus ınıtıatıon were recorded

3 5 2 3 2   Callus dıfferentıatıon studıes

The callus derıved were then transferred to MS basal half strength MS basal MS medıum supplemented wıth varıous cytokınıns (BAP kınetın 2ıP at concentratıons of 0 25 0 50 1 00 2 00 and 3 00 mg 1 ^{1}) alone and ın combınatıon wıth NAA (0 25 0 50 1 00 mg 1 ^{1}) and also MS medıum supplemented wıth coconut water (20 100 150 ml 1 ^{1}) to study ıts ınfluence on organogenesıs Ob servatıons lıke number of days taken for dıfferentıatıon number of shoots and number of roots produced were recorded

3 6   *In vıtro* corm productıon

The elongated shoots from State 2 were used for thıs purpose The plan tlets were ınoculated to MS medıum contaınıng dıfferent levels of sucrose (2 0 3 0 and 5 0%) ın combınatıon wıth NAA (0 0 0 5 and 1 0 mg 1 ^{1}) or IBA (0 0 0 5 and 1 0 mg 1 ^{1}) The cultures were left ın the culture room at 16 h photoperıod Ob servatıons lıke percentage of cultures producıng corms and days taken for corm productıon were recorded The measurements of the corms formed were made after eıght weeks of culturıng

The best treatment ıdentıfıed ın the above experıment was supplemented wıth trıazol (Trıadımefon 1 0 and 5 0 mg 1 ^{1}) and the cultures were left ın the cul ture room as basal portıon exposed to lıght and also under exclusıon of lıght Ob servatıons were recorded as above

The size of the corms were recorded and then the plantlets with corms of uniform size were again subjected to corm enlargement treatments Experiments were carried out with different levels of sucrose (3 0 4 0 5 0 6 0 7 0 8 0 and 9 0%) and the cultures were incubated in 16 h photoperiod and also by excluding light on the basal portions of the tubes Observations were recorded separately on the size of corms after eight weeks and also the number of roots produced and plan tlet survival Full strength semi solid MS salt media was used for this purpose

The same type of trial was also carried out in full strength MS liquid medium with different levels of sucrose (3 0 and 5 0%) in combination with triazols (Triadimefon at the rate of 1 0 2 0 3 0 4 0 and 5 0 mg 1 ^{1}) Observations like plantlet survival size of corms etc were recorded after eight weeks

Results

RESULTS

The results of the studies conducted on the *in vitro* propagation of gladio lus at the Tissue Culture Laboratory of the All India Co ordinated Floriculture Improvement Project attached to the Department of Pomology and Floriculture of the College of Horticulture Vellanikkara are presented in this chapter

4 1 Explant

The explants tried were axillary buds cormel tips inflorescence seg ments flower buds flower bud bracts leaf segments and root pieces

4 1 1 Standardisation of surface sterilisation of explants

The results of the experiments on surface sterilisation of gladiolus ex plants using different chemicals are presented in Table 3

Among the different sterilants mercuric chloride (0 10 and 0 20%) was found to be a better sterilant than sodium hypochlorite solution

A survival percentage of 100 00 was recorded when the corm axillary bud explants were surface sterilised with mercuric chloride (0 10%) for 25 minutes The same rate of survival was also observed in treatments like ethyl alcohol (50 00%) for 3 minutes + mercuric chloride (0 10%) for 25 minutes and in mercur ic chloride (0 20%) for 15 minutes

Table 3 Effect of surface sterilant treatment on the survival of gladiolus explants

Culture period 3 weeks

Medium MS medium with optimum levels of growth regulators

	Treatments	Concentration (%)	Duration (Minutes)	Survival percentage: Corm axillary buds	Cormel tips	Inflorescence segments	Leaf segments	Root pieces ex vitro
1	Mercuric chloride	0 10	1			80 00	30 00	
			2			100 00	80 00	
			3			100 00	100 00	
			10	0 00	0 00			
			15	20 00	15 00			100 00
			20	75 00	100 00			
			25	100 00	100 00			
2	Mercuric chloride	0 20	10	90 00	100 00			
			15	100 00	100 00			
3	Ethyl alcohol followed by mercuric chloride	50 00 / 0 10	3 / 5	0 00	20 00			
			3 / 10	0 00	50 00			
			3 / 15	25 00	60 00			
			3 / 20	[illegible]	100 00			
			3 / 25	100 00	100 00			
4	Sodium hypochlorite	40 00	10	0 00	0 00			
			15	20 00	15 00			
			20	20 00	20 00			
			25	25 0	25 00			

bse a ons ere ta as t e ave e c 0 c ltu e tubes

Treating with ethyl alcohol (50 00%) for 3 minutes followed by mercuric chloride (0 10%) for 20 minutes had a survival of 85 00 per cent for corm axillary bud explants The same sterilants at a different duration ie ethyl alcohol for 3 minutes and mercuric chloride 0 10 per cent for 15 minutes resulted in 25 per cent contamination free cultures Mercuric chloride (0 20%) for 10 minutes resulted in 90 per cent contamination free cultures m corm axillary bud explants

Mercuric chloride (0 10%) treatment of corm axillary buds for 15 and 20 minutes have shown survival percentage of 20 00 and 75 00 respectively

Treatment with 40 per cent sodium hypochlorite solution for 15 20 and 25 minutes resulted in 20 00 20 00 and 25 00 per cent survival of corm axillary buds respectively

Maximum survival percentage (100 00%) for cormel tip explants was observed in the treatments like mercuric chloride (0 10%) for 20 and 25 minutes ethyl alcohol treatment (50 00%) for 3 minutes followed by mercuric chloride (0 10%) for 20 and 25 minutes and also in mercuric chloride (0 20%) treatment for 10 and 15 minutes

Survival rate of cormel tip explants were 60 00 per cent and 50 00 per cent respectively when the explants were treated with ethyl alcohol (50 00%) for 3 minutes + mercuric chloride (0 10%) for 15 minutes and ethyl alcohol (50 00%) for 3 minutes + mercuric chloride (0 10%) for 10 minutes

Low rates of survival were observed in 40 per cent sodium hypochlorite solution for 15 minutes (15 00%) sodium hypochlorite for 20 minutes (20 00%)

sodium hypochlorite treatment for 25 minutes (25 00%) mercuric chloride 0 10 per cent for 15 minutes (15 00%) and also in ethyl alcohol 3 minutes treatment followed by mercuric chloride (0 10%) for 5 minutes (20 00%)

Inflorescence segments flower buds and flower bud bracts when surface sterilised with mercuric chloride (0 10%) for 2 minutes and also for 3 minutes had 100 00 per cent survival A survival percentage of 80 00 was observed when the explant were surface sterilised in mercuric chloride (0 10%) for one minute

Mercuric chloride (0 10%) treatment in leaf explants for 3 minutes resulted in 100 00 per cent contamination free cultures The treatments using the same sterilant for 2 minutes resulted in 80 00 per cent survival and for 1 minute resulted in 30 00 per cent survival

In the case of root pieces 100 per cent contamination free cultures could be obtained when treated with 0 10 per cent mercuric chloride for 15 minutes

4 1 2 Seasonal influence on the *in vitro* establishment of explants of gladiolus

Data regarding the influence of season on explant collection and culture establishment of the explants of gladiolus (corm axillary buds and cormel tips) are presented in Table 4

It is clear from the table that maximum survival percentage (100 00) of the corm axillary buds was obtained during the month of April and minimum rate of survival during the month of July (50 00) The trend represented a gradual increase in the rate of survival as the time proceeded from July to April then a steady decline during the months of May June and July

Table 4 Seasonal influence on the *in vitro* establishment of explants of gladiolus

Basal medium MS with optimum levels of growth regulators
Culture period 3 weeks

Month	Corm axillary buds		Cormel tips	
	Contamination (%)	Survival (%)	Contamination (%)	Survival (%)
January	20 00	80 00	15 00	85 00
February	10 00	90 00	5 00	95 00
March	10 00	90 00	5 00	95 00
April	0 00	100 00	0 00	100 00
May	5 00	95 00	0 00	100 00
June	25 00	75 00	30 00	70 00
July	50 00	50 00	60 00	40 00
August	40 00	60 00	45 00	55 00
September	13 00	87 00	0 00	100 00
October	17 00	83 00	0 00	100 00
November	10 00	90 00	0 00	100 00
December	12 00	88 00	5 00	95 00

In the case of cormel tip explants maximum survival percentage (100 00) was observed during the periods of April May and September November Lowest rate of explant survival and highest rate of contamination was recorded during July (40 00 and 60 00% respectively)

Two peaks of high survival percentage and lowest contamination per centage observed during the periods of April to May and September to November

From November to March a fluctuating tendancy in the survival percent age was observed

During June to August the survival percentage reduced drastically and the minimum percentage of the survival was observed during the month of July (40 00)

4 2 The routes

4 2 1 Enhanced release of axillary buds

4 2 1 1 Culture establishment (Stage 1)

The culture establishment trials were carried out using corm axillary buds and cormel tips (Plate 1 and 2 respectively) in MS medium supplemented with cytokinins (BAP kinetin and 2iP) alone and in combination with NAA The results are presented in Tables 5 to 9

4 2 1 1 1 Effect of BAP on corm axillary buds

Trials were conducted with BAP at different levels (1 0 2 0 3 0 and 4 0 mg l^{1}) alone and in combination with NAA (0 5 and 1 0 mg l^{1}) on culture estab lishment of corm axillary buds at different stages of development and the results are given in Table 5

Stage I (Corm axillary bud explants taken soon after drying of the inflorescence)
Number of days for bud emergence

The average number of days taken for bud emergence after breaking the dormancy of the buds varied from 7 1 to 18 0 in Stage I (soon after drying of in florescence) The minimum period was taken by the treatment BAP 4 0 mg l^{1} (7 1 days) and was significantly different from all other treatments Maximum days (18 0) for bud emergence was taken by the treatment BAP 1 0 mg l^{1} + NAA 1 0 mg l^{1} followed by the treatment BAP 1 0 mg l^{1} + NAA 0 5 mg l^{1} (16 7) days

Number of shoots

The number of shoots ranged from 1 0 to 4 2 under various treatments and the differences were significant

Maximum number of shoots (4 2) was produced by the treatment BAP 3 0 mg l^{1} followed by the treatment BAP 4 0 mg l^{1} (3 1) which was found to be statistically on par with the treatments BAP 1 0 mg l^{1} BAP 4 0 mg l^{1} + NAA 0 5 mg l^{1} BAP 2 0 mg l^{1} and BAP 4 0 mg l^{1} + NAA 1 0 mg l^{1}

Table 5 Effect of BAP and NAA on culture establishment of corm axillary buds of gladiolus at different stages of corm development

Basal medium S
C ture period 4 wee s

Treatments		Stage I			Stage II			Stage III			Stage IV		
BAP (g 1)	NAA (mgl 1)	Time taken for bud emergence (days)	Number of Shoots	Time taken for shoot elongation (days)	Time taken for bud emergence (days)	Number of Shoots	Time taken for shoot elongation (days)	Time taken for bud emergence days)	Number of Shoots	Time taken for shoot elongation (days)	Time taken for bud emergence (days)	Number of Shoots	Time ta o e o at o a)
1 0	0 0	10 7	3	16 1	8 2	3	13 7	6 2	3 2	10 1	3 0	0	
2 0	0 0	10 0	3 0	16 3	8 0	5	5 2	5 1	9	9 0	2 5	5 C	
3 0	0 0	9 8	2	15	5 6	5	11 6	5 0	6	7 7	2 6	5	c
G	0 0	7 1	3 1	1 1	5 3	3	1 4	5	3	8 6	2 5	5 0	
C	0 5	16 7	o	19 1	9 4	3 C	5 8	7 0	6	9 2	7 0	3 0	
C	0 5	10 5	2 0	15 0	6 2	1 5	19	6 9	3 0	9 0	6 9	3 C	
3 0	0 5	9 1	2 3	16 9	10 3	2	1 2	5 2	3 3	7 3	5 2	3	
4 G	0 5	11 5	3 1	16 0	9 1	2	18 1	1		7.6	5 1	3 3	
G	1 0	18 0	1 0	21 0	9 2	2	5 8	7	9	9	7 3	2 1	
C	1 0	10 2	o	16 0	21 0	1	22	7 2	3 0	9 0	6 3	3 C	
3 C	1 0	10 1	2 6	16 3	12 0	2 G	15 6	5 8	3	7 5	5 1	3 6	[illegible]
4 0	1 0	10 8	2 6	16 8	9 c	2 4	19 7	6 2	2 8	7 3	5 2	3 6	o
C (0 05)		1 0	0 5	1 2	1 5	0 5	2 7	0 7	0 6	0 8	0 7	0 6	C 7
+		0 505	0 181	0 615	1 7	0	2 833	0 3 2	0 201	0 399	0 301	0 21	0 2

Stage I Soon a er the d y o i sce ce

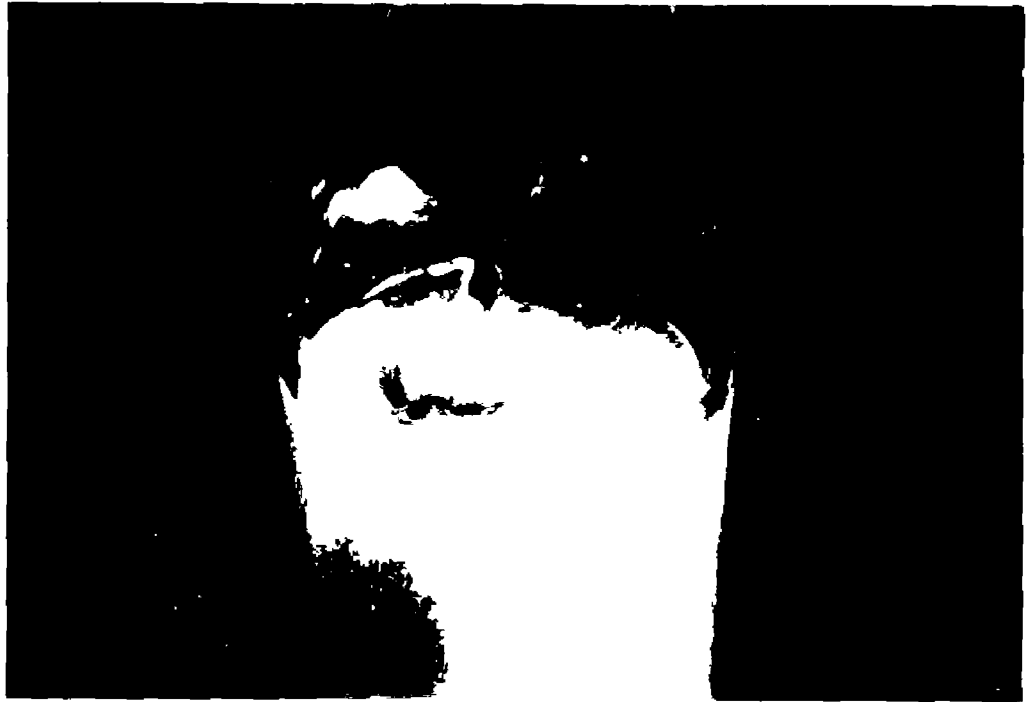
S a e I ca s a e d o i c nce

Stage 60 da s a d g o r sc e

Stage V 60 davs a e d y g o sce e

Plate 1 Corm axillary bud explants of gladiolus after inoculation into MS medium

Plate 2 Cormel tip explant of gladiolus after inoculation into MS medium



Mınımum number of shoots (1 0) was produced by the treatment BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} Treatments lıke BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} BAP 1 0 mg l + NAA 0 5 mg l ^{1} BA2 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} produced 1 9 1 8 2 0 2 3 2 6 shoots respectıvely and these were found to be homogenous and ıntermedıate between the maxımum and mınımum shoot number

Number of days for shoot elongatıon

Number of days taken for shoot elongatıon varıed from 15 0 days to 21 0 days and dıffered sıgnıficantly Mınımum days (15 0) for shoot elongatıon was taken by the treatment BAP 2 0 mg l ^{1} + 0 5 mg l ^{1} NAA (Plate 3) The treatment BAP 3 00 mg l ^{1} BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} BAP 4 0 mg l ^{1} BAP 1 mg l ^{1} whıch took 15 5 16 0 16 0 16 1 1 61 days respectıvely for elongatıon

Maxımum number of days for elongatıon was taken by the treatment BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} (21 0) followed by BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (11 1)

Stage II (30 days after the dryıng of ınflorescence)

Number of days for bud emergence

The average number of days taken for bud emergence ranged from 5 3 days to 21 0 days and dıffered sıgnıficantly The treatment BAP 4 0 mg l ^{1} has taken the mınımum days for bud emergence (5 3) and was found to be statıstıcally on par wıth the treatment BAP 3 0 mg l ^{1} (5 6)

The longest time for bud emergence was taken by the treatment BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} (21 0) followed by BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (16 2) and BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (12 0)

Number of shoots

The average number of shoots per culture differed significantly and varied from 1 4 to 5 7

Maximum number of shoots (5 7) was observed in the medium supple mented with 3 0 mg l ^{1} BAP and was on par with the number shoots produced (5 1) in the treatment with 2 0 mg l ^{1} BAP

Shoot number was minimum (1 4) in the medium supplemented with 2 0 mg l ^{1} BAP and 1 0 mg l ^{1} NAA Treatments like BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} and BAP 3 0 mg l ^{1} and NAA 1 0 mg l ^{1} were on par with this

Number of days for shoot elongation

The data indicated that there were significant differences in the number of days taken for the elongation of buds which ranged from 11 4 days to 22 8 days

The minimum number of days (11 4) was taken in the medium when supplemented with 4 0 mg l ^{1} BAP and the medium supplemented by 3 0 mg l ^{1} BAP (11 6 days) were found to be on par with this

Maximum days for the elongation of shoots (22 8) was taken by the treatment having 2 0 mg l ^{1} BAP + 1 mg l ^{1} NAA followed by the treatments like BAP 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} (19 7) BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1}

(19 2) BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (18 1) these three were statistically homogeneous

Stage III (Corm axillary bud explants taken 60 days after the drying of inflores cence)

Number of days for bud emergence

The number of days taken for bud emergence from the corm axillary buds ranged from 5 0 to 7 6 days in various treatments and were significantly differ ent

The treatment BAP 3 0 mg l ^{1} took the shortest time for bud emergence (5 0 days) and the treatments BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (5 1 days) BAP 2 0 mg l ^{1} (5 1 days) BAP 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} (5 2 days) BAP 4 0 mg l ^{1} (5 5 days) were on par with this

Maximum days (7 6) for bud emergence was taken by the medium supplemented with 1 0 mg l ^{1} BAP and 1 0 mg l ^{1} NAA This was found to be on par with the media supplemented with BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} (7 2 days) BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (7 0 days) BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (6 9 days)

Number of shoots

The average number of shoots produced in various treatments differed significantly and varied from 1 9 to 4 9

The maximum number of shoots was produced by the treatment BAP 2 0 mg l ^{1} (4 9) followed by BAP 3 0 mg l ^{1} (4 6) and were found to be homogeneous

Treatment BAP 4 0 mg l ^{1} produced 3 1 shoots and was found to be homogeneous with the number of shoots produced by BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (3 8) BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (3 4) and BAP 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} (3 3) and BAP 1 0 mg l ^{1} (3 2)

Minimum number of shoots (1 9) was observed when the medium was supplemented with 1 0 mg l ^{1} BAP + 1 0 mg l ^{1} NAA and was found to be signifi cantly different from all other treatments

Number of days for shoot elongation

The average number of days taken for shoot enlongation ranged from 7 3 to 10 1 days and the differences were significant

The treatment BAP 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} recorded the shortest period (7 3 days) for shoot elongation and was found to be statistically homogeneous with treatments having BAP 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} (7 3 days) BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (7 5 days) BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (7 6 days) BAP 3 0 mg l ^{1} (7 7 days)

Maximum days for shoot elongation was taken in the medium when supplemented with BAP 1 0 mg l ^{1} (10 1) The treatment having BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} took 9 8 days for shoot elongation while the medium supplement ed with BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} took 9 4 days for shoot elongation and were statistically on par

Stage IV (Corm axillary bud explants taken 90 days after the drying of inflores cence)

Number of days for bud emergence

Data pertaining to average number of days taken for bud emergence after breaking the dormancy revealed significant differences and varied from 2 5 days to 7 3 days under various treatments

The minimum number of days (2 5) for bud emergence was recorded in MS medium supplemented with 2 0 mg l ^{1} BAP and was found to be homogeneous with the time taken for bud emergence in treatments like BAP 4 0 mg l ^{1} (2 5 days) BAP 3 0 mg l ^{1} (2 6 days) and BAP 1 0 mg l ^{1} (3 0 days)

The longest time for bud emergence was observed in MS medium sup plemented with BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} (7 3 days) and was found to be statistically on par with BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (7 0 days) and BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (6 9 days)

Number of shoots

The average number of shoots produced by the various treatments recorded significant differences and ranged from 2 1 to 5 1 The maximum number of shoots (5 1) was produced in MS medium supplemented with 3 0 mg l ^{1} BAP and was on par with that of treatment having 2 0 mg l ^{1} BAP (5 0) and 4 0 mg l ^{1} BAP (5 0)

Minimum number of shoots (2 1) was observed in treatment BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} and was found to be significantly different from all other treatments

Number of days for shoot elongation

The average days for shoot elongation differed significantly and ranged from 3 9 days to 5 1 days The minimum number of days (3 9) was taken by the medium supplemented with 3 0 mg l^{1} BAP and was found to be on par with those produced by the treatments BAP 4 0 mg l^{1} BAP 1 0 mg l^{1} BAP 2 0 mg l^{1} BAP 1 0 mg l^{1} + NAA 0 5 mg l^{1} BA2 0 mg l^{1} + NAA 0 5 mg l^{1} and BAP 4 0 mg l^{1} + NAA 0 5 mg l^{1} They have taken 4 0 4 0 4 0 4 0 4 2 and 4 5 days respectively for shoot elongation

The maximum days (5 1) for elongation of shoots was taken by the medium supplemented with BAP 1 0 mg l^{1} + NAA 1 0 mg l^{1} and was on par with the treatments like BAP 3 0 mg l^{1} + NAA 0 5 mg l^{1} (5 0 days) BAP 2 0 mg l^{1} + NAA 1 0 mg l^{1} (4 9 days) BAP 4 0 mg l^{1} + NAA 1 0 mg l^{1} (4 8 days) and BAP 3 0 mg l^{1} + NAA 1 0 mg l^{1} (4 7 days)

4 2 1 1 2 Effect of kinetin on corm axillary buds

Influence of kinetin at different levels (1 0 mg l^{1} to 4 0 mg l^{1}) alone and in combination with different levels of NAA (0 5 mg l^{1} and 1 0 mg l^{1}) on culture establishment of corm axillary buds at different stages of development are given in Table 6

Stage I (Corm axillary bud explants taken soon after drying of the inflorescence)

Number of days for bud emergence

Table 6 Effect of kinetin and NAA on culture establishment of corm axillary buds of gladiolus at different stages of corm development

Basal med um S
Culture pe iod 4 weeks

Treat ent m n (gl)	Treat ent NAA (ngl 1)	Stage I Time taken for bud emergence (days)	Stage I Number o s	Stage I Time taken for elongation (days	Stage II Time taken for bud emergence (days)	Stage II Number o Sr s	Stage II Time taken for elongation days	Stage III Time taken for bud emergence (days)	Stage III Number o sh s	Stage III Time taken for elongati n	Stage IV Time taken for bud emergence days	Stage IV Number of Sn s	Stage IV Time ta en o elongation (days)
0	0 0	14 4	1 6	18 5	11 4	3 0	17 3	9 0	2 2	11 0	6 4	2 5	10 0
0	0 0	13 3	2 1	18 1	10 2	3 3	13 0	8 5	2 9	11 0	6 1	2 2	1 1
0	0 0	13 4	2 1	17 3	8 3	3 7	11 9	8 8	2 1	7 6	5 5	1 9	10 5
0	0 0	13 1	2 4	17 7	8 4	3 5	12 0	8 5	2 8	9 7	6 0	2 8	9 8
0	0 5	15 0	1 6	19 2	13 5	3 0	15 1	9 0	2 2	11 0	7 1	3 0	10 2
0	0 5	15 7	1 6	18 2	9 2	2 7	13 1	8 0	2 2	10 5	6 4	2 2	10 0
0	0 5	15 9	2 3	20 3	9 5	3 0	12 0	8 6	2 0	11 6	5 8	2 2	9 2
0	0 5	14 9	2 4	18 8	9 1	2 9	13 0	7 0	1 4	9 4	6 0	2	10 0
0	1 0	14 9	1 4	19 6	14 1	2 5	18 0	10 0	1 5	11 0	6 8	2 1	11 2
0	1 0	16 5	2 3	20 0	9 1	2 2	13 4	8 9	1 9	11 0	6 3	1 9	10 0
0	1 0	14 8	2 3	19 3	9 3	3 1	12 6	8 7	2 2	11 8	5 5	2 3	10 0
0	1 0	16 0	2 3	19 8	9 7	2 8	13 6	7 5	2 8	10 5	5 9	2 2	9 8
D 0 0		1 4	0 5	1 5	1 7	1 2	1 8	0 5	0 4	1 5	0 8	0 5	1 1
		1 226	0 1o3	1 339	1 o50	0 o06	1 979	0 157	0 093	1 00	0 365	0 143	0 758

Stage I 0 n a ter dry ng o n oresc e
Stage II 0 days a ter drying o n lorescence
Stage I I 0 da s a ter dr i g o n loresce ce
Sta e I' 0 d ys a ter dry g o n ore ce ce

The number of days taken for bud emergence varied significantly from 13 1 days to 16 5 days in various treatments Minimum days for bud emergence was taken by the treatment having 4 0 mg l^{1} kinetin (13 1) and was found to be homo geneous with that of kinetin 2 0 mg l^{1} (13 3) 3 0 mg l^{1} (13 4) and 1 0 mg l^{1} (14 4)

Maximum days (16 5) for bud emergence was taken by the medium supplemented with kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} Treatments having 4 0 mg l^{1} kinetin + 1 0 mg l^{1} NAA (16 0 days) 3 0 mg l^{1} kinetin + 0 5 mg l^{1} NAA (15 9 days) 2 0 mg l^{1} kinetin + 0 5 mg l^{1} NAA (15 7 days) were found to be on par with the treatment with kinetin 3 0 mg l^{1} + NAA 1 0 mg l^{1} (14 8 days)

Number of shoots

The average number of shoots varied significantly from 1 4 to 2 4 in various treatments Maximum number of shoots (2 4) was observed in MS medium when supplemented with 4 0 mg l^{1} kinetin and was found to be homogeneous with treatments like kinetin 4 0 mg l^{1} + NAA 0 5 mg l^{1} (2 4) kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} (2 4) kinetin 3 0 mg l^{1} + NAA mg l ^{1} (2 3) kinetin 4 0 mg l^{1} + NAA 1 0 mg l^{1} (2 3) kinetin 3 0 mg l^{1} (2 1) and kinetin 2 0 mg l^{1} (2 1)

The number of shoots was minimum (1 4) when the medium was sup plemented with kinetin 1 0 mg l^{1} + NAA 1 0 mg l^{1} and was found to be homoge neous with those of the treatments kinetin 2 0 mg l^{1} + NAA 0 5 mg l^{1} (1 6) kinetin 1 0 mg l^{1} (1 6) kinetin 1 0 mg l^{1} + NAA 0 5 mg l^{1}) (1 6)

Number of days for shoot elongation

The days taken for shoot elongation varied significantly and were minimum (17 3 days) in the medium supplemented with 3 0 mg l ^{1} kinetin The treatments in which MS medium was supplemented with kinetin 4 0 mg l ^{1} (17 7) kinetin 2 0 mg l ^{1} (18 1) kinetin 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (18 2) and kinetin 1 0 mg l ^{1} (18 5) were found to be on par with this treatment

Maximum days (20 3) for shoot elongation was taken by the treatment having kinetin 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} and was found to be homogeneous with treatments having kinetin 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} (20 0) kinetin 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} (19 8) kinetin 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} (19 6) kinetin 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (19 3) and kinetin 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (19 2)

State II (Corm axillary bud explants taken 30 days after the drying of inflorescence)

Number of days for bud emergence

The average number of days for bud emergence ranged from 8 3 to 14 1 and differed significantly The minimum days (8 3) for bud emergence was taken by the medium supplemented with kinetin 3 0 mg l ^{1} and was statistically homogeneous with the treatments like kinetin 4 0 mg l ^{1} (8 4 days) kinetin 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (9 2 days) kinetin 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (9 3 days) kinetin 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} (9 5 days) and kinetin 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} (9 7 days)

Treatments like kinetin 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} (14 1 days) and kinetin 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (13 5 days) took maximum days for bud emergence

Number of shoots

The shoots produced in various treatments differed significantly and ranged from 2 2 to 3 7 Maximum number of shoots (3 7) was produced in MS medium supplemented with kinetin 3 0 mg l ^{1} and was found to be homogeneous with the treatments involving kinetin 4 0 mg l ^{1} (3 5) kinetin 2 0 mg l ^{1} (3 3) kinetin 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (3 1) kinetin 1 0 mg l ^{1} (3 0) kinetin 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} (3 0) and kinetin 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (3 0)

Minimum number of shoots (2 2) was recorded in medium suppliment ed with kinetin 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} and was found to be homogeneous with the treatments kinetin 1 0 mg l ^{1} + NAA 1 0 mg l ^{1}

Number of days for shoot elongation

Days taken for the elongation of shoots differed significantly among the treatments and ranged from 11 9 to 18 0 in various treatments

Minimum days for shoot elongation (11 9) was taken by the medium supplemented with kinetin 3 0 mg l ^{1} which was on par with the treatments with kinetin 4 0 mg l ^{1} (12 0) kinetin 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} (12 0) kinetin 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (12 6) kinetin 2 0 mg l ^{1} (13 0) kinetin 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (13 0) kinetin 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (13 1) kinetin 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} (13 4) and kinetin 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (13 0)

Maximum days for shoot elongation (18 0) was observed in the medium containing kinetin 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} which was on par with the treat ment in which the medium was supplemented with kinetin 1 0 mg l ^{1} (17 3)

Stage III (Corm axillary bud explants taken 60 days after the drying of inflores cence)

Number of days for bud emergence

The number of days taken for bud emergence ranged from 7 0 to 10 0 showing significant differences among the treatments

The medium supplemented with kinetin 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} has taken the minimum days (7 0) for bud emergence and was significantly different from all other treatments

The maximum days for bud emergence (10 0) was observed in the medium supplemented with kinetin 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} and was signifi cantly different from all other treatments

The other treatments like kinetin 1 0 mg l ^{1} kinetin 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} kinetin 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} kinetin 3 0 mg l ^{1} kinetin 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} kinetin 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} kinetin 2 0 mg l ^{1} and kinetin 4 0 mg l ^{1} have taken 9 0 9 0 8 9 8 8 8 7 8 6 8 5 and 8 5 days respectively for bud emergence

Number of shoots

The average number of shoots produced was maximum (2 9) in the

medium supplemented with kinetin 2 0 mg l ^{1} and was found to be on par with the treatments involving kinetin 3 0 mg l ^{1} (2 9) kinetin 4 0 mg l ^{1} (2 8) and kinetin 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} (2 8)

Minimum number of shoots (1 4) was observed in the medium supple mented with kinetin 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} and was found to be statistically on par with the treatment in which the medium was supplemented with kinetin 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} (1 5)

Number of days for shoot elongation

The average number of days taken for shoot elongation ranged from 7 6 to 11 8

The minimum number of days (7 6) was taken by the treatment in which medium was supplemented with kinetin 3 0 mg l ^{1} and was found to be significantly different from all other treatments

Maximum days (11 8) for shoot elongation was taken by the medium supplemented with kinetin 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} Treatments like kinetin 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} kinetin 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} kinetin 2 0 mg l ^{1} kinetin 1 0 mg l ^{1} kinetin 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} kinetin 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} and kinetin 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} took 11 6 11 0 11 0 11 0 11 0 10 5 10 5 days respectively for shoot elongation and were found to be homogeneous with the treatment with kinetin 3 0 mg l ^{1} + NAA 0 5 mg l ^{1}

State IV (Corm axillary bud explants taken 90 days after the drying of inflorescence)

Number of days for bud emergence

The average number of days taken for bud emergence varied significant ly among the treatments from 5 5 to 7 1

The treatment having kinetin 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} has taken the minimum days (5 5) for bud emergence and was found to be on par with the treatments like kinetin 3 0 mg l ^{1} (5 5) kinetin 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} (5 8) kinetin 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} (5 9) kinetin 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (6 0) kinetin 4 0 mg l ^{1} (6 0) and kinetin 2 0 mg l ^{1} (6 1)

The treatment having kinetin 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} has taken 7 1 days for bud emergence which was the maximum and was on par with the treat ments with kinetin 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} (6 8) kinetin 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (6 4) and kinetin 1 0 mg l ^{1} (6 4)

Number of shoots

The shoots produced were maximum (3 0) when the medium was sup plemented with kinetin 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} and was on par with the shoots produced by the treatments like kinetin 4 0 mg l ^{1} (2 8) kinetin 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (2 7)

Number of shoots were minimum (1 9) under the treatment kinetin 3 0 mg l ^{1} and was found to be homogeneous with the rest of the treatments

Number of days for shoot elongation

The number days taken for shoot elongation differed significantly among the various treatments and ranged from 9 2 to 11 8

The number of days for shoot elongation was 9 2 in the treatment having kinetin 3 0 mg l^{1} + NAA 0 5 mg l^{1} and was found to be homogeneous with the days taken for shoot elongation by various treatments involving kinetin 4 0 mg l^{1} (9 8) kinetin 4 0 mg l^{1} + NAA 1 0 mg l^{1} (9 8) kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} (10 0) kinetin 4 0 mg l^{1} + NAA 0 5 mg l^{1} (10 0) kinetin 2 0 mg l^{1} + NAA 0 5 mg l^{1} (10 0) kinetin 1 0 mg l^{1} (10 0) kinetin 3 0 mg l^{1} + NAA 1 0 mg l^{1} (10 0) and kinetin 1 0 mg l^{1} + NAA 0 5 mg l^{1} (10 2)

Maximum days for shoot elongation (11 2) was observed in the treatment containing 1 0 mg l^{1} kinetin + 1 0 mg l^{1} NAA

4 2 1 1 3 Effect of 2iP on corm axillary buds

Data pertaining to the influence of 2ip at different levels alone and in combination with NAA at different levels on the culture establishment of the corm axillary bud explants and cormel tip explants at different stages of development are given in Table 7

Stage I (Corm axillary bud explants taken soon after drying of inflorescence)

The medium supplemented with 2ip 1 0 mg l^{1} 2 0 mg l^{1} 3 0 mg l^{1} and 4 0 mg l^{1} recorded 20 to 25 days for bud emergence But they failed to elon gate and became pale and slender Treatments with 2ip 1 0 mg l^{1} + NAA 0 5

mg l^{1} 2ip 2 0 mg l^{1} + NAA 0 5 mg l^{1} 2ip 3 0 mg l^{1} + NAA 0 5 mg l^{1} and 2ip 4 0 mg l^{1} + NAA 0 5 mg l^{1} produced swollen buds with pale colour Elongation of the buds or adventitious bud production was not observed

Swelling of the axillary buds and root formation from the basal parts of the explant were observed in treatments supplemented with 2ip 1 0 mg l^{1} + NAA 1 0 mg l^{1} 2ip 2 0 mg l^{1} + NAA 1 0 mg l^{1} 2ip 3 0 mg l^{1} + NAA'1 0 mg l^{1} and 2ip 4 0 mg l^{1} + NAA 1 0 mg l^{1}

Stage II (Corm axillary bud explants taken 30 days after the drying of inflorescence)

Treatments having 2ip 1 0 mg l^{1} 2 0 mg l^{1} 3 0 mg l^{1} and 4 0 mg l^{1} produced small single slender shoots with whitish colouration and the shoots elon gated upto 0 5 cm Further elongation of the shoot was not observed

The medium supplemented with 2ip 1 0 mg l^{1} + NAA 0 5 mg l^{1} however failed to respond

Other treatments of 2ip (1 0 2 0 3 0 and 4 0 mg l^{1}) in combination with NAA (0 5 and 1 0 mg l^{1}) produced swollen buds after 20 25 days of inocula tion and they also produced large number of fleshy roots

Stage III (Corm axillary bud explants taken 60 days after the drying of inflores cence)

Single slender and pale coloured shoots with restricted growth were produced after 15 20 days of inoculation in the MS medium supplemented with 2ip 1 0 mg l^{1} 2ip 2 0 mg l^{1} 2ip 3 0 mg l^{1} and 2ip 4 0 mg l^{1}

Bud emergence was recorded after 15 20 days of inoculation and the emerged buds became swollen with the production of large number of fleshy roots from the basal portion of the explants in all other treatments

Stage IV (Corm axillary bud explants taken 90 days after the drying of inflores cence)

Single slender shoots of length 2 cm was produced by the treatments like 2ip 1 0 mg l ^{1} 2ip 2 0 mg l ^{1} 2ip 3 0 mg l ^{1} and 2ip 4 0 mg l ^{1} after 20 25 days of inoculation

Swollen vitrified shoots were produced by all other treatments of 2ip (1 0 2 0 3 0 and 4 0 mg l ^{1}) in combination with NAA (0 5 and 1 0 mg l ^{1}) Larger number of roots were also recorded

4 2 1 1 4 Effect of 2ip on cormel tips

Cormel tip explant responded to the 2ip in the same way as the corm axillary bud responded (Table 7)

4 2 1 1 5 Effect of BAP on cormel tips

Effect of BAP at different levels alone and in combination with NAA at different levels on the culture establishment of cormel tips taken from the cormels harvested at different stages of development are given in Table 8

Stage I (Cormel tip explants collected soon after drying of inflorescence)

The cormels harvested soon after the drying of the inflorescence were properly surface sterilised and the cormel tips taken from these cormels were incoulated on to

Table 7 Effect of 2ip and kinetin on culture establishment of corm axillary buds and cormel tips of gladiolus taken at different stages of development

Basal medium: S
Culture period: 4 weeks

Treatments 2ip ($mg\ l^{-1}$)	IAA ($mg\ l^{-1}$)	Stage I (Response)	Stage II (Response)	Stage III (Response)	Stage IV (Response)
1 0	0 0	Pale slender buds emerged 20 25 days after inoculation no further elongation	Slender single shoots without adventitious growth Single buds elongated to about 0 5 cm no further elongation shoots shown whitish colouration	Slender single shoots were formed after 15 20 days of inoculation The shoots elongated upto 0 5 cm no further development of the shoot adventitious buds	Slender single shoots which elongated upto 2 cm was formed after 20 25 day of inoculation
2 0	0 0	-do	-do	-do	-do
3 0	0 0	-do	-do	-do	do
4 0	0 0	-do	-do	-do	-do
1 0	0 5	Swelling of the buds and with pale white colour	No response	Bud emergence taken place after 15 20 days buds failed to elongate instead they become swollen and produced large number of fleshy roots after 20 25 days of inoculation	The shoots produced were swollen vitrified and produced large number of fleshy roots
2 0	0 5	No further elongation of the buds	Roots were formed from the basal portion of the explant 20 25 days after elongation bud appeared as swollen and transluscent		
3 0	0 5	-do			
4 0	0 5	-do			
1	1 0	Swelling of the buds and root formation after 20 25 days of inoculation			
3 0	1 0	-do	-do	-do	do
4 0	1 0	-do	-do	-do-	-do

Stage I Soon after drying of inflorescence
Stage II 30 days after drying of inflorescence
Stage III 60 days after drying of inflorescence
Stage IV 90 days after drying of inflorescence

o BAP and NAA on cu ture estab s in e o corm e up exp ants
of gladiolus at different stages of cormel development

basal med um 4S

Culture pe iod 4 weeks

Treatments BAP (mg l[1])	NAA (mg l[1])	Stage I Time taken for bud emergence (days)	Stage I Number of shoots	Stage I Time taken for elongation (days)	Stage II Time taken for bud emergence (days	Stage II Number of shoots	Stage II Time taken for elongation (days)	Stage III Time taken for bud emergence (days	Stage III Number of shoots	Stage III Time taken for elongation (days)	Stage IV Time taken for bud emergence (days)	Stage IV Number of shoots	Stage IV Time taken for elongation (days
0	0 0	12 9	1 8	18 1	10 0	2 0	13 0	9 7	1 6	12 9	8 3	1 8	10 7
0	0 0	12 0	2 3	17 0	9 8	2 3	12 5	9 8	2 1	12 8	8 1	2 0	10 8
0	0 0	10 5	3 0	16 1	9 9	3 1	12 5	9 3	2 1	13 3	8 4	2 3	10 0
0	0 0	10 6	3 1	16 0	9 7	3 9	11 7	9 9	2 9	13 5	8 1	2 2	9 0
0	0 5	12 4	1 7	17 1	9 7	1 7	12 9	10 5	1 5	14 5	8 6	1 6	10 1
0	0 5	16 3	1 3	18 8	15 1	1 4	17 7	12 3	1 3	17 6	8 6	1 7	12 1
0	0 5	16 1	1 4	17 2	13 7	1 4	16 6	14 7	2 5	15 9	9 3	2 3	11 1
0	0 5	16 9	1 6	18 7	14 1	1 4	16 4	14 3	2 1	16 2	8 5	2 1	10 0
0	1 0	17 0	1 1	20 3	12 9	1 7	15 4	13 3	2 5	15 6	10 7	1 1	15 7
0	1 0	16 4	1 4	18 5	15 3	1 3	17 3	14 7	1 4	17 3	9 0	1 6	10 8
0	1 0	16 1	1 8	19 9	14 7	1 2	16 9	14 5	2 0	16 9	9 0	1 9	10 5
0	1 0	18 1	1 3	21 5	15 1	1 4	16 4	13 3	2 3	15 8	8 7	1 9	10 0
(0 05		1 7	0 8	1 7	1 3	0 [illegible]	1 4	1 2	0 8	1 3	0 9	0 6	1 0
		1 863	0 421	1 712	0 [illegible]91	0 222	1 4	0 886	0 358	0 995	0 541	0 232	0 584

Stage I Soon after drying of inflorescence

Stage II 30 days after d ying of inflore ce ce

Stage III 60 days after drying o infloresce ce

Sta e IV 90 days afte c ying of infloresce ce

the MS medium supplemented with various levels of BAP (1 0 2 0 3 0 and 4 0 mg l ^{1}) alone and in combination with NAA (0 5 and 1 0 mg l ^{1})

Number of days for bud emergence

The number of days taken for bud emergence differed significantly in various treatments ranging from 10 5 to 18 1

The minimum days (10 5) for bud emergence was taken by the medium supplemented with BAP 3 0 mg l ^{1} and was found to be on par with the medium supplemented with BAP 4 0 mg l ^{1} (10 6)

The maximum days (18 1) for bud emergence was taken by the medium supplemented with BAP 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} and was on par with media supplemented with BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} (17 0) and BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} (16 9) and BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (16 9)

Number of shoots

The number of shoots produced under various treatments ranged from a minimum of 1 1 to a maximum of 3 1 and showed significant differences

The mean maximum number of shoots (3 1) were recorded in the medium supplemented with BAP 4 0 mg l ^{1} which was statistically homogeneous with the shoot numbers of 3 0 and 2 3 recorded by the media supplemented with BAP 3 0 mg l ^{1} BAP 2 0 mg l ^{1} respectively

The number of shoots produced in the medium supplemented with BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} was only 1 1 which was the minimum and was found

to be statistically on par with the treatments like BAP 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} BAP mg l ^{1} + NAA 0 5 mg l ^{1} BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 1 0 mg l ^{1} and BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} as they produced average shoot numbers of 1 3 1 3 1 4 1 4 1 6 1 7 1 8 and 1 8 respectively

Number of days for shoot elongation

The number of days for shoot elongation was minimum (16 0) when the medium was supplemented with BAP 4 0 mg l ^{1} (Plate 4) and was found to be sta tistically homogeneous with the treatments like BAP 3 0 mg l ^{1} (16 0) BA2 0 mg l ^{1} (17 0) BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (17 1) and BAP 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} (17 2)

The treatment having BAP 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} has taken the maximum number of days for shoot elongation (21 5) and was found to be on par with treatments BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} (20 3) and BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (19 9)

Stage II (Cormel tip explants collected 30 days after the drying of the inflores cence)

Number of days for bud emergence

The number of days taken for bud emergence varied significantly

The minimum number of days taken by the treatment involving BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (9 7) was found to be on par with the treatments BAP 4 0 mg l ^{1} (9 7 days) BAP 2 0 mg l ^{1} (9 8 days) BAP 3 0 mg l ^{1} (9 9 days) and BAP 1 0 mg l ^{1} (10 0 days)

Plate 3 Elongated buds in corm axillary bud explant of gladiolus (Stage I) in MS medium supplemented with BAP 2 0 mg l ^{1} and NAA 0 5 mg l ^{1}

Plate 4 Elongated bud in cormel tip explant of gladiolus (Stage I) in MS medium supplemented with BAP 3 0 mg l ^{1}

The maximum number of days for bud emergence (15 3) was recorded by the treatment in which the medium was supplemented with BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} and this was on par in the treatments BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} as they had taken 15 1 15 1 14 7 14 1 days respectively for bud emergence

Number of shoots

The number of shoots produced was maximum (3 9) when the medium was supplemented with BAP 4 0 mg l ^{1} and was found to differ significantly from all other treatments and was followed by the medium containing BAP 3 0 mg l ^{1} (3 1 shoots)

Lowest number of shoots were recorded in the treatment having BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (1 2) and was found to be on par with the treatments like BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} BAP 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 3 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} and BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} as they produced 1 3 1 4 1 4 1 4 1 4 1 7 and 1 7 shoots respec tively

Number of days for shoot elongation

The number of days taken for shoot elongation varied from 11 7 to 17 7 and showed significant differences among the treatments

The minimum days (11 7) for shoot elongation was taken by the medium supplemented with BAP 4 0 mg l^{1} and was on par with the treatments like BAP 3 0 mg ^{1} (12 5) BAP 2 0 mg l^{1} (12 5) and BAP 1 0 mg l^{1} + NAA 0 5 mg l^{1} (12 9)

Maximum days (17 7) for bud elongation was recorded in the medium supplemented with BAP 2 0 mg l^{1} + NAA 0 5 mg l^{1} followed by treatments BAP 2 0 mg l^{1} + NAA 1 0 mg l^{1} (17 3) BAP 3 0 mg l^{1} + NAA 1 0 mg l^{1} (16 9) BA3 0 mg l^{1} + NAA 0 5 mg l^{1} (16 6) BAP 4 0 mg l^{1} + NAA 0 5 mg l^{1} (16 4) BAP 4 0 mg l^{1} + NAA 1 0 mg l^{1} (16 4) and these were found to be homogeneous

Stage III (Cormel tip explants collected 60 days after the drying of inflorescence)

Number of days for bud emergence

The number of days taken for bud emergence ranged from 9 3 to 14 7 under various treatments and differed significantly

The minimum number of days (9 3) was taken in the medium supple mented with BAP 3 0 mg l^{1} The bud emergence in the medium containing BAP 1 0 mg l^{1} BAP 2 0 mg l^{1} and BAP 4 0 mg l^{1} was observed after 9 7 9 8 9 9 days respectively and these were found to be homogeneous with that of the medium supplemented with BAP 3 0 mg l^{1}

Number of shoots

The number of shoots ranged from 1 3 to 2 9 in various treatments and

showed sıgnıficant dıfferences among the treatments The maxımum number of shoots (2 9) was observed ın the medıum supplemented wıth BAP 4 0 mg l^{1} followed by the treatments lıke BAP 3 0 mg l^{1} + NAA 0 5 mg l^{1} (2 5) BAP 1 0 mg l^{1} + NAA 1 0 mg l^{1} (2 4) BAP 4 0 mg l^{1} + NAA 1 0 mg l^{1} (2 3) BAP 3 0 mg l^{1} (2 1) and these were found to be on par wıth the treatment BAP 4 0 mg l^{1}

Mınımum number of shoots (1 3) was recorded ın the medıum supple mented wıth BAP 2 0 mg l^{1} + NAA 0 5 mg l^{1} and was on par wıth the remaınıng treatments

Number of days for shoot elongatıon

The number of days taken for shoot elongatıon varıed sıgnıficantly and ranged from 12 8 to 17 6 days

The medıum supplemented wıth BAP 2 0 mg l^{1} recorded the mınımum number of days for shoot elongatıon (12 8) and ıt dıd not dıffer sıgnıficantly wıth the treatments lıke BAP 1 0 mg l^{1} (12 9 days) BAP 3 0 mg l^{1} (13 3 days) and BAP 4 0 mg l^{1} (13 5 days)

Maxımum days for the elongatıon of shoots (17 6) was recorded ın the medıum supplemented wıth BAP 2 0 mg l^{1} + NAA 0 5 mg l^{1} The number of days taken for bud elongatıon ın the treatments lıke BAP 2 0 mg l^{1} + NAA 1 0 mg l^{1} (17 3 days) BAP 3 0 mg l^{1} + NAA 1 0 mg l^{1} (16 9 days) dıd not vary sıgnıf ıcantly wıth that of BAP 2 0 mg l^{1} + NAA 0 5 mg l^{1}

Stage IV (Cormel tip explants collected 90 days after the drying of the inflores cence)

Number of days for bud emergence

The average number of days taken for bud emergence ranged from 8 1 to 10 7 The medium supplemented with BAP 4 0 mg l ^{1} recorded the minimum days for bud emergence (8 1 days) which was followed by the treatments like BAP 2 0 mg l ^{1} (8 1 days) BAP 1 0 mg l ^{1} (8 3 days) BAP 3 0 mg l ^{1} (8 4 days) BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (8 5 days) BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (8 6 days) BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (8 6 days) BAP 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} (8 7 days) BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (9 0 days) and BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} (9 0 days) They were found to be homogeneous with the treatment having BAP 4 0 mg l ^{1} with respect to the number of days taken for bud emergence

Maximum days for bud emergence (10 7) was taken by the medium supplemented with BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1}

Number of shoots

The number of shoots produced by various treatments varied significant ly and ranged from 1 1 to 2 3

The mean number of shoots produced was maximum (2 3) in the medium supplemented with BAP 3 0 mg l ^{1} and was found to be homogeneous with the shoots produced by the treatments like BAP 3 0 mg l ^{1} + NAA 0 5 mg l ^{1}

(2 3) BAP 4 0 mg l ^{1} (2 2) BAP 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (2 1) BAP 2 0 mg l ^{1} (2 0) BAP 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} (1 9) BAP 3 0 mg l ^{1} + NAA 1 0 mg l ^{1} (1 9) and BAP 1 0 mg l ^{1} (1 8)

Shoots produced were mınımum ın treatments lıke BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} (1 1) BAP 2 0 mg l ^{1} + NAA 1 0 mg l ^{1} (1 6) BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (1 6) and BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (1 7)

Number of days for shoot elongatıon

The average number of days taken for shoot elonga tıon was mınımum m the medıum supplemented wıth BAP 4 0 mg l ^{1} (9 0) and was found to be sıgnıficantly dıfferent from all other treatments

The maxımum days for shoot elongatıon (15 7) was recorded m the medıum supplemented wıth BAP 1 0 mg l ^{1} + NAA 1 0 mg l ^{1} and was sıgnıficant ly dıfferent from all other treatments Thıs was closely followed by the treatment contaınıng BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (12 1 days) whıch also varıed sıgnıf ıcantly from the rest of the treatments

When the cultures were retaıned wıthout subculturıng the basal portıon of the elongated shoots developed callus and multıple axıllary buds after 5 weeks especıally ın the medıum supplemented wıth BAP and lower levels of NAA (Plate 5) regardless of the stage of development of the cormels

4 2 1 1 6   Effect of kınetın on cormel tıps

Data showıng the ınfluence of kınetın at dıfferent levels alone and ın

combination with different levels of NAA on the culture establishment of cormel tip explants at different stages of development are given in Table 9

Stage I (Cormel tip explants collected soon after the drying of inflorescence)

Number of days for bud emergence

The time taken for bud emergence varied significantly (from 17 0 days to 24 8 days) among the different treatments

The minimum days (17 0) for bud emergence was taken by the treatment containing kinetin 2 0 mg l ^{1} and kinetin 1 0 mg l ^{1} and was found to be on par with the treatment involving kinetin 3 0 mg l ^{1} (17 5 days) and kinetin 4 0 mg l ^{1} (18 4 days)

Medium containing kinetin 4 0 mg l ^{1} + NAA 1 0 mg l ^{1} had taken 24 8 days for bud emergence and the treatment was found to be on par with the treatment having kinetin 4 0 mg l ^{1} + NAA 0 5 mg l ^{1} (23 9 days)

Number of shoots

The number of shoots produced varied from 1 0 to 1 3 in different treatments and did not vary significantly

Number of days for shoot elongation

The treatments differed significantly among themselves with respect to the number of days taken for shoot elongation and ranged from 22 5 to 27 0 The treatment containing kinetin 4 0 mg l ^{1} had taken the minimum days (22 5) for elongation and was found to be statistically homogeneous with the number of days

Table 9 Effect of kinetin and NAA on culture establishment of cormel tips explants of gladiolus at different stages of cormel development

Basal medium S

Culture period 4 weeks

Treatments		Stage I			Stage II			Stage III			Stage IV		
Kinetin (mgl 1)	NAA (mgl 1)	Time taken for bud emergence (days	Number of shoots	Time taken for elongation days	Time taken for bud emergence days	Number of shoots	Time taken for elongation days	Time taken for bud emergence days	Number of shoots	Time taken for elongation days	Time taken for bud emergence days)	Number of shoots	Time taken for elongation days
1 0	0 0	17 0	1 1	22 6	16 3	1 1	9 5	16 3	1 1	20 2	15 5	1 3	18 1
2 0	0 0	17 0	1 0	23 9	16 4	1	9 9	16 5	1 0	20 8	15 4	1 3	17 5
3 0	0 0	17 5	1 3	23 0	16 3	1 3	9 6	15 8	1 4	19 7	14 2	1 5	16 7
4 0	0 0	18 4	1 3	22 5	16 1	1 4	19 8	15 6	1 5	19 4	14 8	1 5	17 1
1 0	0 5	19 4	1 2	23 7	19 4	1 2	20 4	17 1	1 0	21 6	16 9	1 5	18 5
2 0	0 5	20 8	1	24 7	19 4	1 2	22 9	18 4	1 2	23 3	18 1	1 3	20
3 0	0 5	19 8	1 2	24 7	18 6	1 4	23 2	18 1	1	23 4	16 9	1 5	9 2
4 0	0 5	23 9	1 2	26 2	18 2	1 4	22 8	18 4	1 0	22 4	17 3	1 7	18 9
1 0	1 0	20 6	1 1	24 6	19 4	1 3	23 0	21 8	0	23 8	18 5	1 5	19 9
2 0	1 0	2 0	1 1	25 3	19 4	1 4	23 5	18 7	1 1	23 1	17 7	1	20 2
3 0	1 0	20 4	1 1	25 0	18 2	1 3	22 8	18 7	1 0	22 9	17 5	1 4	19 4
4 0	1 0	24 8	1 3	27 0	18 2	1 5	24 1	18 8	1 0	23 7	16 9	1 5	19 3
CD 0 05		2 0	NS	1 7	1 7	NS	1 5	1 4	0 2	1 5	1 1	0 5	1 5
SEm +		2 471	0 069	1 72	1 777	0 139	1 367	1 268	0 227	1 351	0 682	0 162	0 813

Stage I Soon after the drying of inflorescence

Stage II 30 days after drying of inflorescence

Stage III 60 days after drying of inflorescence

Stage IV 90 days after drying of inflorescence

taken by the treatments like kinetin 1 0 mgl^{1} (22 6) kinetin 3 0 mg l^{1} (23 0) kinetin 1 0 mg l^{1} + NAA 0 5 mg l^{1} (23 7) and kinetin 2 0 mg l^{1} (23 9)

Maximum days for shoot elongation was recorded in medium supple mented with kinetin 4 0 mg l^{1} + NAA 1 0 mg l^{1} (27 0) The treatments having kinetin 4 0 mg l^{1} + NAA 0 5 mg l^{1} (26 2 days) kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} (25 3 days) were found to be on par

Stage II (Cormel tip explants collected 30 days after the drying of inflorescence)

Number of days for bud emergence

The number of days taken for bud emergence ranged from 16 1to 19 4 and varied significantly among the treatments

The medium supplemented with kinetin 4 0 mg l^{1} had taken the mini mum days for bud emergence (16 1) and this was found to be homogeneous with the days taken by the treatments containing kinetin 1 0 mg l^{1} (16 3) kinetin 3 0 mg l^{1} (16 3) and kinetin 2 0 mg l^{1} (16 4)

Maximum days for bud emergence (19 4) was recorded in the treatments having kinetin 1 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 2 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 1 0 mg l^{1} + NAA 1 0 mg l^{1} and kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} and were on par with the rest of the treatments

Number of shoots

The number of shoots produced by the various treatments ranged from 1 1 to 1 5 and all the treatments were found to be on par

Days taken for shoot elongation

The treatments showed significant differences among themselves with respect to the time taken for shoot elongation and ranged from 19 5 days to 24 1 days The medium supplemented with kinetin 1 0 mg l^{1} had taken 19 5 days for shoot elongation and the treatments like kinetin 3 0 mg l^{1} (19 6 days) kinetin 4 0 mg l^{1} (19 8 days) kinetin 2 0 mg l^{1} (19 9 days) kinetin 1 0 mg l^{1} + NAA 0 5 mg l^{1} (20 4 days) were found to be on par Maximum days (24 1) for shoot elonga tion was taken by the treatment containing kinetin 4 0 mg l^{1} + NAA 1 0 mg l^{1}

Stage III (Cormel tip explants collected 60 days after the drying of inflorescence)

Number of days for bud emergence

The number of days taken for bud emergence ranged from 15 6 days to 21 8 days and showed significant variation among the treatments The medium containing kinetin 4 0 mg l^{1} had taken the minimum days (15 6) for the bud emergence and was found to be on par with the treatments containing kinetin 3 0 mg l^{1} kinetin 1 0 mg l^{1} and kinetin 2 0 mg l^{1} which took 15 8 16 3 and 16 5 days respectively

The maximum number of days (21 8) for bud emergence was reported in MS medium containing kinetin 1 0 mg l^{1} + NAA 1 0 mg l^{1} and was significantly different from the rest of the treatments

Number of shoots

Maximum shoot number was observed in treatment having kinetin 4 0 mg l^{1} (1 5) and was found to be on par with the treatment kinetin 3 0 mg l^{1} which produced an average shoot number of 1 4 All other treatments were on par and differed significantly from these two treatments

Number of days for shoot elongation

The number of days taken for shoot elongation showed significant variation ranging from 19 4 to 23 8 days

The minimum days for shoot elongation was taken by the medium supplemented with kinetin 4 0 mg l^{1} (19 4) followed by kinetin 3 0 mg l^{1} (19 7) kinetin 1 0 mg l^{1} (20 2) and kinetin 2 0 mg l^{1} (20 8) which did not vary significantly

Maximum days for shoot elongation was recorded in the treatment having kinetin 1 0 mg l^{1} + NAA 1 0 mg l^{1} (23 8) and was found to be statistically homogeneous with treatments involving kinetin 4 0 mg l^{1} + NAA 1 0 mg l^{1} (23 7) kinetin 3 0 mg l^{1} + NAA 0 5 mg l^{1} (23 4) kinetin 2 0 mg l^{1} + NAA 0 5 mg l^{1} (23 3) kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} (23 1) kinetin 3 0 mg l^{1} + NAA 1 0 mg l^{1} (22 9) and kinetin 4 0 mg l^{1} + NAA 0 5 mg l^{1} (22 4)

Stage IV (Cormel tip explants collected 90 days after the drying of inflorescence

Number of days for bud emergence

Significant differences were observed among the treatments with respect to the time taken for bud emergence ranging from 14 3 days to 18 5 days The days taken was minimum (14 3) in the medium supplemented with kinetin 3 0 mg l^{1} and was found to be on par with that of the medium supplemented with kinetin 4 0 mg l^{1} (14 8 days)

Maximum days for bud emergence (18 5) was recorded in the medium supplemented with kinetin 1 0 mg l^{1} + NAA 1 0 mg l^{1} This was found to be on par with the treatments containing kinetin 2 0 mg l^{1} + NAA 0 5 mg l^{1} (18 4 days) kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} (17 7 days) and kinetin 3 0 mg l^{1} + NAA 1 0 mg l^{1} (17 5 days)

Number of shoots

The number of shoots produced ranged from 1 3 to 1 7 in various treat ments All the treatments were found to be homogeneous

Number of days taken for shoot elongation

The treatments differed significantly with respect to the time taken for shoot elongation The treatment containing kinetin 3 0 mg l^{1} had taken 16 7 days for shoot elongation which was the minimum and treatments involving kinetin 4 0 mg l^{1} (17 1) kinetin 2 0 mg l^{1} (17 5) and kinetin 1 0 mg l^{1} (18 1) were found to be on par

Maximum days for shoot elongation was recorded the medium supple mented with kinetin 2 0 mg l^{1} + NAA 0 5 mg l^{1} (20 4) and was found to be homogeneous with treatments like kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} (20 2 days) kinetin 1 0 mg l + NAA 1 0 mg l^{1} (19 9 days) kinetin 3 0 mg l^{1} + NAA 1 0 mg l^{1} (19 9 days) kinetin 3 0 mg l^{1} + NAA 1 0 mg l^{1} (19 4 days) kinetin 4 0 mg l^{1} + NAA 1 0 mg l^{1} (19 3 days) kinetin 3 0 mg l^{1} + NAA 0 5 mg l^{1} (19 2 days) and kinetin 4 0 mg l^{1} + NAA 0 5 mg l^{1} (18 9 days)

When the cultures were retained without subculturing in the medium supplemented with higher levels of kinetin and lower levels of NAA multiple axillary buds and callus were produced after 5 weeks from the base of the elongated shoots (Plate 6) regardless of the stage of development of the cormels

4 2 1 1 7 Effect of media on culture establishment

Of the different media (White s SH and MS) tried to screen out the best basal medium for the culture establishment of gladiolus explants the following results were obtained (Table 10 and Plate 7)

Number of days for bud emergence

The number of days taken for bud emergence differed significantly

The minimum number of (9 3 days) for bud emergence was taken by the MS medium supplemented with 3 0 mg l^{1} BAP and was on par with SH medium supplemented with 3 0 mg l^{1} BAP (10 2 days)

Maximum days (15 5) for bud emergence taken in White s medium supplemented with 3 0 mg l^{1} BAP

Number of shoots

The number of shoots produced differed significantly and varied from 1 1 (White s + BAP 3 0 mg l^{1}) to 3 2 (MS + BAP 3 0 mg l^{1})

Plate 5 Production of multiple axillary buds and callus from cormel tip explant of gladiolus in culture establishment medium (MS + BAP 2.0 mg l^{-1} + NAA 0.5 mg l^{-1})

Plate 6 Production of multiple axillary buds from cormel tip explant of gladiolus in culture establishment medium (MS + kinetin 3.0 mg l^{-1} + NAA 0.5 mg l^{-1})

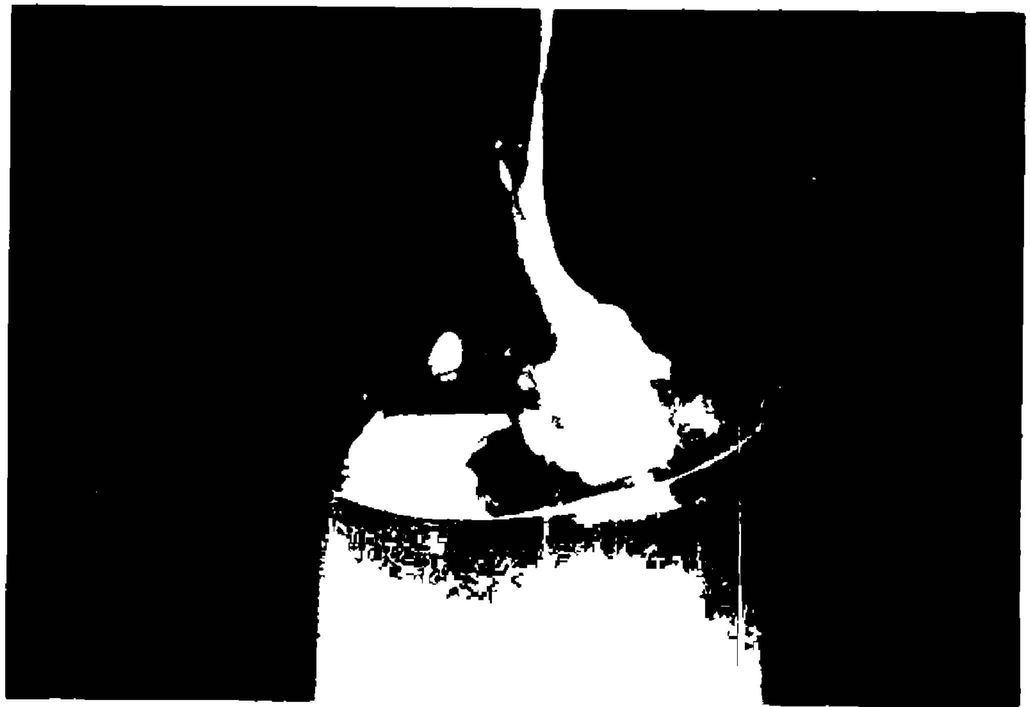


Table 10 Effect of different media on the culture establishment of corm axillary buds

Basal medium supplemented with 3 0 mg l^{1} BAP
Culture period 3 weeks

Basal media	Number of days taken for sprouting	Number of shoots/ culture	Number of days taken for elongation
Whites	15 5	1 1	23 4
SH	10 2	2 4	16 8
MS	9 3	3 3	15 9
CD (0 05)	1 1	0 6	1 3
SEm+	0 090	0 170	0 900

Number of days for bud elongation

The number of days taken for elongation varied from 15 9 to 23 4 days and differed significantly

Minimum days for elongation (15 9) was recorded in MS medium supplemented with 3 0 mg l ^{1} BAP and was on par with SH medium supplemented with 3 0 g l ^{1} BAP (16 8)

Maximum days (23 4) for elongation of the shoots was taken by White s medium having 3 0 mg l ^{1} BAP

4 2 1 2 Shoot proliferation (Stage 2)

The elongated buds derived from corm axillary buds and cormel tips of gladiolus in Stage 1 were cultured in MS medium containing various levels of cytokinins (BAP kinetin and 2ip) alone and in combination with NAA The responses observed were the production of multiple axillary buds and callus which are presented in Table 11 to 13

4 2 1 2 1 Effect of BAP

The organogenic responses of elongated shoots as influenced by BAP (1 0 2 0 3 0 and 4 0 mg l ^{1}) alone and in combination with NAA (0 5 1 0 and 2 0 mg l ^{1}) in MS medium are given in Table 11

A very high rate of axillary bud production was observed when the MS medium was supplemented with BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} and BAP 2 0 mg l ^{1} + NAA 0 5 mg l ^{1} (Plate 8) High rate of axillary bud production was

Table 11 Organogenic responses of elongated shoots derived from corm axillary buds and cormel tips of gladiolus as influenced by BAP and NAA

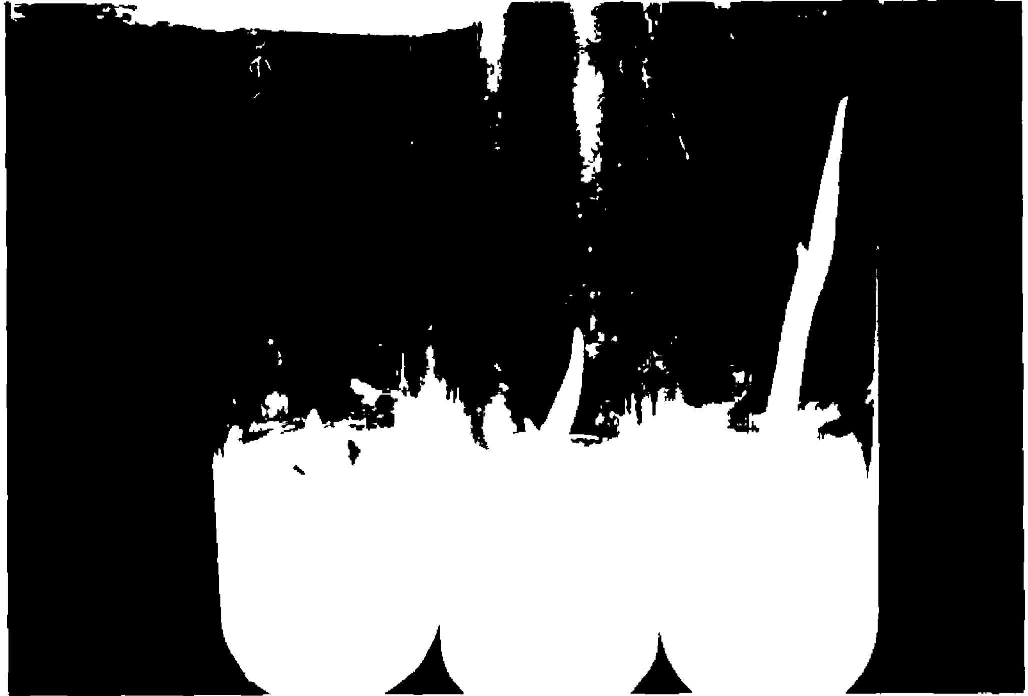
Basal medium: MS
Culture period: 3 weeks

Treatment BAP (mg l^{1})	Treatment NAA (mg l^{1})	Nature of response: Multiple axillary buds*	Nature of response: Callus growth
1 0	0 0	++	
2 0	0 0	++	
3 0	0 0	+++	++
4 0	0 0	+++	+++
1 0	0 5	++++	
2 0	0 5	++++	
3 0	0 5	+++	++
4 0	0 5	+++	++
1 0	1 0	+++	+++
2 0	1 0	+++	+++
3 0	1 0	++	++
4 0	1 0		+++
1 0	2 0	+++	++
2 0	2 0	++	+++
3 0	2 0	++	+++
4 0	2 0		

+ Low rate of production (10 to 20 buds)*
++ Medium rate of production (20 to 30 buds)*
+++ High rate of production (30 to 40 buds)*
++++ Very high rate of production (more than 40 buds)*
No response

Plate 7 Effect of media (White's medium SH medium and MS medium) on culture establishment of corm axillary bud explants of gladiolus

Plate 8 Multiple axillary bud production from elongated buds of gladiolus in MS medium supplemented with BAP 2 0 mg l^{1} + NAA 0 5 mg l^{1}



observed in several treatments viz BAP 3 0 mg l^{1} BAP 4 0 mg l^{1} BAP 3 0 mg l^{1} + NAA 0 5 mg l^{1} BAP 4 0 mg l^{1} + NAA 0 5 mg l^{1} BAP 1 0 mg l^{1} + NAA 1 0 mg l^{1} BAP 2 0 mg l^{1} + NAA 1 0 mg l^{1} BAP 1 0 mg l^{1} + NAA 2 0 mg l^{1}

Medium rate of axillary bud production could be noticed in treatments when MS medium was supplemented with BAP 1 0 mg l^{1} BAP 2 0 mg l^{1} BAP 3 0 mg l^{1} + NAA 1 0 mg l^{1} BAP 2 0 mg l^{1} + NAA 2 0 mg l^{1} and BAP 3 0 mg l^{1} + NAA 2 0 mg l^{1} The treatments BAP 4 0 mg l^{1} + NAA 1 0 mg l^{1} and BAP 4 0 mg l^{1} + NAA 2 0 mg l^{1} could not produce multiple axillary buds

Callus production was high in treatments involving BAP 4 0 mg l^{1} BAP 1 0 mg l^{1} + NAA 1 0 mg l^{1} BAP 2 0 mg l^{1} + NAA 2 0 mg l^{1} and BAP 3 0 mg l^{1} + NAA 2 0 mg l^{1} (Plate 9) Medium rate of callus production was observed in treatments with BAP 3 0 mg l^{1} BAP 3 0 mg l^{1} + NAA 0 5 mg l^{1} BAP 4 0 mg l^{1} + NAA 0 5 mg l^{1} BAP 3 0 mg l^{1} + NAA 0 5 mg l^{1} and BAP 1 0 mg l^{1} + NAA 2 0 mg l^{1}

Callus production was not observed in treatments involving BAP 1 0 mg l^{1} BAP 2 0 mg l^{1} BAP 1 0 mg l^{1} + NAA 0 5 mg l^{1} BAP 2 0 mg l^{1} + NAA 0 5 mg l^{1} and BAP 4 0 mg l^{1} + NAA 2 0 mg l^{1}

4 2 1 2 2 Effect of kinetin

Data showing the effect of kinetin (1 0 mg l^{1} 2 0 mg l^{1} and 4 0 mg l^{1}) alone and in combination with NAA (0 5 mg l^{1} 1 0 mg l^{1} and 2 0 mg l^{1}) on elongated shoots of gladiolus in MS medium are presented in Table 12

Table 12 Organogenic responses of elongated shoots derived from corm axillary buds and cormel tips of gladiolus as influenced by kinetin and NAA

Basal medium MS
Culture period 3 weeks

Treatments (mg l ^{1}) Kinetin	NAA	Multiple axillary buds	Nature of response: Callus growth	Others if any
1 0	0 0	++		
2 0	0 0	++		
3 0	0 0	++	+	
4 0	0 0	++	+	
1 0	0 5	++	+	
2 0	0 5			Swelling at the shoot base
3 0	0 5	+	+	Rhizogenesis and shoot elongation
4 0	0 5			
1 0	1 0	+	++	Rhizogenesis
2 0	1 0			Swelling at the shoot base and rhizogenesis
3 0	1 0			
4 0	1 0			
1 0	2 0	+	++	Rhizogenesis
2 0	2 0		+	
3 0	2 0	+	+	Rhizogenesis swelling of shoot base
4 0	2 0		+	

+ Low rate of production (10 20 buds)*
++ Medium rate of production (20 30 buds)*
No response

Medium rate of multiple axillary bud production was observed in MS medium supplemented with kinetin 1 0 mg l^{1} kinetin 2 0 mg l^{1} kinetin 3 0 mg l^{1} kinetin 4 0 mg ^{1} and kinetin 1 0 mg l^{1} + NAa 0 5 mg l^{1} (Plate 10) The rate of production of multiple axillary buds was low in treatments viz kinetin 3 0 mg l^{1} + NAA 0 5 mg l^{1} and kinetin 1 0 mg l^{1} + NAA 2 0 mg l^{1}

Treatments like kinetin 2 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 4 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 2 0 mg l^{1} + NAA 2 0 mg l^{1} and kinetin 4 0 mg l^{1} + NAA 2 0 mg l^{1} could not induce axillary buds

Callus production was medium in treatments like kinetin 1 0 mg l^{1} + NAA 1 0 mg l^{1} and kinetin 1 0 mg l^{1} + NAA 2 0 mg l^{1} The rate of production of callus was low in treatments like kinetin 3 0 mg l^{1} kinetin 4 0 mg l^{1} kinetin 1 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 3 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 2 0 mg l^{1} + NAA 2 0 mg l^{1} kinetin 3 0 mg l^{1} + NAA 2 0 mg l^{1} and kinetin 4 0 mg l^{1} + NAA 2 0 mg l^{1}

Callus production was not observed in treatments involving kinetin 1 0 mg l^{1} kinetin 2 0 mg l^{1} kinetin 2 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 4 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} kinetin 3 0 mg l^{1} + NAA 1 0 mg l^{1} and kinetin 4 0 mg l^{1} + NAA 1 0 mg l^{1}

Of the different treatments kinetin 2 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 2 0 mg l^{1} + NAA 1 0 mg l^{1} kinetin 3 0 mg l^{1} + NAA 1 0 mg l^{1} kinetin 4 0 mg l^{1} + NAA 0 5 mg l^{1} and kinetin 4 0 mg l^{1} + NAA 1 0 mg l^{1} could produce neither multiple axillary buds nor callus They showed swelling of the shoot base and rhizogenesis

Plate 9 Callus induction from the base of elongated buds of gladiolus in MS medium supplemented with BAP 3 0 mg l^{1} and NAA 2 0 mg l^{1}

Plate 10 Multiple axillary bud production at medium rate from the base of elongated buds of gladiolus in MS medium supplemented with kinetin 4 0 mg l^{1}

Rhizogenesis was also noted when MS medium was supplemented with kinetin 1 0 mg l^{1} + NAA 1 0 mg l^{1} kinetin 3 0 mg l^{1} + NAA 0 5 mg l^{1} kinetin 1 0 mg l^{1} + NAA 2 0 mg l^{1} and kinetin 2 0 mg l^{1} + NAA 2 0 mg l^{1}

4 2 1 2 3 Effect of 2ip

Organogenic response of elongated shoots derived from corm axillary buds and cormel tips of gladiolus as influenced by 2ip (1 0 mg l^{1} 2 0 mg l^{1} 3 0 mg l^{1} and 4 0 mg l^{1}) either alone or in combination with NAA (0 5 mg l^{1} 1 0 mg l^{1} and 2 0 mg l^{1}) in MS medium is given in Table 13

Low rate of multiple axillary bud production was observed at higher levels of 2ip (3 0 mg l^{1} and 4 0 mg l^{1}) and low rate of callus production in treatments with 2 ip 4 0 mg l^{1} 2ip 1 0 mg l^{1} + NAA 0 5 mg l^{1} 2ip 2 0 mg l^{1} + NAA 0 5 mg l^{1} 2ip 1 0 mg l^{1} + NAA 1 0 mg l^{1} 2ip 2 0 mg l^{1} + NAA 1 0 mg l^{1} 2ip 3 0 mg l^{1} + NAA 1 0 mg l^{1} 2ip 1 0 mg l^{1} + NAA 2 0 mg l^{1} and 2ip 2 0 mg l^{1} + NAA 2 0 mg l^{1}

Single shoot elongation was noticed at lower levels of 2ip (1 0 and 2 0 mg l^{1}) At higher level of 2ip (4 0 mg l^{1}) shoot elongation combined with swelling of the base and rhizogenesis was observed (Plate 11)

Rhizogenesis was noticed in all the treatments having 2ip and NAA

4 2 1 2 4 Influence of frequent subculturing

Of the three cytokinines tried viz BAP kinetin and 2ip a very high rate of multiple axillary bud production was noticed in treatments involving BAP

Table 13 Organogenic responses of elongated shoots derived from corm axillary buds and cormel tips of gladiolus as influenced by 2ip and NAA

Basal medium MS
Culture period 3 weeks

Treatments (mg l 1)		Multiple* axillary buds	Nature of response	
2ip	NAA		Callus growth	Others if any
1 0	0 0			Shoot elongation
2 0	0 0			
3 0	0 0	+		
4 0	0 0	+	+	Shoot elongation Rhizogenesis
1 0	0 5		+	Rhizogenesis
2 0	0 5		+	
3 0	0 5			
4 0	0 5			
1 0	1 0		+	
2 0	1 0		+	
3 0	1 0		+	
4 0	1 0			
1 0	2 0		+	
2 0	2 0		+	
3 0	2 0			
4 0	2 0			

\+ Low rate of production (10 20 buds)*
No response

Hence the studies on the influence of frequent subculturing on multiple axillary bud production were carried out in MS medium supplemented with BAP (2 0 and 3 0 mg l^{-1}) alone and in combination with NAA (0 0 0 5 and 1 0 mg l^{-1}) and are given in Table 14

Very high rate of production of bud aggregates could be noticed in all the subcultures when MS medium was supplemented with BAP 2 0 mg l^{-1} and NAA 0 5 mg l^{-1} (Plate 12) and also with BAP 2 0 mg l^{-1} In the case of other treatment combinations the rate of production of multiple axillary buds increased upto fourth subculture but callus production also could be noticed

4 2 1 2 5 Elongation of multiple axillary buds

The bud aggregates obtained from the Stage 2 have been subjected to elongation treatments in MS full strength (MS_a) and half strength medium (MS_b) supplemented with various levels and combinations of BAP and NAA The response are given in Table 15 and in Plates 13 and 14)

The basal media (both MSa and MSb) without growth regulators produced elongated shoots with roots More elongated shoots and normal roots have been obtained in full strength MS medium (MS_a devoid of growth regulators)

Further multiplication of axillary buds have been observed in treatments containing BAP 1 0 mg l^{-1} in both full strength MS (MS_a) and half strength (MS_b) media The treatment involving BAP 1 0 mg l^{-1} + NAA 1 0 mg l^{-1} produced multiple axillary buds and callus in both the media

Plate 11 Single shoot elongation and swelling of the base of elongated buds of gladiolus in the MS medium supplemented with 2ip 4 0 mg l^{-1}

Plate 12 Effect of frequent subculturing on multiple axillary bud production from elongated buds of corm axillary bud explants of gladiolus in MS medium supplemented with BAP 2 0 mg l^{-1} and NAA 0 5 mg l^{-1}

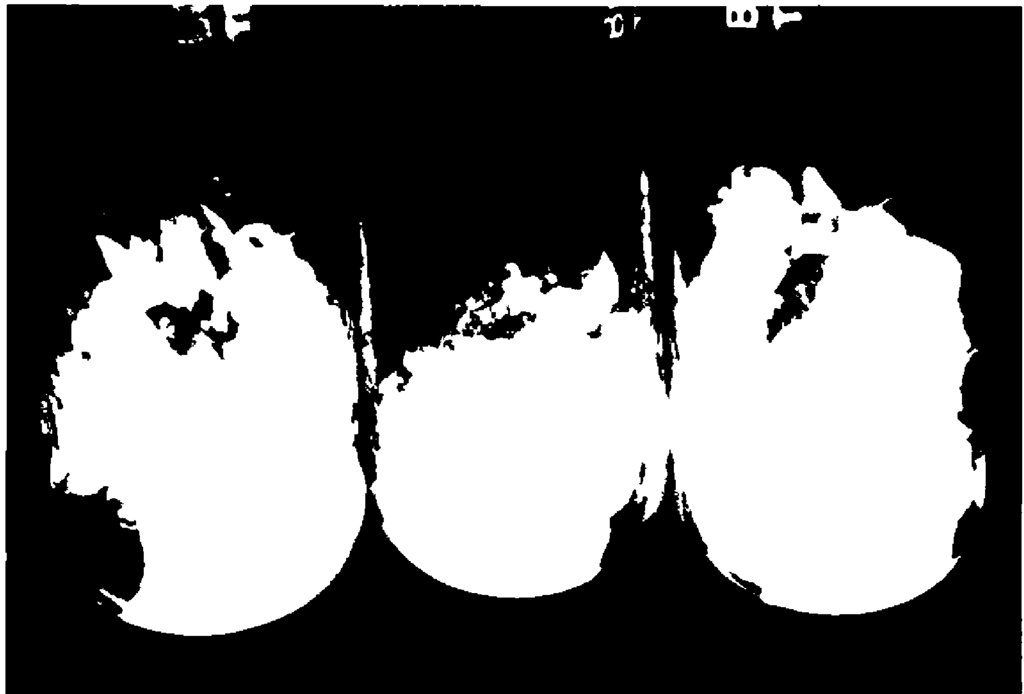


Table 14 Frequency of multiple axillary bud formation during four generations of subculture on MS medium

Culture period 3 weeks

Treatments BAP	NAA (mg l $^{-1}$)	Sub culture I	II	III	IV	Remarks
2 0	0 0	+++	+++	++++	ND	Rate of production of bud aggregates increased
3 0	0 0	+++	++++	++++	ND	Rate of production of bud aggregates increased + callus growth
2 0	0 5	++++	++++	ND	ND	Very high rate of production of bud aggregates
3 0	0 5	+++	+++	++++	ND	Increased bud aggregates production + Callus growth
2 0	1 0	++++	++++	++++	ND	
3 0	1 0	++	++++	++++	++++	

++ Medium rate multiple axillary bud formation
+++ High rate multiple axillary bud formation
++++ Very high rate multiple axillary bud formation
ND Not determined

Table 15 Differences in shoot elongation from multiple axillary bud aggregates with BAP and NAA

Culture period 3 weeks

Medium	Growth regulators BAP (mg l^{-1})	NAA	Response
MS_a	0	0	Shoot elongation normal root formation more elongated shoots
	0	1	Callus growth
	1	0	Further multiplication
	1	1	Further multiplication of bud aggregates and callus growth
MS_b	0	0	Shoot elongation root formation
	0	1	Callus growth
	1	0	Further multiplication
	1	1	Further multiplication of bud aggregates and callus growth

MS_a MS medium with full concentration of inorganic salts

MS_b MS medium with ½ the concentration of inorganic salt

Plate 13 Repeated multiplication and callusing of multiple axillary buds in full strength MS medium supplemented with BAP 1 0 mg l^{1} (a) Elongation of multiple axillary buds in full strength MS basal medium (b)

Plate 14 Repeated multiplication and callusing of multiple axillary buds in half strength MS medium supplemented with BAP 1 0 mg l^{1} (a) Elongation of multiple axillary buds in half strength MS medium (b)

The treatments having NAA 1 0 mg l ^{1} in both media have shown rapid conversion of the multiple axillary buds to callus

4 2 1 2 6 Effects of media

The bud aggregates taken from the stage 2 were subjected to elongation studies in different basal media viz full strength MS salt medium (MS_a) half strength MS salt medium (MS_b) SH salt medium and White s salt medium and the observations are presented in Table 16

Days taken for shoot elongation

The number of days taken for shoot elongation varied significantly in different media It ranged from 7 5 days (MS half strength mediaum MS_b) to 20 2 days (White s medium) Days taken for shoot elongation in full strength MS medium (MS_a) and SH medium were 9 6 and 15 0 respectively

Days taken for root initiation

The number of days taken for root initiation varied singnificantly ranging from 17 4 days (SH medium) to 22 6 days (White s medium) The minimum days for root initiation (17 4) in SH medium and was on par with that of full strength MS medium (18 4 days) and half strength MS medium (19 6 days)

Number of shoots

The number shoots produced in different media also varied significantly

Table 16 Dıfferences ın shoot elongatıon from multıple axıllary bud agregates on dıfferent medıa
Culture perıod 3 weeks

Medıa	Tıme taken for		Observatıons after 4 weeks			
	Shoot elongatıon (days)	Rootıng (days)	Shoot number	Shoot length (cm)	Root number	Nature of roots
MS_a	9 6	18 4	17 4	12 40	5 0	Normal roots wıth root haırs
MS_b	7 5	19 6	6 9	12 00	5 5	
SH medıum	15 0	17 4	10 0	11 90	10 6	Thıck and straıght roots
Whıte s medıum	20 2	22 6	6 0	4 90	5 4	Short medıum thıck roots
CD (0 05)	1 8	2 1	2 4	1 30	3 2	
SEm±	1 727	2 550	2 325	0 997	5 765	

MS_1 MS medıum wıth full concentratıon of ınorganıc salts

MS_b MS medıum wıth ½ concentratıon of ınorganıc salts

ranging from 6 0 (White s medium) to 17 4 (full strength MS medium) The number of shoots produced m White s medium (6 0) was found to be on par with that of half strength MS medium (6 9)

Length of shoots

Shoot length also varied significantly in different media It ranged from 4 90 cm (White s medium) to 12 40 cm (MS full strength medium) The maximum shoot length recorded in full strength MS medium (12 40 cm) was found to be on par with that of half strength MS medium (12 00 cm)

Number of roots

Number of roots produced in different media also varied significantly ranging from 5 0 (full strength MS medium) to 10 6 (SH medium) The number of roots produced in full strength MS medium (5 0) was found to be homogeneous with that of number of roots produced in White s medium (5 4) and half strength MS medium (5 5)

Nature of roots

The roots produced in different media differed considerably Normal roots with root hairs were observed m full strength MS medium and half strength MS medium In White s medium the roots produced were short and medium thick SH medium produced thick unbranched roots tapering in nature

4 2 1 2 7 Effect of medium supplements

Influence of medium supplements (coconut water and activated charcoal)

on shoot elongation was studied on MS basal medium with multiple axillary buds taken from the Stage 2 and the results are presented in Tables 17 and 18 respective ly

4 2 1 2 7 1 Effect of coconut water

The multiple axillary bud aggregates were subjected to the treatments with various levels of coconut water (0 0 2 5 5 0 7 5 10 0 and 15 0%) in MS medium and the results are given in Table 17 and Plate 15

Days taken for shoot elongation

Significant variation among the treatments were observed with respect to the number of days taken for shoot elongation and ranged from 9 6 in control to 16 8 in the medium containing coconut water 10 0 per cent and 15 0 per cent which were found to be on par with coconut water 7 5 per cent (16 1 days) and 2 5 per cent (15 4 days)

Days taken for root initiation

Significant variation was observed between the treatments with different levels of coconut water and the control Days taken for root initiation varied from 15 1 days (coconut water 10%) to 18 4 days (control) The MS medium supple mented with 10 0 per cent coconut water took the minimum days (15 10 days) for root initiation and was found to be on par with all other treatments containing coconut water

Table 17 Effect of coconut water on elongation of bud aggregates

Basal medium MS
Culture period 3 weeks

Coconut water (%)	Time taken for shoot bud elongation (days)	Time taken for root initiation (days)	Number of shoots	Length of shoots (cm)	Number of roots	Nature of shoots
0 0 (Control)	9 6	18 4	17 4	12 40	5 0	Normal roots with root hairs
2 5	15 4	15 2	9 8	11 60	7 2	Slender roots without root hairs
5 0	14 8	15 8	12 4	11 80	7 7	
7 5	16 1	15 6	13 6	11 40	8 5	
10 0	16 8	15 1	12 5	11 20	8 4	
15 0	16 8	15 3	9 3	11 00	9 5	
CD (0 05)	1 6	1 4	2 2	NS	1 4	
SEm±	1 575	1 079	2 714	1 383	1 201	

Number of shoots

The different treatments varied significantly with respect to the production of shoots which ranged from 9 8 in 2 5 per cent coconut water to 17 4 in control Maximum number of shoots (17 4) was produced by the treatment devoid of coconut water The number of shoots produced by the medium containing 5 0 7 5 10 0 and 15 0 per cent coconut water were 12 4 13 6 12 5 and 9 3 days respectively

Length of shoots

Significant variation could not be observed with respect to the length of shoots and the value ranged from 11 90 cm in the treatment containing 15 0 per cent coconut water to 12 40 cm in control

Number of roots

The treatments differed significantly with respect to the number of roots produced It varied from 5 0 (control) to 9 5 (coconut water 15%) The number of shoots produced in the medium containing 15 0 per cent and was on par with treatments with 10 0 per cent (8 4) and 7 5 per cent (8 5) coconut water

Nature of roots

In all the treatments coconut water produced very slender roots without root hairs Normal roots were produced in the MS medium devoid of coconut water (control)

4 2 1 2 7 2 Effect of activated charcoal

Multiple axillary buds taken from stage 2 were subjected to various levels of activated charcoal (0 0 0 1 0 2 0 3 and 0 4%) in MS medium and the results are given in Table 18

Days taken for shoot elongation

The treatments did not differ significantly with respect to the number of days taken for shoot elongation the minimum (9 6 days) being in control and maximum (10 7 days) in the treatment involving 0 4 per cent activated charcoal

Days taken for root initiation

The number of days taken for root initiation varied from 15 5 (activated charcoal 0 3%) to 18 4 days (control) and the differences were significant All the other treatments viz activated charcoal 0 4 0 2 0 1 per cent took 15 6 16 2 and 16 8 days respectively for root initiation

Number of shoots

Significant variation was obtained with respect to the number of shoots produced Maximum number of shoots (17 4) was produced in the control and the minimum number (8 2) in charcoal 0 2 per cent (Plate 16) All other treatments like charcoal 0 1 0 3 and 0 4 per cent produced 8 5 9 3 and 8 7 shoots respectively which were homogeneous with that of charcoal 0 2 per cent

Table 18 Effect of activated charcoal on elongation of bud aggregates

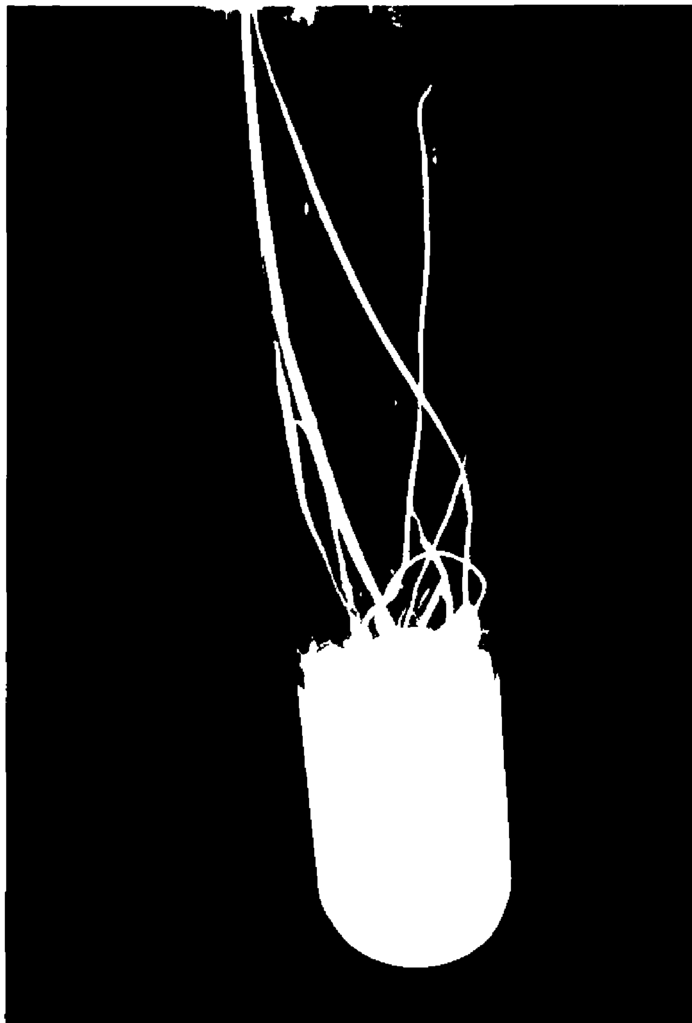
Basal medium MS

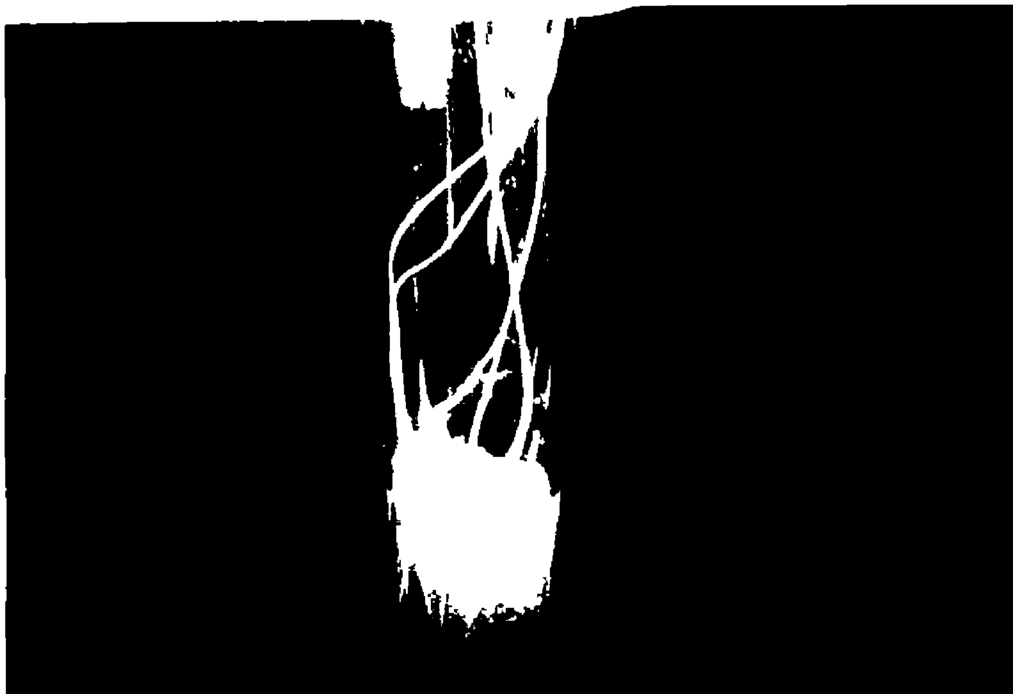
Culture period 3 weeks

Activated charcoal	Time taken for shoot elongation (days)	Time taken for root initiation (days)	Number of shoots	Length of shoots (cm)	Number of roots	Nature of roots
0 0	9 6	18 4	17 4	12 40	5 0	Normal roots with root hairs
0 1	10 0	16 8	8 5	8 00	6 8	Long white coloured slender roots without root hairs
0 2	10 4	16 2	8 2	8 60	7 2	
0 3	10 0	15 5	9 3	7 96	6 9	
0 4	10 7	15 6	8 7	8 14	6 9	
CD (0 05)	NS	1 4	1 8	1 50	1 3	
SEm±	2 458	1 155	1 774	1 289	0 966	

Plate 15 Effect of coconut water on the elongation of multiple axillary buds

Plate 16 Effect of activated charcoal on the elongation of multiple axillary buds





Length of shoots

Maximum shoot length (12 40 cm) was produced in control which was significantly superior to all other treatments

Number of roots

The number of roots produced varied from 5 0 (control) to 7 2 (activated charcoal 0 2%) Treatments with charcoal 0 1 0 3 and 0 4 per cent produced 6 8 6 9 and 6 9 roots respectively and were on par

Nature of roots

All the treatments with activated charcoal produced long white coloured slender roots without root hairs The roots produced in MS medium without activat ed charcoal (control) were normal with root hairs

4 2 1 3 *In vitro* rooting (Stage 3)

Elongated shoots from Stage 2 were used for *in vitro* rooting studies and the results are presented in Tables 19 to 23

4 2 1 3 1 Effect of media

To study the effect of different basal media on rooting a trial was con ducted with solid full strength MS (MS_a) half strength MS (MS_b) liquid MS_a liquid MS_b and solid SH media The results are given in Table 19

Table 19 Effect of dıfferent medıa on rootıng of elongated shoots of gladıolus

Medıa	Tıme taken for root ınıtıatıon (days)	Number of roots per culture	Length of roots (cm)	Nature of roots
Solıd MS_a	9 5	12 0	3 60	Normal wıth branchıng habıt and root haırs
Solıd MS_b	10 6	8 7	3 00	Slender wıth branch ıng habıt and root haırs
Lıquıd MS_a	8 3	8 0	3 30	Roots wıth secondar ıes and root haırs
Lıquıd MS_b	8 5	7 9	3 60	
Solıd SH	14 0	11 6	2 90	Thıck strong straıght and taperıng
CD (0 05)	1 2	1 3	0 62	
SEm±	0 877	1 035	0 220	

Number of days taken for root initiation

The number of days taken for rooting varied from 8 3 to 14 0 The minimum number of days (8 3) was taken by liquid full strength MS medium fol lowed by liquid half strength MS medium (8 5) These two did not vary significant ly Maximum days for root initiation was taken by solid SH medium (14 0) which differed significantly from all other treatments

Solid MS medium (full strength) took 9 5 days for rooting and was found to be on par with that of solid half strength MS medium (10 6 days)

Number of roots

Number of roots produced in different media differed significantly and ranged from 7 9 to 12 0 Maximum number of roots (12 0) was observed in solid MS medium and was found to be homogeneous with that of solid SH medium (11 6)

Liquid half strength MS medium produced the minimum number of roots (7 9) and the number of roots produced by liquid full strength MS medium (8 0) solid half strength MS medium (8 7) were found to be on par with this

Length of roots

Significant differences were observed among various treatments with respect to the length of roots as it ranged from 2 9 cm to 3 6 cm The longest root (3 6 cm) were produced by solid full strength MS medium and was found to be on par with that of liquid half strength MS medium (3 6 cm) Minimum root length was

observed in solid SH medium (2 9 cm) The root lengths produced by solid half strength medium (3 00 cm) and liquid full strength MS medium (3 30 cm) were found to be homogeneous with that of solid SH medium (2 90 cm)

Nature of roots

Normal roots with branching habit and with root hairs were produced by solid full strength MS medium (Plate 17a)

Solid half strength MS medium produced slender roots with root hairs and branching nature

Liquid MS (Plate 17b) and half strength MS produced roots with sec ondaries and root hairs

Thick strong straight growing roots with tapering nature without branching habit was produced by the SH medium (Plate 18)

4 2 1 3 2 Effect of auxins and sucrose levels on rooting

To study the effect of auxins and different levels of sucrose trials were conducted with IBA and NAA (each at 0 5 1 0 2 0 mg l^{-1}) in combination with different levels of sucrose (1 2 and 3%) and the results are presented in Tables 20 and 21 respectively

4 2 1 3 2 1 Effect of IBA

Number of days taken for root initiation

The days taken for rooting of *in vitro* shoot ranged from 8 7 days to 21 8 days in different treatments The medium supplemented with 3 0 per cent sucrose

Plate 17a *In vitro* rooting of elongated shoots of gladiolus in MS medium

Plate 17b *In vitro* rooting of elongated shoots of gladiolus in liquid MS medium

and 0 5 mg l^{1} IBA took the minimum days for rooting (8 7) and was on par with that of the medium supplemented with 3 0 per cent sucrose and 1 0 mg l^{1} IBA and the medium supplemented with 3 0 per cent sucrose and IBA 2 0 mg l^{1} which took 9 2 and 10 0 days respectively (Table 20)

Maximum days (21 8) for root initiation was taken by the treatment having 1 0 per cent sucrose and 1 0 mg l^{1} IBA The treatment having 1 0 per cent sucrose and 2 0 mg l^{1} IBA was found to be on par with this (21 5 days)

Number of roots

The number of roots varied from 1 1 to 15 8 and showed in significant variation among treatments Treatment having sucrose 3 0 per cent and IBA 0 5 mg l^{1} was found to be superior with maximum number of roots (15 8) and was on par with the treatment having sucrose 3 0 per cent and IBA 1 0 mg l^{1} which had an average root number of 15 0

Minimum number of roots (1 1) were noticed in the medium supple mented with sucrose 1 0 per cent and IBA 1 0 mg l^{1} and was found to be homoge neous with the treatments like sucrose 1 0 per cent + IBA 0 5 mg l^{1} and sucrose 1 0 per cent + IBA 2 0 mg l^{1} which produced 1 6 and 1 8 roots respectively

Length of roots

The length of roots ranged from 0 28 cm to 4 60 cm and differed signifi cantly in various treatment The maximum length of roots (4 60 cm) was observed in the treatment having 3 0 per cent sucrose and 0 5 mg l^{1} IBA which was on par with that of the medium supplemented with 3 0 per cent sucrose and 1 mg l^{1} IBA (3 90 cm)

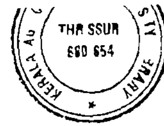


Table 20 Effect of sucrose and IBA at different levels on rooting of elongated shoots

Basal medium MS
Culture period 3 weeks

Treatments		Time taken for root initiation (days)	Number of roots per culture	Length of roots (cm)	Nature of roots
Sucrose (%)	IBA (mg l^{-1})				
1	0 5	20 4	1 6	0 80	Short and slender
2	0 5	20 6	3 6	1 66	Medium thick
3	0 5	8 7	15 8	4 60	Normal with branching habit
1	1 0	21 8	1 1	0 34	Slender
2	1 0	20 2	4 4	2 00	Short and medium thick
3	1 0	9 2	15 0	3 90	Short and slender
1	2 0	21 5	1 8	0 28	Short and slender
2	2 0	21 2	2 7	1 26	Medium thick
3	2 0	10 0	5 4	1 60	Thick fleshy and abnormal
CD (0 05)		1 1	1 1	0 72	
SEm±		0 682	0 758	0 319	

The minimum root length (0 28 cm) was observed in the treatment having 1 0 per cent sucrose + 2 0 mg l ^{1} IBA and was on par with that of the media having 1 0 per cent sucrose + 1 0 mg l ^{1} IBA (0 34 cm) 1 0 per cent sucrose + 0 5 mg l ^{1} IBA (0 80 cm)

Nature of roots

The roots produced were normal with branching habit in the case of medium supplemented with IBA 0 5 mg l ^{1} or IBA 1 0 mg l ^{1} in combination with sucrose 3 0 per cent and slender m the medium supplemented with IBA 1 0 mg l ^{1} + sucrose 2 0 per cent

The treatment having IBA 2 mg l ^{1} and sucrose 3 0 per cent produced thick fleshy abnormal roots (Plate 19) while medium thick root were observed in the medium supplemented with IBA 0 5 mg l ^{1} + sucrose 2 0 per cent Short medium thick roots were observed in the treatments having 1 0 mg l ^{1} IBA + su crose 2 0 per cent IBA 2 mg l ^{1} + sucrose 2 0 per cent

Short slender roots were observed in the treatments having 1 0 per cent sucrose and also m different concentrations of IBA

4 2 1 3 2 2 Effect of NAA

Number of days taken for root initiation

The days taken for root initiation showed significant differences and ranged from 8 5 to 20 4 (Table 21)

Plate 18 *In vitro* rooting ot elongated shoots of gladiolus as influenced by SH medium (a) half strength MS medium (b)

Plate 19 *In vitro* rooting of elongated shoots of gladiolus as influenced by sucrose 3 0 per cent and IBA 2 0 mg l^{1}

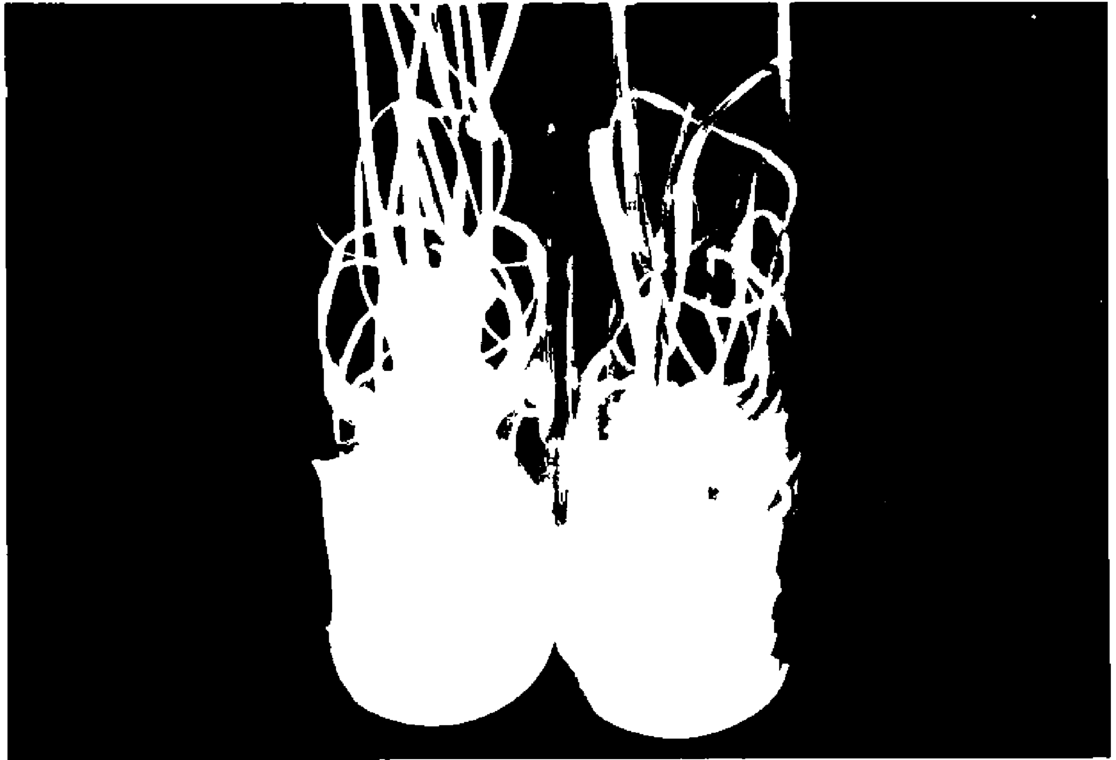


Table 21 Effect of sucrose and NAA at different levels on rooting of elongated shoots of gladiolus

Basal medium MS
Culture period 3 weeks

Treatments		Time taken for root initiation (days)	Number of roots per culture	Length of roots (cm)	Nature of roots
Sucorse (%)	NAA ($mg\ l^{-1}$)				
1	0 5	19 6	1 1	0 14	Medium thick
2	0 5	14 2	3 7	2 80	Normal with branching habit
3	0 5	8 9	8 6	3 70	Normal with branching habit
1	1 0	20 4	1 1	0 14	Short and thick
2	1 0	16 3	2 2	0 30	Short and thick
3	1 0	8 5	9 2	2 90	Normal with branching habit
1	2 0	19 1	1 2	0 10	Short and thick
2	2 0	19 6	1 5	0 13	Short and thick
3	2 0	10 1	6 6	1 30	Medium thick
CD (0 05)		1 3	0 9	0 49	
SEm±		1 049	0 515	0 143	

The minimum day (8 5) for rooting was taken by the medium supple mented with sucrose 3 0 per cent and NAA 1 0 mg l ^{1} and was on par with that of sucrose 3 0 per cent + NAA 0 5 mg l ^{1} (8 9)

Maximum days (20 4) for rooting was taken in the treatment having 1 0 per cent sucrose 1 0 mg l ^{1} NAA which was on par with treatments having sucrose 1 0 per cent + NAA 2 0 mg l ^{1} sucrose 2 0 per cent + NAA 2 0 mg l ^{1} and sucrose 1 0 per cent + NAA 0 5 mg l ^{1} which took 19 1 19 6 19 6 days respec tively

Number of roots

The number of roots produced was the highest (9 2) in the medium having 3 0 per cent sucrose and 1 0 mg l ^{1} NAA which was on par with the roots (8 6) produced in the medium supplemented with 3 0 per cent sucrose and NAA 0 5 mg l ^{1}

The minimum number of roots was noticed in medium supplemented with 1 0 per cent sucrose and 0 5 mg l ^{1} NAA and also in medium having 1 0 per cent sucrose + 1 0 mg l ^{1} NAA This was on par with the medium containing 1 0 per cent sucrose + 2 0 mg l ^{1} NAA (1 2)

Length of roots

The length of roots produced differed significantly and was found to be maximum (3 70 cm) in the treatment having sucrose 3 0 per cent and NAA 0 5 mg l ^{1} (Table 21)

The minimum root length (0 10 cm) was observed in the treatment having sucrose 1 0 per cent and NAA 2 0 mg l ^{1} which was on par with the length of roots observed in the treatments having 2 0 per cent sucrose + 2 0 mg l ^{1} NAA (0 13 cm) 1 0 per cent sucrose + 1 0 mg l ^{1} NAA (0 14 cm) 1 0 per cent sucrose + 0 5 mg l ^{1} NAA (0 14) and 2 0 per cent sucrose + 1 0 mg l ^{1} NAA (0 30 cm)

Nature of roots

Normal slender roots with secondaries and tertiaries were produced by the treatments having 3 0 per cent sucrose and 0 5 mg l ^{1} or 1 0 mg l ^{1} NAA and also in the treatment having 2 0 per cent sucrose + 0 5 mg l ^{1} NAA (Plate 20)

Medium thick roots without root hairs were observed in the treatment having 1 0 per cent sucrose and 0 5 mg l ^{1} NAA

Roots were short thick and lack further elongation in the case of the treatments having 1 0 per cent sucrose or 2 0 per cent sucrose with different levels of NAA (1 0 mg l ^{1} or 2 0 mg l ^{1}) (Table 21 and Plates 20)

4 2 1 3 3 Effect of light on rooting

The elongated shoots were kept under two levels of IBA (1 0 and 2 0 mg l ^{1}) under light and under exclusion of light to study the effect light on the rooting The results obtained are given in Table 22

Number of days taken for root initiation

Treatments with IBA 1 0 mg l ^{1} and IBA 2 mg l ^{1} kept in light and the

Table 22 Effect of IBA and lıght on rootıng of elongated shoots of gladıolus

Treatments	IBA (mg l $^{-1}$)	Tıme taken for root ınıtıatıon (days)	Number of roots per culture	Length of roots (cm)	Nature of roots
Darkness	1 0	9 2	19 4	7 20	Normal long and branchıng wıth root haırs
Lıght	1 0	9 2	15 0	3 90	Short and normal
Darkness	2 0	9 1	6 0	2 10	Short thıck wıth root haırs
Lıght	2 0	10 0	5 4	1 50	Thıcker wıthout root haırs
CD (0 05)		NS	1 5	0 86	
SEm+		0 769	1 306	0 411	

treatments IBA 1 0 mg l ^{1} and IBA 2 0 mg l ^{1} kept under darkness took 9 2 10 0 9 2 and 9 2 days respectively for root initiation These did not differ significantly

Number of roots

The number of roots produced ranged from 5 4 to 19 4 and differed significantly among the treatments Maximum number of roots was observed in treatment supplemented with 1 0 mg l ^{1} IBA and kept in exclusion of light

Minimum number of roots (5 4) was recorded in the medium supple mented with 2 0 mg l ^{1} IBA and kept m light The number of roots produced the medium having 2 0 mg l ^{1} IBA and kept under exclusion of light produced a root number of 6 0 and was on par with the treatment produced minimum number of roots

Root number was intermediate (15 0) when the medium was supplement ed with 1 0 mg l ^{1} IBA and kept under light

Length of roots

The length of roots varied significantly ranging from 1 50 cm to 7 20 cm Longest roots (7 20 cm) were observed in medium supplemented with 1 0 mg l ^{1} IBA and kept in exclusion of light The smaller roots were observed in the medium supplemented with 2 0 mg l ^{1} IBA and kept under light

The treatment having 2 0 mg l ^{1} IBA and kept under the exclusion of light produced roots with an average length of 2 10 cm which was on par with the minimum root length (1 50 cm)

In the presence of light the treatment having 1 0 mg l ^{1} IBA produced roots of intermediate length (3 90 cm)

Nature of roots

The roots produced were normal branched and with root hairs in the case of medium supplemented with 1 0 mg l ^{1} IBA and kept under exclusion of light

The treatment with 1 0 mg l ^{1} IBA and kept under light produced short normal and slender roots

The treatment having 2 0 mg l ^{1} IBA kept under exclusion of light produced roots with short and thick nature with root hairs (Plate 21) Under light the same treatment have shown much thicker roots without root hairs (Table 22)

4 2 1 3 4 Effect of activated charcoal

The elongated shoots from Stage 2 were separated and kept for rooting in MS medium containing different levels of activated charcoal to study the effect of charcoal on rooting and the results are presented in Table 23

Number of days taken for root initiation

The number of days taken for rooting ranged from 8 8 to 9 0 under various levels of charcoal and the difference were not significant

Plate 20 *In vitro* rooting of elongated shoots of gladiolus in MS medium supplemented with sucrose 3 0 per cent and NAA 0 5 mg l^{-1} (a) and in MS medium supplemented with sucrose 2 0 per cent and NAA 2 0 mg l^{-1} (b)

Plate 21 Influence of etiolation on *in vitro* rooting of elongated shoots of gladiolus in MS medium containing NAA 2 0 mg l^{-1}

Table 23 Effect of charcoal on rooting of elongated shoots of gladiolus

Activated charcoal (%)	Time taken for root initiation (days)	Number of roots per culture	Length of roots (cm)	Nature of roots
0 1	9 0	8 6	3 96	White slender and long
0 2	8 8	9 2	4 12	
0 3	8 9	10 0	4 30	
0 4	8 9	7 6	3 76	
CD (0 05)	NS	1 7	NS	
SEm±	0 473	1 585	0 542	

Number of roots

The average number of roots ranged from 7 6 to 10 0 and differed signif icantly Maximum number of roots (10 0) were observed in the medium supple mented with 0 3 per cent activated charcoal

Minimum number of roots (7 6) was produced by the medium contained 0 4 per cent activated charcoal

The treatments having 0 1 per cent activated charcoal and 0 2 per cent activated charcoal were on par with the treatment having 0 4 per cent activated charcoal and number of roots produced were 8 6 9 2 and 7 6 respectively

Length of roots

The length of roots in various treatments ranged from 3 76 cm to 4 30 cm and differed significantly The maximum length was observed in treatment having 0 3 per cent activated charcoal and the minimum root length in the medium supplemented with 0 4 per cent activated charcoal The treatments having 0 1 per cent and 0 2 per cent activated charcoal produced 3 96 and 4 12 cm long roots respectively and these were found to be homogeneous with the minimum root length

Nature of roots

The roots produced in all treatments were identical white slender and long (Table 23 and Plate 22)

4 2 1 4 Plantıng out and acclımatısatıon (Stage 4)

The rooted plantlets (Plate 23) were carefully removed from the tubes and morphologıcal observatıons recorded The data gıven ın Table 24

The heıght of the plantlets ranged from 17 5 to 19 5 cm wıth a mean heıght of 18 4 cm

The number of leaves at the tıme of plantıng out ranged from 3 0 to 5 0 wıth a mean value of 3 9

Number of roots ranged from a mınımum of 15 0 to a maxımum of 19 0 roots and the mean number of roots were 17 3 The length of the roots also ranged from 7 0 to 8 5 cm wıth a mean value of 8 0 cm

The plantlets were treated wıth 0 1 per cent solutıon of Bavıstın for five mınutes These plantlets were then planted out and subjected to dıfferent acclımatısa tıon treatments and the results are presented ın Table 25

Observatıons were recorded on the survıval percentage after one week two weeks four weeks sıx weeks eıght weeks ten weeks and twelve weeks

When the plantlets were kept ın open condıtıon all the treatments except the mud pot and coarse sand recorded complete death of the plants wıthın a week Twenty per cent survıval was recorded ın mud pot + coarse sand when gıven a pre plantıng treatment wıth 0 2 per cent Dıthane M 45 and Norfloxacın treatment and post plantıng treatment wıth 1/10 MS solutıon These survıved plantlets faıled to grow further and collapsed wıthın two weeks

Table 24 Growth characters of the rooted plantlets of gladiolus at the time of planting out

Growth characters	Range *
Plant height (cm)	17 5 19 5 (18 4)
Number of leaves	3 0 5 0 (3 9)
Number of roots	15 0 19 0 (17 3)
Length of roots (cm)	7 0 8 50 (8 0)

Observations were taken at the time of transplanting
Values in the paranthesis are the mean of ten observations
*Range shows the minimum and maximum values

Plate 22 *In vitro* rooting of elongated shoots of gladiolus as influenced by activated charcoal

Plate 23 *In vitro* plantlet before planting out

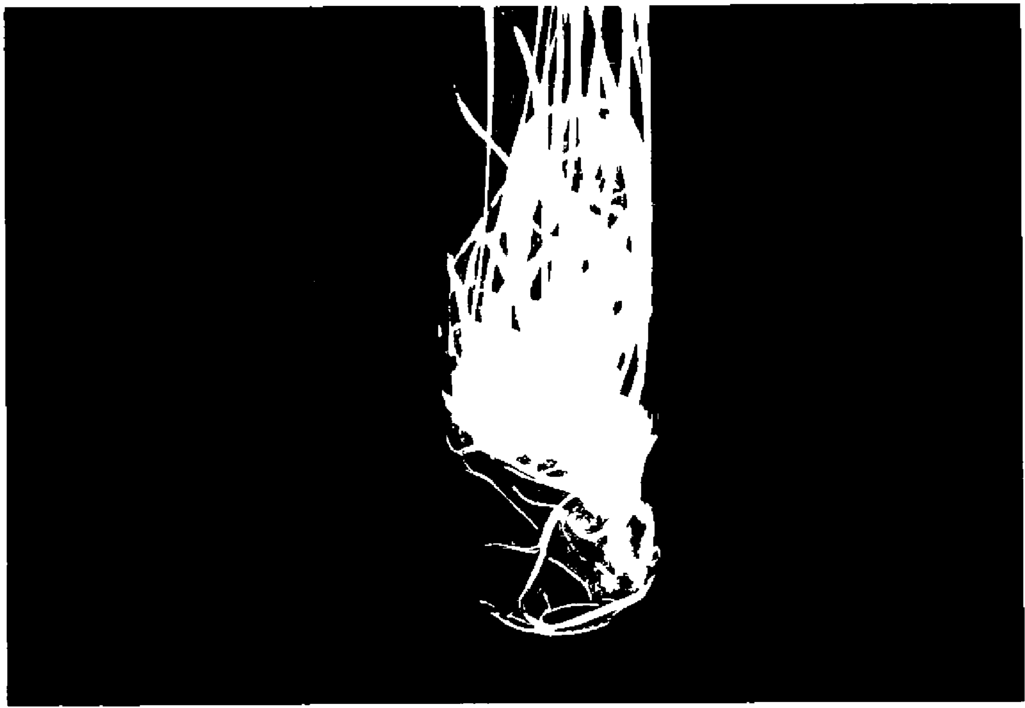


Table 25 Effect of different hardening techniques on post transplanting survival of tissue cultured gladiolus plantlets

Treatmen s		Pre planting treat ents	Pos o anting treatme t	Surv						
				A te 1 ee	fter 2 eeks	fte 4 eeks	f 6 e ks	Af e 8 weeks	te 0 ee s	A te 2 ee
A Open condition										
Conta e	ed a									
ud ot	ine sand	Nil	Drench ng ith / 0 S so ution	0 0						
		Dithane 45 0 2 + Norr oxacin treatment		0 0						
ud pot	Pot ing mi tu e	Nil		0 0						
		Ditha e 45 0 2 + Norf oxacin treatment		0 0						
ud pot	Coco peat	Nil		0 0						
		Dithane 45 0 2 Nor o acin t eatme t		0 0	0 0					
ud po	Coarse sand	Nil		0 0						
		Dithane 45 0 2 + orfloxacin treatment		20 0	0 0					
ud po	Perlite	il		0 0						
		Di hane 45 0 2 + Norfloxacin treatment		0 0						
B Plas c cover										
ud pot	Fine sand	Nil		0 0						
		Dit ane 45 0 2 orfloxacin treatment		0 0						
ud pot	Potting mix ure	Ni		0 0						
		Dithane 45 0 2 + orfloxacin treatme t		10 0	30 0					
ud po	Coco peat	Nil		0 0						
		Ditha e 45 0 2 orfloxacin treatment		0 0						
ud pot	Coarse sand	l		0 0						
		Dithane 45 0 2 + orfloxacin treat ent		20 0	20 0	0 0				
Mud po	Perli e	Nil		0 0						
		Di hane 45 0 2 + Norfloxacin treat ent		0 0						
C st c a ber (i pro sed										
ud po	Fin sand	il		0 0						
		Dit a e 45 0 2 + o loxacin reat e t		10 0	0 0					
uc p	Po ing mixture	i		0 0						
		D ha e 5 0 2 + or lo acin t eatme t		40 0	30 0	0 0				

Treatments		Pre planting treatments	Post o anting treatme t	Survival (%						
				fter 1 eek	er 2 eeks	Afte 4 we ks	er 6 eeks	Afte 8 wee	Af er 10 eeks	er 12 eeks
ud pot	Coco peat	il	Drenching with 1/10 MS solution	0 0						
		Dithane M 45 0 2 + Norfloxacin treatment		0 0						
ud pot	Coarse sand	Nil		0 0						
		Dithane 45 0 2% + orfloxacin treatment		50 0	30 0	20 0				
ud pot	Perlite	Nil		0 0						
		Dithane M 45 0 2% + Norfloxacin treatment		0 0						
D Microscope cover										
Mud pot	Fine sand	Nil		0 0						
		Dithane M 45 0 2% + Norfloxacin treatment		30 0	0 0					
Mud pot	Potting mixture	Nil		0 0						
		Dithane M 45 0 2% + Norfloxacin treatment		10 0	10 0	0 0				
Mud pot	Coco peat	Nil		0 0						
		Dithane M 45 0 2 + Norfloxacin treatment		0 0						
Mud pot	Coarse sand	il		0 0						
		Dithane M 45 0 2% + Norfloxacin treatment		10 0	0 0					
ud pot	Perlite	Nil		0 0						
		Dithane 45 0 2% + Norfloxacin treatment		0 0						
E Mist chamber (Improvised)										
Mud pot	Fine sand	Dithane M 45 0 2% + Norfloxacin treatment	Drenching with 1/10 MS solution and Drenching with Tria dimefon 20 mgl[1] solution at 3 days interval	60 0	50 0	40 0	10 0 corm o- duction 3	10 0 Corm pro duction (5%	The leaves drie o f	
Mud pot	Potting mixture	-do		60 0	40 0	30 0	10 0	0 0		
Mud pot	Coco peat	-do	-do	30 0	0 0					
Mud pot	Coarse sand	-do	-do	80 0	60 0	60 0	60 0	50 0	Corm prod duction (10 0	Corm pro duction (10 0)
Mud pot	Perlite	-do	-do	0 0						
Plastic pot	Fine sand	-do	-do	60 0	60 0	50 0	30 0	10 0	Corm prodn (10 0)	Leaves dried o
Plastic pot	Potting mixture	-do	-do	50 0	50 0	40 0	40 0	0 0		
Plastic po	Coco peat	-do	-do	10 0	0 0					
Plastic pot	Coarse sand	-do	-do	80 0	70 0	70 0	50 0	30 0	30 0 corm p odn 15	20 0
Plastic o	Perlite	-do	-do	0 0						

When the pots were covered with polythene cover to maintain high relative humidity survival percentage of 20 0 was recorded in mud pot + coarse sand treated (with Dithane M 45 2 0 per cent + Norfloxacin treatment even after two weeks Under same condition mud pot + potting mixture treatment showed 10 0 per cent plantlet survival for one week

When plantlets were kept under microscope cover survival percentages of 30 0 10 0 and 10 0 were reported upto one week in the case of treatments mud pot + fine sand mud pot + potting mixture and mud + coarse sand (all treated with Dithan M 45 (2%) + Norfloxacin) respectively

The treatment mud pot + potting mixture had also recorded 10 0 per cent survival of plantlets upto two weeks

The plantlets transplanted in different media (fine sand potting mixture coco peat coarse sand perlite) in mud pots were kept under improvised mist chamber The percentage of survival was 10 0 per cent upto one week in the case of fine sand and 40 0 per cent in potting mixture and 50 0 in coarse sand upto one week

Upto four weeks 10 0 per cent survival was reported in potting mixture and 20 0 in coarse sand

The treatments with different media in mud pot (fine sand potting mixture cocopeat coarse sand perlite) and in plastic pot (fine sand potting mixture cocopeat coarse sand perlite) were kept under improvised mist chamber and post planting treatment with 20 0 mg l ^{1} triadimefon at 3 days interval along with 1/10th MS solution (on alternate days) was given

The maximum survival percentage (50 0%) after 8 weeks was recorded in mud pot + coarse sand followed by plastic pot + coarse sand (30 0%) (Plate 24) Mud pot + fine sand and plastic pot + fine sand had a survival percentage of 10 0 at the end of 8 weeks

Mud pot + potting mixture and plastic pot + potting mixture recorded 10 0 and 40 0 per cent survival respectively upto 6 weeks

Mud pot + coco peat recorded 30 % plantlet survival upto one week and plastic pot + cocopeat recorded 10 00 per cent survival upto one week

Corm production was observed in 3 0 per cent plantlets after six weeks in the case of mud pot + find sand and 5 0 per cent plantlets after eight weeks (Plate 25)

Plantlet survival upto one week was 80 0 per cent in the case of mud pot + coarse sand and plastic pot + coarse sand

Survival percentage of 60 00 per cent was recorded in the treatments like mud pot + fine sand mud pot + potting mixture and plastic pots + fine sand upto one week

4 2 2 Somatic organogenesis

4 2 2 1 Explant choice

The results of the trial conducted to find out the response of various explants of gladiolus to somatic organogenesis are given in the Table 26

Plate 24 Performance of micropropagated plantlet

Plate 25 Corm production in planted out plantlet after six weeks

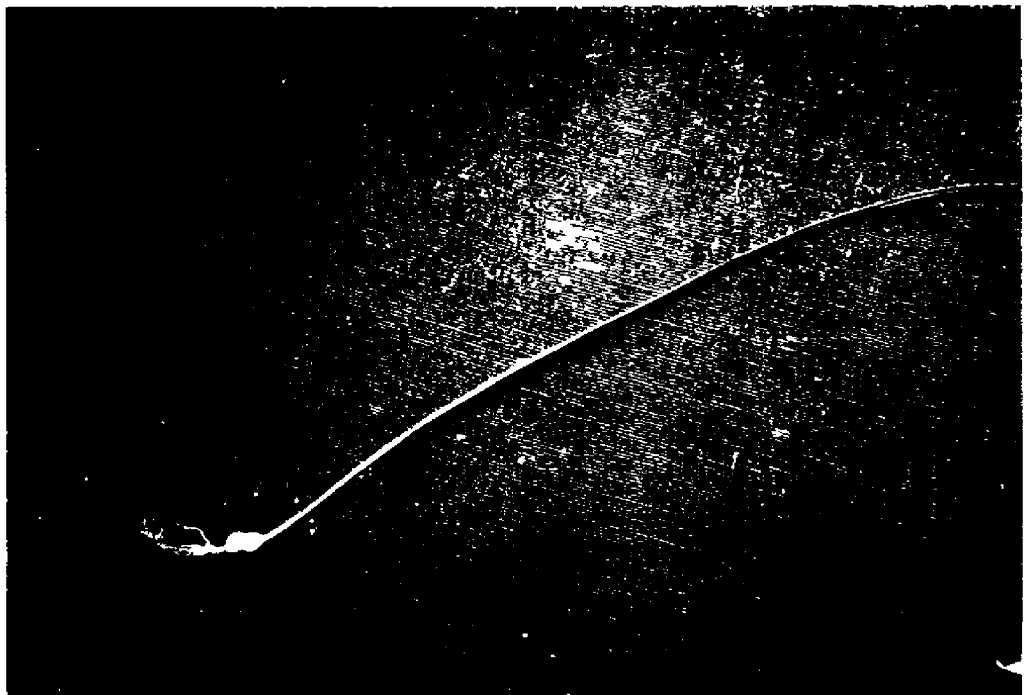


Table 26 Response of various explants of gladiolus on callus initiation
Basal medium MS

Treatments		Percentage of culture produced callus*					
NAA (mg l [1])	BAP	Infloresence** explants	Flower** bud explants	Flower** bud bracts	*In vitro* roots	*In vivo* roots	Corm inter nodal pieces and leaf explants
1	1				10 0	0 0	
2 0	1 0				30 0	25 0	
5 0	0 0	80 0	0 0				
5 0	0 5	90 0	100 0				
5	1 0	100 0	100 0				
5	2 0	80 0	80 0				
10	0 0	100 0	0 0	0 0			
10	0 5	100 0	80 0	60 0			
10	1 0	90 0	80 0	80 0			
10	2 0	100 0	0 0	60 0			
15 0	0 0	100 0		10 0			
15	0 5	100 0		80 0			
15	1 0	100 0		60 0			
15	2 0	100 0		70 0			
20	0 0	80 0	0 00	10 0			
20	0 5	80 0	100 0	80 0			
20	1 0	10 0	100 0	80 0			
20	2 0	40 0	80 0	80 0			

* Observations were taken from ten cultures
** Basal medium used was modified MS

The inflorescence axis explants collected before the emergence were found to be responding in 100 per cent of the cultures in many treatments like modified MS medium supplemented with NAA 5 0 + BAP 1 0 mg l^{1} NAA 10 mg l^{1} NAA 10 mg l^{1} + BAP 0 5 mg l^{1} NAA 10 + 2 0 mg l^{1} NAA 15 0 mg l^{1} NAA 15 + BAP 0 5 mg l^{1} NAA 15 + BAP 1 0 mg l^{1} NAA 15 + BAP 2 0 mg l^{1}

Flower bud explants (100 0% cultures) initiated callus in treatments containing 5 0 mg l^{1} NAA + 0 5 mg l^{1} BAP 5 0 mg l^{1} NAA + 1 0 mg l^{1} BAP 20 0 mg l^{1} NAA + 0 5 mg l^{1} BAP and 20 0 mg l^{1} NAA + 1 0 mg l^{1} BAP

The response of bracts of the flower buds was 80 0 per cent in treatments containing NAA 10 0 mg l + BAP 1 0 mg l^{1} NAA 15 0 mg l^{1} + BAP 0 5 mg l^{1} NAA 20 0 mg l^{1} + BAP 0 5 mg l^{1} NAA 20 0 mg l^{1} + BAP 1 0 mg l^{1} and NAA 20 0 mg l^{1} + BAP 2 0 mg l^{1}

The *in vitro* produced root pieces when inoculated on to MS medium supplemented with BAP 1 0 mg l^{1} + NAA 2 mg l^{1} 30 0 per cent of cultures produced callus *In vivo* roots when inoculated to the same media induced callus on 25 0 per cent cultures only

4 2 2 2 Direct organogenesis

Among the different explants tried for direct organogenesis only the inflorescence axis explants taken before the emergence of the inflorescence was found to be responding

4 2 2 2 1 Effect of NAA and BAP

Data pertaining to the effect of NAA and BAP on the direct organogene sis on the inflorescence axis explants are presented in Table 27 and Plate 26

Maximum morphogenesis (50 0%) was observed in the medium supple mented with NAA 15 0 mg l ^{1} + BAP 3 0 mg l ^{1} followed by the treatments NAA 10 0 mg l ^{1} + BAP 0 5 mg l ^{1} NAA 10 0 mg l ^{1} + BAP 3 0 mg l ^{1} NAA 15 0 mg l ^{1} + BAP 0 5 mg l ^{1} and NAA 15 0 mg l ^{1} + BAP 2 0 mg l ^{1} as they produced morphogenesis in 30 0 per cent of the cultures Morphogenesis could be observed upto an extent of 20 0 per cent cultures in media supplemented with NAA 15 0 mg l ^{1} + BAP 1 0 mg l ^{1} and NAA 20 0 mg l ^{1} + BAP 3 0 mg l ^{1} Mini mum rate of morphogenesis (10 0%) was observed in treatments like NAA 10 0 mg l ^{1} NAA 10 0 mg l ^{1} + BAP 2 0 mg l ^{1} NAA 20 0 mg l ^{1} + BAP 0 5 mg l ^{1} and NAA 20 0 mg l ^{1} + BAP 2 0 mg l ^{1} The rest of the treatments failed to induce morphogenesis

Rhizogenesis was the maximum (80 0% cultures) in the treatments having NAA (15 0 mg l ^{1} and NAA 20 0 mg l ^{1}) About 60 0 per cent cultures produced direct rhizogenesis in the medium supplemented with NAA 10 0 mg l ^{1} (Plate 27)

Treatments like NAA 10 0 mg l ^{1} + BAP 0 5 mg l ^{1} NAA 20 0 mg l ^{1} + BAP 0 5 mg l ^{1} and NAA 15 0 mg l ^{1} + BAP 2 0 mg l ^{1} showed rhizogenesis in 30 0 per cent 20 0 per cent and 10 00 per cent cultures respectively

Table 27 Effect of dıfferent levels of NAA and BAP on dırect organogenesıs of the ınflorescence axıs segments of gladıolus

Basal medıum Modıfıed MS
Culture perıod 5 weeks

Treatments NAA (mg l⁻¹)	BAP (mg l⁻¹)	Tıme taken to respond (days)	Cultures showıng morphogenesıs (%)	Cultures showıng rhızogenesıs (%)
5 0	0 0	0 0	0 0	0 0
5 0	0 5	0 0	0 0	0 0
5 0	1 0	0 0	0 0	0 0
5 0	2 0	0 0	0 0	0 0
5 0	3 0	0 0	0 0	0 0
5 0	4 0	0 0	0 0	0 0
10 0	0 0	16 4	10 0	60 0
10 0	0 5	12 6	30 0	30 0
10 0	1 0	18 4	10 0	0 0
10 0	2 0	17 8	0 0	0 0
10 0	3 0	17 8	30 0	0 0
15 0	0 0	11 6	0 0	80 0
15 0	0 5	11 4	30 0	0 0
15 0	1 0	10 2	20 0	0 0
15 0	2 0	11 6	30 0	10 0
15 0	3 0	12 0	50 0	0 0
20 0	0 0	15 2	0 0	80 0
20 0	0 5	11 2	10 0	20 0
20 0	1 0	27 0	0 0	0 0
20 0	2 0	16 6	10 0	0 0
20 0	3 0	15 8	20 0	0 0

Observatıons were taken from ten cultures

Plate 26 Production of shoot buds from inflorescence axis segments of gladiolus (Direct organogenesis) as influenced by NAA 15 0 mg l ^{1} and BAP 3 0 mg l ^{1} in modified MS medium

Plate 27 Rhizogenesis of infloresence axis segments in the modified MS medium supplemented with NAA 15 0 mg l ^{1} (a) and complete morphogenesis of inflorescence axis segments in modified MS medium supplemented with NAA 15 0 mg l ^{1} and BAP 3 0 mg l ^{1} (b)

Tıme taken to respond ranged from 10 2 days (NAA 20 0 mg l ^{1} to 18 4 days (NAA 10 0 mg l ^{1})

4 2 2 2 2 Effect of NAA and kınetın

Date pertaınıng to the results of the trıal conducted on the dırect organo genesıs ın the medıa supplemented wıth dıfferent combınatıons of NAA and kınetın are presented ın Table 28

The medıum supplemented wıth NAA 10 0 mg l ^{1} + kınetın 3 0 mg l ^{1} NAA 15 0 mg l ^{1} + kınetın 0 5 mg l ^{1} could ınduce morphogenesıs ın about 20 0 per cent of the cultures (Plate 28) The medıum supplemented wıth NAA 5 0 mg l ^{1} + BAP 3 0 mg l ^{1} could ınduce morphogenesıs only ın 15 0 per cent of the cul tures About 10 0 per cent of the cultures showed morphogenesıs when the medıum was supplemented only wıth NAA 10 0 mg l ^{1} and BAP 0 5 mg l ^{1}

Rhızogenesıs was maxımum (upto 80 0 per cent of the cultures) when the medıum was supplemented wıth NAA alone at 15 0 mg l ^{1} 20 0 mg l ^{1} and 60 0 per cent of the cultures could ınduce rhızogenesıs ın medıum contaınıng 10 0 mg l ^{1} NAA

When the medıum was supplemented wıth 0 5 mg l ^{1} kınetın along wıth 10 0 mg l ^{1} NAA 40 0 per cent of the cultures showed rhızogenesıs The treatment contaınıng 15 0 mg l ^{1} NAA + 0 5 mg l ^{1} kınetın could ınduce rhızogenesıs on 20 0 per cent cultures Lower rates of rhızogenesıs (10 0%) could be observed ın treatments lıke NAA 20 0 mg l ^{1} + kınetın 2 0 mg l ^{1} NAA 20 0 mg l ^{1} + kınetın 1 0 mg l ^{1}

Table 28 Effect of different levels of NAA and kinetin on direct organogenesis of infloresence axis segments of gladiolus

Basal medium Modified MS
Culture period 5 weeks

Treatments NAA (mg l 1)	Kinetin	Time taken to respond (days)	Cultures showing morphogenesis (%)	Cultures showing rhizogenesis (%)
5 0	0 0	0 0	0 0	0 0
5 0	0 5	0 0	0 0	0 0
5 0	1 0	0 0	0 0	0 0
5 0	2 0	0 0	0 0	0 0
5 0	3 0	19 3	15 0	0 0
10 0	3 0	16 4	10 0	60 0
10 0	0 5	20 0	0 0	40 0
10 0	1 0	0 0	0 0	0 0
10 0	2 0	0 0	0 0	0 0
10 0	3 0	15 8	20 0	0 0
15 0	0 0	11 4	0 0	80 0
15 0	0 5	16 2	20 0	20 0
15 0	1 0	0 0	0 0	0 0
15 0	2 0	0 0	0 0	0 0
15 0	3 0	0 0	0 0	0 0
20 0	0 0	15 2	0 0	80 0
20 0	0 5	13 5	0 0	10 0
20 0	1 0	18 0	0 0	10 0
20 0	2 0	0 0	0 0	0 0
20 0	3 0	0 0	0 0	0 0

Observations were taken from ten cultures

Time taken for response varied from 11 4 (NAA 1 50 mg l^{1}) to 20 0 days (NAA 10 0 mg l^{1} + BAP 0 5 mg l^{1})

4 2 2 3 Callus mediated organogenesis

4 2 2 3 1 Callus initiation

The explants like corm internodal pieces leaves and inflorescence axis segments inoculated to the medium containing various levels of 2 4 D and 2 4-5 T failed to induce callus The different explants like inflorescence axis segments leaf pieces flower buds and bracts inoculated to modified MS medium contained different cytokinins and auxins at various levels The results are presented in explant wise as follows

4 2 2 3 1 1 Inflorescence segments

4 2 2 3 1 1 1 Effect of NAA and BAP under 16 h photoperiod

Data relating to the influence of NAA and BAP on callus induction and growth on inflorescence segments of gladiolus in 16 h photoperiod are presented in Table 29 and Plate 29)

Days taken for callus induction

The average time taken for callus production ranged from 10 2 days 27 0 days Minimum days for callus initiation was taken by the medium supplemented with NAA 15 0 mg l^{1} + BAP 1 0 mg l^{1} Maximum days for callus induction (27 0) was taken by the medium supplemented with NAA 20 0 mg l^{1} + BAP 1 0 mg l^{1}

Table 29 Influence of NAA and BAP on callus induction and growth on inflorescence axis segments of gladiolus under 16 h photoperiod

Basal medium Modified MS
Culture period 6 weeks

Treatments NAA (mg l⁻¹)	BAP	Time taken for callusing (days)	Callusing (%) (P)	Growth score (G)	Callus index (CI)	Nature of callus
5 0	0 0	15 0	80 0	2	160	Friable
5 0	0 5	15 2	90 2	2	180	
5 0	1 0	17 2	100 0	2	200	Watery
5 0	2 0	17 0	80 0	1	160	Crinkled
10 0	0 0	16 4	100 0	2	200	Friable
10 0	0 5	12 6	100 0	3	300	Watery & yellowish
10 0	1 0	18 4	90 0	2	180	
10 0	2 0	17 8	100 0	2	200	Greenish
15 0	0 0	11 6	100 0	4	400	Friable
15 0	0 5	11 4	100 0	3	300	
15 0	1 0	10 2	100 0	2	200	Watery
15 0	2 0	11 6	100 0	3	300	Watery greenish yellow
20 0	0 0	15 2	80 0	2	160	Friable
20 0	0 5	11 2	80 0	1	80	
20 0	1 0	27 0	10 0	1	10	
20 0	2 0	16 0	40 0	1	40	

Observations were taken from ten cultures

Plate 28 Direct organogenesis on inflorescence segments in modified MS medium supplemented with NAA 15 0 mg l^{-1} and kinetin 5 0 mg l^{-1}

Plate 29 Callus production on inflorescence segments of gladiolus in modified MS medium supplemented with NAA 10 0 mg l^{-1} and BAP 0 5 mg l^{-1}

Callusing percentage

Maximum cultures (100 0%) callused in the treatments with NAA 5 0 mg l ^{1} + BAP 1 0 mg l ^{1} NAA 10 0 mg l ^{1} NAA 10 0 mg l ^{1} + BAP 0 5 mg l ^{1} NAA 10 0 mg l ^{1} + BAP 2 0 mg l ^{1} NAA 15 0 mg l ^{1} NAA 15 0 mg l ^{1} + BAP 2 0 mg l ^{1} followed by the treatment having NAA 5 0 mg l ^{1} + BAP 0 5 mg l ^{1} (90 0%) NAA 10 0 mg l ^{1} + BAP 1 0 mg l ^{1} (90 0%) NAA 5 0 mg l ^{1} NAA 5 0 mg l ^{1} + BAP 2 0 mg l ^{1} (80 0%) NAA 20 0 mg l ^{1} + BAP 0 5 mg l ^{1} (80 0%) NAA20 0 mg l ^{1} (80 0%)

Minimum callusing (10 0%) was observed in the medium supplemented with 20 0 mg l ^{1} NAA + BAP 1 0 mg l ^{1}

Callus index

Callus index was maximum (400) when the medium was supplemented with NAA 15 0 mg l ^{1} (Plate 30) followed by the callus index of 300 each in the treatments NAA15 0 mg l ^{1} + BAP 0 5 mg l ^{1} NAA 15 0 mg l ^{1} + BAP 0 5 mg l ^{1} and NAA 15 0 mg l ^{1} + BAP 2 0 mg l ^{1}

Callus index of 200 was recorded in treatments with NAA 15 0 mg l ^{1} + BAP 1 0 mg l ^{1} NAA 10 0 mg l ^{1} + BAP 2 0 mg l ^{1} and NAA 15 0 mg l ^{1} + BAP 1 0 mg l ^{1}

Minimum callus index (10 0) was noticed in the medium containing NAA 20 0 mg l ^{1} and BAP 1 0 mg l ^{1}

Effect of NAA and kinetin under 16 h photoperiod

Influence of NAA and kinetin on callus induction and growth on inflor esence segments of gladiolus under 16 h photoperiod are given in the Table 30 and Plate 31

Days taken for callus induction

The average days taken for callus induction ranged from 10 5 days to 21 0 days

The minimum days was taken when the medium was supplemented with 20 0 mg l ^{1} NAA and kinetin 2 0 mg l ^{1} Maximum days for callus induction was taken by the treatment supplemented with NAA 15 0 mg l ^{1} + kinetin 2 0 mg l ^{1}

Callusing percenting

Callusing percentage ranged from 10 0 to 50 0 Maximum callusing (50 0%) was obtained when the medium was supplemented with NAA 10 0 mg l ^{1} followed by the treatments like NAA 10 0 mg l ^{1} + kinetin 0 5 mg l ^{1} (40 0) NAA 10 0 mg l ^{1} + kinetin 1 0 mg l ^{1} (40 0) and NAA 15 0 mg l ^{1} + 0 5 mg l ^{1} (40 0)

About 10 0 percentage of cultures callused when the explants were inoculated into the media containing NAA 5 0 mg l ^{1} NAA 5 0 mg l ^{1} + kinetin 0 5 mg l ^{1} NAA 5 0 mg l ^{1} + kinetin 1 0 mg l ^{1} NAA 5 0 mg l ^{1} + kinetin 2 0 mg l ^{1} NAA 15 0 mg l ^{1} + kinetin 1 0 mg l ^{1} NAA 15 0 mg l ^{1} + kinetin 2 0 mg l ^{1} NAA 20 0 mg l ^{1} NAA 20 0 mg l ^{1} + kinetin 0 5 mg l ^{1} NAA 20 0 mg l ^{1} + kinetin 1 0 mg l ^{1} and NAA 20 0 mg l ^{1} + kinetin 2 0 mg l ^{1}

Table 30 Influence of NAA and kınetın on callus ınductıon and growth on ınflorescence axıs segments of gladıolus under 16 h photoperıod

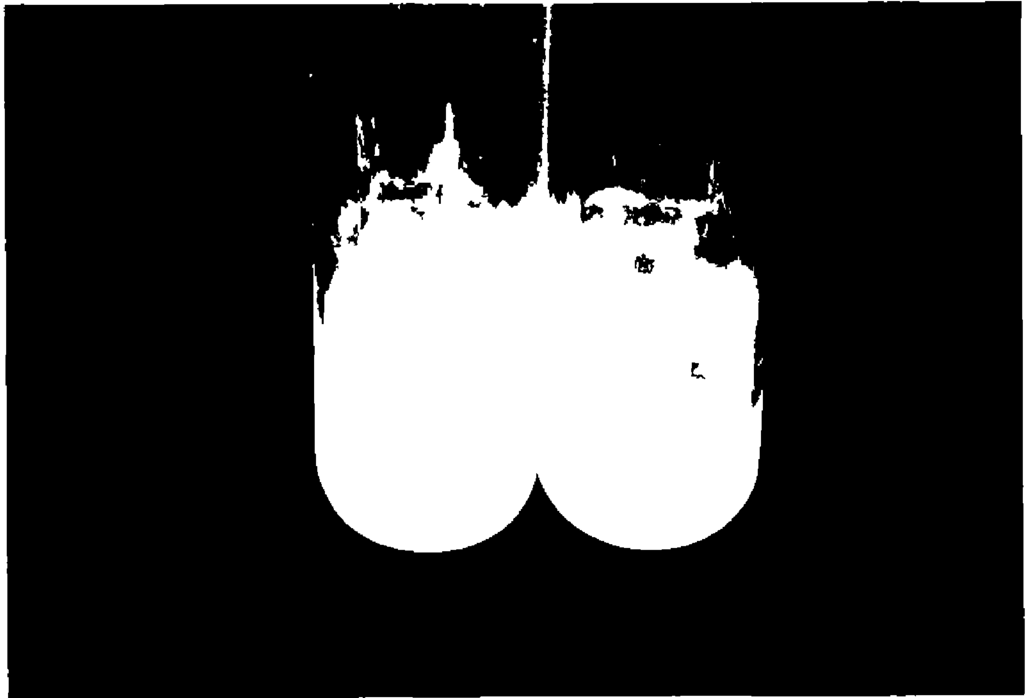
Basal medıum Modıfied MS
Culture perıod 6 weeks

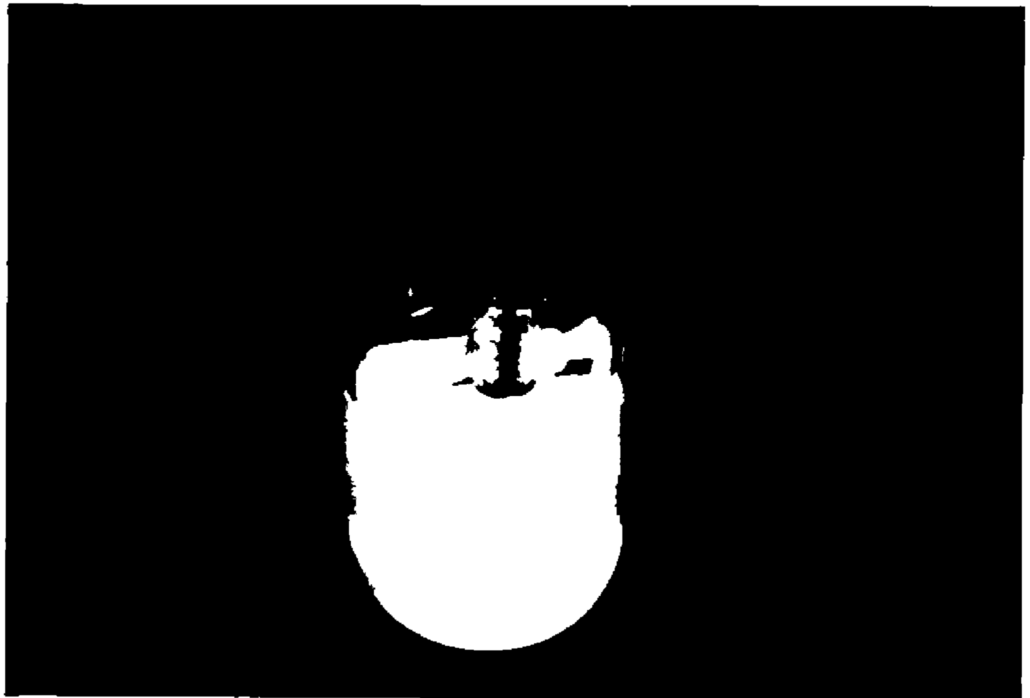
Treatments NAA (mg l^-1)	Kınetın	Tıme taken for callusıng (days)	Callusıng (%) (P)	Growth score (G)	Callus ındex (CI)	Nature of callus
5 0	0 0	17 8	10 0	1	10	Frıable
5 0	0 5	18 4	10 0	1	10	
5 0	1 0	19 0	10 0	1	10	
5 0	2 0	20 0	10 0	1	10	
10 0	0 0	20 0	50 0	2	100	Watery & whıtısh
10 0	0 5	20 0	40 0	2	80	
10 0	1 0	16 4	40 0	2	80	
10 0	2 0	16 6	20 0	2	40	
15 0	0 0	16 2	30 0	1	30	Frıable & yellowısh
15 0	0 5	16 0	40 0	1	40	
15 0	1 0	20 0	10 0	1	10	
15 0	2 0	21 0	10 0	1	10	
20 0	0 0	15 0	10 0	1	10	
20 0	0 5	13 5	10 0	1	10	
20 0	1 0	18 0	10 0	1	10	
20 0	2 0	10 5	10 0	1	10	

Observatıons were taken from ten cultures

Plate 30 Callus growth on inflorescence segments of gladiolus in the modified MS medium supplemented with NAA 15 0 mg l^{-1}

Plate 31 Callus production on inflorescence segments of gladiolus in modified MS medium supplemented with NAA 10 0 mg l^{-1} and kinetin 1 0 mg l^{-1}





Callus index

Maximum callus index (100) was recorded m the medium supplemented with NAA 10 0 mg l^{1} followed by NAA 10 0 mg l^{1} + kinetin 0 5 mg l^{1} (80) NAA 10 0 mg l^{1} + kinetin 1 0 mg l^{1} NAA 15 0 mg l^{1} + kinetin 0 5 mg l^{1} (40) NAA 10 0 mg l^{1} + kinetin 2 0 mg l^{1} (40) and NAA 15 0 mg l^{1} (30)

Callus index was minimum (10) m all the other treatments

4 2 2 3 1 1 3 Effect of BAP and NAA under dark condition

Data on the influence of BAP and NAA at different levels on the callus induction and growth on infloresence segments incubated in dark condition are given in the Table 31

Days taken for callus induction

The days taken for callus induction ranged from 9 8 to 23 0 Minimum days for callus induction was taken by the treatment having 5 0 mg l^{1} NAA and devoid of BAP Maximum days for callus induction was taken by the medium supplemented with NAA 20 0 mg l^{1} and BAP 2 0 mg l^{1}

Callusing percentage

The percentage of cultures callused in various treatments ranged from 10 0 to 100 0 per cent

Maximum callusing ures (100 0%) was obtained in the modified MS medium supplemented with NAA 5 0 mg l^{1} + BAP 1 0 mg l^{1} NAA 5 0 mg l^{1}

Table 31 Influence of NAA and BAP on callus induction and growth on inflorescence axis segments of gladiolus under exclusion of light

Basal medium: Modified MS
Culture period: 6 weeks

Treatments NAA (mg l^{-1})	Treatments BAP (mg l^{-1})	Time taken for callusing (days)	Callusing (%) (P)	Growth score (G)	Callus index (CI)	Nature of callus
5 0	0 0	9 8	10 0	1	10	Watery
5 0	0 5	13 8	90 0	2	180	Whitish & watery
5 0	1 0	13 0	100 0	1	100	
5 0	2 0	13 0	100 0	1	100	Yellowish
10 0	0 0	14 5	90 0	2	180	Yellowish & watering
10 0	0 5	14 6	100 0	1	100	Greenish & friable
10 0	1 0	13 3	90 0	2	180	
10 0	2 0	14 0	100 0	2	200	
15 0	0 0	12 0	100 0	3	300	Friable calli with roots and root hairs
15 0	0 5	12 0	100 0	3	300	Watery & yellowish
15 0	1 0	12 2	100 0	3	300	
15 0	2 0	11 8	100 0	4	400	Friable prolific & greenish yellow
20 0	0 0	12 6	80 0	3	240	Friable with roots
20 0	0 5	12 2	50 0	2	100	Watery & yellowish
20 0	1 0	11 8	40 0	2	80	Whitish & watery
20 0	2 0	23 0	10 0	1	10	Whitish & watery

Observations were taken from ten cultures

+ NAA 2 0 mg l^{1} NAA 10 0 mg l^{1} + BAP 0 5 mg l^{1} NAA 10 0 mg l^{1} + BAP 2 0 mg l^{1} NAA 15 0 mg l^{1} NAA 15 0 mg l^{1} + BAP 0 5 mg l^{1} NAA 15 0 mg l^{1} + BAP 1 0 mg l^{1} and NAA 15 0 mg l^{1} + BAP 2 0 mg l^{1}

Percentage of cultures callused were the lowest (10 0%) in the medium supplemented with NAA 5 0 mg l^{1} and NAA 20 0 mg l^{1} + NAA 2 0 mg l^{1}

Callus mdex

Callus index ranged from a minimum of 10 to 400

The highest callus index (400) was obtained in modified MS medium supplemented with NAA 15 0 mg l^{1} + BAP 2 0 mg l^{1} followed by treatments having 15 0 mg l^{1} NAA NAA 15 0 mg l^{1} + BAP 0 5 mg l^{1} NAA 15 0 mg l^{1} + BAP 1 0 mg l^{1} as these treatments have shown a callus index rating of 300

Callus mdex was minimum in the modified MS medium supplemented with NAA 5 0 mg l^{1} (10) and NAA 20 0 mg l^{1} + BAP 2 0 mg l^{1} (10)

4 2 2 3 1 1 4 Effect of NAA and kinetin under dark condition

Data on the influence of NAA and kinetin on the callus induction and growth under complete exclusion of light are presented in Table 32

Number of days taken for callus induction

The days taken for callus induction ranged from a minimum of 9 8 to 26 5 The minimum days for callus induction was taken by the modified MS medium supplemented with NAA 5 0 mg l^{1} Maximum days for callus induction was taken by the modified MS medium supplemented with NAA 5 0 mg l^{1} + kinetin 0 5 mg l^{1}

Table 32 Influence of NAA and kinetin on callus induction and growth on inflorescence axis segments of gladiolus under exclusion of light

Basal medium Modified MS
Culture period 6 weeks

Treatments NAA (mg l⁻¹)	kinetin	Time taken for callusing (days)	Callusing (%) (P)	Growth score (G)	Callus index (CI)	Nature of callus
5 0	0 0	9 8	10 0	1	10	Watery
5 0	0 5	26 5	20 0	1	20	Watery & whitish
5 0	1 0	24 0	40 0	1	40	Friable & whitish coloured
5 0	2 0	14 0	40 0	1	40	Friable & whitish
10 0	0 0	14 0	90 0	1	90	Yellowish and watery
10 0	0 5	14 6	60 0	1	60	Whitish & friable
10 0	1 0	12 2	60 0	2	120	Swollen & whitish
10 0	2 0	12 0	60 0	2	160	Friable & whitish
15 0	0 0	12 0	100 0	3	300	Friable with root sand root hairs
15 0	0 5	18 0	10 0	1	10	Watery & crinkled
15 0	1 0	18 0	10 0	1	10	Translucent
15 0	2 0	19 5	10 0	1	10	Watery & whitish
20 0	0 0	12 6	80 0	3	240	Friable with roots
20 0	0 5	20 0	40 0	1	40	Whitish & watery with thick roots
20 0	1 0	20 0	40 0	1	40	Swollen
20 0	2 0	16 3	20 0	1	20	

Observations were taken from ten cultures

Callusing percentage

The percentage of cultures producing callus ranged from 10 0 to 100 0 Maximum callus production was observed in the treatment having NAA 15 0 mg l ^{1} The lowest rate of callus production percentage was observed in the treat ments having NAA 15 0 mg l ^{1} + kinetin 0 5 mg l ^{1} NAA 15 0 mg l ^{1} + kinetin 1 0 mg l ^{1} and NAA 15 0 mg l ^{1} + NAA 2 0 mg l ^{1}

Callus index

Callus index ranged from 10 to 300 Callus index was maximum m the treatment having NAA 15 0 mg l ^{1} and devoid of kinetin Minimum callus index was recorded in the treatments havmg NAA 15 0 mg l ^{1} NAA 5 0 mg l ^{1} + kinetin 0 5 mg l ^{1} NAA 15 0 mg l ^{1} + kinetin 1 0 mg l ^{1} and NAA 15 0 mg l ^{1} + kinetin 2 0 mg l ^{1}

4 2 2 3 1 1 5 Callus differentiation

The callus derived from the infloresence segments were inoculated in MS medium supplemented with lower levels of cytokinins and combination of cytokinin and auxins

4 2 2 3 1 1 5 1 Effect of cytokinins

Data pertaining to the effect of lower levels of different cytokinins on the differentiation of the callus are shown m the Table 33 Plate 32 and 33

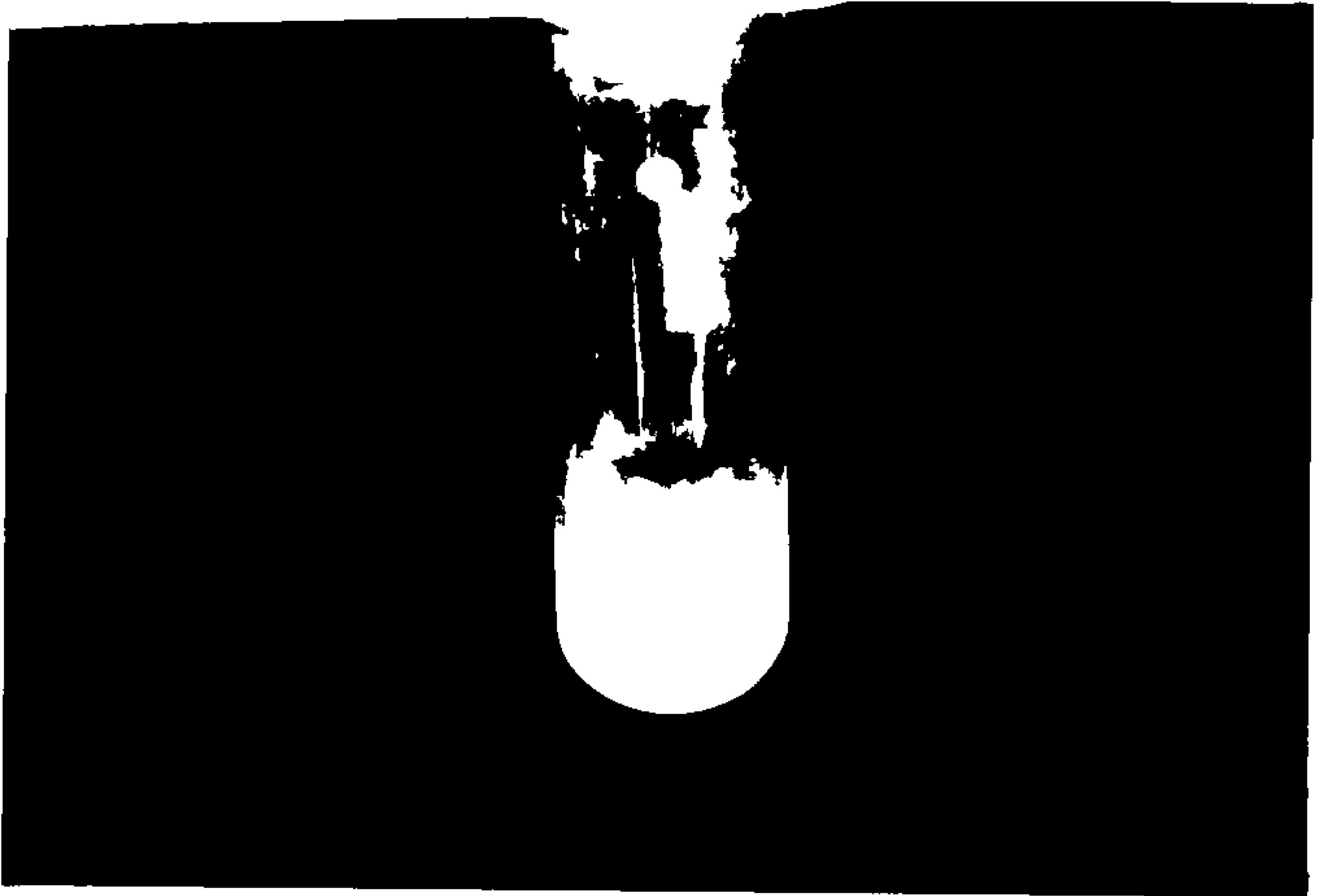
Table 33 Effect of different levels of cytokinins on the differentiation of inflorescence derived callus

Basal medium MS
Culture period 4 weeks

Cytokinins (mg l^{-1})		Time taken for differentiation (days)	Number of shoots	Number of roots
Kinetin	0 25	20 0	4 0	6 8
	0 50	20 4	3 4	7 0
	1 0	19 8	2 6	7 2
2ip	0 25	20 0	3 2	8 8
	0 50	18 2	4 8	9 8
	1 00	22 0	1 6	9 8
BAP	0 25	18 8	10 0	8 8
	0 50	19 3	8 8	6 0
	1 00	19 2	4 2	3 0
	2 00	18 6	12 0	1 8
	3 00	17 6	13 6	1 4
CD (0 05)		1 7	2 0	1 2
SEm±		1 87	2 558	0 93

Plate 32 Regeneration of shoot buds from callus derived from inflorescence segments of gladiolus in MS medium supplemented with BAP 3 0 mg l^{-1}

Plate 33 Regeneration of shoot buds from the callus derived from inflorescence segments of gladiolus in MS medium supplemented with kinetin 0 5 mg l^{-1}



Days taken for differentiation

The average number of days taken for the differentiation of the callus ranged from 17 6 to 22 0 and were significantly different

MS medium supplemented with 3 0 mg l ^{1} BAP has taken the minimum days (17 6 days) for differentiation and this was on par with the treatments having 2ip 0 5 mg l ^{1} (18 2) BAP 2 0 mg l ^{1} (18 6) BAP 0 25 mg l ^{1} (18 8) BAP 1 0 mg l ^{1} (19 2) and BAP 0 5 mg l ^{1} (19 3)

Maximum days for differentiation of callus was taken by the medium supplemented with 2ip 1 0 mg l ^{1} (22 0) and with kinetin 1 0 mg l ^{1} (20 4)

Number of shoots

Signifcant variation could be observed among the treatments with respect to the number of shoots produced

The average number of shoot buds ranged from 1 6 to 13 6 Maximum shoot number was observed when the MS medium was supplemented with BAP 3 0 mg l ^{1} (13 6) and it was found to be homogeneous with the number of shoots (12 0) in the medium supplemented with 2 0 mg l ^{1} BAP

The number of shoots produced was minimum (1 6) in the medium having 1 0 mg l ^{1} 2ip and was on par with the media having kinetin 1 0 mg l ^{1} (2 6) 2ip 0 25 (3 2) and kinetin 0 5 mg l ^{1} (3 4)

Number of roots

The roots produced ın varıous treatments ranged from 1 4 to 9 8 and were sıgnıficantly dıfferent The maxımum number of roots were produced by the medıum supplemented by 2ıp 1 0 mg l ^{1} and thıs dıd not dıffer sıgnıficantly from the treatments havıng 2ıp 0 5 mg l ^{1} (9 8) BAP 0 25 mg l ^{1} (8 8) and 2ıp 0 25 mg l ^{1} (8 8)

The roots produced were the lowest m number when the medıum was supplemented wıth 3 0 mg l ^{1} BAP Thıs was on par wıth the root numbers pro duced by the medıum havıng 2 0 mg l ^{1} BAP (1 8)

4 2 2 3 1 1 5 2 Effect of cytokınıns and auxın

Trıals were conducted wıth lower levels of cytokımns (BAP and kınetın) m combınatıon wıth lower levels of NAA The results are presented m Table 34 and Plate 34

Number of days taken for dıfferentıatıon

The average number of days taken for dıfferentıatıon of callus ranged from 17 4 to 20 6 and dıffered sıgnıficantly

The mınımum days for callus dıfferentıatıon has been taken by the medıum supplemented wıth BAP 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (17 4) whıch dıd not vary sıgnıficantly from the treatments kınetın 0 5 mg l ^{1} + NAA 0 25 mg l ^{1} (18 0 days) kınetın 1 0 mg l ^{1} + NAA 0 5 mg l ^{1} (19 4 days) The medıum havıng BAP 0 50 mg l ^{1} + NAA 0 25 mg l ^{1} had taken maxımum days (20 6) for dıfferen cıatıon

Table 34 Effect of cytokinins at lower levels in combination with NAA on the differentiation of inflorescence derived callus

Basal medium MS
Culture period 4 weeks

Treatments (mg l $^{-1}$)	Time taken for differentiation (days)	Number of shoots	Number of roots
BAP 0 50 + NAA 0 25	20 6	14 6	2 4
BAP 1 00 + NAA 0 50	17 4	13 4	6 0
Kinetin 0 50 + NAA 0 25	18 0	5 0	6 6
Kinetin 1 00 + NAA 0 50	19 4	5 8	8 0
CD (0 05)	2 2	2 7	1 6
SEm±	2 60	4 05	1 40

Number of shoots

Significant variation was observed among the treatments with respect to the number of shoots and the average number of shoots produced ranged from 5 0 to 14 6 in different treatments Maximum number of shoots was observed in treatment having BAP 0 5 mg l^{1} + NAA 0 25 mg l^{1} and found to be homogeneous with the treatments having BAP 1 0 mg l^{1} + NAA 0 5 mg l^{1} (13 4) Minimum number of shoots was observed in the medium supplemented with kinetin 0 5 mg l^{1} + NAA 0 25 mg l^{1} (5 0) and also in the medium supplemented with kinetin 1 0 mg l^{1} + NAA 0 5 mg l^{1} (5 8) and they did not differ significantly

Number of roots

The treatments showed significant differences with respect to the number of roots and ranged from 2 4 to 8 0 The number of roots produced was found to be maximum (8 0) when the medium was supplemented with kinetin 1 0 mg l^{1} + NAA 0 50 mg l^{1} and was on par with the number of roots in the medium supplemented with kinetin 0 5 mg l^{1} + NAA 0 25 mg l^{1} (6 6)

Minimum number of roots (2 4) was observed in the medium having BAP 0 50 mg l^{1} + NAA 0 25 mg l^{1} and it differed significantly from all the other treatments

4 2 2 3 1 2 Flower buds

4 2 2 3 1 2 1 Effect of NAA and BAP

Data pertaining to the results of the trial conducted with the flower bud explants are given in the Table 35 and Plate 35

Table 35 Influence of different levels of NAA and BAP on callus induction and growth on flower bud explants of gladiolus

Basal medium Modified MS
Culture period 6 weeks

Treatments NAA (mg l^{-1})	BAP	Time taken for callusing (days)	Percentage of cultures callused (P)	Growth score (G)	Callus index (CI)	Nature of callus
5 0	0 0		0 0	0	0	
5 0	0 5	19 0	100 0	1	100	Seen at the base of the buds and friable nature
5 0	1 0	19 0	100 0	1	100	
5 0	2 0	19 2	80 0	1	80	
10 0	0 0		0 0	0	0	
10 0	0 5	18 0	80 0	1	80	
10 0	1 0	17 0	80 0	1	80	
10 0	2 0	16 0	80 0	1	80	
20 0	0 0		0 0	0	0	
20 0	0 5	17 0	100 0	1	100	
20 0	1 0	16 0	100 0	1	100	
20 0	2 0	17 0	80 0	1	80	

Observations were taken from ten cultures

Plate 34 Regeneration of shoot buds from the callus derived from inflorescence segments of gladiolus in medium supplemented with BAP 0 5 mg l ^{1} and NAA 0 25 mg l ^{1}

Plate 35 Callus induction on gladiolus flower buds in modified MS medium supplemented with NAA 5 0 mg l ^{1} and BAP 0 5 mg l ^{1}

Number of days for callus induction

The days ranged from 16 0 to 19 2 The minimum number of days (16 0) for callus induction was taken by the treatment having NAA 10 0 mg l^{1} + BAP 2 0 mg l^{1} NAA 20 0 mg l^{1} + BAP 1 0 mg l^{1} The maximum days (19 2) for callus induction was taken by the medium containing NAA 5 0 mg l^{1} + BAP 2 0 mg l^{1}

Callusing percentage

Callusing percentage ranged from 80 0 to 100 0 in different cultures Cent per cent callusing of the cultures were obtained in the modified MS medium supplemented with NAA 5 0 mg l^{1} + BAP 0 5 mg l^{1} NAA 5 0 mg l^{1} + BAP 1 0 mg l^{1} NAA 20 0 mg l^{1} + BAP 0 5 mg l^{1} and NAA 20 0 mg l^{1} + BAP 1 0 mg l^{1} The treatments devoid of cytokinins failed to induce callus The rest of the treatments have shown 80 0 per cent callusing

Callus index

Callus index ranged from 80 0 to 100 0 The maximum callus index was observed in the treatments having NAA 5 0 mg l^{1} + BAP 0 5 mg l^{1} NAA 5 0 mg l^{1} + BAP 1 0 mg l^{1} NAA 20 0 mg l^{1} + BAP 0 5 mg l^{1} and NAA 20 0 mg l^{1} + BAP 1 0 mg l^{1}

The medium supplemented with NAA alone at 5 0 mg l^{1} 10 0 mg l^{1} 20 0 mg l^{1} failed to induce callus and hence the callus index was zero The rest of the treatments viz NAA 5 0 mg l^{1} + BAP 2 0 mg l^{1} NAA 10 0 mg l^{1} + BAP

0 5 mg l^{1} NAA 10 0 mg l^{1} + BAP 1 0 mg l^{1} NAA 10 0 mg l^{1} + BAP 2 0 mg l^{1} NAA 20 0 mg l^{1} + BAP 2 0 mg l^{1} have shown a callus index of 80

The callus produced in all the treatments were whitish and friable type and produced from the stalk side

4 2 2 3 1 2 2 Callus differentiation

The callus produced by the flower buds failed to differentiate

4 2 2 3 1 3 Flower bud bracts

4 2 2 3 1 3 1 Effect of NAA and BAP

Data showing the influence of NAA and BAP on the callus initiation of the flower bud bracts are given in the Table 36 and Plate 36

Days taken for callus induction

Days taken for callus induction of the flower bud bracts ranged from 16 0 to 21 0 The minimum days (16 0) for callus production was taken by the medium supplemented with NAA 20 0 mg l^{1} and NAA 20 0 mg l^{1} + BAP 0 5 mg l^{1}

Maximum days (21 0) for callus initiation was observed in treatment having 10 0 mg l^{1} NAA + BAP 1 0 mg l^{1} NAA

Callusing percentage

The percentage of cultures callused ranged from 10 0 to 80 0 Maximum percentage (80 0) of callusmg was observed in the cultures in the treatments having

Table 36 Influence of NAA and BAP on callus induction and growth on the bracts of flower buds of gladiolus

Basal medium Modified MS
Period 6 weeks

Treatments NAA (mg l⁻¹)	BAP	Time taken for callusing (days)	Percentage of cultures (P)	Growth score (G)	Callus index (CI)
10 0	0 0	0 0	0 0	0	
10 0	0 5	20 0	60 0	1	60
10 0	1 0	21 0	80 0	1	80
10 0	2 0	20 0	60 0	1	60
15 0	0 0	18 0	10 0	1	10
15 0	0 5	19 0	80 0	1	80
15 0	1 0	19 5	60 0	1	60
15 0	2 0	20 0	40 0	1	40
20 0	0 0	16 0	10 0	1	10
20 0	0 5	16 0	80 0	1	80
20 0	1 0	17 0	80 0	1	80
20 0	2 0	20 0	80 0	1	80

Observations were taken from ten cultures

modified MS medium supplemented with 20 0 mg l ^{1} NAA + 0 5 mg l ^{1} BAP NAA 10 0 mg l ^{1} + BAP 1 0 mg l ^{1} NAA 15 0 mg l ^{1} + BAP 0 5 mg l ^{1} 20 0 mg l ^{1} NAA + BAP 0 5 mg l ^{1} NAA 20 0 mg l ^{1} + BAP 1 0 mg l ^{1} and NAA 20 0 mg l ^{1} + BAP 2 0 mg l ^{1}

The medium supplemented with 15 0 mg l ^{1} NAA and 20 0 mg l ^{1} NAA resulted in callus induction 10 0 per cent cultures The treatment having 10 0 mg l ^{1} NAA failed to induce callus

Callus index

Callus mdex was maximum (80) in treatments containing NAA 10 0 mg l ^{1} + BAP 1 0 mg l ^{1} NAA 1 50 mg l ^{1} + BAP 0 5 mg l ^{1} NAA 20 0 mg l ^{1} + BAP 0 5 mg l ^{1} NAA 20 00 mg l ^{1} + BAP 1 0 mg l ^{1} and NAA 20 0 mg l ^{1} + BAP 2 0 mg l ^{1}

4 2 2 3 1 4 Corm internodal pieces and leaf pieces

The corm internodal pieces failed to induce callus under various treat ments The leaf pieces taken at different stages of its development also failed to induce callus

4 2 2 3 1 5 Corm axillary buds and cormal tips

The callus obtained m Stage 2 (Plate 37 and 38) of the first route (Table 11) were subjected to differentiation and the data are presented in Tables 37 to 39

4 2 2 3 1 5 1 Effect of media on callus differentiation

Data on the influence of various media on callus differentiation are given in Table 37 and Plate 39

Table 37 Effect of media on the differentiation of callus derived from corm axillary buds and cormel tip cultures of gladiolus

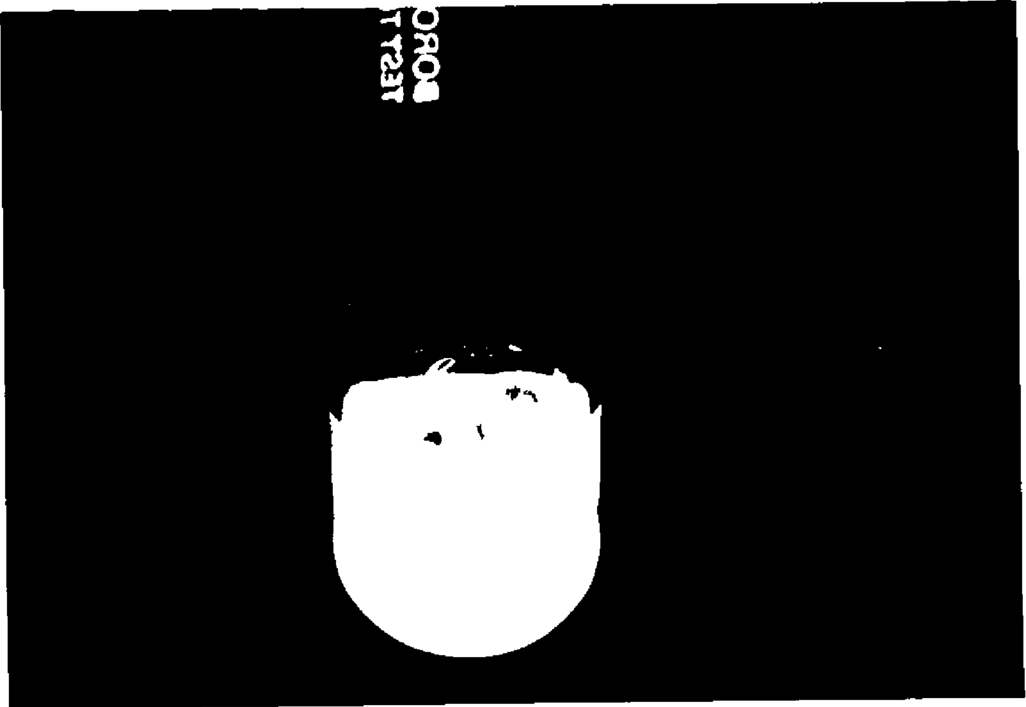
Culture period 3 weeks

Treatment	Time taken for differentiation (days)	Number of shoots	Number of roots produced
MS_a	18 4	3 1	8 8
MS_b	20 0	2 6	14 2
SH	22 0	2 5	15 0
White s	0 0	0 0	0 0

Observations were taken as an average of twenty cultures

Plate 36 Callus induction on flower bud bracts of gladiolus in modified MS medium supplemented with NAA 20 0 mg l $^{-1}$ and BAP 0 5 mg l $^{-1}$

Plate 37 Callus derived from corm axillary bud explants of gladiolus



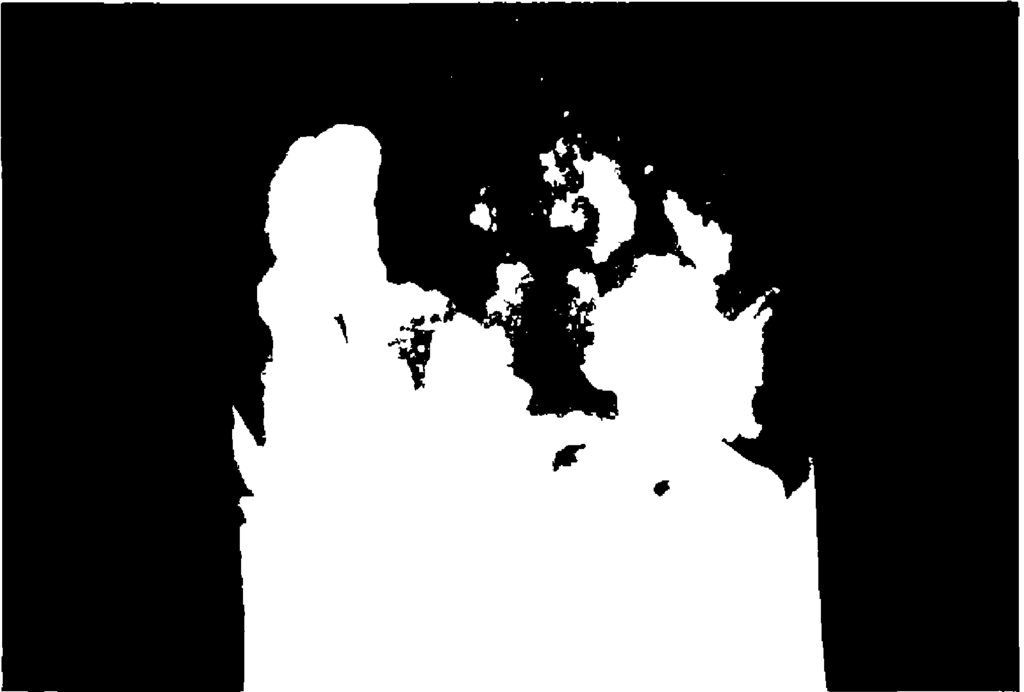
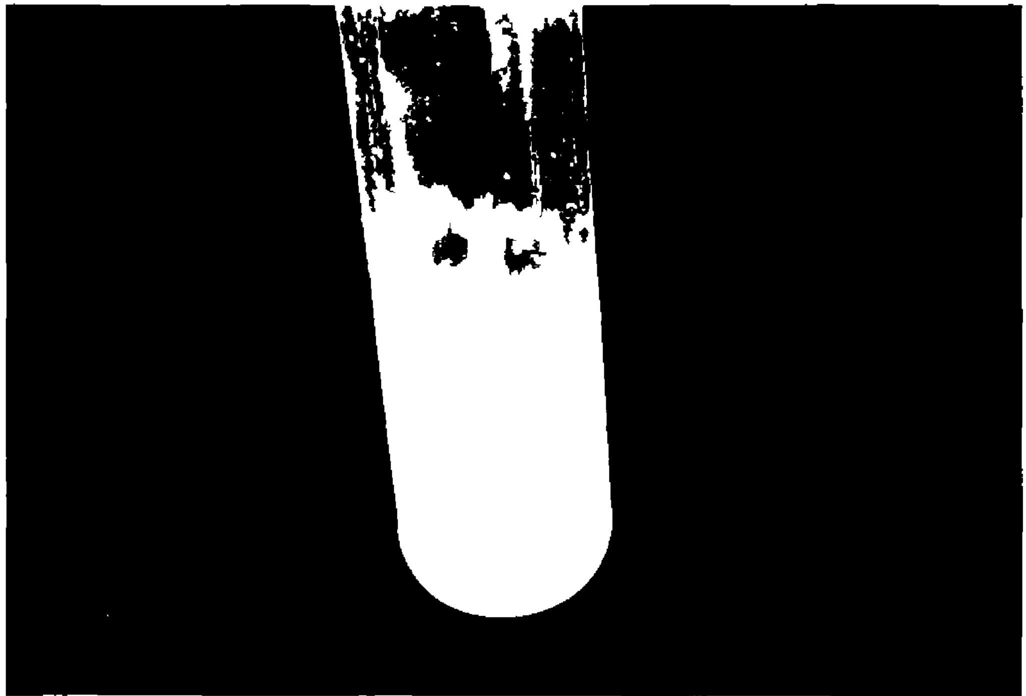


Plate 38 Callus derived from cormel tip explants of gladiolus

Plate 39 Regeneration of shoot buds from corm axillary bud derived callus of gladiolus in MS basal medium



Number of days taken for differentiation

Treatment showed significant variation with respect to the days taken for differentiation

The average number of days taken for callus differentiation ranged from 18 4 to 22 0 The MS full strength medium has shown the earliest differentiation (18 4 days) The SH medium has taken the maximum days (22 0) followed by half strength MS medium (20 0 days)

Number of shoots

The number of shoots produced in various treatments ranged from 2 5 to 3 1 The maximum number of shoots (3 1) was observed in MS full strength medium and the minimum number in SH medium (2 5) The number of shoots produced by half strength MS was 2 6

Number of roots

Treatments showed significant variation and the average number of roots varied from 8 8 to 15 0 and the maximum number of roots observed in SH medium and the minimum in full strength MS MS half strength medium produced an average root number of 14 2

The callus failed to differentiate in White s medium (Table 37)

4 2 2 3 1 5 2 Effect of medium supplements

As the MS full strength salt medium was observed to be an ideal basal

medium for callus differentiation the trials were conducted with coconut water at different levels in this medium The influence of coconut water on callus differentia tion of the corm axillary bud derived callus are given in Table 38 and Plate 40

Number of days taken for differentiation

The average number of days taken for differentiation ranged from 12 2 days to 18 4 days and showed significant variation

The minimum days for callus differentiation was taken by the MS medium having 20 0 ml l ^{1} coconut water and was found to be on par with the medium having 50 0 ml l ^{1} coconut water (14 6) Maximum days was taken by the control ie the medium without coconut water (18 4)

Number of shoots

The number of shoots ranged from 2 0 to 6 4 and showed significant variation among treatments Maximum number of shoots (6 4) observed in full strength MS medium supplemented with 20 0 ml l ^{1} coconut water was found to be on par with that of media having 50 0 ml l ^{1} coconut water (4 6) The minimum number of shoots were observed in the medium having 150 0 ml l ^{1} coconut water and was on par with control (3 1)

Number of roots

With respect to the number of roots all the treatments differed signifi cantly Average number of roots ranged from 6 2 to 19 0 Maximum number of roots (19 0) were observed in medium having 150 0 ml l ^{1} coconut water and the minimum in the medium having 20 0 ml l ^{1} coconut water and it differed signifi cantly from all other treatments

Table 38 Effect of coconut water on differentiation of the callus derived from corm axillary buds and cormel tip cultures of gladiolus

Basal medium MS
Culture period 3 weeks

Coconut water (ml l $^{-1}$)	Time taken for differentiation (days)	Number of shoots	Number of roots
20 0	12 2	6 4	6 2
50 0	14 6	4 6	15 6
150 0	15 4	2 0	19 0
Control	18 4	3 1	8 8
CD (0 05)	2 4	1 9	2 2
SEm+	3 25	1 96	2 80

4 2 2 3 1 5 3 Effect of cytokinins

Number of days for differentiation

The average number of days taken for differentiation of callus ranged from 18 4 to 28 8 (Table 39) and differed significantly

The minimum days (18 4) for differentiation was recorded in the medium devoid of growth regulators and was on par with the treatments having BAP 0 5 mg l ^{1} and BAP 0 25 mg l ^{1} as they took 19 4 and 20 9 days respectively to differentiate

The number of days taken for differentiation was maximum (28 8) when MS medium was supplemented with 2ip 0 50 mg l ^{1} and was homogeneous with the MS medium supplemented with 2ip 0 25 mg l ^{1} (28 6)

Number of shoots

The average number of shoots produced in various treatments ranged from 3 1 to 14 1 and showed significant variation among treatments

The maximum number of shoots (14 1) was observed in the medium supplemented with BAP 0 50 mg l ^{1} and it differed significantly from the rest of the treatments

The medium devoid of growth regulators produced the minimum number of shoots (3 1) and was homogeneous with the treatments having 2ip 0 25 mg l ^{1} (3 6) 2ip 0 50 mg l ^{1} (3 8) and kinetin 0 50 mg l ^{1} (5 8)

The medium having 0 25 mg l^{1} BAP (7 0) was on par with the media having kinetin 0 25 mg l^{1} (5 8) and kinetin 0 50 mg l^{1} (4 7)

Number of roots

The number of roots ranged from 1 8 to 8 8 in various treatments with significant variation among treatments The maximum number of roots were found in the control (8 8) and differed significantly from other treatments The roots produced were minimum (1 8) in the treatment having 0 5 mg l^{1} kinetin and was on par with the root numbers produced by kinetin 0 25 mg l^{1} (2 4) 2ip 0 5 mg l^{1} (2 5) and 2ip 0 25 mg l^{1} (2 8)

The medium supplemented with 0 25 mg l^{1} BAP and 0 5 mg l^{1} BAP failed to produce roots (Table 39)

4 2 2 3 1 6 Root pieces

Root pieces collected from *in vitro* cultures as well as from the field after surface sterilisation were inoculated to MS medium containing various levels of growth regulators The results are presented in Tables 40 to 42

4 2 2 3 1 6 1 Effect of BAP and NAA

Data pertaining to the response of the root piece explants (*ex vitro* and *in vitro*) are given m the Table 40

Days taken for callus induction

The days taken for callus induction of the root segments ranged from 28 to 34 The explants taken from the *in vitro* produced roots took 28 days to develop

Table 39 Effect of lower levels of cytokinins on the differentiation of callus derived from axillary buds and cormel tip cultures of gladiolus

Basal medium MS

Culture period 4 weeks

Treatments (mg l $^{-1}$)		Time taken for differentiation (days)	Number of shoot buds	Number of roots
Control		18 4	3 1	8 8
BAP	0 25	20 9	7 0	0 0
	0 50	19 4	14 1	0 0
Kinetin	0 25	23 2	5 8	2 4
	0 50	21 6	4 7	1 8
2ip	0 25	28 6	3 6	2 8
	0 50	28 8	3 8	2 5
CD (0 05)		3 0	2 7	1 3
SEm±		5 51	4 37	0 984

Plate 40 Effect of coconut water on regeneration of shoot buds from corm axillary bud derived callus

Plate 41 Regeneration of shoot buds from corm axillary bud derived callus in MS medium containing BAP 0 5 mg l^{-1} (a) and in MS medium containing kinetin 0 25 mg l^{-1} (b)

Table 40 Effect of BAP and NAA on the callus initiation and growth of gladiolus roots

Basal medium MS
Culture period 6 weeks

Treatments BAP	NAA (mg l-1)	*In vitro* roots Callusing (%)	Amount of callus	Days taken	*In vivo* roots Callusing (%)	Amount of callus	Days taken
0 0	0 0	0 0	0	0	0 0	0	0
0 0	1 0	0 0	0	0	0 0	0	0
0 0	2 0	0 0	0	0	0 0	0	0
1 0	0 0	0 0	0	0	0 0	0	0
1 0	1 0	40 0	+	34	0 0	0	0
1 0	2 0	50 0	++	28	10 0	+	32
2 0	0 0	0 0	0	0	0 0	0	0
2 0	1 0	0 0	0	0	0 0	0	0
2 0	2 0	0 0	0	0	0 0	0	0

Observations were taken as an average of ten cultures

0 No response
\+ Only the initiation of callus
++ Medium callus growth

callus when the MS medium was supplemented with 1 0 mg l ^{1} BAP and 2 0 mg l ^{1} NAA (Plate 43) In the same combination *ex vitro* root explants has taken 32 days In the MS medium supplemented with 1 0 mg l ^{1} BAP and 1 0 mg l ^{1} NAA the *in vitro* produced root explants had taken 34 days to respond

Percentage of callusing

In the case of *in vitro* root explants the maximum percentage of cultures callused in the treatment having 1 0 mg l ^{1} BAP and 2 0 mg l ^{1} NAA (50 0%) followed by the MS medium supplemented with BAP 1 0 mg l ^{1} and NAA 1 0 mg l ^{1} (40 0%) *Ex vitro* root explants initiated callus only in 10 0 per cent cultures m the MS medium supplemented with 1 0 mg l ^{1} BAP and 2 0 mg l ^{1} NAA (Table 40)

Callus growth

The callus produced had medium growth in MS medium supplemented with 1 0 mg l ^{1} BAP and 2 0 mg l ^{1} NAA for *in vitro* root explants (Plate 44)

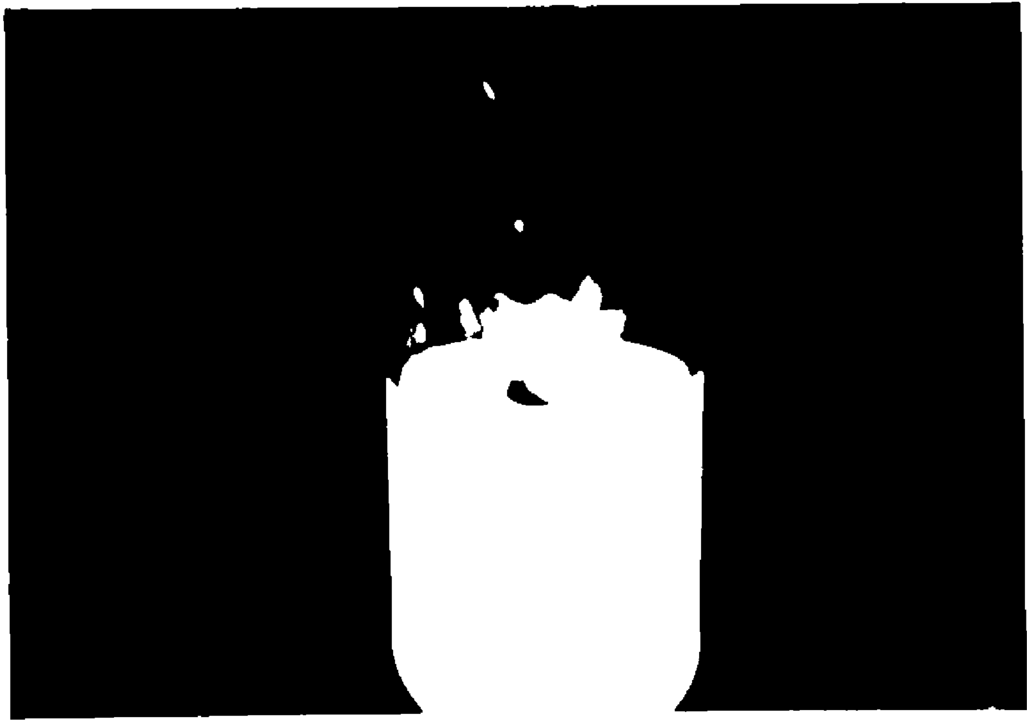
In vivo root explants have only shown the initiation of callus in the MS medium having 1 0 mg l ^{1} BAP and 2 0 mg l ^{1} NAA Initiation of callus on the *in vitro* root explants was also noticed m the MS medium having 1 0 mg l ^{1} NAA 1 0 mg l ^{1} BAP

4 2 2 3 1 6 2 Callus differentiation

The two month old callus derived from the root tissues were subcultured on a series of MS medium supplemented with varying levels of NAA and BAP for organogenesis (Table 41) The callus when cultured on MS medium without growth

Plate 42 Regeneration of shoot buds from the callus derived from the cormel tip explants in MS medium supplemented with BAP 0 5 mg l^{-1}

Plate 43 Callus derived from root segments of gladiolus in MS medium supplemented with NAA 2 0 mg l^{-1} and BAP 1 0 mg l^{-1}



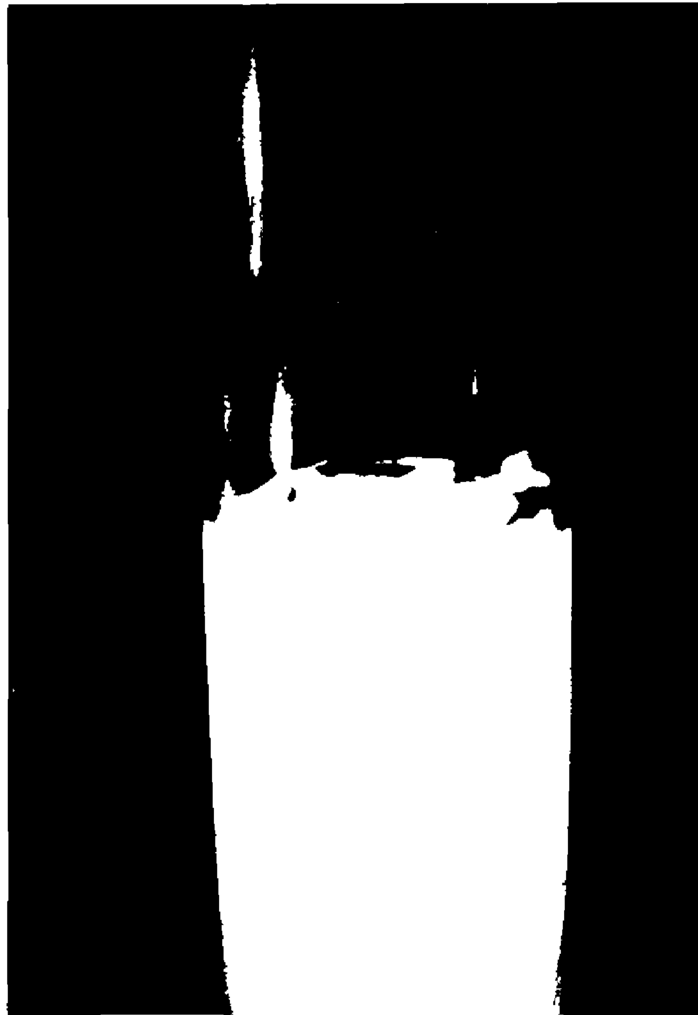


Table 41 Morphogenic response of root derived callus in gladiolus to NAA and BAP

Basal medium MS
Culture period 5 weeks

Treatments (mg l⁻¹) NAA	BAP	Response
0 0	0 0	Rhizogenesis
0 0	1 0	No response
0 0	3 0	No response
1 0	0 0	Low callus growth
1 0	1 0	Medium callus growth
1 0	3 0	Shoot organogenesis

Observations were as an average of ten cultures

Plate 44 Growth of root derived callus in MS medium containing NAA 2 0 mg l^{-1} and BAP 1 0 mg l^{-1}

Plate 45 Rhizogenesis from the root derived callus in MS basal medium

regulators resulted in rhizogenesis (Plate 45) BAP or NAA when used alone did not result in organogenesis even after 3 4 cycles of subculture When MS medium with 3 mg l^{-1} BAP and 1 0 mg l^{-1} NAA was used shoot primodia were developed within 30 days (Plate 46)

The medium sequence for regeneration of root derived callus in gladiolus is given in Table 42 The elongation of these shoot primordia resulted when the growth regulators were completed removed from the medium within 21 days Root development also took place in the basal medium (Plate 47) Extensive root development occurred in the MS medium supplemented with 2 0 mg l^{-1} IBA

4 2 3 Somatic embryogenesis

Data showing results of the trial with boot leaf explants are presented Table 43 and Plate 48

About 10 0 per cent of the cultures produced somatic embryoes in the modified MS medium supplemented with 15 0 mg l^{-1} NAA and 1 0 mg l^{-1} BAP Embryogenesis and callus production was observed after 3 months of dark culture The embryoes later turned to callus when the sub culturing was delayed (Plate 49)

4 3 *In vitro* corm production

4 3 1 Influence of sucrose concentration and auxin levels on *in vitro* corm production in gladiolus

The elongated shoots from the Stage 2 were separated and individually inoculated to liquid MS medium containing different levels of sucrose (2 0 3 0 5 mg l^{-1}) alone and in combination with NAA (0 5 and 1 0 mg l^{-1}) and also with IBA

Table 42 Media sequence for regeneration of root derived callus in gladiolus

Media	Stages of regeneration
MS + BAP 1.0 mg l^{-1} NAA 2.0 mg l^{-1}	Callus initiation and maintenance
MS + BAP 3.0 mg l^{-1} NAA 2.0 mg l^{-1}	Bud initial differentiation
MS basal media	Elongation of buds and root production
MS + IBA 2.0 mg l^{-1}	Root development

Observations were taken from ten cultures

Plate 46 Organogenesis of root derived callus in MS medium containing BAP 3.0 mg l^{-1} and NAA 1.0 mg l^{-1}

Plate 47 Regeneration of shoot buds from root derived callus MS basal medium

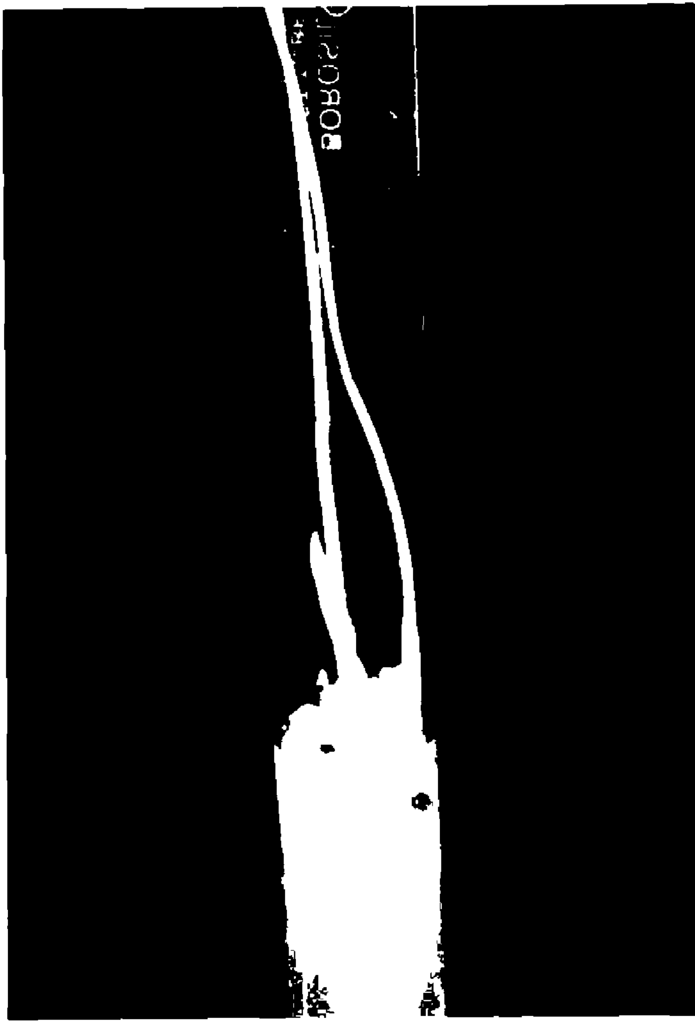


Table 43 Effect of NAA and BAP on somatic embryogenesis on the boot leaf explants of gladiolus

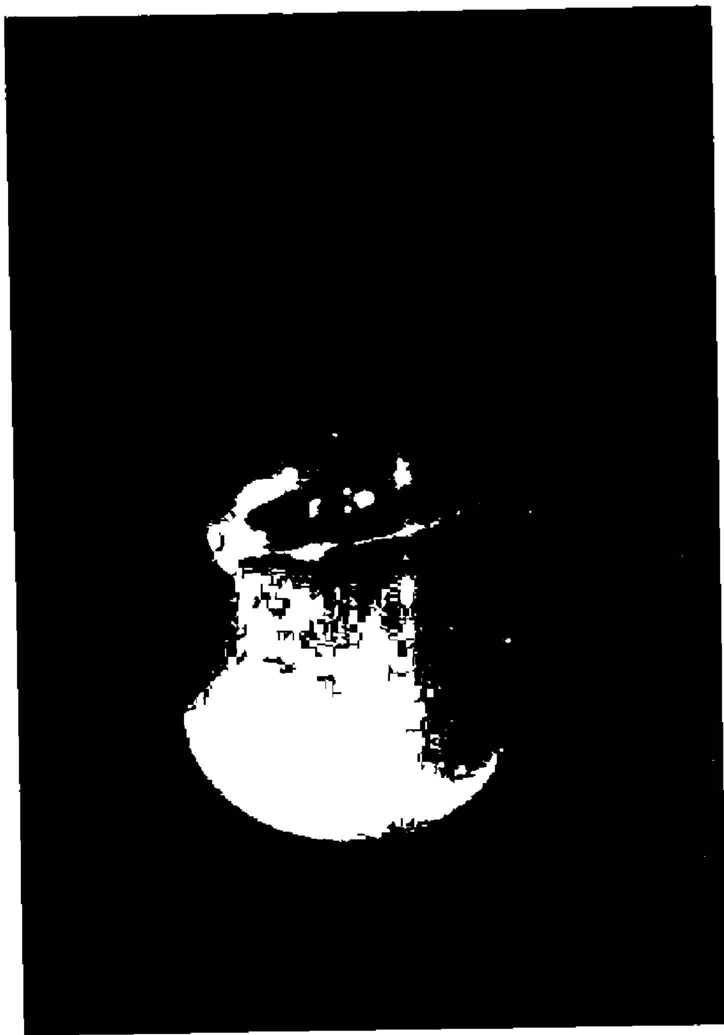
Basal medium Modified MS
Culture period 4 months

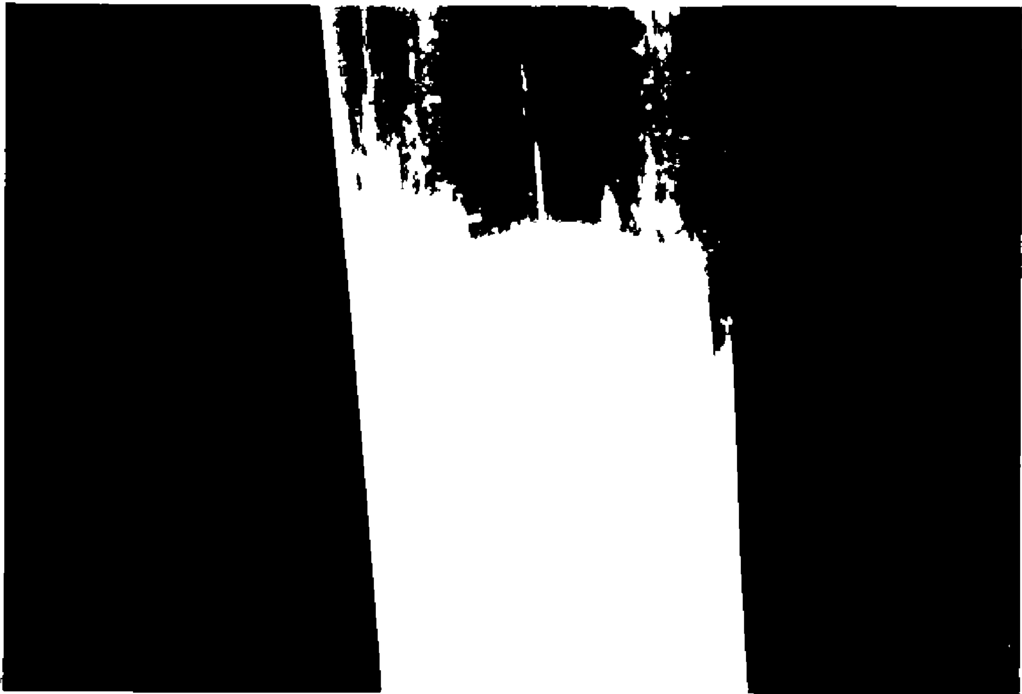
Treatments NAA (mg l^{-1})	BAP	Time taken for response	Cultures responded (%)	Nature of response
10 0	1 0		Nil	
15 0	1 0	3 months	10 0	Somatic embryogenesis and callus production
20 0	1 0		Nil	
10 0	2 0		Nil	
15 0	2 0		Nil	
20 0	3 0		Nil	

Observations were taken from ten cultures

Plate 48 Somatic embryogenesis on leaf segments of gladiolus in modified MS medium containing NAA 15.0 mg l^{-1} and BAP 3.0 mg l^{-1}

Plate 49 Somatic embryo derived from leaf segments of gladiolus kept for germination





(0 5 mg l^{1} and 1 0 mg l^{1}) for corm production The results are presented in Table 44

With respect to the average number of days taken for corm induction the treatments varied from 26 0 to 28 8 Maximum number of days (28 8) was taken by the treatment having 5 0 per cent sucrose and also the treatment containing 5 0 per cent sucrose + 0 5 mg l^{1} NAA

Minimum days (26 0) for the corm induction was recorded in the medium having 3 0 per cent sucrose and 0 5 mg l^{1} NAA

The rate of corm production ranged from 0 0 to 70 0 per cent in different treatments

The treatments containing 2 0 per cent sucrose with and without auxins (IAA and NAA) failed to produce corms

Corm production was maximum (70 0%) when MS medium was supplemented with 5 0 per cent sucrose and 0 5 mg l^{1} NAA This was followed by the medium containing 5 0 per cent sucrose without growth regulators (60 0%)

MS medium supplemented with 5 0 per cent sucrose + 1 0 mg l^{1} NAA 5 0 per cent sucrose + 1 0 mg l^{1} IBA 5 0 per cent sucrose + 0 5 mg l^{1} IBA produced corms in 50 0 per cent of the culture

The corm production was 40 0 per cent in MS medium containing 3 0 per cent sucrose + 0 5 mg l^{1} NAA and with 3 0 per cent sucrose + 1 0 mg l^{1} NAA

Table 44 Influence of sucrose concentration and auxin levels on *in vitro* corm production in gladiolus

Basal medium: MS
Explant: Elongated shoots from Stage 2
Culture period: 4 weeks

Treatments			Cultures producing corms (%)	Time taken for corm production (days)	Corm size (4 weeks after production) (mm)
Sucrose concentration (%)	Auxin levels ($mg\ l^{-1}$)				
	IBA	NAA			
2	0.0	0.0	0.0	0.0	0.0
2	0.5	0.0	0.0	0.0	0.0
2	1.0	0.0	0.0	0.0	0.0
2	0.0	0.5	0.0	0.0	0.0
2	0.0	1.0	0.0	0.0	0.0
2	0.0	0.0	20.0	28.0	2.3
3	0.5	0.0	30.0	26.4	4.0
3	1.0	0.0	30.0	27.1	3.8
3	0.0	0.5	40.0	26.0	4.5
3	0.0	1.0	40.0	26.8	4.6
5	0.0	0.0	60.0	28.8	4.4
5	0.5	0.0	50.0	26.8	4.[illegible]
5	1.0	0.0	50.0	27.4	4.8
5	0.0	0.5	70.0	28.8	6.4
5	0.0	1.0	50.0	28.1	5.0

Mean values are taken from ten observations

MS medium containing 3 0 per cent sucrose and 0 5 mg l^{-1} IBA 1 mg l^{-1} IBA produced corms in 30 0 per cent cultures

MS medium containing 3 0 per cent sucrose without growth regulator induced corms only in 20 0 per cent of the cultures

Size of corms ranged from 2 3 mm to 6 4 mm The corm size was maximum (6 4 mm) when the MS medium containing 5 0 per cent sucrose was supplemented with 0 5 mg l^{-1} NAA (Plate 50) This was followed by the treatment having 5 0 per cent sucrose and supplemented with 1 0 mg l^{-1} NAA (5 0 mm Minimum corm size (2 3 mm) was observed in the treatment containing 3 0 per cent sucrose with growth regulators (2 3 mm)

4 3 2 Influence of triazol and etiolation on *in vitro* corm production in gladiolus

The elongated shoots from Stage 2 were subjected to corm induction treatments For this the shoots were separated and inoculated to MS medium containing 5 0 per cent sucrose + 0 5 mg l^{-1} NAA (as it was found to be superior in the previous treatment) supplemented with 0 1 and 5 mg l^{-1} triadimeton and kept etiolated and in open conditions The results are presented in Table 45

Average number of days taken for corm production ranged from 20 0 to 28 8 The minimum days for corm development was taken by the medium supplemented with 5 0 mg l^{-1} triadimeton and kept under etiolated condition

Days taken for corm production was maximum (28 8 days) when the medium without triadimeton was kept in open condition

Table 45 Influence of triadimefon and etiolation on *in vitro* corm production in gladiolus

Basal medium : MS

Explant : Elongated shoots from Stage 2

Treatments	Cultures producing corm (%)	Time taken for corm production (days)	Corm size (4 weeks after production (mm)
MS + 5% sucrose + NAA 0.5 $mg\ l^{-1}$	70.0	28.8	5.4
+ Etiolation	80.0	25.7	[illegible]
+ Triadimefon 1.0 $mg\ l^{-1}$	80.0	28.0	8.8
+ + etiolation	80.0	25.5	[illegible] 4
+ Triadimefon 5.0 $mg\ l^{-1}$	100.0	21.6	11.2
+ + etiolation	100.0	20.0	12.1

Mean values are taken on ten observations

The percentage of cultures which produced corms was maximum (100 0%) when the medium was supplemented with 5 0 mg l^{-1} triadimefon both in the open and etiolated conditions

About 80 0 per cent of the cultures induced corms in treatments like medium without triadimefon kept under etiolation and medium with 1 0 mg l^{-1} triad imefon kept in open and etiolated condition

Minimum number of cultures (70 0%) produced the corms when the control (medium devoid of triadimefon) kept in open condition

Size of the corms were maximum (12 1 mm) in the medium supplement ed with 5 0 mg l^{-1} triadimefon and kept under etiolated condition (Plate 51) Minimum corm size (5 4 mm) was recorded in the medium devoid of triad mefon and kept in open condition

4 3 3 Corm enlargement studies

The effect of different levels of sucrose on the enlargement of *in vitro* produced corms are given in Table 46 The different levels of sucrose (3% 4% 5% 6% 7% 8% and 9%) tried on MS medium for increasing the size of *in vitro* produced corms showed significant variation after eight weeks The size of the corms ranged from 1 02 cm to 1 82 cm in various treatments and showed signifi cant differences among treatments The corm size was maximum (1 82 cm) in the MS medium supplemented with 8 0 per cent sucrose (Plate 52) which was on par

Plate 50 Effect of sucrose 5 0 per cent and NAA 0 5 mg l ^{1} on *in vitro* corm production on elongated shoots of gladiolus in MS medium

Plate 51 Effect of sucrose 5 0 per cent NAA 0 5 mg l ^{1} and Triadimefon 5 0 mg l ^{1} on *in vitro* corm production

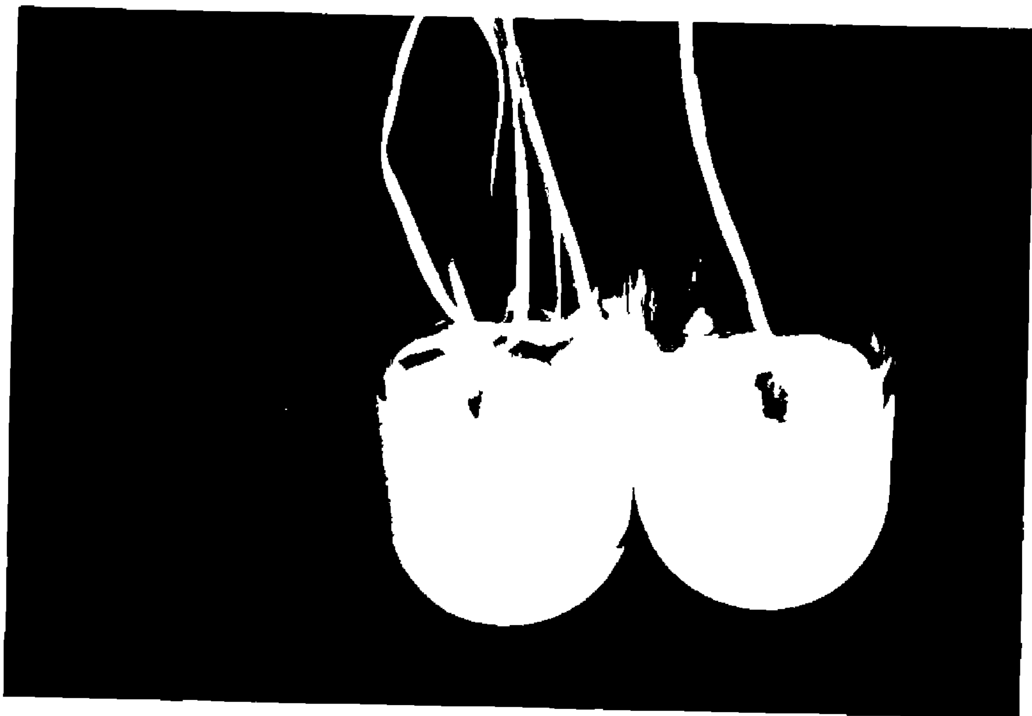


Table 46 Effect of sucrose at different levels on the corm enlargement

Basal medium MS
Culture period 8 week

Levels of sucrose (%)	Corm size at the time of inoculation (cm)	Number of roots produced	Corm size after 8 weeks (cm)
3 0	0 2	5 0	1 04
4 0	0 2	5 0	1 02
5 0	0 2	6 8	1 30
6 0	0 2	7 4	1 30
7 0	0 2	7 2	1 30
8 0	0 2	5 0	1 82
9 0	0 2	2 8	1 60
CD (0 05)		1 3	0 34
SEm+		1 010	0 069

with the medium c ntaining) 0 per cent sucrose (1 60 cm) C rm size was mini mum (1 02 cm) in the medium containing 4 0 per cent sucrose and this did not differ significantly from the corm sizes in media having 3 0 per cent sucrose (1 04 cm) 5 0 per cent sucrose (1 30) 6 0 per cent sucrose (1 3) and 7 0 per cent sucr se (1 30)

Significant differences were also noticed among the number of r ots pro duced in various treatments as it ranged from 2 8 (medium containing 9 0% sucrose) to 7 4 (medium containing 6 0% sucrose) which was on par with the root numbers 7 2 6 8 produced by the media having 7 0 per cent sucrose and 5 0 per cent su crose respectively

The plantlets in all the treatments dried 8 weeks after in oculation (Tal le 46)

In another trial light was excluded from the basal portion of the cultures containing different levels of sucrose and the data recorded are presented in Table 47 The number of roots produced varied from 6 2 t) 9 0 in different treatı e t showing significant differences among treatments Maximum number () 0) w ı recorded in the medium containing 6 0 per cent sucrose and was n par w tl numbers of medium having sucrose 7 0 per cent (8 8) 5 0 per cent (7 6) and 8 per cent (8 8) sucrose

The minimum number of roots were observed in the treatment l avii g 3 0 per cent sucrose (6 2) followed by 4 0 per cent sucrose (6 9) 9 0 per sucrose (6 8) 8 0 per cent sucrose (7 6) and 5 0 per cent sucr se (7 6) wh l w ı on par

Table 47 Effect of sucrose at different levels and exclusion of light at the basal portion of culture tubes on corm enlargement

Basal medium MS
Culture period 8 week

Levels of sucrose (%)	Corms size at the of inoculation (cm)	Number of roots produced	Corm size after 8 weeks (cm)
3 0	0 2	6 2	1 10
4 0	0 2	[illegible]	1 52
5 0	0 2	7 [illegible]	1 [illegible]
6 0	0 2	9 0	1 40
7 0	0 2	8 8	1 50
8 0	0 2	7 6	1 66
9 0	0 2	6 8	1 76
CD (0 05)		1 5	0 31
SEm+		1 4	0 058

The size of corms also differed significantly in various treatments Maximum corm size (1 76 cm) was recorded in the medium supplemented with 9 0 per cent sucrose (Plate 53) and was homogeneous with corm sizes in the medium supplemented with 8 0 per cent sucrose (1 66 cm) 4 0 per cent sucrose (1 52 cm) and 7 0 per cent sucrose (1 50 cm) The corm size was minimum (1 10 cm) in the medium containing 3 0 per cent sucrose and it did not vary significantly from the size of corms produced in the media containing 5 0 per cent sucrose (1 2 cm) and 6 0 per cent sucrose (1 4 cm)

The plantlets in different cultures dried after 8 weeks

4 3 3 1 Effect of sucrose and triazols (triadimefon) on corm enlargement

The trials were conducted with liquid MS medium and the results are presented in Table 48

The average size of the corms after 8 weeks of culture ranged fr m 1 30 to 2 37 cm with significant variation among treatments

The maximum (2 37 cm) size of the corms was observed in the MS liquid medium supplemented with sucrose 5 0 per cent and triadimefon 3 0 mg l (Plate 54) and was on par with the corm sizes of 2 24 cm (Sucrose 5 0% + Tr ad mefon 4 0 gm l ^{1}) 2 18 cm (Sucrose 5 0% + Triadimefon 5 0 mg l ^{1}) 2 1 (Sucrose 5 0 % + Triadimefon 2 0 mg l ^{1}) and 2 0 cm (Sucrose 5 0 mg l ^{1} Triadimefon 1 0 mg l ^{1})

The corm size was minimum (1 30 cm) in the medium supplem t d w th 3 0 per cent sucrose and 3 0 mg l ^{1} triazol and was ho n geneous w t e

Plate 52 Effect of different levels of sucrose on corm enlargement (8 0% (a) 7% (b) 6% (c) 5% (d))

Plate 53 *In vitro* corm enlargement of gladiolus in MS medium supplemented with 9 0 per cent sucrose and kept under exclusion of light

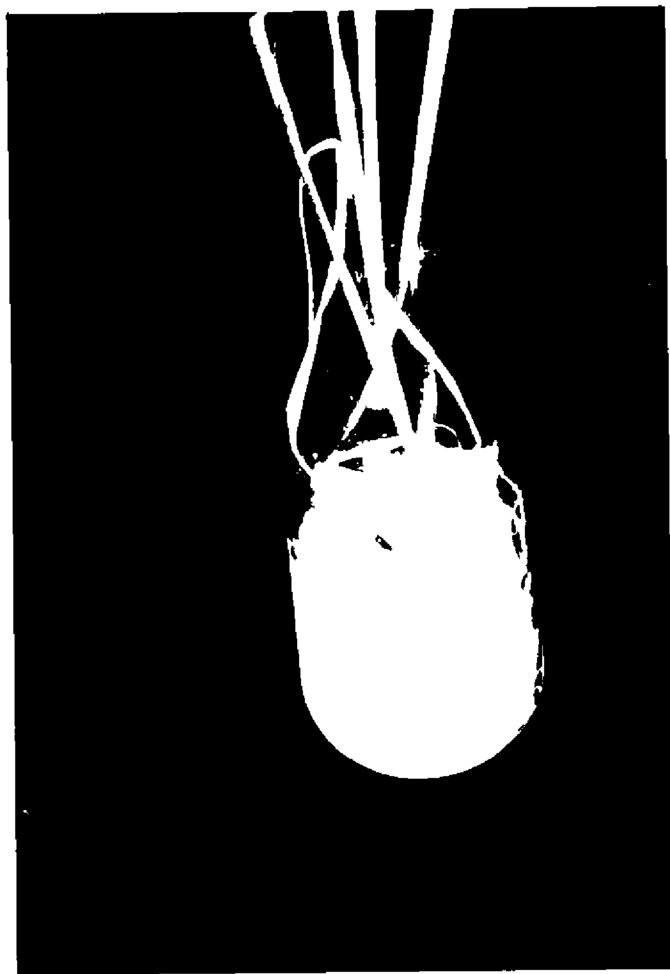


Table 48 Effect of different levels of sucrose and triadimefon on corm enlargement

Basal medium Liquid MS
Culture period 8 week

Treatments		Size of corm at the time of inoculation (cm)	Corm size after 8 weeks (cm)
Sucrose (%)	Triadimefon (mg l⁻¹)		
3 0	1 0	0 2	1 50
3 0	2 0	0 2	1 60
3 0	3 0	0 2	1 30
3 0	4 0	0 2	1 60
3 0	5 0	0 2	1 60
5 0	1 0	0 2	2 00
5 0	2 0	0 2	2 10
5 0	3 0	0 2	2 37
5 0	4 0	0 2	2 [illegible]4
5 0	5 0	0 2	2 18
CD (0 05)			0 31
SEm+			0 06

Table 48 Effect of different levels of sucrose and triadimefon on corm enlargement

Basal medium Liquid MS
Culture period 8 weeks

Treatments		Size of corm at the time of inoculation (cm)	Corm size after 8 week (cm)
Sucrose (%)	Triadimefon ($mg\ l^{-1}$)		
3 0	1 0	0 2	1 50
3 0	2 0	0 2	1 60
3 0	3 0	0 2	1 30
3 0	4 0	0 2	1 60
3 0	5 0	0 2	1 60
5 0	1 0	0 2	2 00
5 0	2 0	0 2	2 10
5 0	3 0	0 2	2 37
5 0	4 0	0 2	2 24
5 0	5 0	0 2	2 18
CD (0 05)			0 31
SEm+			0 06

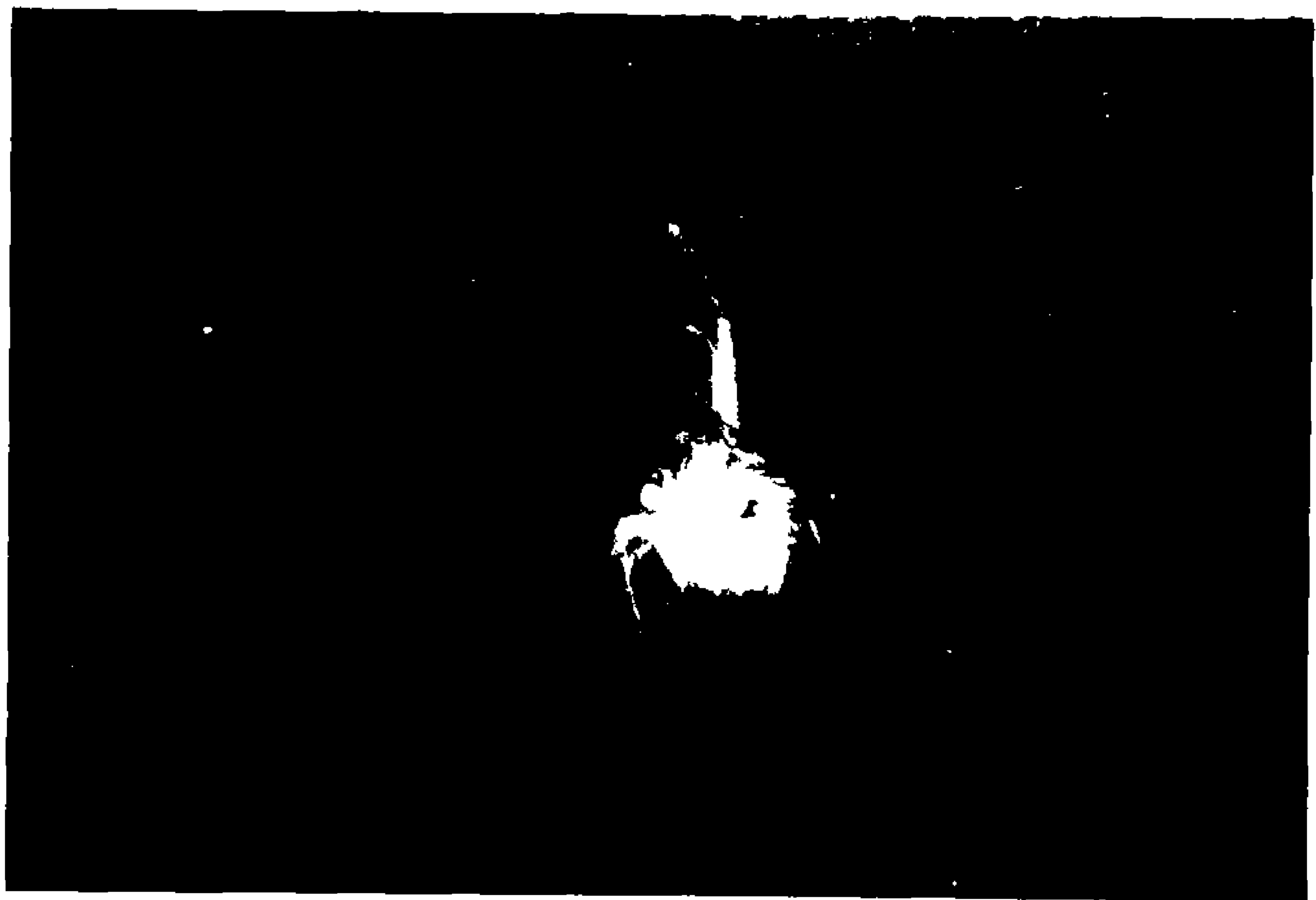
treatment having 3 0 per cent sucrose + 1 0 mg l ^{1} NAA (1 50 cm) sucr se 3 0 mg l ^{1} + Triadimefon 2 0 mg l ^{1} (1 60 cm) sucrose 3 0 per cent + triadimefon 4 0 mg l ^{1} (1 60 cm) sucrose 3 0 per cent +Triadimefon 5 0 mg l ^{1} (1 60 cm)

One notable feature was that the plantlets survived without drying even after 15 weeks

The *in vitro* produced corms germinated in fine sand (Plate 55) and also under *in vitro* conditions (Plate 56)

Plate 54 Corm enlargement in MS liquid medium supplemented with sucrose 5 0 per cent and Triadimefon 3 0 mg l^{-1}

Plate 55 *In vitro* produced corm germinated in fine sand



Discussion

DISCUSSION

The present investigations on the response of gladiolus to rapid cloning through *in vitro* techniques were carried out at the Plant Tissue Culture Laboratory attached to the All India Co ordinated Floriculture Improvement Project (AICFIP) of Department of Pomology and Floriculture College of Horticulture Vellanikkara during 1992 94 The results of the study are discussed in this chapter

Gladiolus belongs to the family Iridaceae of monocotyledon and is an important commercial flower cultivated for its attractive spike

Propagation of gladiolus is normally carried out through corms and cormels By this conventional method the rate of multiplication is extremely slow as the cormels or daughter corms produced by the mother corm are very less in number and also due to their smallness in size they require 3 4 seasons to develop into an ideal size to produce a marketable spike as the spike size is highly influenced by the corm size

Other problems faced by the *in vivo* propagation are the presence of dormancy which last for 3 4 months and non availability of disease free planting materials These problems necessitated an alternative method of propagation which helps in bye passing dormancy bulking up of new cultivars or disease free stocks and production of sufficient materials in a short period for field planting

As the *in vitro* propagation methods were standardised for many of the bulbous plants investigation on these lines also were started in gladiolus by various

workers (Hussey 1975 1976a and 1977 Ziv 1971 and 1979 Simonson and Hil derbrandt 1971 Bajaj *et al* 1983) But compared to other crops the reported works are little The present investigations have thus been carried out to find out the most ideal explant medium medium supplements and hardening techniques for gladiolus

The corms were directly taken from the field and hence the possibilities of impurities contaminants etc were there In order to make the corm and cormel explants free of contaminants surface sterilization was done

Of the various surface sterilants tried mercuric chloride (0 1 and 0 2 %) was found to be ideal Though in literature use of sodium hypochlorite is more common in the surface sterilisation of bulbous plants like garlic (Nagakubo *et al* 1993) narcissus (Hol and Van der Linde 1992) allium (Van der Valk *et al* 1992) and gladiolus (De Bruyn and Ferreira 1992) in the present investigations it was found to be less efficient than mercuric chloride The duration of the treatments varied and it was found that for higher concentration of mercuric chloride the duration of treatment required was less The suitability of mercuric chloride as an ideal surface sterilant has been reported in banana (Bhaskar 1991) ginger (Babu *et al* 1992) and in lily (Priyadarshi and Sen 1992) for the surface sterilisation of rhizomatous and aerial explants

The explants like cormel tips inflorescence axis root pieces leaves etc were also surface sterilized with mercuric chloride and 100 per cent survival at lower duration of treatment was recorded (Table 3)

The season of explant collection has also profound influence on the rate of contamination of the culture and rate of survival in the case of corm and cormel tip explants This is mainly because of the availability of congeneal conditions for growth and development of microorganisms in certain months due to heavy rainfall (Appendix II)

A study was conducted in this regard and it was found that in the case of corm axillary bud explants minimum percentage of contamination and maximum percentage of survival was during April The percentage of contamination was higher during the months from May to August (Table 4)

In the case of cormel tip explants maximum survival rate was obtained during the months of April to May and again during September to November

One of the problems associated with the propagation of gladiolus is the dormancy of the corms Sachs and Thimman (1964) have tried cytokinins to break the dormancy of corms The ability of cytokinins to break the dormancy and to promote their outgrowth under *in vitro* condition has been reported in various dicoty ledons (Murashige *et al* 1974 Boxus 1974 and Earle and Langhans 1975)

The influence of cytokinins on breaking the dormancy of axillary buds under *in vitro* conditions and proliferation of axillary buds have also been reported in various bulbous plants like iris freesia hyacinth lilium and narcissus (Hussey 1975 1976a) The cytokinin BAP prevented the dormancy and promoted the development of resting corms (Hussey 1977)

In the present investigations studies were conducted to find out the effect of various cytokinins like BAP kinetin and 2ip alone and in combination with NAA on breaking the dormancy of corm axillary bud explants and cormel tips and on culture establishment The explants were collected at various stages of corm development ie immediately after the drying of inflorescence one month after the drying of inflorescence two months after the drying of inflorescence and three months after drying of inflorescence The results showed that BAP and its combina tions with NAA were superior with respect to the early bud break and bud elonga tion as compared to the kinetin and its combination with NAA (Tables 5 to 9) Though 2ip was considered to be the most efficient cytokinin (Murashige 1974) and the favourable effect of 2ip over kinetin and BAP have been reported (Johnson and Burchette 1991) on the multiple shoot production of *Blandfordia grandiflora* (Lilia ceae) different levels of 2ip and their combinations with different levels of NAA resulted in delayed bud break and delayed elongation of the buds in the case of axi llary bud explants and cormel tip explants of gladiolus

The days taken for the bud emergence were reduced drastically as the corms matured after the drying of inflorescence Higher levels of BAP recorded lesser days for bud emergence after breaking the dormancy

The inclusion of NAA however could not induce early bud emergence often resulted in swelling of the buds and inhibited shoot growth A similar response has also been reported by Hussey (1976a)

The number of axillary shoots from the axillary buds also varied in dif ferent cytokinins and also in explants taken at different stages of corm development

BAP at higher concentrations produced maximum number of shoots in all the Stages and in combination with NAA however have shown reduction in the number of shoots Similar effect was reported by Hussey (1976a) too

The effect of kinetin and NAA on the growth responses of the axillary bud and the cormel tips cultured in nutrient media was reported by Bajaj *et al* (1983) The present investigations with kinetin and NAA have also shown that kinetin could break the dormancy and could enhance the release of axillary buds but as compared to BAP and its combination with NAA the efficiency was less

The effect of 2ip and its combination with NAA was not identifical with that of BAP and kinetin At lower concentrations of 2ip the axillary buds emerged only after 20 25 days in Stage I The production of single shoots and subsequent swelling at the base of the shoots were also observed in Stages II III and IV In combination with NAA 2ip produced fleshy roots and inhibited the elongation of shoots

The cormel tip explants also followed the trend of corm axillary bud explants in breaking dormancy and in producing axillary shoots The production of multiple axillary buds also could be observed when auxins were added to the medium along with BAP Higher levels of BAP and kinetin in combination with lower levels of NAA resulted in high frequency of multiple axillary bud production Bajaj *et al* (1983) could produce only six plantlets from one cormel through *in vitro* techniques

The culture media also influenced the success of *in vitro* propagation Normally it is possible to employ the same medium throughout Stages 1 and 2 The

medium in Stage 1 should enable the survival *in vitro* The medium m Stage 2 should enable the multiplication of the propagule The medium in Stage 3 should enable the process of rhizogenesis of the propagules (Murashige 1974)

Murashige and Skoog s (1962) medium developed for tobacco has been used for the *in vitro* culture of different plant species (Bonga 1980)

The medium used for the micropropagation of gladiolus varied with the explants and routes tried Ziv *et al* (1970) used modified MS medium for inflores cence segments for callus production and subsequent organogenesis The present investigations carried out using MS medium and modified MS medium gave similar results Simonson and Hilderbrandt (1971) also used the modified MS medium for callus production from corm and cormel tip explants

For the micropropagation of the members of the family Iridaceae Lilia ceae and Amaryllidaceae Hussey (1975 and 1976b) employed full strength MS medium Half strength MS medium in which iron was added as ferrous ethylenedia mine sulphate (25 0 mg l ^{1}) was used by Hussey (1977) for *in vitro* release of axi llary buds from corm explants of gladiolus Ziv (1979) used full strength MS medium supplemented with 100 0 mg l ^{1} myoinositol 0 4 mg l ^{1} thiamine hydro chloride 1 0 mg l ^{1} nicotinic acid 1 0 mg l ^{1} pyrodoxine 2 0 mg l ^{1} kinetin and 0 1 mg l ^{1} NAA for the propagule multiplication and half strength MS medium supplemented with 0 4 mg l ^{1} thiamine hydrochloride 0 5 mg l ^{1} NAA and sucrose (half the requirement ie 15 0 mg l ^{1}) for the pretransplanting medium The root growth and number of roots were more in the half strength MS medium which lacked the cytokinin

MS full strength medium for the micropropagation of gladiolus was suggested by various workers (Bajaj *et al* 1983 Dickens *et al* 1986 Lilien kipnis and Kochba 1987 Dantu and Bhojwani 1987 Kim *et al* 1988 Kamo *et al* 1990 Ziv 1990 Arora and Grewal 1990 Steinitz *et al* 1991 and De Bruyn and Ferreira 1992)

The present investigations were carried out with three media viz MS White s and SH The culture establishment trial was conducted in these media with corm axillary bud explants supplemented with BAP 3 0 mg l ^{1} MS medium was found to be superior with respect to the time taken for sprouting for elongation of shoots and the number of shoots produced The favourable effects of bud bursting and multiple shoot production by cytokinins had been reported by Murashige (1974)

Hussey (1976a) reported enhanced release of axillary buds by incorporat mg BAP 0 008 to 32 000 mg l ^{1} into the MS medium The maximum rate of branching observed was 1 to 5 per plantlet at lower concentrations of BAP At higher concentrations ie 2 000 mg l ^{1} highly distorted plantlets with numerous branches were produced and crowns later became swollen and callus like This indicated that the outgrowths were truely axillary as only few branches were pro duced with distichous arrangement at lower levels of BAP But higher levels of BAP promoted secondary axillary bud production as the apical dominance was progressively reduced in both the main shoot and released laterals as a result of pile up of growing axillaries leading to crowding and non distichous arrangement Ziv (1979) obtained enhanced release of axillary buds in MS medium supplemented with kinetin 2 0 mg l ^{1} and NAA 0 1 mg l ^{1} without callus production

Repeated proliferation of axillary buds was observed by Lilien kipnis and Kochba (1987) when the apical and lateral buds of gladiolus corms and cormels were inoculated to MS medium supplemented with low levels of NAA and BAP or kine tin

In the present investigation when the elongated buds from Stage 1 were inoculated to MS medium containing different levels of cytokinins (BAP Kinetin 2ip) and their combinations with different levels of NAA production of multiple axillary buds and callus were observed (Tables 11 12 and 13) The treatments having lower levels of BAP and their combinations with lower levels of auxin produced only multiple axillary buds and a very high rate of multiple axillary bud production could be observed with lower levels of BAP in combination with lower levels of NAA without any callus production As the concentration of BAP and NAA increased callus production was also increased with gradual reduction in the rate of multiple axillary bud production This is in confirmation with the findings of Hussey (1976)

It was also found that the rate of axillary bud production and callusing was lesser with kinetin and NAA Medium rate of multiple axillary bud production was also observed in all the levels of kinetin Inclusion of auxin (NAA) at higher levels with kinetin induced callus Higher levels of kinetin with lower levels of NAA resulted in the swelling of the shoot base and rhizogenesis Ziv 1979 and Bajaj *et al* (1983) also observed rhizogenesis when combination of higher levels of kinetin and lower levels of NAA was used in the media

Low rate of multiple axillary buds production and callus production were observed at higher concentrations of 2ip Callus production was also observed when the auxins were incorporated with 2ip Lower levels of 2ip resulted in the elongation of single shoots while its combination with NAA resulted in rhizogenesis

Frequent subculturing in MS medium containing BAP 2 0 mg l^{1} and NAA 0 5 mg l^{1} and BAP 2 0 mg l^{1} induced very high rate of multiple axillary bud production (Table 14)

Combination of higher levels of BAP and NAA also produced multiple axillary buds and at the same time callusing was also noticed Contrary to this the bud aggregates obtained when transferred to MS medium containing low concentra tion of BAP produced normal shoots (Hussey 1976a) By subculturing successively with reduced levels of BAP he obtained normal rooted plantlets

Investigations were carried out for the elongation of the bud aggregates using MS basal medium with different levels of BAP and NAA Both MS and half strength MS medium without the growth regulators induced elongation of shoots and production of normal roots (Table 15) This was found to be in confirmation with the findings of Kim *et al* (1988) The medium containing the growth regulators enhanced further multiplication of the multiple axillary buds and callus production

The treatments having NAA in both the media resulted in rapid conver sion of the multiple axillary buds to callus This is in confirmation with the findings of Hussey (1975) who also reported the role of auxin in enhancing callus produc tion (Hussey 1975) The addition of NAA inhibited shoot growth and enhanced

callus formation the extent of callus formation was higher when the level of auxin was near to that of BAP (Hussey 1977)

In the present study with the different basal media like full strength MS salt medium half strength MS salt medium SH salt medium and White s salt medium the elongation of multiple axillary buds were observed in all the media tested Elongation of buds were earlier in half strength MS medium The elongation of bud aggregates into normal shoots in MS medium has also been reported by Hussey (1977) and Ziv (1979) The production of roots were earlier in SH half strength MS and full strength MS medium More days for initiation of roots were taken by White s medium (Table 16)

The highest number of shoots was formed in full strength MS medium Similar results have been obtained in Pineapple (Prabha 1993) The maximum shoot length was also observed in MS full strength medium However the number roots were maximum in SH medium

The media further differed in the nature of roots produced Normal roots with root hairs were obtained in full strength MS medium and half strength MS medium Ziv (1979) also obtained normal roots in the half strength MS medium The roots produced in SH medium were found be to thick unbranched and tapering nature

Medium supplements like coconut water and activated charcoal have been used for the growth and differentiation of excised tissues and organs of several crops The beneficial effect of coconut water has been attributed to the presence of cytokinin like substances (Straus and Rodney 1960) Coconut water contains a

number of cell division factors and free aminoacids (Shantz and Steward 1952) In the present investigation when the MS full strength medium was supplemented with coconut water to study the effect of coconut water on the production of normal shoots from multiple bud aggregates it has been observed that as the concentration of the coconut water increased the days taken for shoot elongation also increased Though the presence of coconut water in the medium enhanced early rooting and increased the number of roots all the roots produced were devoid of root hairs Coconut water also reduced the number of shoots (Table 17)

Addition of activated charcoal to the medium resulted in early induction of roots and also increased number of roots (Table 17) This is in confirmation with the finding of Ziv (1979) as he could improve the root production and growth by adding 0 3 per cent activated charcoal to the pre transplanting medium However the present investigation revealed the reduction in shoot length and shoot number in the presence of activated charcoal

Investigations conducted in the field of plant tissue culture had shown that *in vitro* rooting could be successfully achieved by reducing the salt concentration in the media particularly in high salt media like MS and its derivatives (Kartha *et al* 1974 Lane 1979 Skirvin and Chu 1979) Half the concentration of MS medium was found to be inducing rooting in banana without affecting the shoot growth unlike in certain species (Wang 1978 Gupta *et al* 1981) Balachandran (1993) reported cent per cent rooting of banana cultures in both half and full concentration of MS medium Elongated shoots of gladiolus rooted easily when transferred to half strength MS medium supplemented with 0 5 mg l^{1} NAA and 15 0 g l^{1} sucrose (Ziv 1979)

In the present study where the experiments were conducted on the elongated shoots of Stage 2 using different media (full strength MS half strength MS liquid full strength MS liquid half strength MS and SH) earlier root induction was observed in liquid full strength MS and liquid half strength MS medium (Table 19) This is in line with the findings of Arora and Grewal (1990) However the number of roots were higher in full strength MS medium and SH medium Length of the roots was also found to be more in solid full strength MS medium

Normal roots with branching habit were observed in MS full strength and half strength semi solid media In liquid full strength and half strength media roots with more root hairs were observed Thick roots were observed in SH medium

Hussey (1976a and 1977) reported the inhibition of root growth in gladiolus when BAP concentration was increased above 0 12 mg l ^{1} The present investigation also revealed that the rooting of elongated shoots is not possible in cytokinin rich media This coincides with the statement of Yeoma (1986) According to him all cytokinins inhibit root induction and BAP which is widely used for shoot multiplication does so particularly strongly that roots are delayed even after tranferring to a cytokinin free medium

Ancora *et al* (1981) reported the effectiveness of IBA and NAA on root induction of *in vitro* produced plants In the present study conducted with different levels of sucrose in combination with various levels of NAA and IBA lower levels of auxins (0 5 mg l ^{1} or 1 0 mg l ^{1} of IBA and NAA) induced early rooting (Tables 20 and 21)

Number of roots were found to be more in media having lower levels of IBA in combination with higher levels of sucrose than the treatments having lower levels of NAA and higher levels of sucrose

Length of roots was also found to be superior in the treatments having lower levels of IBA and NAA in combination with higher levels of sucrose

Normal roots with branching habit were observed when the medium was supplemented with lower levels of IBA and higher levels sucrose (IBA 0 5 mg l^{1} + sucrose 3 0 per cent) or in the combination of 3 0 per cent sucrose with 0 5 mg l^{1} IBA or 1 0 mg l^{1} IBA

The role of NAA at lower levels alone or in combination with kinetin on the rooting of gladiolus cultures was reported by many workers (Ziv 1979 Bajaj *et al* 1983 and Dickens *et al* 1986) In the present study it has been found that lower levels of IBA is most ideal for rooting According to the present study lower levels of sucrose adversely affected rooting as more number of roots early root initiation etc were recorded in treatments having higher levels of sucrose and lower levels of auxins This contradict the findings of Ziv (1979) asshe has observed profuse rooting of elongated gladiolus shoots with the half concentration of sucrose in the media

According to Ziv (1979) the rooting of *in vitro* shoots of gladiolus was faster when the shoots inoculated to pre transplanting media were kept under high light intensities In this study the exclusion of light increased the number of roots length of roots etc The roots produced were normal at lower concentrations of auxin (IBA) under etiolated condition

Rumynin *et al* (1990) used BAP free medium supplemented with IAA 0 1 mg l ^{1} and activated charcoal (AC 5 0 g l ^{1}) for rooting of *in vitro* produced shoots In the present study conducted with different concentrations of activated charcoal that at 0 3 per cent induced more number of roots (Table 23) This is in confirmation with the findings of Ziv (1979)

The success of tissue culture depends on the establishment of *in vitro* produced plants in natural condition Under *in vitro* condition the plants will be heterotrophs and they have to gradually get converted into autotrophs The harden ing is the process of making *in vitro* raised plantlets adapted to the outside environ ment Brainered and Fuchigami (1981) and Fabbri *et al* (1984) have reported the improper development of cuticle on the tissue cultured plants Another problem was the improper connection between the root and shoots of *in vitro* plantlets The plants have to undergo both morphological and physiological adaptation so as to enable them the typical terrestrial plant water control mechanism for which a period of humidity acclimatisation was considered necessary (Grout and Aston 1977 Sutter *et al* 1985)

Bhaskar (1991) and Balachandran (1993) have modified the techniques for hardening of *in vitro* banana plants Better results could be obtained when the roots of the plantlets were dipped in 0 1 per cent Bavistin for 5 minutes and the plantlets covered with microscope cover after planting Poor survival of transplanted plants of gladiolus was mainly because of the poorly developed roots orginating actually from the basal part of the original explant and not from the newly formed plantlets and also because of the absence of direct vascular connection between the

roots and shoots (Ziv *et al* 1970) Hardening of the plantlets could be possible by transferring the plantlets into a pre transplanting medium with half strength MS salt mixture 15 0 per cent sucrose 0 5 mg l ^{1} NAA and 0 3 per cent activated charcoal (Ziv 1979) which increased the survival percentage of plantlet by preventing desic cation after transplanting The method also helped in the production of non dormant corms

In this present investigation transplanting studies have shown that the maximum survival percentage of plantlets was observed in the case of plantlets treat ed with 0 2 per cent Bavistin soon after the removal from the culture vessels fol lowed by treatment with 0 2 per cent Mancozeb and norfloxacin at the time of plant ing post planting treatment with 1/10th strength MS solution in alternate days drenching with triadimefon 20 0 mg l ^{1} solution at 3 days interval and then keeping the plantlets under an improvised mist chamber Under this conditions up to 50 0 per cent survival was observed in the case of plantlets planted in coarse sand in mud pots Later corm production were also reported Fifty per cent of the survived plantlets produced corms of sufficiently larger size when they were planted in the plastic pots using coarse sand as the medium (Table 25)

Among the various explants tried for somatic organogenesis inflores cence axis segments taken before the actual emergence from the plants were found to be ideal with respect to the callus initiation and growth direct organogenesis and callus differentiation (Table 27 to 32) Ziv *et al* (1970) used the explants from inflorescence axis before emergence for callus induction and regeneration Bajaj *et al* (1983) and Kamo *et al* (1990) have also reported best callus production from segments of the flower stalks

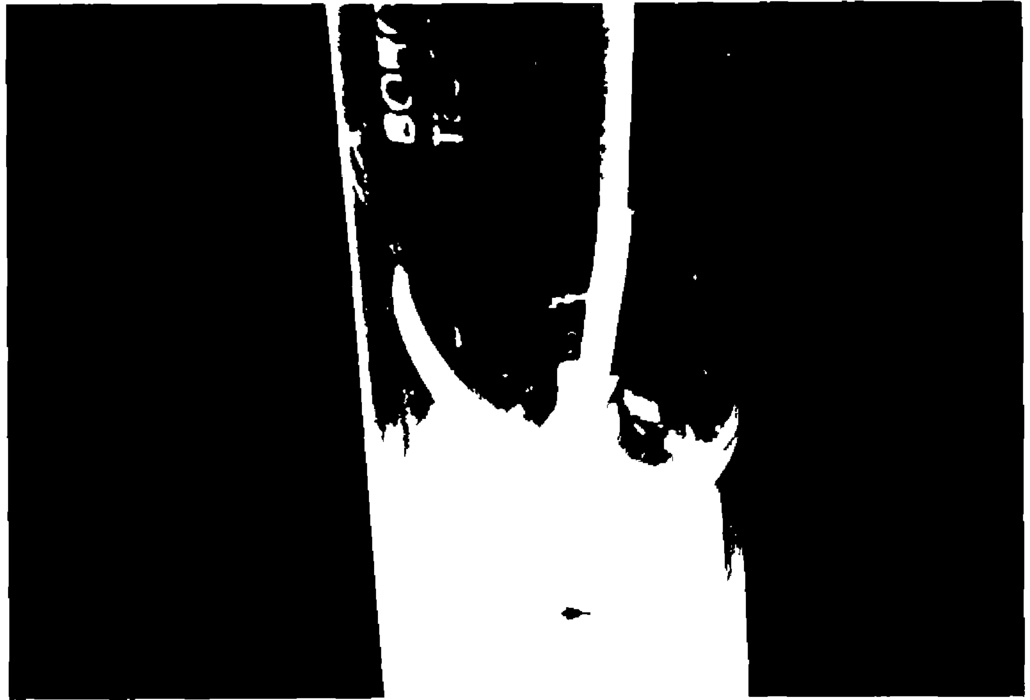
Bajaj *et al* (1983) could induce callus from bracts denuded flower etc In the present study also cent per cent cultures of flower buds and 80 0 per cent cultures of bracts induced callus (Tables 32 and 33 respectively) *In vitro* and *in vivo* root pieces also induced callus but the percentage of cultures responded was very less (Table 37) Leaf explants according to Bajaj *et al* (1983) failed to induce callus Callus induction from innermost leaf explants was recorded by Babu *et al* (1992) in ginger In the present study conducted in gladiolus also the innermost leaf explants responded (Table 40) It is reported that the success in callus induction of the flower stem axis of tulip depends on the stage of development of floral stems (Alderson *et al* 1983) In gladiolus too the stage of development of the mflores cence was important for callus induction Bajaj *et al* (1983) and Ziv *et al* (1970) have also reported proliferation of callus from the immature stage of the inflores cence In the present study conducted with the immature and mature inflorescence axis the mature inflorescence axis segment produced large number of roots from the basal callus (Plate 57) and it failed to induce morphogenetic response while culture of immature inflorescence segment responded well

Direct organogenesis from the inflorescence axis segments was recorded in the presence of higher levels of NAA and lower levels of BAP and kinetin Direct morphogenesis in about 50 0 per cent cultures was reported when the modified MS medium was supplemented with 15 0 mg l^{1} NAA and 3 0 mg l^{1} BAP (Table 27)

Direct rhizogenesis was also observed in many treatments the highest being m the modified MS medium having high levels of auxins alone Combinations of NAA with kinetin also resulted in the same response but the rate or per cent

Plate 56 *In vitro* produced corm germinated under *in vitro* condition

Plate 57 Response of mature inflorescence segments to somatic organogenesis



cultures which pr duced m rph genesis were lesser (Table 25) Direct rg n g i sis especially morphogenesis was not reported in gladiolus so far In ginger direct organogenesis from the immature inflorescence was reported in revised MS medium supplemented with 10 0 mg l^{-1} BAP and 0 2 mg l^{-1} 2 4 D (Babu *et al* 1992)

The cultures of inflorescence segments produced callus in the m dified MS medium supplemented with various levels of NAA and cytokinins (BAP and kinetin) both in 16 h photoperiod and in complete darkness The percentage of cultures which developed callus were increased as the concentration of the NAA increased Combinations of NAA with BAP resulted in high callus index c mpared to the combinations of NAA with kinetin (Tables 29 to 32) The results of this study agree with those of Ziv *et al* (1970) and Bajaj *et al* (1983) as they could induce callus from the inflorescence segments in MS medium supplemented with NAA and kinetin

Cultures kept in the darkness also had the same trend but the response was early

Differentiation of inflorescence derived callus was made p ssible by transferring this callus to MS medium supplemented with NAA 0 1 mg l^{-1} and kinetin 2 0 mg l^{-1} (Bajaj *et al* 1983) The regeneration of leaf derived callus in ginger was observed in MS medium supplemented with 2 4 D and cytokinins BAP or kinetin The cultures when transferred to the basal MS medium resulted in rhiz genesis

In the present study earlier differentiation of callus int sh t bud wa found in the medium supplemented with different levels of BAP (0 5 t 3 mg l^{-1}

Different levels of kinetin in the media could also differentiate the callus but took more time In ginger also it is reported that the differentiation of callus on MS medium supplemented with low levels of BAP and the transferring of these shoot buds to media containing different concentrations of NAA and BAP developed shoots (Malamug *et al* 1991)

Different levels of 2ip also could differentiate the callus but the rate of rhizogenesis and shoot production was very little The number of shoots were greater in the medium having BAP compared to that having kinetin and 2ip

Differentiation of the inflorescence derived callus was also made possible by the combinations of NAA and cytokinins (BAP and kinetin)

Flower buds and bracts taken from the immature inflorescence of gladiolus also callused from the stalk portion and gave a callus index of 100 00 and 80 00 respectively in some of the combinations of high levels of NAA with lower level of BAP Production of callus from flower stalk and bracts was reported in gladiolus by Bajaj *et al* (1983) too in MS medium supplemented with NAA 10 0 mg l^{-1} kinetin 0 5 mg l^{-1}

Callus obtained from the corm axillary bud and cormel tip explants differentiated into shoots and roots in various treatments Among the various basal media tried MS full strength medium was found to be the most effective one with respect to early differentiation and number of shoot buds produced However addition of coconut water into the medium at a rate of 20 0 ml l^{-1} resulted in more number of shoot buds and earlier differentiation Further increase in the concentration of the coconut water did not increase the number of shoot (Tables 37 to 39)

Supplementing the medium with lower levels of cytokinins (BAP kinetin and 2ip) however increased the number shoots during callus differentiation Among the cytokinins BAP was found to be most effective This contradicts the findings of Kim *et al* (1988) suggesting a cytokinin free medium for the successful regeneration of gladiolus callus derived from the corm explants cultured in MS medium supplemented with 10 0 mg l ^{1} 2 4 D and 1 0 mg l ^{1} kinetin Cytokinin free media for regeneration of inflorescence derived callus was also proposed by Kamo *et al* (1990) as they found the differentiation of inflorescence derived callus of gladiolus cultivars Blue Isle and Hunting Song cultured in MS basal medium But the positive effect of BAP and kinetin on callus differentiation in combination with auxins have been reported in ginger (Babu *et al* 1992) with more number of shoot buds in medium supplemented BAP

So far there are no reports of organogenesis from the root explants of the gladiolus Present study indicated that in MS medium high auxin (NAA) in combination with cytokinin (BAP) can induce callus under 16 h photoperiod (Table 40 Regeneration of root derived callus also made possible (Tables 41 and 42)

Somatic embryogenesis was observed in banana shoot tip callus cultured in MS basal medium supplemented with 2 4 D on prolonged incubation and immediate transfer to MS liquid medium devoid of growth regulators (Banerjee *et al* 1985) In gladiolus somatic embryogenesis has not so far been reported In the present study with leaf explants taken from the inner most leaves however developed callus under prolonged incubation in darkness and it later developed globular bodies These later turned to callus when the subculture was delayed (Table 43)

The gladiolus cultures if left in sucrose medium without subculturing to the pre transplanting medium were found to develop dormant cormels (Ziv 1979) Dickens *et al* (1987) could induce corm production in hardening media introducing an auxin (NAA) at rate of 0 5 mg l $^{-1}$

Transplanting and establishing of *in vitro* produced gladiolus plantlets was not easy and the survival percentage was very less Attempts were carried out to produce *in vitro* corms and to transplant the *in vitro* produced corms directly to planting media Production of the corms in the culture tubes was reported by Ziv (1979) when she retained the cultures without subculturing Similar results were also obtained in the present study (Plate 58) The formation of corms on the basal portion of the *in vitro* shoots was observed even in the presence of auxin (NAA) in the hardening medium (Dickens *et al* 1986) Dantu and Bhojwani (1987) produced *in vitro* corms from axillary buds both in liquid and solid media and observed increased rate of corm production as the concentration of sucrose increased from 3 0 per cent to 6 0 per cent The role of sucrose as an important carbohydrate in the *in vitro* bulb development have also been reported by Taeb and Alderson (1990) in tulips

Ziv (1991) reported the formation of cormlets from the multiple buds of gladiolus derived from bud explants of cormels in the hardening phase with the addition of growth retardent paclobutrazol The formation of corms from the elongated shoots in the basal MS medium supplemented with 1 0 mg l $^{-1}$ IBA was reported by Rao *et al* (1991) In the present study attempt was made to produce large sized corms under *in vitro* conditions It was observed that the presence of sucrose higher level resulted in corm induction majority number of the cultures (Table 44

Plate 58 Corm formation in the culture medium when retained without subculturing

This was in confirmation with the findings of Dantu and Bhojawani (1987) It was also observed that the size of corms induced were larger in media containing auxin (NAA) This may be due to the antogonising action of auxin over residual cytoki nins as the presence of cytokinins in the medium is deleterious for corm formation as reported by Lilien kipnis and Kochba (1987) and De Bruyn and Ferriera (1992) The presence of auxins (NAA or IBA) at lower levels in the medium was also found to favour root production and corm formation (Dickerns *et al* 1986 and Rao *et al* 1991)

Effect of growth retardants in promoting corm production on the elon gated shoots of *in vitro* gladiolus cultures had been reported by Ziv (1990 1991) the proliferating axillary buds when transferred to hardening phase media in presence of paclobutrazol resulted in formation of corms Steinitz *et al* (1991) found that initial treatment with BAP in the medium and successively culturing on medium without growth regulators but with inclusion of paclobutrazol promoted corm development and fresh weight of the corms In the present study conducted with the inclusion of triazol (Triadimefon) 5 0 mg l^{1} into the MS medium supplemented with 5 0 per cent sucrose and NAA 0 5 mg l^{1} resulted in corm production in all the cultures Etiolation of the basal portion of the tubes resulted in early corm induction and increase in corm size upto 12 1 mm (Table 45) The maximum size of *in vitro* corms in the available literature is 8 10 mm (Ziv 1990)

Favourable effect of sucrose on bulb formation in tulip (Alderson and Thaeb 1990) and for corm formation in gladiolus (Dantu and Bhojawani 1987) have been reported Chow *et al* (1992) reported increased percentage of bulbils formation in narcissus from 49 0 per cent to 71 0 per cent by increasing the sucrose

concentration from 3 0 per cent to 6 0 per cent or 9 0 per cent Growth of bulbils was also favoured by increasing sucrose concentration and the presence of low levels of NAA had no deleterious effect In the investigation conducted to study the effect of sucrose on corm size improvement it has been found that increasing the concentration of sucrose has a direct effect on the size of corms (Table 46) The corm size could be increased from 1 04 mm (3 0% sucrose) to 1 82 cm (8 0% sucrose)

The production of roots increased as the concentration of sucrose increased in the medium upto 7 0 per cent thereafter a decrease in the number of roots was observed This contradicts the findings of Ziv (1979) as she observed high rate of root production in the medium having half the normal concentration of sucrose The exclusion of light from the basal portion of the culture vessels however had little effect on the corm elongement although the number of roots increased (Table 47)

In the present study with different levels of sucrose and triazol (Triadimefon) on corm enlargement the corm size could be increased from 0 2 cm to 2 3 cm in liquid MS medium supplemented with 5 0 per cent sucrose and 3 0 mg l^{-1} triazol (Table 48) This is in confirmation with the findings of Steinitz *et al* (1991) who reported that the growth retardant paclobutrazol at 10 0 mg l^{-1} had a positive effect on corm formation and development in the presence of high concentration of sucrose as the presence of paclobutrazol shifted the assimilate allocation towards the growing corm The presence of the triazol also resulted in the production of adventitious corms and the plantlets survived for a long time

The foregoing discussions on the results generated from the present studies indicated the possibility of efficient and faster multiplication of elite clones of

gladiolus *in vitro* circumventing the undesirable effect of dormancy Further stud ies however are necessary especially to refine *ex vitro* techniques and to evaluate field performance

Callus mediated organogenesis offers immense scope for developing variants through induced mutagenesis This would turn to be a highly beneficial tool in breeding of gladiolus *in vitro*

The results of the present studies also point to the possibility of somatic embryogenesis in gladiolus Feasibility of enhancing size of corms *in vitro* is also highly desirable since it reduces the time delay in production of commercially ac ceptable corms

Summary

SUMMARY

Investigations on the response of gladiolus to rapid cloning through *in vitro* techniques were carried out at the Plant Tissue Culture Laboratory attached to the All India Co ordinated Floriculture Improvement Project (AICFIP) College of Horticulture Vellanikkara The main objective was to study the response of various explants of gladiolus and to identify the most suitable explant and media combination for *in vitro* cloning Attempts were also made to increase the size of *in vitro* pro duced corms The results and salient findings are summarised hereunder

The response of various explants of gladiolus was attempted through enhanced release of axillary buds organogenesis and somatic embryogenesis in the present study The different explants tried were corm axillary buds cormel tips inflorescence segments leaves buds bracts and root segments The corm axillary buds and cormel tips were used for the enhanced release of axillary buds and others were tried for somatic organogenesis (direct or indirect)

Treatment with 0 1 per cent mercuric chloride for 20 to 25 minutes or 0 2 per cent mercuric chloride for 15 minutes was ideal for surface sterilisation of corm axillary buds and cormel tip explants

For inflorescence segments and leaf segments treatment with 0 1 per cent mercuric chloride for two minutes and three minutes respectively was found to be ideal

The most ideal season for corm explant collection was April when the percentage survival was 100 00 and in the case of cormel explants the ideal season of collection was April to May and also from September to November

The culture establishment of corm axillary buds was tried at various stages of corm development MS medium supplemented with BAP 4 0 mg l^{1} was found to be ideal for early bud break after breaking dormancy under *in vitro* condi tion Maximum shoot proliferation was observed when MS medium was supple mented with 3 0 mg l^{1} BAP in the case of corms taken out soon after the drying of inflorescence (Stage I)

MS medium supplemented with BAP 3 0 mg l^{1} or BAP 4 0 mg l^{1} was found to be ideal for breaking the dormancy under the *in vitro* condition and resulted in early (5 3 and 5 6 days respectively) bud break Maximum number of shoots (5 7) were produced in the treatment having 3 0 mg l^{1} of BAP in the case of corms harvested after 30 days of drying of inflorescence (Stage II)

In the case of corms harvested after 60 days of drying of inflorescence (Stage III) BAP 3 mg l^{1} induced early bud break (5 0 days) under *in vitro* condi tion Maximum number of shoots (4 6) was obtaianed when 2 mg l^{1} BAP was used

In the case of the axillary buds taken from the corms harvested 90 days after the drying of inflorescence (Stage IV) MS medium supplemented with 2 0 mg l^{1} BAP took less time (2 5 days) for the emergence of bud after breaking the dormancy Maximum number of adventitious shoots (5 10) was obtained when MS medium was supplemented with 3 0 mg l^{1} BAP

In the case of cormels harvested soon after the drying of inflorescence early bud emergence was reported m medium supplemented with 3 0 mg l^{1} BAP and maximum number of shoots was obtaianed in medium supplemented with BAP 4 0 mg l^{1}

In cormel tips taken from the cormels harvested 30 days after drying of inflorescence early bud emergence was obtained when the medium was supplement ed with 1 0 mg l^{1} BAP and 0 5 mg l^{1} NAA (9 68 days) A shoot number of 3 86 was observed in the medium having 4 0 mg l^{1} BAP whereas BAP 3 0 mg l^{1} was found to be ideal for breaking *in vitro* dormancy of cormels harvested sixty days after drying of inflorescence and the number of shoots was the maximum (2 94) m the medium having 4 0 mg l^{1} BAP In the case of cormels taken 90 days after drying of inflorescence BAP 4 0 mg l^{1} was found to be ideal for early bud emer gence whereas for maximum adventitious shoot production BAP 3 0 mg l^{1} was found to be ideal

When the cormel tip cultures were retaianed without subculturing higher levels of BAP and kinetin in combination with NAA at lower levels resulted in the production of multiple axillary buds in all the stages of cormel growth

Very high rate of multiple axillary bud growth was obtained in MS medium supplemented with BAP 1 mg l^{1} and NAA 0 5 mg l^{1} or BAP 2 0 mg l^{1} and NAA 0 5 mg l^{1} when elongated shoots from the cormel tips or corm axillary bud cultures were subcultured Frequent subculturmg into the medium containing BAP 2 0 mg l^{1} and NAA 0 5 mg l^{1} continued production of multiple axillary buds were noticed

Some of the combinations also produced callus High rate of callus production was observed in MS medium when supplemented with 1 0 mg l ^{1} BAP and 0 5 mg l ^{1} NAA or 2 0 mg l ^{1} BAP and 0 5 mg l ^{1} NAA

Elongation of the multiple axillary buds with normal shoot growth and root growth was recorded in full strength MS medium devoid of growth regulators Liquid full strength MS medium took the minimum days (8 3) for induction of roots on elongated shoots Maximum number of roots were observed in solid MS and SH medium and normal roots with branching habit were produced in MS media (both full and half strength)

Inclusion of auxins and different levels of sucrose into the media could not induce early rooting However they could increase the number of roots Maxi mum number of roots (15 8) was observed in treatment having 3 0 per cent sucrose and 0 5 mg l ^{1} NAA Exclusion of light and addition of 1 0 mg l ^{1} IBA also result ed in high rate of root production (19 4)

Maximum survival percentage of the plantlets was obtained when the rooted plantlets were treated with 0 2 per cent Bavistin soon after the removal from the culture vessels followed by treatment with 0 2 per cent mancozeb and norfloxa cin at the time of planting and post planting treatment with 1/10th strength MS salt solution on alternate days and drenching with triazol (Triadimefon) 20 0 mg l ^{1} at three days interval and later on keeping in an improvisied mist chamber Fifty per cent survival of plantlets were obtained under this condition when planted in mud pots containing coarse sand

Inflorescence segments taken before the actual emergence were found to be the most ideal explants for somatic organogenesis

Direct organogenesis from the immature inflorescence axis segments was obtained in modified MS medium supplemented with 15 mg l ^{1} NAA and 3 0 mg l ^{1} BAP

Callus production was maximum in modified MS medium supplemented with high levels of NAA and low levels of BAP compared to the medium supple mented with NAA and kinetin Keeping the cultures under exclusion of light induced early callus production

Differentiation of the callus derived from the inflorescence segments was made possible in MS medium supplemented with lower levels of cytokinins (BAP kinetin 2ip) alone and m combination with NAA Earliest differentiation of callus was possible in MS medium supplemented with 3 0 mg l ^{1} BAP and also m MS medium supplemented with BAP mg l ^{1} + NAA 0 5 mg l ^{1} (17 4 days in both the cases)

Maximum number of shoots was obtained in the medium supplemented with BAP 0 5 mg l ^{1} + NAA 0 25 mg l ^{1} (14 6) while maximum number of roots (9 8) was obtained in the medium supplemented with 2ip 1 0 mg l ^{1}

The callus obtained during the culture of corm axillary buds in the shoot proliferation stage (Stage 2) could differentiate m basal full strength MS medium half strength MS and SH medium devoid of growth regulators The MS full strength medium was found to be superior with respect to earliness and number of shoots Addition of 20 ml l ^{1} coconut water to the medium further increased the earliness

and number of shoot buds Addition of BAP 0 5 mg l ^{1} to the basal medium also could increase the number of shoot buds

Callusing of *ex vitro* and *in vitro* roots was observed in MS medium supplemented with 1 0 mg l ^{1} BAP and 2 0 mg l ^{1} NAA The explants collected from *in vitro* produced roots took 28 days and *ex vitro* root explants took 32 days to develop callus The differentiation of these callus could be obtained when 3 0 mg l ^{1} BAP and 1 0 mg l ^{1} NAA was added to the medium and subsequent transfer of the cultures to medium devoid of growth regulators

Explants collected from the innermost leaves covering the inflorescence underwent somatic embryogenesis when cultured in modified MS medium supple mented with 15 0 mg l ^{1} NAA and 1 0 mg l ^{1} BAP and incubated under darkness Further development of the embryos could not be obtained

In vitro corm induction was maximum when the concentration of the sucrose was increased in the medium Presence of NAA or Triadimefon increased the size of *in vitro* produced corms

Maximum corm size (12 1 mm) was obtained in MS medium supple mented with 5 0 per cent sucrose 0 5 mg l ^{1} NAA and 5 0 mg l ^{1} triazol (Triadi mefon) after four weeks in etiolated condition Minimum corm size (5 4 mm) was recorded in the medium devoid of triadimefon in open condition

The effect of sucrose levels on the enlargement of *in vitro* produced corms was significant The corm size was maximum (1 82 cm) in MS medium supplemented with 8 0 per cent sucrose and was on par with the medium containing 9 0 per cent sucrose (1 60 cm) under 16 hour photoperiod

When light was excluded from the basal portion of the culture tubes maximum corm size (1 76 cm) was obtained in the medium supplemented with 9 0 per cent sucrose and was on par with corm size produced in the medium with 8 0 per cent sucrose (1 66 cm)

The maximum enlargement of *in vitro* produced corms (2 37 cm) was observed in the MS liquid medium supplemented with sucrose 5 0 per cent and triadimefon 3 0 mg l^{1} The plantlets survived without drymg even after 15 weeks The *in vitro* produced corms sprouted both under *in vitro* and *in vivo* conditions

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* Originals not seen

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Appendices

APPENDIX I
Chemical composition of the media

Chemical	Quantity (mg l 1)			
	MS	Modified MS	SH	White s
Macronutrients				
KNO_3	1900 000	1900 000	2500 00	80 0000
NH_4NO_3	1650 000	1650 00		
KH_2PO_4	170 000			
KCl				65 0000
NAH_2PO_4 H_2O		300 700		19 0000
$NH_4H_2PO_4$			300 00	
$MgSO_4$ $7H_2O$	370 000	370 000	400 00	737 0000
$CaCl_2$ $2H_2O$	440 000	440 000	200 00	
$Ca(NO_3)_2$ $4H_2O$				288 0000
Na_2SO_4 $10H_2O$				460 0000
Micronutrients				
H_3BO_3	6 200	6 200	5 00	
$MnSO_4$ $4H_2O$	22 300	22 300		1 5000
$MnSO_4$ H_2O			10 00	0 7500
$ZnSO_4$ $7H_2O$	8 600	8 600	1 00	
$NaMoO_4$ $2H_2O$	0 250	0 250	0 10	
$CuSO_4$ $5H_2O$	0 025	0 025	0 20	0 0010
$CoCl_2$ $6H_2O$	0 025	0 025	0 10	
KI	0 830	0 830	1 00	
$FeSO_4$ $7H_2O$	27 800	27 800	15 00	27 8000
NaEDTA	33 600	33 600	20 00	2 6700
MoO_3				0 0001

Vıtamıns				
Thıamıne HCl	0 100	0 100	5 00	0 1000
Pyrıdoxıne HCl	0 500	0 500	0 50	0 1000
Nıcotınıc acıd	0 500	0 500	5 00	0 5000
Others				
Glycıne	2 000	2 000		3 0000
Myo ınosıtol	100 00	100 00	1000 00	
Sucrose	30 00	30 00	30 00	20 00
Adenene sulphate		10 00		
pH	5 8	5 8	5 5	5 5

MS (Murashıge and Skoog 1962)
SH (Shenk and Hılderbrandt 1972)
Whıte s (Whıte 1943)

APPENDIX II

Monthly mean rainfall distribution during the period of January 1993 to December 1994

Month	Monthly rainfall (mm)
January	0 0
February	6 6
March	0 0
April	32 1
May	131 1
June	700 3
July	661 6
August	288 7
September	85 3
October	519 0
November	74 6
December	18 0

APPENDIX III

Abstract of analysis of variance for the effect of different treatments during culture establishment

Sl No	Characters	Treatment mean squares		Error mean squares	
		Corm axillary buds	Cormel tips	Corm axillary buds	Cormel tips
1	2	3	4	5	6
	Degrees of freedom	11		48	
	1) Effect of BAP and NAA				
	a) Stage I				
	i) Time taken for bud emergence	71 03	38 34	0 565*	1 86*
	ii) Number of shoots	3 56	2 23	0 181*	0 421*
	iii) Time taken for shoot elongation	116 70	15 72	0 815*	1 712*
	b) Stage II				
	i) Time taken for bud emergence	96 12	30 07	1 374*	0 991*
	ii) Number of shoots	8 79	3 36	0 538*	0 222*
	iii) Time taken for shoot elongation	64 75	22 49	2 833*	1 142*
	c) Stage III				
	i) Time taken for bud emergence	4 38	26 63	0 322*	0 886*
	ii) Number of shoots	3 46	1 44	0 201*	0 358*
	iii) Time taken for shoot elongation	5 01	14 87	0 399*	0 995*
	d) Stage IV				
	i) Time taken for bud emergence	1 20	2 514	0 301*	0 541*
	ii) Number of shoots	4 45	0 57	0 215*	0 232*
	iii) Time taken for shoot elongation	1 12	14 10	0 26*	0 584*
	2) Effect of Kinetin and NAA				
	a) Stage I				
	i) Time taken for bud emergence	6 23	30 84	1 226*	2 471*
	ii) Number of shoots	0 73	0 06	0 163*	0 069*
	iii) Time taken for shoot elongation	4 48	9 54	1 339*	1 712*

Contd

Appendix III Continued

1	2	3	4	5	6
b)	Stage II				
i)	Time taken for bud emergence	17 40	8 79	1 850*	1 777*
ii)	Number of shoots	0 80	0 082	0 806*	0 139*
iii)	Time taken for shoot elongation	20 73	16 40	1 979*	1 367*
c)	Stage III				
i)	Time taken for bud emergence	2 98	14 89	0 157*	1 268*
ii)	Number of shoots	1 01	0 132	0 093*	0 027*
iii)	Time taken for shoot elongation	6 58	13 02	1 300*	1 351*
d)	Stage IV				
i)	Time taken for bud emergence	1 11	9 14	0 365*	0 682*
ii)	Number of shoots	0 61	12 00	0 143*	0 162*
iii)	Time taken for shoot elongation	1 54	14 10	0 758*	0 584*
3)	Effect of media				
	Degrees of freedom	2 0		12 0	
i)	Time taken for bud emergence	55 71		0 690	
ii)	Number of shoots	6 25		0 170	
iii)	Time taken for shoot elongation	84 40		0 900	

* Significant at 5% level

APPENDIX IV

Abstract of analysis of variance for the effect of different treatments

Sl No	Character	Treatment mean squares			Error mean squares		
		Effect of media	Effect of coconut water	Effect of acti vated char coal	Effect of media	Effect of coconut water	Effect of acti vated char coal
1)	Elongation of multiple axillary buds						
	Degree of freedom	3	5	4	16	24	20
i)	Time taken for shoot elongation	161 97	37 10	0 91	1 727*	1 575*	2 458
ii)	Time taken for root initiation	25 40	7 73	6 97	2 550*	1 079*	1 155*
iii)	Number of shoots	82 51	23 85	40 55	3 325*	2 714*	1 779*
iv)	Length of shoots	58 72	1 23	18 44	0 997*	1 38	1 289*
v)	Number of roots	35 19	12 12	3 98	5 764*	1 201*	0 966*

Sl No	Character	Effect of media	Effect of IBA and sucr ose	Effect of NAA and sucr ose	Effect of light	Effect of acti vated char coal	Effect of media	Effect of IBA and sucr ose	Effect of NAA and sucr ose	Effect of light	Effect of acti vated char coal
2)	In vitro rooting										
	Degree of freedom	4	8	8	3	3	20	36	36	16	16
i)	Time taken for root initiation	26 91	171 18	121 04	0 88	0 038	0 87*	0 682*	1 049*	0 769	0 473
ii)	Number of roots	20 01	163 67	55 64	236 85	4 39	1 035*	0 758*	0 515*	0 306*	1 585*
iii)	Length of roots	0 54	11 37	10 71	38 04	0 26	0 220*	0 319*	0 143*	0 414*	0 542

Contd

Appendix IV Continued

	Effect of cyto kinins	Effect of cyto kinins and auxin	Effect of cyto kinins	Effect of cyto kinins and auxin
3) Callus differentiation				
a) Inflorescence derived callus				
Degree of freedon	10	3	44	16
i) Time taken for differentiation	7 25	10 31	1 744*	2 600*
ii) Number of shoots	85 68	125 00	2 558*	4 050*
iii) Number of roots	46 80	28 40	0 927*	1 40*

	Effect of cyto kinins	Effect of coconut water	Effect of cyto kinins	Effect of coconut water
b) Corm axillary bud and corm tip derived callus				
Degree of freedom	6	3	28	16
i) Time taken for differentiation	88 08	32 70	5 510*	3 250*
ii) Number of shoots	71 79	18 08	4 370*	1 910*
iii) Number of roots	5 5		3 920*	2 800*

	Effect of sucr ose in light	Effect of sucr- ose in dark	Effect of Triadi mefon	Effect of sucr ose in light	Effect of sucr ose in dark	Effect of Triadi mefon
4) Crom enlargement						
Degree of freedom	6	6	9	28	28	40
i) Corm size	0 23	0 28	0 80	0 069*	0 058*	0 06*
ii) Number of roots	13 47	5 50		1 01	1 400	

*Significant at 5% level

RESPONSE OF GLADIOLUS TO RAPID CLONING THROUGH *IN VITRO* TECHNIQUES

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ABSTRACT OF A THESIS

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ABSTRACT

Investigations were carried out to study the response of gladiolus to rapid cloning through *in vitro* techniques at the Department of Pomology and Floricul ture and Plant Tissue Culture Laboratory of All India Co ordinated Floriculture Improvement Project College of Horticulture Vellanikkara during 1992 94 The main objective was to identify the most suitable explant and media combination for *in vitro* cloning

The explants used were corm axillary buds cormel tips inflorescence nodal segments (for enhanced release of axillary buds) inflorescence internodal segments flower buds flower bud bracts root segments (for somatic organogenesis) and leaf segments (for somatic embryogenesis) The best season for the collection of corm axillary buds and cormel tips was from September to May Surface sterilization of the explants could be effectively done with 0 1 or 0 2 per cent mercuric chloride and the duration of treatment varied from 1 to 25 minutes

Culture establishment of the corm axillary bud cormel tip explants were better in MS medium supplemented with BAP ranging from 1 0 mg l^{1} to 4 0 mg l^{1} The concentration of BAP required varied according to the stage of development of corms and cormels Higher levels of BAP was ideal during early stages of develop ment of corm and cormels Of the different media (White s SH and MS) tried MS medium was found to be the best culture establishment (Stage 1) when supplemented with 3 0 mg l^{1} BAP

Elongated shoots of Stage 1 were subjected to shoot proliferation (Stage 2) Multiple axillary bud production was very high when the MS medium was supple mented with BAP 1 0 mg l^{1} and NAA 0 5 mg l^{1} or BAP 2 0 mg l^{1} and NAA 0 5 mg l^{1} Callus production from the base of the elongated shoots were observed when the concentration of NAA increased in the medium

Of the different cytokinins (BAP kinetin and 2ip) tried BAP was found to be the best in Stage 2 Frequent subculturmg onto the MS medium containing BAP 2 0 mg l^{1} and NAA 0 5 mg l^{1} increased the production of multiple axillary buds These when transferred to the MS medium devoid of growth regulators resulted m elongation of shoots

The elongated shoots produced maximum number of roots in the MS medium containing 1 0 mg l^{1} IBA under the exclusion of light However early root ing was obtained m MS liquid medium devoid of growth regulators

Plantlet survival was maximum when treated with 0 2 per cent Bavistin immediately after removing from the culture vessels followed by treatment with 0 2 per cent mancozeb and norfloxacin at the time of transplanting and post planting treatment with 1/10 MS solution and drenching with triadimefon 20 0 mg l^{1} at three days interval inside improvised mist chamber

Direct organogenesis could be obtained from immature inflorescence segments in modified MS medium supplemented with 15 0 mg l^{1} NAA and 3 0 mg l^{1} BAP

Among the various explants tried for callus mediated organogenesis callus index was the maximum (400) when immature inflorescence segments were

inoculated to the modified MS medium supplemented with NAA 15 0 mg l ^{1} in 16 h photoperiod and also in the medium supplemented with 15 0 mg l ^{1} NAA + 2 0 mg l ^{1} BAP and kept under exclusion of light The callus derived from inflorescence segments differentiated into shoots m the MS medium supplemented with 3 0 mg l ^{1} BAP and also in the medium supplemented with 1 0 mg l ^{1} BAP and 0 5 mg l ^{1} NAA Callus also could be obtained from flower buds and flower bud bracts

The callus derived from the corm axillary buds and cormel tip explants in Stage 2 differentiated in the basal MS medium devoid of growth regulators or supplemented with 20 0 ml l ^{1} coconut water and also m the medium with 0 5 mg l ^{1} BAP

The root segments (both *in vitro* and *in vivo*) produced callus in MS medium supplemented with 1 0 mg l ^{1} BAP and 2 0 mg l ^{1} NAA and the differentia tion was obtained in the medium containing 3 0 mg l ^{1} BAP an 1 0 mg l ^{1} NAA

Leaf segments failed to develop callus However the explants collected from the leaf covering the inflorescence (boot leaf) when cultured in modified MS medium supplemented with 15 0 mg l ^{1} NAA and 1 0 mg l ^{1} BAP and incubated under darkness for three months developed somatic embryoes

In vitro corm production was noticed in the cultures if planting out was delayed Earliest and large sized corm induction was made possible in elongated shoots of gladiolus from Stage 2 in MS medium containing 5 0 per cent sucrose 0 5 mg l ^{1} NAA and 5 0 mg l ^{1} triadimefon kept under etiolated condition The size of the *in vitro* produced corms enlarged from 0 2 cm to 2 3 cm in the MS liquid medium con taining 5 0 per cent sucrose and 3 0 mg l ^{1} triadimefon